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The effect of genetic alterations detected by the circulating tumor DNA-based next-generation sequencing technique on prognosis and survival in metastatic colorectal cancer

Metastatik kolorektal kanserde dolaşan tümör DNA'sına dayalı yeni nesil dizileme tekniği ile tespit edilen genetik değişikliklerin prognoz ve sağ kalım üzerine etkisi

Ahmet Ünlü, Atike Gökçen Demiray, Aydın Demiray, Arzu Yaren, Hakan Akça

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Abstract

Purpose: Studies conducted to date showed that circulating tumor DNA (ctDNA)-based next generation sequencing (NGS) panels are beneficial in the treatment strategies of patients with metastatic colorectal cancer (mCRC). In this study, we planned to determine the frequencies of various genetic alterations in patients with mCRC by ctDNA-based NGS analyses, evaluate the concordance rates by comparing these results with the results in standard polymerase chain reaction (PCR) analyses, and investigate the effect of the detected alterations on overall survival and progression-free survival.

Materials and methods: The study was conducted by retrospective screening and analysis of the data on 48 patients, who were followed up with a diagnosis of mCRC and who received chemotherapy and/or biological agents. The data were analyzed using SPSS 25.0 [IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)] package program.

Results: In this study, ctDNA-based NGS analyses, compared to the quantitative PCR-based gold standard method, were found to have a sensitivity rate of 64.7%, specificity rate of 55.6% and concordance rate of 59.1% for KRAS mutation; a sensitivity rate of 100%, specificity rate of 86.7% and concordance rate of 87.1% for NRAS mutation; a sensitivity rate of 50%, specificity rate of 96.4% and concordance rate of 90.6% for BRAF mutation. In addition, concordance rates were evaluated based on the time elapsed between the time of taking the liquid biopsy and tissue biopsy samples. As a result, concordance rates for KRAS, NRAS, and BRAF mutations were found to be 60.9%, 100%, and 100% respectively, in cases where this elapsed time was less than 6 months; and were found to be 57.1%, 78.9%, and 85% respectively, in cases where this elapsed time was more than 6 months. Furthermore, the comprehensive analyzes revealed that the frequency of many molecular changes in mCRC as well as the relationship of these changes with clinicopathological features and survival times.

Conclusion: Our study demonstrates the clinical benefit of ctDNA-based NGS analyzes in patients with mCRC.

Keywords: Colorectal cancer, ctDNA, gene sequencing, liquid biopsy, next generation sequencing.

Unlu A, Demiray AG, Demiray A, Yaren A, Akca H. The effect of genetic alterations detected by the circulating tumor DNA-based next-generation sequencing technique on prognosis and survival in metastatic colorectal cancer. Pam Med J 2025;18:1-14.

Öz

Amaç: Şu ana kadar yapılan çalışmalar; dolaşan tümör DNA'sı (ctDNA) tabanlı next-generation sequencing (NGS) panellerinin, metastatik kolorektal kanserli (mCRC) hastalarda tedavi stratejilerinde yarar sağladığını göstermiştir. Biz de bu çalışmamızda; mCRC'li hastalarda ctDNA tabanlı NGS analizleriyle çeşitli gen değişikliklerinin sıklıklarını saptamayı, bu sonuçları standart polimeraz zincir reaksiyonu (PCR) analizlerindeki sonuçlarla karşılaştırarak uyum oranlarını değerlendirmeyi ve saptanan değişikliklerin genel sağ kalım ve progresyonsuz sağ kalıma etkisini araştırmayı planladık.

Gereç ve yöntem: Çalışma; mCRC tanısı ile takip edilen, kemoterapi ve/veya biyolojik ajan alan 48 hastaya ait bilgilerin retrospektif olarak taranması ve analiz edilmesi ile hazırlandı. Veriler SPSS 25.0 [IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)] paket programı kullanılarak analiz edildi.

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Bulgular: Çalışmada; ctDNA tabanlı NGS analizlerinin, kantitatif PCR tabanlı altın standart yönteme kıyasla KRAS mutasyonu için %64,7 duyarlılık, %55,6 özgüllük ve %59,1 uyum oranına; NRAS mutasyonu için %100 duyarlılık, %86,7 özgüllük ve %87,1 uyum oranına; BRAF mutasyonu için %50 duyarlılık, %96,4 özgüllük ve %90,6 uyum oranına sahip olduğu gösterildi.
Ayrıca, likit biyopsi ile doku biyopsisi örneklerinin alınma zamanları arasındaki süreye göre uyum oranları da değerlendirildi. Sonuçta; uyum oranları bu süre 6 aydan kısa olanlarda KRAS, NRAS, BRAF mutasyonları için sırasıyla %60,9, %100, %100 olurken; süre 6 aydan uzun olanlarda sırasıyla %57,1, %78,9, %85 olarak saptandı. Bunun yanında, yapılan kapsamlı analizler sonucunda; mCRC'de birçok moleküler değişikliğin sıklığı ve bu değişikliklerin klinikopatolojik özellikler ve sağ kalım süreleriyle ilişkisi ortaya koyuldu.
Sonuç: Çalışmamız; mCRC'li hastalarda, ctDNA tabanlı NGS analizlerinin klinik yararını göstermektedir.

Anahtar kelimeler: ctDNA, gen dizileme, kolorektal kanser, likit biyopsi, yeni nesil dizileme.

Ünlü A, Demiray AG, Demiray A, Yaren A, Akça H. Metastatik kolorektal kanserde dolaşan tümör DNA'sına dayalı yeni nesil dizileme tekniği ile tespit edilen genetik değişikliklerin prognoz ve sağ kalım üzerine etkisi. Pam Tıp Derg 2025;18:1-14.

Introduction

With 1.9 million new cases each year, colorectal cancer is the 3rd most common and the 2nd most deadly type of cancer worldwide [1]. Approximately 20% of patients with colorectal cancer have a metastatic disease at the time of diagnosis, and the 5-year survival rate in these patients is 13% [2, 3]. Therefore, the focus in a significant part of cancer research has been on new diagnostic and therapeutic approaches for metastatic colorectal cancers (mCRC) in recent years [2, 4].

In recent years, many targetable molecular changes have been detected; especially after the next generation sequencing (NGS) technique's coming into use for cancer patients. This technique offers the advantages of preventing delays for patients and the ability to direct patients to the most appropriate clinical research by making it possible to sequence multiple genes at once instead of performing multiple sequential single tests [5].

NGS analyzes can be performed directly on samples taken from tumor tissue, as well as on materials taken by liquid biopsy from peripheral blood. This is because peripheral blood contains ctDNA (circulating tumor DNA) that offers a great opportunity for the use of detailed molecular techniques. Some studies have shown that mutations in ctDNA correspond exactly to mutations from the primary tumor. For this reason, it has been reported that ctDNA-based molecular analyses can be conducted to detect targetable molecular changes [6, 7]. The US Food and Drug Administration (FDA) has approved the use of ctDNA-based Guardant360CDx and FoundationOne Liquid CDx tests in many types of cancer [8, 9]. Studies conducted to date showed that circulating tumor DNA (ctDNA)-based next generation sequencing (NGS) panels are beneficial in the treatment strategies of patients with mCRC [10].

Therefore, we planned in our study to determine the frequency of various gene changes in mCRC patients by conduction ctDNA-based NGS analyses; to evaluate concordance rates by comparing the results with the results of standard polymerase chain reaction (PCR) analyses; and to investigate the effect of the detected changes on overall survival and progression-free survival. With all this information, we aimed at contributing to the literature, in terms of identifying ideal personalized treatment procedures for mCRC patients and enhancing overall survival (OS) and progression-free survival (PFS).

Materials and methods

Study design and participants

The study was conducted by retrospective screening and analyzing the anamnesis, examination, laboratory and imaging data on 48 patients aged 18 years and over, who were being followed up with a diagnosis of mCRC and being treated with chemotherapy and/or a biological agent at Pamukkale University Faculty of Medicine, Medical Oncology Clinic. The study involved data from patients who were in the metastatic stage at the time of diagnosis or who developed metastases during follow-up. Patients whose medical records were not fully accessible, patients diagnosed with cancer in an

external center, and patients whose NGS and PCR analyzes were performed in an external center were not included in the study.

In our study; the results of quantitative PCR-based gold standard genomic DNA analyzes on tumor tissue samples were compared with the results of ctDNA-based NGS analyzes on peripheral blood samples. In our clinic, quantitative PCR-based genomic DNA analysis are conducted routinely only for the KRAS, NRAS, and BRAF genes in the group of patients with mCRC; and therefore, comparison was made based on the mutation rates in these genes. In addition, concordance rates of the results were evaluated based on the time elapsed between the time of taking the liquid biopsy and tissue biopsy samples (less than 6 months vs more than 6 months).

Demographic characteristics of the patients including age, gender, family history, smoking, and alcohol consumption were evaluated. The age variable is grouped as under 65 years of aged, and 65 years of age and above. As family history, it was questioned whether there was a history of solid cancer in first-degree relatives. For the variable smoking, patients were classified into groups of those who had no history of smoking and those with a history of smoking (who were still an active smoker or who used to smoke but then quitted). For the variable alcohol consumption, patients were classified into groups of those who had consumed alcohol, without specifying the amount, and those who had not.

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. The study protocol was approved by the Medical Ethics Committee of Pamukkale University Faculty of Medicine with No. 21 on 12.11.2020. Patients alive at the time of data collection provided informed consent.

Molecular analysis

Samples taken from the patients were studied in the molecular laboratory of Pamukkale University, Department of Medical Genetics. 7 ml of peripheral blood samples taken from the patients were collected in pax gene tubes. Samples were then centrifuged for 20 minutes at 1600 xg and for 20 minutes at 4000 xg, in order to separate the plasmas. Circulating free DNA (cfDNA) isolation from approximately 5 ml plasma samples was performed using Qiagen Qiamp Circulating Nucleic Acid Kit (kat:55114 Germany). Samples were measured in Nanodrop and were then involved in the study. From the obtained cfDNAs, a library was prepared using Accel-Amplicon 56 G Oncology Panel Kit for next-generation DNA sequencing. The prepared libraries were run on the Illumina MiSeq platform. The resulting Fastq files were analyzed on the Sophia DDM platform.

Statistical analysis

The data were analyzed using SPSS 25.0 [IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.)] package program. Continuous variables were represented as mean ± standard deviation (S.D.), while categorical variables were represented as frequencies and percentages. Pearson chi-square test was used in analyzing the relationships between categorical variables. Survival curves were calculated using Kaplan-Meier method, and were compared using the log-rank test. $p<0.05$ was considered to be statistically significant. Concordance analyses were performed using Cohen's kappa test.

Results

Study population

In the study, data belonging to a total of 48 patients diagnosed with mCRC, who met the inclusion criteria, were studied. Demographic and clinical characteristics of patients are shown in Table 1.

Results of NGS analysis

In our study, we determined mutation frequencies in genes by conducting NGS analyses on ctDNA materials isolated from peripheral blood samples (Table 2).

The study also involved questioning whether there was a significant difference between the frequency of gene mutations, on the basis of the demographic and clinical characteristics of patients. Table 3 shows the groups with a statistically significant difference.

Table 1. Demographic and clinical characteristics (n=48)

Characteristics	n (%)	Characteristics	n (%)
Age (year)		**Metastasectomy**	
<65	34 (70.8)	Yes	9 (18.8)
≥65	14 (29.2)	No	39 (81.3)
Gender		**Radiofrequency ablation**	
Female	12 (25)	Yes	3 (6.3)
Male	36 (75)	No	45 (93.8)
Family history of cancer		**TAKE**	
Yes	11 (22.9)	Yes	8 (16.7)
No	37 (77.1)	No	40 83.3)
Smoking*		**HIPEC**	
Yes	13 (37.1)	Yes	3 (6.3)
No	22 (62.9)	No	45 (93.8)
Alcohol consumption**		**Liver metastasis**	
Yes	3 (8.6)	Yes	26 (54.2)
No	32 (91.4)	No	22 (45.8)
Tumor histology		**Lung metastasis**	
Adenocarcinoma	38 (79.2)	Yes	23 (47.9)
Mucinous adenocarcinoma	10 (20.8)	No	25 (52.1)
Primary tumor localization		**Peritoneal metastasis**	
Right-sided colon	13 (27.1)	Yes	7 (14.6)
Left-sided colon	15 (31.3)	No	41 (85.4)
Rectum	20 (41.7)		
Lymphovascular invasion		**Bone metastasis**	
Yes	20 (64.5)	Yes	3 (6.3)
No	11 (35.5)	No	45 (93.8)
Perineural invasion		**CEA level**	
Yes	13 (43.3)	Normal	19 (40.4)
No	17 (56.7)	High (>4.7 ug/l)	28 (59.6)
Microsatellite instability		**CA 19-9 level**	
Yes	6 (26.1)	Normal	27 (57.4)
No	17 (73.9)	High (>27 u/ml)	20 (42.6)
Resection of primary tumor		**First-line regimen**	
Yes	35 (72.9)	Capecitabine	1 (2.6)
No	13 (27.1)	Xelox+bevacizumab	13 (34.2)
Adjuvant therapy		Xelox+cetuximab	1 (2.6)
Yes	25 (52.1)	Folfox+bevacizumab	3 (7.9)
No	23 (47.9)	Folfox+cetuximab	4 (10.5)
Adjuvant therapy regimen		Folfox+panitumumab	5 (13.2)
Xelox	20 (80)	Folfiri+Bevacizumab	3 (7.9)
Folfox	3 (12)	Folfiri+Cetuximab	4 (10.5)
De gramont	1 (4)	Folfiri+panitumumab	2 (5.3)
Capecitabine	1 (4)	İrinotecan+cetuximab	1 (2.6)
Local therapy		Oxaliplatin+panitumumab	1 (2.6)
Yes	29 (60.4)		
No	19 (39.6)		

Table 1. Demographic and clinical characteristics (n=48) (continued)

Characteristics	n (%)	Characteristics	n (%)
Radiotherapy		**First-line biological agent**	
Yes	14 (29.2)	Bevacizumab	19 (51.4)
No	34 (70.8)	Cetuximab	10 (27.0)
		Panitumumab	8 (21.6)

*Smoking data could not be obtained for 13 patients, **Alcohol consumption data could not be obtained for 13 patients

Table 2. Mutant gene rates obtained by NGS analysis (n>48)

Gene	n (%)	Gene	n (%)
ALK	0 (0)	GNAQ	1 (2.1)
ATM	8 (16.7)	GNAS	4 (8.3)
BRAF	4 (8.3)	JAK2	1 (2.1)
EGFR	37 (77.1)	JAK3	26 (54.2)
ERBB2	11 (22.9)	NOTCH1	34 (70.8)
HRAS	17 (35.4)	NPM1	0 (0)
IDH1	0 (0)	PIK3CA	37 (77.1)
IDH2	2 (4.2)	SMAD4	29 (60.4)
KDR	38 (79.2)	ABL1	16 (33.3)
KIT	18 (37.5)	AKT1	29 (60.4)
KRAS	26 (54.2)	CDHİ	3 (6.3)
MAP2K1	20 (41.7)	CSF1R	32 (66.7)
MET	9 (18.8)	CTNNB1	1 (2.1)
NRAS	6 (12.5)	DDR2	0 (0)
PTEN	11 (22.9)	EZH2	6 (12.5)
RB1	25 (52.1)	FBXW7	25 (52.1)
RET	38 (79.2)	FOXL2	8 (16.7)
TP53	46 (95.8)	HNF1A	4 (8.3)
APC	27 (56.3)	MLH1	2 (4.2)
CDH1	5 (10.4)	MPL	4 (8.3)
CDKN2A	9 (18.8)	MSH6	8 (16.7)
DNMT3A	10 (20.8)	PDGFRA	16 (33.3)
ERBB4	29 (60.4)	SMARCB1	15 (31.3)
FGFR1	2 (4.2)	SMO	4 (8.3)
FGFR2	31 (64.6)	SRC	0 (0)
FGFR3	30 (62.5)	STK11	22 (45.8)
FLT3	18 (37.5)	TSCI	0 (0)
GNA11	17 (35.4)	VHL	35 (72.9)

Table 3. Groups with significant differences in gene mutation frequencies according to demographic and clinical characteristics

	Age				
Mutation	**<65**		**≥65**		***p* value**
	n	**%**	**n**	**%**	
KDR exon 30	2	5.9	6	42.9	0.005* X^2=9.761
	Smoking				
Mutation	**No**		**Yes**		***p* value**
	n	**%**	**n**	**%**	
EGFR exon 20	0	0	3	23.1	0.044* X^2=5.553
MET	1	4.5	5	38.5	0.019* X^2=6.618
	Family history of cancer				
Mutation	**No**		**Yes**		***p* value**
	n	**%**	**n**	**%**	
TP53	37	100	9	81.8	0.049* X^2=7.020
TP53 exon 5	12	32.4	0	0	0.044* X^2=4.757
ERBB4	26	70.3	3	27.3	0.016* X^2=6.555
ERBB4 exon 9	17	45.9	1	9.1	0.035* X^2=4.914
	Tumor histology				
Mutation	**Adenocarcinoma**		**Mucinous adenocarcinoma**		
	n	**%**	**n**	**%**	***p* value**
DNMT3A	5	13.2	5	50	0.022* X^2=6.515
TP53 exon 5	12	31.6	0	0	0.039* X^2=4.211
	Primary tumor localization						
Mutation	**Right-sided colon**		**Left-sided colon**		**Rectum**		
	n	**%**	**n**	**%**	**n**	**%**	***p* value**
KRAS	5	38.5	12	80	9	45	0.050* X^2=6.001
ERBB4	4	30.8	11	73.3	14	70	0.037* X^2=6.593
RET exon 10	8	61.5	4	26.7	15	75	0.015* X^2=8.339
	Lymphovascular invasion				
Mutation	**No**		**Yes**		
	n	**%**	**n**	**%**	***p* value**
CDKN2A	0	0	9	45	0.012* X^2=6.975
PIK3CA	4	36.4	16	80	0.023* X^2=5.903
PIK3CA exon 14	0	0	8	40	0.028* X^2=5.930
	Perineural invasion				
Mutation	**No**		**Yes**		
	n	**%**	***n***	**%**	***p* value**
MAP2K1	6	35.3	10	76.9	0.024* X^2=5.129
JAK3	6	35.3	11	84.6	0.007* X^2=7.298
SMO	0	0	4	30.8	0.026* X^2=6.036
	Microsatellite instability				
Mutation	**No**		**Yes**		
	n	**%**	**n**	**%**	***p* value**
KDR	16	94.1	3	50	0.040* X^2=6.008
PDGFRA	3	17.6	5	83.3	0.009* X^2=8.435
EGFR exon 21	3	17.6	4	66.7	0.045* X^2=5.033

Table 3. Groups with significant differences in gene mutation frequencies according to demographic and clinical characteristics (continued)

Liver metastasis					
Mutation	**No**		**Yes**		***p* value**
	n	**%**	**n**	**%**	
EGFR	20	90.9	17	65.4	0.036* X^2=4.395
EGFR exon 7	13	59.1	5	19.2	0.004* X^2=8.078
KDR exon 11	7	31.8	16	61.5	0.040* X^2=4.218
ERBB4 exon 9	13	59.1	5	19.2	0.004* X^2=8.078
FBXW7	18	81.8	7	26.9	0.000* X^2=14.389
VHL exon 2	11	50	5	19.2	0.024* X^2=5.077
Lung metastasis					
Mutation	**No**		**Yes**		***p* value**
	n	**%**	**n**	**%**	
MAP2K1	14	56	6	26.1	0.036* X^2=4.410
MAP2K1 exon 6	8	32	1	4.3	0.024* X^2=6.013
PTEN exon 2	0	0	4	17.4	0.046* X^2=4.743
TP53 exon 5	3	12	9	39.1	0.030* X^2=4.703
ERBB4 exon 9	6	24	12	52.2	0.044* X^2=4.057
FGFR3	11	44	19	82.6	0.006* X^2=7.619
FGFR3 exon 9	3	12	9	39.1	0.030* X^2=4.703
FBXW7	9	36	16	69.6	0.020* X^2=5.408
FBXW7 exon 9	8	32	14	60.9	0.045* X^2=4.022
Peritoneal metastasis					
Mutation	**No**		**Yes**		***p* value**
	N	**%**	**n**	**%**	
KDR exon 11	23	56.1	0	0	0.010* X^2=7.540
Bone metastasis					
Mutation	**No**		**Yes**		***p* value**
	n	**%**	**n**	**%**	
KRAS exon 2	6	13.3	3	100	0.005* X^2=13.867
PTEN exon 8	1	2.2	2	66.7	0.008* X^2=19.935
APC exon 16	15	33.3	3	100	0.047* X^2=5.333
VHL	35	77.8	0	0	0.017* X^2=8.615
CEA level					
Mutation	**Normal**		**High**		***p* value**
	n	**%**	**n**	**%**	
KRAS	6	31.6	19	67.9	0.014* X^2=5.983
RB1	14	73.7	11	39.3	0.020* X^2=5.379
CA 19-9 level					
Mutation	**Normal**		**High**		***p* value**
	n	**%**	**n**	**%**	
FLT3 exon 11	0	0	4	20	0.027* X^2=5.902

*Pearson chi-square test

Liquid biopsy-tissue biopsy concordance analysis

In our study; the results of quantitative PCR-based gold standard genomic DNA analyzes on tumor tissue samples were compared with the results of ctDNA-based NGS analyzes on peripheral blood samples. In our clinic, quantitative PCR-based genomic DNA analysis are conducted routinely only for the KRAS, NRAS, and BRAF genes in the group of patients with mCRC; and therefore, comparison was made based on the mutation rates in these genes. During the investigation of the KRAS gene, 11 of the 17 patients, who were found to have a KRAS mutation in their PCR analyses, were also found to have mutations in their NGS analyses, while no mutations were detected in NGS analyses of 6 among them (64.7% sensitivity). Of the 27 patients who were not found to have a KRAS mutation in their PCR analyses, 15 patients were found to have no mutation while 12 were found to have KRAS gene mutation in their NGS analyses (55.6% specificity). In general, NGS analyzes performed for the KRAS gene showed a result compatible with the PCR analyzes in 26 of 44 patients (59.1% concordance rate). During the investigation of the NRAS gene, the PCR analysis detected NRAS mutation in only 1 patient, who was also found to have NRAS mutation in his NGS analysis (100% sensitivity). Of the 30 patients who were not found to have a NRAS mutation in their PCR analyses, 26 patients were found to have no mutation while 4 were found to have NRAS gene mutation in their NGS analyses (86.7% specificity). In general, NGS analyzes performed for the NRAS gene showed a result compatible with the PCR analyzes in 27 of 31 patients (87.1% concordance rate). During the investigation of the BRAF gene, 2 of the 4 patients, who were found to have a BRAF mutation in their PCR analyses, were also found to have mutations in their NGS analyses, while no mutations were detected in NGS analyses of 2 among them (50% sensitivity). Of the 28 patients who were not found to have a BRAF mutation in their PCR analyses, 27 patients were found to have no mutation while 1 were found to have BRAF gene mutation in their NGS analyses (96.4% specificity). In general, NGS analyzes performed for the BRAF gene showed a result compatible with the PCR analyzes in 29 of 32 patients (90.6% concordance rate) (Table 4).

In addition, concordance rates of the results were evaluated based on the time elapsed between the time of taking the liquid biopsy and tissue biopsy samples. During the investigation of KRAS mutation, NGS analysis gave results compatible with PCR analysis in 14 (60.9%) of 23 cases where this elapsed time was less than 6 months, while results compatible with PCR analysis in 12 (57.1%) of 21 cases, where this elapsed time was more than 6 months. During the investigation of NRAS mutation, NGS analysis gave results compatible with PCR analysis in 12 (100%) of 12 cases where this elapsed time was less than 6 months, while results compatible with PCR analysis in 15 (78.9%) of 19 cases, where this elapsed time was more than 6 months. During the investigation of BRAF mutation, NGS analysis gave results compatible with PCR analysis in 15 (100%) of 15 cases where this elapsed time was less than 6 months, while results compatible with PCR analysis in 17 (85%) of 20 cases, where this elapsed time was more than 6 months. As a result, the concordance rate for cases, in which this elapsed time was less than 6 months, was 60.9% for KRAS, 100% for NRAS, and 100% for BRAF, while for cases, in which this elapsed time was more than 6 months, the concordance rate was 57.1% for KRAS, 78.9% for NRAS, and 85% for BRAF (Table 5).

Survival analysis

In our study, the mean overall survival time of the all patient group was calculated to be 59.3 months (±11.0 95% confidence interval [CI]: 37.7-80.9), while the progression-free survival time was calculated to be 18.8 months (±3.5 95% CI: 11.9-25.7). In the study, analyses on overall survival and progression-free survival were conducted according to the mutation status in the genes, and the groups found to have statistically significant differences were shown in Table 6 and Table 7. Survival curves were shown in Figure 1 and Figure 2.

Table 4. Liquid biopsy-tissue biopsy concordance analysis

Gene	Concordance	Sensitivity	Specificity	Kappa	*p* value
KRAS	59.1%	64.7%	55.6%	0.190	0.190
NRAS	87.1%	100%	86.7%	0.295	0.020*
BRAF	90.6%	50%	96.4%	0.520	0.003*

*Cohen's kappa test

Table 5. Concordance rates of analysis results according to the time between collection of liquid biopsy and tissue biopsy samples

Gene	Time interval (months)	Concordance	Kappa	*p* value
KRAS	<6	60.9%	0.219	0.292
	>6	57.1%	0.160	0.407
NRAS	<6	100%	1.000	0.001*
	>6	78.9%	-	-
BRAF	<6	100%	1.000	0.001*
	>6	85%	0.318	0.144

*Cohen's kappa test

Table 6. Groups with significant differences in overall survival according to mutation status

Gene	Mutation	Mean	S. D.	95% CI	*p* value
ERBB4 exon8	No	61.714	11.475	39.224-84.204	0.022*
	Yes	16.233	6.890	2.729-29.737	
FGFR1	No	61.882	11.537	39.269-84.496	0.034*
	Yes	18.017	4.183	9.817-26.216	
FGFR3 exon 14	No	62.941	11.619	40.166-85.715	0.001*
	Yes	11.542	3.240	5.191-17.892	
FLT3 exon 11	No	64.297	12.018	40.742-87.852	0.002*
	Yes	15.800	7.346	1.401-30.199	
NOTCH1	No	25.290	3.711	18.017-32.562	0.012*
	Yes	71.545	14.016	44.075-99.016	
CDHİ	No	61.581	11.407	39.223-83.938	0.017*
	Yes	11.889	3.219	5.581-18.197	
CSF1R exon 22	No	62.890	11.768	39.824-85.956	0.013*
	Yes	16.456	10.348	0.000-36.737	
MSH6	No	66.624	12.715	41.702-91.547	0.011*
	Yes	22.610	6.062	10.729-34.491	
SMARCB1 exon 5	No	67.046	12.963	41.638-92.454	0.032*
	Yes	25.045	4.990	15.264-34.826	
STK11	No	38.268	9.354	19.933-56.602	0.009*
	Yes	80.539	16.472	48.254-112.825	
PIK3CA exon 10	No	65.711	12.327	41.550-89.872	0.005*
	Yes	18.817	3.393	12.166-25.468	

* log-rank test

Table 7. Groups with significant differences in progression-free survival according to mutation status

Gene	Mutation	Mean	S. D.	95% CI	*p* value
EGFR exon 20	No	19.693	3.676	12.488-26.898	0.014*
	Yes	5.367	4.833	0.000-14.840	
KIT exon 10	No	15.887	2.770	10.459-21.316	0.034*
	Yes	22.320	2.159	18.089-26.551	
PTEN exon 8	No	19.747	3.671	12.553-26.942	0.002*
	Yes	5.956	3.131	0.000-12.091	
JAK3	No	11.815	1.941	8.010-15.621	0.017*
	Yes	26.340	5.402	15.751-36.929	
PIK3CA exon 10	No	20.044	3.763	12.667-27.420	0.002*
	Yes	6.993	1.147	4.745-9.242	
FBXW7	No	13.030	1.704	9.689-16.370	0.042*
	Yes	25.710	6.924	12.138-39.282	
STK11	No	10.907	1.261	8.436-13.378	0.001*
	Yes	27.207	6.252	14.954-39.460	

* log-rank test

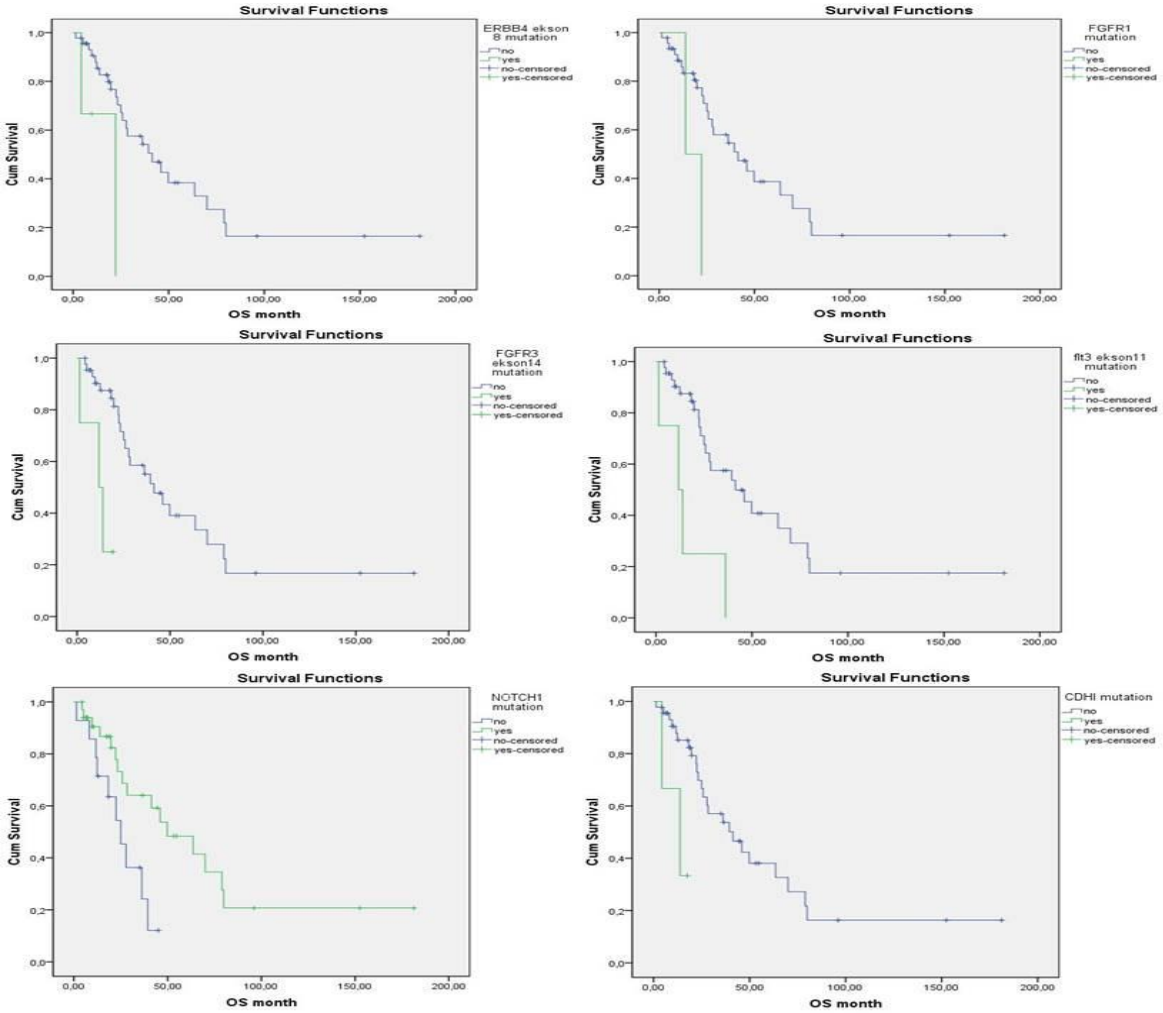

Figure 1. Survival curves of groups with significant differences in overall survival according to mutation status

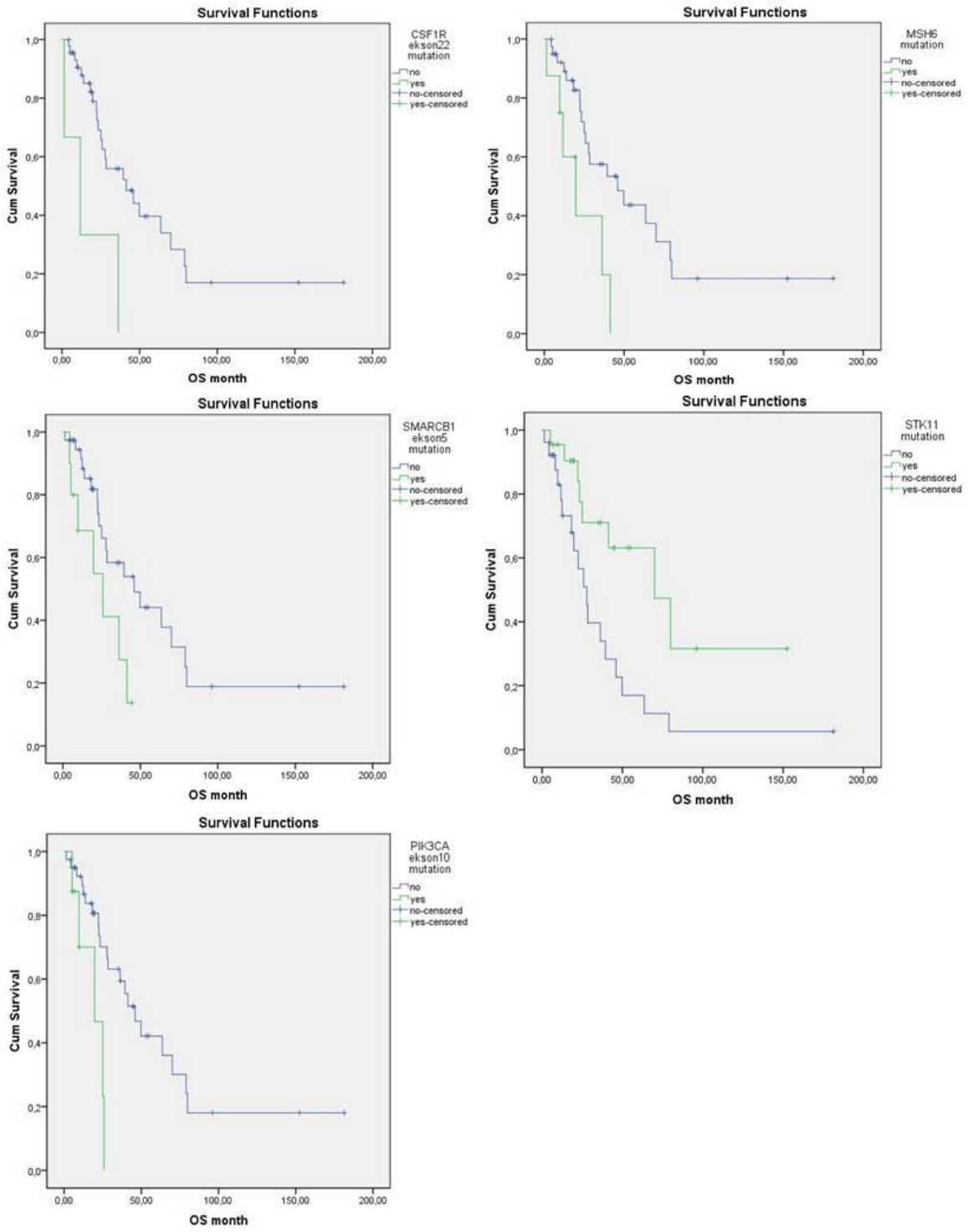

Figure 2. Survival curves of groups with significant differences in progression-free survival according to mutation status

Discussion

Mutations is detected in the KRAS gene in 35-45% of all CRC cases [11]. In a study; disease-free survival times were observed to be shorter in patients with KRAS exon 2 mutation than in patients without this mutation [12]. In our study, the frequency of mutations in the KRAS gene was found to be 54.2%. However, no difference statistically significant in terms of OS and PFS was observed according to the KRAS mutation status. As for the NRAS gene, mutations are detected in 5% of all CRC cases [13]. In the studies, it has been determined that the overall survival time of patients with NRAS mutations is significantly lower than that of patients with RAS wild type [14]. In our study, the NRAS gene was detected as a mutant gene in 12.5% of patients. However, no difference statistically significant in terms of OS and PFS was observed according to the NRAS mutation status.

Mutations in the BRAF gene are detected in approximately 8-12% of mCRC cases [15]. In a CRYSTAL study, BRAF mutation in mCRC was shown to be associated with a poor prognosis [16]. Consistent with the literature, the BRAF gene was found to be mutant in 8.3% of patients in our study. However, no difference statistically significant in terms of OS and PFS was observed according to the BRAF mutation status.

The PIK3CA gene is found to be mutated in about 80% of CRC cases [13]. Some studies have provided evidence that PIK3CA mutation is associated with resistance to anti-EGFR therapy [17]. Consistent with the literature, the PIK3CA gene was found to be mutant in 77.1% of patients in our study. In addition, the presence of PIK3CA exon 10 mutation was found to be associated with statistically significantly worse overall survival and progression-free survival times.

In the TCGA (Tumor Cancer Genome Atlas) dataset published in 2012, findings were obtained that suggest that mutations in the ERBB4 gene create a survival disadvantage in CRC [18]. In our study, the frequency of ERBB4 gene mutation was calculated to be 60.4%. In addition, the presence of ERBB4 exon 8 mutation was found to be associated with statistically significantly worse overall survival times.

Mutations in FBXW7, EGFR, JAK3, KIT, CSF1R, CDHI, FLT3, FGFR1, FGFR3, SMARCB1, PTEN, MSH6, NOTCH1, STK11 genes, which are among the genes analyzed in this study, have not clearly known roles in the pathogenesis and prognosis of CRC. Our study showed that mutations in the CDHI, MSH6, FGFR1, FGFR3 exon 14, CSF1R exon 22, FLT3 exon 11, and SMARCB1 exon 5 genes were associated with statistically significantly worse overall survival times; mutations in the NOTCH1 and STK11 genes were associated with better overall survival times; mutations in the EGFR exon 20 and PTEN genes were associated with worse progression-free survival times; and mutations in the STK11, FBXW7, JAK3, and KIT exon 10 genes were associated with better progression-free survival times.

In a study published in 2014, tissue samples taken from 106 patients were analyzed using the quantitative PCR-based gold standard method, while blood samples were analyzed using the ctDNA-based NGS technique, and the analysis of ctDNA showed 98% specificity, 92% sensitivity, and 96% concordance rates for KRAS mutation [19]. In a similar study conducted in 2018 showed 67% sensitivity, 90% specificity and 81% concordance rates for KRAS mutation [20]. In a study published in Cancer Medicine in 2019, which enrolled 101 patients with mCRC, the overall concordance rate between ctDNA and tissue analyzes was calculated to be 77.2%, in terms of the RAS mutation status [21]. In our study, ctDNA-based NGS analyses conducted on peripheral blood samples, compared to the quantitative PCR-based gold standard method used with tissue samples, were found to have a sensitivity rate of 64.7%, specificity of 55.6% and concordance rate of 59.1% for KRAS mutation; a sensitivity rate of 100%, specificity of 86.7% and concordance rate of 87.1% for NRAS mutation; and a sensitivity rate of 50%, specificity of 96.4% and concordance rate of 90.6% for BRAF mutation. As a result, it was revealed that ctDNA-based NGS analyses can be a good option for detecting molecular changes in patients with mCRC.

The findings of the limited number of studies in the literature suggested that the time elapsed between the sampling times of the liquid biopsy and tissue biopsy procedures has an effect on the concordance between the results of the

molecular analyzes conducted on the samples. In a 2020 study that enrolled 54 patients with mCRC, the rate of concordance between the results of the liquid biopsy and tissue biopsy procedures was found to be 50% in cases where the time elapsed between the sampling times of these procedures was more than 6 months and 83.1% in cases where this elapsed time was less than 6 months [22]. In another study that compared the results of the ctDNA analyses performed on samples taken from peripheral blood of 101 patients and the results of the genomic DNA analyses on samples taken from tumor tissues of these patients, the concordance rates were found to be 63% for TP53, 69% for EGFR, 85% for PIK3CA, and 87% for ERBB2 in cases where the time elapsed between the sampling times of the procedures was more than 6 months; while the concordance rates were found to be 82.1% for TP53, 71% for EGFR, 90% for PIK3CA, and 97% for ERBB2 in cases where this elapsed time was less than 6 months [23]. In the light of these data, in our study, concordance of the rates were also evaluated based on the time elapsed between the time of taking the liquid biopsy and tissue biopsy samples. The concordance rate for cases, in which this elapsed time was less than 6 months, was 60.9% for KRAS, 100% for NRAS, and 100% for BRAF, while for cases, in which this elapsed time was more than 6 months, the concordance rate was 57.1% for KRAS, 78.9% for NRAS, and 85% for BRAF. As a result, it was revealed that the time elapsed between the sampling times of the liquid biopsy and tissue biopsy procedures affects the concordance between the results of molecular analyses of the samples and that ensuring this elapsed time to be shorter would contribute to the ability to achieve better results. Limitations of our study: the number of patients is relatively small, and the study is retrospective and single-center.

In conclusion, our study is one of the studies in the literature, which show the clinical benefit of ctDNA-based NGS analyses for mCRC patients. Our study showed that ctDNA-based NGS analyzes gives results highly consistent with the results of quantitative PCR-based gold standard genomic DNA analyzes conducted on tumor tissue samples; and that this concordance is much higher in cases where the time elapsed between the sampling times of these procedures was less than 6 months. In addition, the comprehensive analyzes revealed that the frequency of many molecular changes in mCRC as well as the relationship of these changes with clinicopathological features and survival times.

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A.G.D.; Designed study, agreed to be accountable for all aspects of the work.

A.D.; Analysed data.

A.Y.; Searched literature, collected data.

H.A.; Developed the theoretical framework.

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Research Article

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Do spinopelvic parameters affect the severity of thoracolumbar trauma differently between in-vehicle traffic accidents and falling from a height?

Spinopelvik parametreler torakolomber travmanın şiddetini araç içi trafik kazası ve yüksekten düşme arasında farklı şekilde etkiler mi?

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Abstract

Purpose: Although there is a comprehensive characterization of the impact of spinopelvic parameters on outcomes after degenerative spine surgery, the impact of spinopelvic parameters on thoracolumbar trauma has not yet been defined. In the present study, it was aimed to reveal the correlation between the severity of vertebral fractures developing after trauma according to the mechanism of occurrence and sagittal spinopelvic parameters.

Materials and methods: Patients with thoracolumbar vertebra fractures were evaluated retrospectively. The patients were divided into two groups: in-vehicle traffic accident (sitting group) and fall from height (standing group). The pelvic incidence (PI), pelvic tilt (PT), sacral slope (SS) and vertebral Hounsfield unit (HU) values were measured on computed tomography (CT) scans.

Results: The results of the multivariate logistic regression analysis performed in the study revealed that a one-unit increase in PI reduced the risk of more comminuted fractures (A2 and above) by 0.90 times in sitting position trauma (Odds ratio (OR): 0.90; 95% CI: 0.84-0.96; *p*=0.002) and by 0.96 times in standing position trauma (OR: 0.96; 95% CI: 0.93-0.99; *p*=0.040).

Conclusions: It was observed that in vertebral fractures developed after trauma, the fact that the vertebral column of patients with low PI is more rigid increased the severity of the fracture.

Keywords: Vertebral fracture, Hounsfield unit, pelvic parameters, AO Spine thoracolumbar classification.

Kiyak V, Astan S. Do spinopelvic parameters affect the severity of thoracolumbar trauma differently between in-vehicle traffic accidents and falling from a height? Pam Med J 2025;18:17-30.

Öz

Amaç: Spinopelvik parametrelerin dejeneratif omurga cerrahisi sonrası sonuçlar üzerindeki kapsamlı bir etkisinin karakterizasyonu olmasına rağmen, spinopelvik parametrelerin torakolomber travma üzerindeki etkisi henüz tanımlanmamıştır. Bu çalışmada travma sonrası gelişen vertebra kırıklarının oluşma mekanizması ve tipine göre sagittal spinopelvik parametreler ile arasındaki ilişkinin ortaya konulması amaçlandı.

Gereç ve yöntem: Torakolomber vertebra kırığı olan hastalar retrospektif olarak değerlendirildi. Hastalar araç içi trafik kazası (oturarak travmaya maruz kalan grup) ve yüksekten düşme (ayakta travmaya maruz kalan grup) olmak üzere iki gruba ayrıldı. Bilgisayarlı tomografi görüntülerinde pelvik insidans, pelvik tilt, sakral slop ve vertebral Hounsfield ünitesi değerleri ölçüldü.

Bulgular: Çalışmada yapılan çok değişkenli lojistik regresyon analizi sonuçları, PI'deki bir birimlik artışın, oturan grup travmalarında daha fazla parçalı kırık (A2 ve üzeri) riskini 0,90 kat azalttığını ortaya koydu (Risk oranı: 0,90; %95 GA: 0,84-0,96; *p*=0,002) ve ayakta durma pozisyonu travmasında 0,96 kat (Risk oranı: 0,96; %95 GA: 0,93-0,99; *p*=0,040).

Sonuç: Travma sonrası gelişen vertebra kırıklarında düşük PI'li hastaların vertebral kolonunun daha rijit olmasının kırığın şiddetini arttırdığı görüldü.

Anahtar kelimeler: Vertebral kırık, Hounsfield ünitesi, pelvik parametreler, AO Spine torakolomber sınıflandırması.

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Introduction

Vertebral fractures usually take place in the thoracolumbar and lower lumbar regions and have adverse effects on the patient's quality of life [1]. Vertebral fractures occur in conditions affecting the bone such as inappropriate axial or rotational loading during trauma, osteoporosis, metastasis and infection [2, 3]. A successful classification system both facilitates the communication between physicians and guides prognosis and treatment by determining the severity of injury [4].

The AO Spine classification, a commonly used system for the classification of thoracolumbar injuries, attempts to facilitate fracture classification and guide treatment, establishing hierarchical and morphological criteria [4]. A type injuries indicate compression; B type injuries indicate distraction, and C type injuries indicate translation [5]. The AO Spine classification system is descriptive rather than determining the treatment and holds more options to describe the fracture morphology. The major mechanisms in the occurrence of the vertebral fracture are mostly unknown. Vertebral fractures vary according to the trauma type, forces that the spine and pelvis are exposed to, and anatomical and biological characteristics of the patient [6]. Lumbosacral sagittal balance and pelvic parameters play a significant role in maintaining the physiological function of the spine and compensatory mechanisms [7-9].

Spinopelvic parameters and sagittal balance are hot topics in spine surgery. Being one of the sagittal spinopelvic parameters, pelvic incidence (PI), independent of the position of the pelvis, is a constant value [10]. Although PI falls short to indicate pelvis width and whole spine balance, it helps us to get an idea about the pelvis to encounter in case the balance between the pelvis and the spine is disturbed [11-13]. Abnormal spinopelvic values take a part in the occurrence of pathologies such as low back pain, lumbar disc herniation, degenerative disc disease, degenerative and isthmic spondylolisthesis, and hip osteoarthrosis [14-17]. Among vertebral fractures, osteoporotic fractures hold a significant place. Quantitative computed tomography (QCT) and dual energy X-ray absorptiometry (DXA) are used in detecting bone mineral density (BMD). Spatial and volumetric BMD (sBMD and vBMD) measured with DXA and QCT are generally utilized to estimate the risks of vertebral fracture [18, 19]. However, due to high cost of equipment and personnel in BMD measurements, for osteoporosis, vertebral bone attenuation in HU value, which is measured from CT images, has been suggested to be used instead of these examinations [18]. The results of vertebral HU values have been shown to be affirmative in the detection of osteoporosis [18-21].

Objective

The literature has not elucidated the relationship between PI and severity of spinal fracture. The objective of the current study is to establish the correlation between the sagittal spinopelvic parameters and the severity of vertebral fractures assessed by the AO Spine classification and to identify possible risk factors in patients with vertebral fracture occurred after trauma.

Material and method

Study design

Permission was obtained from Tokat Gaziosmanpasa University Clinical Research Ethics Committee for the study (date: 18.04.2022 and number: 83116987). The patients who were admitted to the emergency department of our hospital between 01/01/2016 and 12/31/2020 after trauma and were diagnosed with thoracolumbar and lower lumbar vertebral fractures were retrospectively reviewed. Since the study was retrospective in nature, informed consent was waived. Age, gender, height, weight and BMI of the patients were collected. The diagnosis was made with clinical examination and radiographic assessment, whereas the study data were retrieved from the medical files of the patients with the help of the electronic health record system (ENLIL hospital information management system, version v2.19.46 20191118). The patients were separated into two groups as in-vehicle traffic accidents (IVTA) and falling from height. Our aim in dividing patients into standing and sitting groups; to evaluate the effect of changing and

unchanging spinopelvic parameters on the type of fracture by changing the way the force that causes the fracture is applied. Vertebral fracture was considered to occur in standing position in patients who fall from height and in sitting position in those injured in IVTA. All measurements were performed on preoperative CT scans of the patients using the patient archiving computer system (PACS) software (Sectra Workstation IDS7, Version 21.2.11.6289, ©2019 Sectra AB).

Patients aged over 18 years with acute thoracolumbar and lower lumbar vertebral fractures occurred due to trauma were included in the study. Fractures were judged as acute when the patient had been detected to have a fracture with spine BT in our hospital within 7 days of fracture occurrence. Patients whose thoracolumbar and lower lumbar spine can be clearly evaluated in PACS, those with CT scans allowing multi-plane reconstruction (MPR) imaging, those with a clear evaluation of the femoral head and pelvis for the measurement of sagittal spinal parameters and those without anatomical changes in the pelvis detected on CT scans were included into the study.

Patients having a past history of spine or pelvis surgery or fractures, those with pathological, chronic or multiple vertebral fractures, those having congenital spinal deformity and those who had a hip anomaly disrupting the proximal femoral anatomy such as developmental hip dysplasia and prosthesis or who underwent hip surgery were left out from the study.

The patients' medical records were reviewed by an orthopedist and a neurosurgeon, both with at least 5 years of experience. All measurements were made separately by the two observers, and the average of their results was calculated to minimize measurement errors. The neurosurgeon performed the measurements again to assess the intra-observer variability one month after the first measurement.

For all patients, the CT scan that includes the pelvis acquired while the patient lies in the supine position with hip and knee joints extended was examined. The patients received no additional radiation as the CT scans had been taken during routine treatment.

Measurement of vertebral HU

As defined in previous studies, the L1 vertebra were mostly selected in measuring HU, since the HU value of L1 provides better results in the detection of osteoporosis compared to other vertebrae [20]. However, if L1 was fractured, the HU value of T12 or L2 was used as stated by the literature [19]. All data were manually evaluated after performing MPR of the CT data images by using the PACS software [18]. The oval region of interest (ROI) was placed in the axial section of the trabecular part of the vertebral body [22].

Vertebral fracture classification

The vertebral fractures between T12 and L5 were graded according to the commonly used "the AO Spine classification of thoracolumbar injuries". The types of fractures according to the AO Spine classification are shown in Figures 1A-1E.

Measurement of spinal and pelvic parameters

PI, PT and sacral slope(SS) angle were measured to evaluate the sacropelvic balance. For the measurement of PI, in MPR images of the CT, the angle formed by the intersection of the two lines was calculated; the first line was drawn, if the femoral heads overlap, from the midpoint of the femoral head, if not, from the middle of the line connecting the midpoint of both femoral heads to the midpoint of the upper endplate of S1; and the second line was drawn at a 90-degree angle from the midpoint of the upper endplate of S1 to the bottom.

SS is the angle between the line drawn from the upper endplate of S1 and the horizontal line extending from the midpoint of the upper sacral endplate.

PT is described as the measurement in degrees of the angle between a line connecting the midpoint of sacral endplate to the center of the femoral heads and the vertical axis (Figure 2).

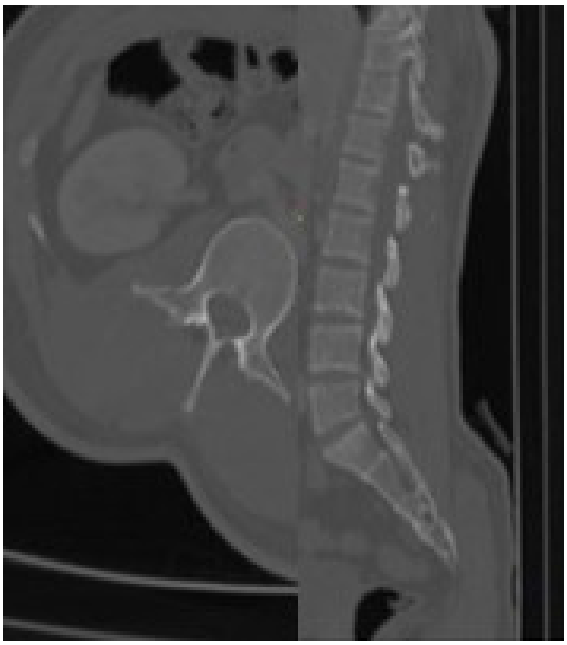

Figure 1A. A0: No fracture or clinically insignificant fracture of the spinous or transverse processes

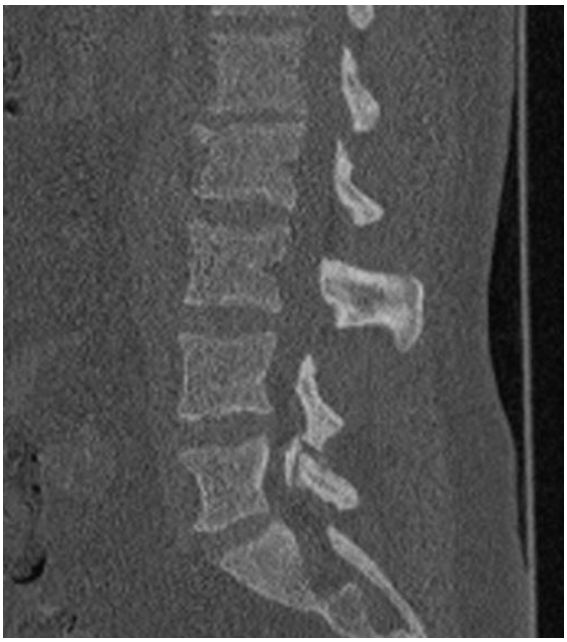

Figure 1B. A1: Wedge compression or impaction fracture, which involves a single endplate of the vertebral body without involvement of the posterior vertebral Wall

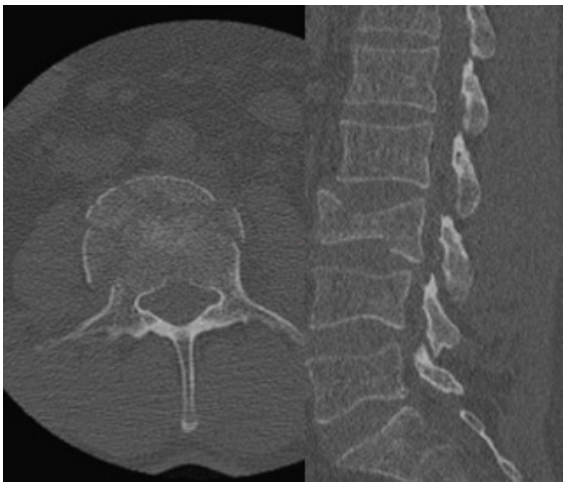

Figure 1C. A2: Split or pincer type fracture, which involves both endplates without the involvement of the posterior Wall

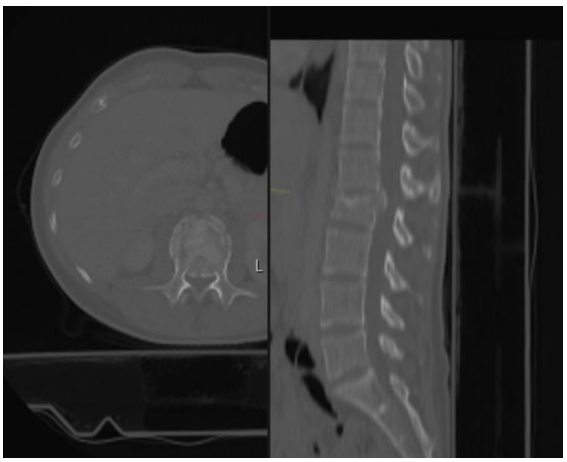

Figure 1D. A3: Incomplete burst fracture, which involves a single endplate along with the posterior vertebral Wall

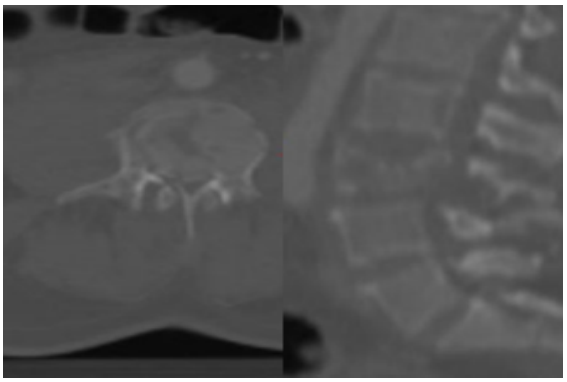

Figure 1E. A4: Complete burst fracture, which involves both endplates along with the posterior vertebral wall: Split fractures that involve the posterior vertebral wall are also included

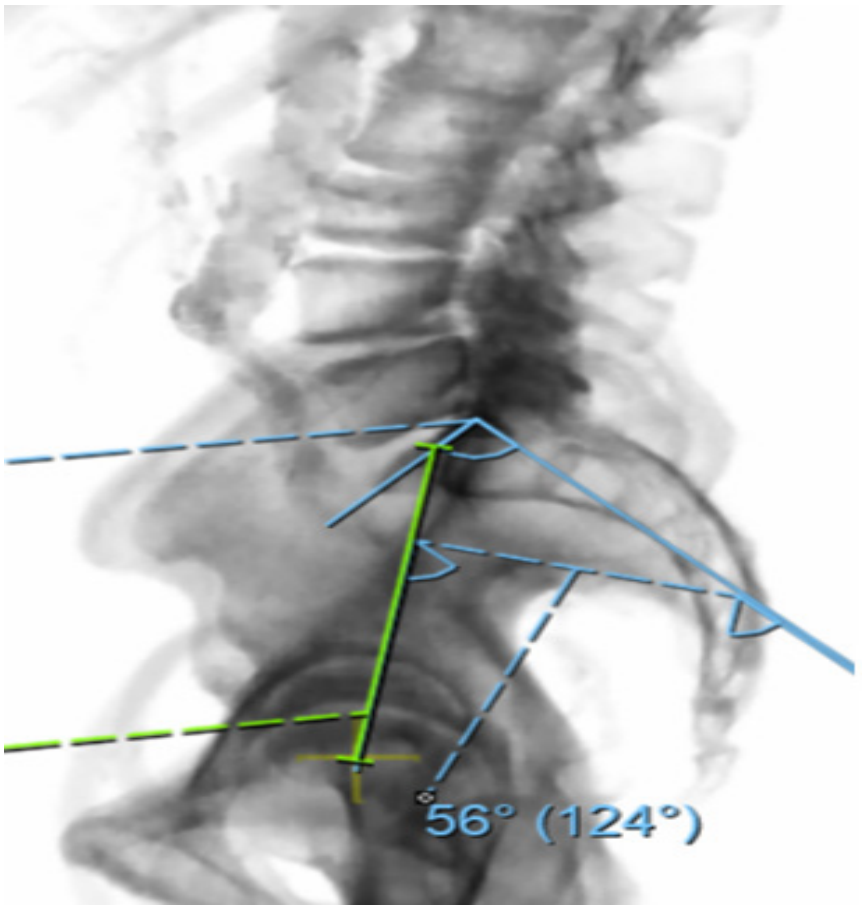

Figure 2. Pelvic incidence measurement

Statistical analysis

In the study, the quantitative variables were reported in mean and standard deviation, whereas frequency and percentage were used to express the qualitative variables. The differences between groups in terms of the means of quantitative variables were determined using the *independent samples t test* and one-way analysis of variance, when there were two groups in which the normality assumption was met. Tukey HSD was used for multiple comparisons. In the qualitative variables, the chi-square test was employed in assessing the relationships between related variables by creating contingency tables. The *p*-values less than 0.05 was regarded as statistically significant. The Pearson correlation coefficient was utilized to find the relationship between quantitative variables. Univariate and Multivariate logistic regression analysis was used to examine the effect of more than one independent variable on the dependent variable. All statistical calculations were conducted using a commercially available statistical software (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY).

Results

There were 82 (33.6%) female and 162 (66.4%) male patients in the study, and the mean age was 51.52±15.99 (20–88) in the study. The female patients in the study were older than males (55.22±17.3, 49.6±14.97, respectively; t=2.605; p=0.010). The male patients were taller ($p \leq 0.001$). The female vertebrae were more osteoporotic. The vertebral HU value was significantly higher in males (178.06±55.9) than in females (158.4±65.29) (p=0.015). The difference between males and females was not statistically significant in terms of PI, PT, SS, weight and BMI (t=0.519; p=0.604, t=0.947; p=0.344, t=1.661; p=0.098, t=0.824; p=0.411, t=1.683; p=0.094, respectively, Table 1).

Table 1. Distribution of quantitative variables by gender

	Gender		*t*	*p*
	Female	**Male**		
	Mean	**Mean**		
Age (years)	55.22±17.33	49.64±14.97	2.605	0.010*
HU level (hounsfield Units) (up)	158.48±65.29	178.06±55.94	2.439	0.015*
SS	34.57±9.76	37.03±11.46	1.661	0.098
PI	50.45±9.9	51.19±10.77	0.519	0.604
Pelvik Tilt	15.87±12.45	14.16±13.65	0.947	0.344
Weight (kg)	72.94±3.97	73.64±7.12	0.824	0.411
Height (m)	1.66±0.03	1.69±0.05	3.992	<0.001*
BMI (kg/m²)	26.41±1.66	25.91±2.41	1.683	0.094

HU: Hounsfield Units, SS: Sacral Slope, PI: Pelvic incidence, BMI: Body mass index, The independent samples t test used, *: $p<0.05$

According to the AO Spine thoracolumbar classification system, there were 231 patients with type A compression injury and 13 patients with Type B tension band injury. Of the patients, 89 (36.5%) had been exposed to trauma in sitting position and 155 (63.5%) in standing position. The patients injured in sitting positions had a lower mean age (p=0.009). There was no statistically significant difference observed between the sitting and standing groups regarding HU, SS, PI, PT, weight, height and BMI results (t=0.958; p=0.339, t=0.924; p=0.356, t=0.914; p=0.057, t=1.564; p=0.119, t=0.372; p=0.710, t=1.577; p=0.116, t=0.739; p=0.461, t=0.986; p=0.325, respectively, Table 2). The female/male ratio was similar with respect to injury position (χ^2=0.094; p=0.759) (Table 3). Distribution of quantitative variables according to the AO Spine classification sitting and standing group in Table 4 and 5.

Among the patients, 44.3% had fractures in L1, 18.0% in L2 and 16.8% in T12 vertebrae. In terms of the AO Spine classification, of the fractures, 26.6% was A1, 24.2% was A2, 20.9% was A0 and 23.4% was A4. No statistically significant correlation was detected between the AO Spine classification fracture types in terms of weight, height, and BMI (F=1.411; p=0.231, F=2.063; p=0.087, F=0.833; p=0.505, respectively).The AO group had a significantly higher PI value compared to the other groups (A0=59.05±10.47, A1=49.86±10.08, A2=48.64±9.13, A4:47.95±7.92; F=11.705;_ $p\leq0.001$) (Figure 3).

Table 2. Distribution of quantitative variables by injury position

Variables	**Total Mean±SD**	**Position**		*t*	*p*
		Sitting (IVTA)	**Standing (Falling from height)**		
		Mean±SD	**Mean±SD**		
Age (years)	51.52±15.99	47.99±15.79	53.54±15.8	2.644	0.009*
HU level (hounsfield Units) (up)	171.48±59.83	176.32±62.11	168.7±58.51	0.958	0.339
HU level(down)	163.44±65.08	168.52±66.17	160.52±64.48	0.924	0.356
Sacral Slope	36.21±10.96	34.44±9.65	37.22±11.56	1.914	0.057
Pelvic incidence	50.94±10.47	49.56±9.88	51.73±10.74	1.564	0.119
Pelvic tilt	14.74±13.26	15.15±13.65	14.5±13.07	0.372	0.710
Weight (kg)	73.4±6.24	72.57±5.91	73.88±6.39	1.577	0.116
Height (m)	1.68±0.05	1.68±0.04	1.68±0.05	0.739	0.461
BMI (kg/m²)	26.07±2.2	25.89±2.41	26.18±2.06	0.986	0.325

IVTA: in-vehicle traffic accidents, BMI: body mass index, The independent samples t test used, *: $p<0.05$

Table 3. Distribution of qualitative variables by injury position

Variables		Position		χ^2	*p*
		Sitting (1)	Standing (2)		
		n (%)	n (%)		
Gender	**Female**	31 (34.8)	51 (32.9)	0.094	0.759
	Male	58 (65.2)	104 (67.1)		
Type	**1**	80 (89.9)[a]	151 (97.4)[b]	6.358	0.012*
	2	9 (10.1)[a]	4 (2.6)[b]		
Segment	**L1**	36 (40.4)	72 (46.5)	9.546	0.089
	L2	16 (18)	28 (18.1)		
	L3	9 (10.1)	22 (14.2)		
	L4	8 (9)	2 (1.3)		
	L5	4 (4.5)	6 (3.9)		
	T12	16 (18)	25 (16.1)		
AO Spine Classification	**A0**	22 (24.7)	29 (18.7)	5.561	0.230
	A1	21 (23.6)	44 (28.4)		
	A2	19 (21.3)	40 (25.8)		
	A4	19 (21.3)	37 (23.9)		
	B2	8 (9)	5 (3.2)		

Pearson chi-square test was used, [a-b]: means with the different letter in the rows indicates statistical significance, *: $p<0.05$

Table 4. Distribution of quantitative variables according to the AO Spine classification (sitting)

Variables	AO Spine Classification (sitting)					*F*	*p*
	A0	A1	A2	A4	B2		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Age (years)	49.18±14.19[ab]	50.05±14.78[ab]	58.21±16.72[b]	37.63±12.68[a]	39.63±10.13[a]	5.728	<0.001*
HU level	213±59.61[a]	178.91±73.19[ab]	137.44±58.26[b]	168.37±37.61[ab]	179.88±40.28[ab]	4.488	0.002*
Sacral Slope	33.02±9.03[abc]	31.81±10.34[ab]	39.26±10.36[ac]	30.68±7.11[b]	42.75±2.55[c]	4.525	0.002*
Pelvic incidence	54.8±9.13[a]	51.62±10.52[ab]	47.71±9.88[ab]	44.58±7.95[b]	46.01±7.63[ab]	3.844	0.006*
Pelvic Tilt	21.77±13.42[a]	19.81±12.92[a]	8.6±10.34[b]	13.89±14.29[ab]	3.26±6.3[b]	5.490	0.001*
Weight (kg)	74.27±5.92[a]	69.81±6.36[b]	73.37±5.84[ab]	74.16±5.3[ab]	69.5±2.67[ab]	2.777	0.032*
Height (m)	1.68±0.04	1.68±0.03	1.69±0.05	1.66±0.03	1.67±0.04	1.015	0.405
BMI (kg/m²)	26.29±2.09	24.91±2.66	25.92±2.71	26.88±2.21	24.98±1.2	2.219	0.074

HU: hounsfield unit, One-way analysis of variance was used, [a-d]: the same letter in the rows indicates statistical insignificance
*: $p<0.05$, BMI: body mass index

Table 5. Distribution of quantitative variables according to the AO Spine classification (standing)

Variables	AO Spine Classification (standing)					*F*	*p*
	A0	A1	A2	A4	B2		
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
Age (years)	51.62±19[a]	53.39±15.16[ab]	61.9±12.09[b]	44.92±13.29[a]	63±8.25[ab]	7.079	<0.001*
HU level	200.25±58.84[ac]	154.51±68.06[b]	126.87±58.71[c]	170.15±54.77[ab]	181±29.53[abc]	2.694	<0.001*
Sacral Slope	36±10.24	37.19±11.45	39.99±13.78	34.2±8.94	44.7±13.67	1.850	0.122
Pelvic incidence	62.27±10.41)[a]	49.01±9.88[b]	49.09±8.85[b]	49.69±7.42[b]	50.84±19.39[ab]	10.848	<0.001*
Pelvic Tilt	26.27±14.16[a]	11.8±9.16[b]	9.13±12.47[b]	15.41±11.28[b]	6.14±11.88[b]	10.783	<0.001*
Weight (kg)	72.21±5.4	73.32±6.05	75.3±7.25	73.78±6.46	77.8±4.6	1.573	0.184
Height (m)	1.68±0.04	1.67±0.04	1.69±0.06	1.68±0.04	1.66±0.04	1.418	0.231
BMI (kg/m²)	25.56±1.92	26.22±2.14	26.25±2.09	26.25±1.97	28.31±1.58	2.073	0.087

HU: hounsfield unit, One-way analysis of variance was used, [a-d]: the same letter in the rows indicates statistical insignificance
*: $p<0.05$, BMI: body mass index

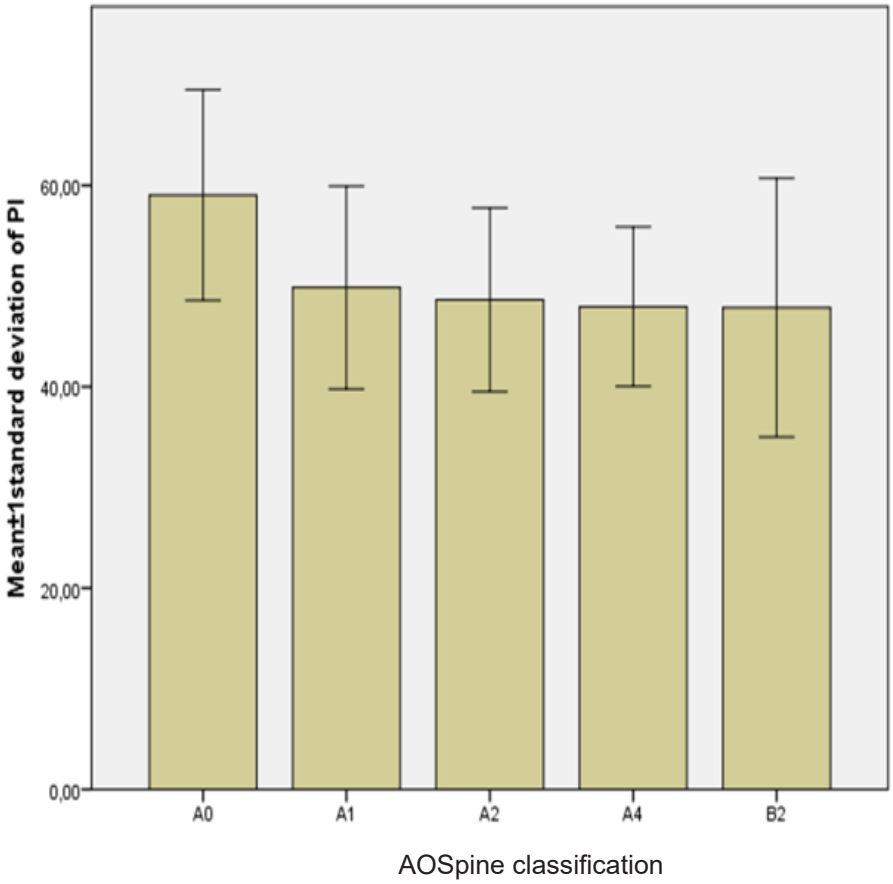

Figure 3. Bar graph of pelvic incidence values according to the AO Spine classification (Mean±1 SD)

Between the age of the patients and their HU values, there was a negative correlation considering all patients as well as in the sitting group (Table 6) and in the standing group (Table 7).

The univariate logistic regression analysis results revealed that for every one-unit increase in the PI value, the risk of more comminuted fractures (A2 and above) decreased by 0.919 times in sitting position trauma (OR:0.919; 95% CI:0.87-0.96; *p*=0.001) and by 0.957 times in standing position trauma (OR:0.957; 95% CI:0.92-0.98; *p*=0.006) (Table 8).

The univariate logistic regression analysis conducted in the study indicated that a one-unit increase in the HU value decreased the risk of more comminuted fractures (A2 and above) by 0.994 times in standing position trauma (OR:0.994; 95% CI:0.989-0.999; *p*=0.028) and by 0.989 times in sitting position trauma (OR:0.989; 95% CI:0.981-0.997; *p*=0.005) (Table 9).

As a result of the multivariate logistic regression analysis performed in the study, it was observed that for every one-unit increase in PI, the risk of more comminuted fractures (A2 and above) decreased by 0.901 times in sitting position trauma (OR:0.901; 95% CI:0.843-0.963; *p*=0.002) (Table 8) and by 0.965 times in standing position trauma (OR:0.965; 95% CI:0.933-0.998; *p*=0.040) (Table 9).

Table 6. Pairwise correlation between quantitative variables (sitting n=89)

Variables		HU level (up)	HU level (down)	Sacral Slope	Pelvic incidence	Pelvic Tilt	Weight (kg)	Height (m)	BMI (kg/m²)
Age (years)	**r**	-0.576*	-0.607*	0.082	0.092	0.011	-0.067	0.219*	-0.160
	p	<0.001	<0.001	0.442	0.393	0.915	0.533	0.040	0.135
HU level(up)	**r**	1	0.850*	-0.012	0.021	0.020	0.049	-0.022	0.047
	p		<0.001	0.909	0.844	0.855	0.646	0.841	0.659
HU level (down)	**r**		1	0.019	0.103	0.058	0.079	-0.078	0.099
	p			0.862	0.335	0.587	0.462	0.470	0.355
Sacral Slope	**r**			1	0.025	-0.688*	-0.025	0.107	-0.078
	p				0.813	<0.001	0.817	0.319	0.467
Pelvic incidence	**r**				1	0.708*	0.157	-0.066	0.164
	p					<0.001	0.143	0.536	0.126
Pelvic Tilt	**r**					1	0.133	-0.123	0.175
	p						0.216	0.251	0.102
Weight (kg)	**r**						1	-0.024	0.874*
	p							0.826	<0.001
Height (m)	**r**							1	-0.502*
	p								<0.001

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

Table 7. Pairwise correlation between quantitative variables (standing n=155)

Variables		HU level (up)	HU level (down)	Sacral Slope	Pelvic incidence	Pelvic Tilt	Weight (kg)	Height (m)	BMI (kg/m²)
Age (years)	**r**	-0.669*	-0.677*	-0.075	-0.014	0.051	0.236*	0.135	0.159*
	p	<0.001	<0.001	0.357	0.866	0.528	0.003	0.093	0.049
HU level(up)	**r**	1	0.902*	0.075	0.127	0.040	-0.190*	-0.103	-0.135
	p		<0.001	0.354	0.116	0.623	0.018	0.201	0.093
HU level(down)	**r**		1	0.099	0.212*	0.089	-0.229*	-0.117	-0.165*
	p			0.220	0.008	0.271	0.004	0.146	0.040
Sacral Slope	**r**			1	0.311*	-0.625*	-0.031	-0.038	-0.005
	p				<0.001	<0.001	0.699	0.640	0.952
Pelvic incidence	**r**				1	0.547*	-0.049	0.063	-0.096
	p					<0.001	0.541	0.435	0.237
Pelvic Tilt	**r**					1	-0.008	0.092	-0.074
	p						0.921	0.254	0.361
Weight (kg)	**r**						1	0.454*	0.763*
	p							<0.001	<0.001
Height (m)	**r**							1	-0.227*
	p								0.004

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

Table 8. Univariate and Multivariate logistic regression results (Sitting)

Variables	Univariate				Multivariate			
	p	**Odds Ratio**	**95% C.I.for Odds Ratio**		***p***	**Odds Ratio**	**95% C.I.for Odds Ratio**	
			Lower	**Upper**			**Lower**	**Upper**
Gender	0.649	1.225	0.511	2.932	0.690	1.246	0.423	3.668
PI	0.001*	0.919	0.873	0.968	0.002*	0.901	0.843	0.963
Age	0.349	0.987	0.961	1.014	0.009*	0.941	0.899	0.985
ÜSH	0.005*	0.989	0.981	0.997	0.018*	0.975	0.955	0.996
ASH	0.026*	0.992	0.985	0.999	0.974	1.000	0.980	1.021

Reference category for Gender: Female

Table 9. Univariate and Multivariate logistic regression results (Standing)

Variables	Univariate				Multivariate			
	p	**Odds Ratio**	**95% C.I.for Odds Ratio**		***p***	**Odds Ratio**	**95% C.I.for Odds Ratio**	
			Lower	**Upper**			**Lower**	**Upper**
Gender	0.174	1.596	0.813	3.133	0.066	1.985	0.957	4.118
PI	0.006*	0.957	0.927	0.988	0.040*	0.965	0.933	0.998
Age	0.523	1.007	0.987	1.027	0.543	0.991	0.961	1.021
HU level(up)	0.219	0.997	0.991	1.002	0.169	1.010	0.996	1.025
HU level(down)	0.028*	0.994	0.989	0.999	0.026*	0.985	0.971	0.998

Reference category for Gender: Female

Discussion

Lumbosacral sagittal balance has a significant role in maintaining the normal physiological function of the spine [7, 23]. Independent factors such as spinal curvature and spinal loading contribute the risk of vertebral fracture [24, 25]. Very little is known about how spinopelvic balance and spinal malalignment affect spinal load distribution. Once a vertebral fracture occurs, the risk of subsequent fracture substantially increases within the first year [26, 27]. To be able to prevent vertebral fractures, there is a need to elucidate the mechanical, morphological and biological mechanisms underlying fracture occurrence [6]. The present study demonstrated that in patients with acute vertebral fracture, the cases with more severe fracture according to the AO Spine classification are characterized by lower vertebral HU and PI values, and that HU and PI values can be utilized to identify the risk of high-grade fractures. So far as we are aware, this study is the first in the literature to show that PI is an important determinant of fracture severity in acute vertebral fractures due to trauma. Because of the relationship between spinal sagittal parameters and vertebral fracture risk, which we detected in our study in trauma patients, taking these variations in the spinal sagittal parameter into account will help better understand vertebral fracture types. To understand fully the types of vertebral fractures will make a contribution to resolve a number of question marks in the classification and treatment of thoracolumbar vertebral fractures. Our study will guide clinical and biomechanical studies to be conducted in the future to better understand vertebral fracture types.

PI is an important link between the pelvis and mobile spinal vertebral structures that determines the ability of the pelvis to rotate around the axis of the femoral head, which is the optimal compensation for sagittal alignment [28]. Because the compensatory characteristic of the pelvic incidence on the sagittal alignment, we hypothiesed that pelvic parameters may effect the vertebral fracture severity.

It is known that pelvic incidence is an individual fixed feature and does not change with body positions. An increase in the PI causes a horizontal sacrum, while a decrease in the PI causes a vertical sacrum [29, 30]. A vertical sacrum transmits the load more directly to the vertebrae. In the literature, we could not find any other study examining the relation between the PI and vertebral fracture severity. However, studies examining the relationship between degenerative spine diseases and PI have previously shown the relationship between low PI and disease severity [31, 32]. For example, Imagama et al. [31] and Strube et al. [32] showed the relationship between low PI and increased disc degeneration. Kobayashi et al. [33] reported that while high PI was associated with flexible vertebrae, low PI was associated with a more rigid spine. The presence of rigidity that is related to the decreased PI and/or the more vertical sacrum seen at decreased pelvic incidence that is related to the more direct load transfer to the vertebrae may cause a more severe fracture in response to trauma.

Relevant studies in the literature indicate that spinopelvic parameters are generally evaluated in terms of compensations and complications occurring in elderly osteoporotic vertebral fractures or after treatment. It has been stated that sagittal spinal alignment takes a significant role in the biomechanical adaptation of the spine [34, 35]. The sacrum has been recognized as the first vertebra of the spine by Dubousset [36, 37]. PI, a parameter of the sagittal spine profile, defines the angulation of the sacrum in the pelvis with respect to the hip joints [35]. Bao et al. [38] in their study evaluating osteoporotic vertebral fractures, concluded that spinal malalignment that develops after fracture will cause elevated PI, which in turn will increase the L5–S1 bending moment. In the study on osteoporotic vertebral fractures, Fechtenbaum et al. [8] reached a conclusion that pelvic parameters contribute in the development of the compensatory mechanism. The study in which Kobayashi et al. [33] evaluated the lumbo-pelvic complex showed that patients with physiologically low PI and high anatomical acetabular anteversion (anatomical AA) have a spine that indicates low lumbar lordosis (LL) when standing. They also noted that in daily life activities, low PI is associated with low vertebral sagittal flexibility, which is in turn compensated by using hip joint mobility. The detection of more severe fractures in the patients with low PI also in our study confirms that low PI leads to a more rigid spine.

Albeit it has been reported that changes occur during adolescence in PI and pelvic morphology, PI is regarded to remain anatomically constant throughout an individual's life. The normative value of PI has been stated to be 50°-55° [39]. In our study, the mean PI of the patients was found to be 50.9°, which is in accordance with the literature. No significant correlation between PI and age was found in the present study, however, there existed a close correlation between PI and fracture type.

PI impacts the force transmission and has been associated with spondylolisthesis [13]. A large PI value corresponds a horizontal sacrum located anteriorly, while a low PI value corresponds a vertical sacrum located posteriorly high [9]. Chau et al. [40] showed that spinopelvic parameters such as thoracic kyphosis (TK), PT, and PI increase after vertebral fracture. Ru et al. [41] indicated that sacral anatomical parameters show strong correlations with lumbopelvic parameters and that the specific lumbar shape may be affected by the sacral morphology. Lordosis of the spinal segments adjacent to the fracture, posterior tilting of the pelvis, hip extension, knee flexion and even ankle dorsiflexion may develop to compensate for the kyphosis that may occur in the sagittal plane after vertebral fractures [42]. Previous studies demonstrated that patients with vertebral fracture have higher TK and lower LL [8]. On the contrary, Smorgick et al. [43] found no significant correlation between PI, PT, SS, fracture type, or fracture height loss in their study in which 124 patients were included.

Vertebral HU has been reported to be an effective parameter for the detection of osteoporosis [19, 44]. In elderly patients with osteoporotic fractures, much lower HU values have been detected than in those without fracture [45]. Our study confirms these results.

Our study bears certain limitations. First, there was a limited number of cases in the study, and all of which were from a single center. Second, the study was a retrospective analysis. For this reason, as the tomographies were taken only in the supine position, the variation of sagittal spinal parameters in other positions was not able to be examined. Besides, mechanisms such as knee flexion and ankle extension that may affect the fracture mechanism were not taken into account. Further prospective studies are necessary to better elucidate the association between spinal parameters and fracture.

In conclusion low PI causes a more rigid vertebral column, which indicates that patients with low PI are at increased risk in terms of high degree fractures according to the AO Spine classification, and that pelvic parameters play a role in compensatory mechanisms. The lower the vertebral HU value, the more likely patients are to have a high-grade vertebral fracture.

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Authors contributions: V.K. and S.A. constructed the main idea and hypothesis of the study. V.K. and S.A. developed the theory and arranged/edited the material and method section. V.K. and S.A. have done the evaluation of the data in the results section. Discussion section of the article written by V.K. and S.A.

V.K. and S.A. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Research Article

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Evaluation of knowledge and attitudes of female university students about ovarian reserve awareness and technologies for Ovarian Reserve: a cross-sectional study

Kız üniversite öğrencilerinin over rezervi farkındalığı ve buna yönelik teknolojiler hakkındaki bilgi ve tutumlarının değerlendirilmesi: kesitsel çalışma

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Abstract

Purpose: The aim of this study was to evaluate the knowledge and attitudes of female university students about ovarian reserve awareness and technologies for ovarian reserve.

Materials and methods: This study was designed as a descriptive and cross-sectional study and was conducted on 660 female university students. The data were collected by using a questionnaire form based on the literature on ovarian reserve and related technologies. The mean, standard deviation and percentage distributions of the data obtained through the WEB page and face-to-face interview technique were analysed in the SPSS programme.

Results: 86.2% of the students wanted to have children in the future and 75.8% planned to have children between the ages of 26-30. 56.7% of the students were aware of the availability of tests related to ovarian reserve and 86.6% of them demanded the development of new tests. In case of low ovarian reserve, 65.6% of the students stated that they could have children earlier, 68% could freeze their eggs, 51.2% could freeze their embryos, 73.8% could adopt a child and 84.7% could continue their work/education.

Conclusion: It is remarkable that the majority of the students did not make any attempt for ovarian reserve evaluation although they wanted to have a child. Among the reasons for this situation, besides the fact that the students are still receiving education, they have false beliefs that their ovarian reserves will be sufficient at the age when they want to have children, that a healthy lifestyle and activity protect the ovarian reserve, and that they can have children with assisted reproductive techniques even if their ovarian reserves are low. It is important to evaluate the ovarian reserves of young women at an early stage in order for them to make a more conscious career and family planning.

Keywords: Ovarian reserves, fertility, infertility, female, students.

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Öz

Amaç: Bu araştırmanın amacı, kız üniversite öğrencilerinin over rezervi farkındalığı ve buna yönelik teknolojiler hakkındaki bilgi ve tutumlarının değerlendirilmesidir.

Gereç ve yöntemler: Bu araştırma tanımlayıcı ve kesitsel tipte tasarlanmış olup,660 kız üniversite öğrencisi üzerinde yapılmıştır. Araştırmanın verileri over rezervi ve buna yönelik teknolojiler hakkında literatüre dayanarak hazırlanan bir anket formu kullanılarak toplanmıştır. WEB sayfası üzerinden ve yüz yüze görüşme tekniğiyle elde edilen verilerin aritmetik ortalama, standart sapma ve yüzde dağılımları SPSS programında analiz edilmiştir.

Bulgular: Öğrencilerin %86,2'si gelecekte çocuk sahibi olmayı istemekte ve %75,8'i ise 26-30 yaş arasında çocuk sahibi olmayı planlamaktadır. Öğrencilerin %56,7'si over rezervi ile ilgili testlerin olduğunun farkında olup, %86,6'sı ise yeni testler geliştirilmesini talep etmektedir. Öğrenciler over rezervlerinin düşük olması durumunda %65,6'sı daha erken çocuk sahibi olabileceğini, %68'si yumurtalarını dondurabileceğini, %51,2'si embriyolarını dondurabileceğini, %73,8'i evlat edinebileceğini ve %84,7'si iş/eğitimine kaldığı yerden devam edebileceğini belirtmiştir.

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Sonuç: Öğrencilerin büyük bir çoğunluğunun çocuk sahibi olmayı istemelerine rağmen over rezervi değerlendirilmesi için herhangi bir girişimde bulunmaması dikkat çekicidir. Bu durumun nedenleri arasında ise, öğrencilerin henüz eğitim alıyor olmalarının yanında, çocuk sahibi olmak istedikleri yaşlarda over rezervlerinin yeterli olacağına, sağlıklı yaşam biçimi ve aktivitenin over rezervini koruduğuna, over rezervleri düşük olsa bile yardımcı üreme teknikleri ile çocuk sahibi olabileceklerine yönelik yanlış inanışlarıdır. Genç kadınların over rezervlerinin erken dönemde değerlendirilmesi daha bilinçli kariyer ve aile planlaması yapabilmeleri açısından önemlidir.

Anahtar kelimeler: Yumurtalık rezervleri, doğurganlık, infertilite, kadın, öğrenciler.

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Introduction

As the age of the woman increases, the number of follicles (the spherical structure in the ovary in which the egg develops, which varies in size according to the stages of development, filled with fluid, with a cavity) and egg quality decrease. In the medical literature, this is referred to as a decrease in fertility, that is, a decrease in ovarian reserve (the number of eggs in the ovaries) [1-3]. This decrease may vary between women due to some biological differences. For example, when a girl is born, the follicles in her ovaries are lower than normal, and the proportion of follicles that undergo atresia (some of the follicles that are congenital in the ovary lose their function during the developmental stages) and follicles that start to grow are related to this situation [4, 5]

Primary ovarian insufficiency (POI) is a condition in which a woman stops menstruating (menstruation stops) or menstruation is less or less frequent than normal before the age of 40, the follicles in the ovaries are depleted or the follicles in the ovaries lose their function due to the increase in FSH (follicle-stimulating hormone) hormone. POI is also known as POF (prematureovarianfailure) [6]. The decrease in the number of ovaries can occur with a condition such as menstrual irregularity or without any symptoms. Primary Ovarian Failure has a significant impact on the health of the ovaries. Early diagnosis of Primary Ovarian Failure is important for women who want to have children (conceive) [7, 8].

Although the information on age-related fertility decline is quite abundant in the literature, what is currently discussed is that the decline in ovarian reserves varies and women postpone having children at the age when their fertility is at its peak [9-11]. Women may postpone their first pregnancy because they want to increase their level of education and pursue their career, they want to reach a certain maturity before having children, they cannot find the right mate, and they think that their independence will be limited [9-13].

In Türkiye, there is not enough literature evaluating women's awareness of ovarian reserve and their knowledge and attitudes about the technologies related to it. It is estimated that the future pregnancy plans of women who can use such screening technologies and have sufficient knowledge on this subject will be positively affected. This study will enable the determination of female university students' attitudes towards fertility and childbearing before they have children. It is also aimed to increase the awareness of young women about ovarian reserves and screening technologies used.

Matherials and methods

Type of research

This study was planned as descriptive and cross-sectional type.

Population and sample of the study

The population of the study consisted of undergraduate, graduate and doctoral students at Pamukkale University, aged 18 and over, who can read and understand Turkish. The sample calculation was made by the method of sample size calculation (99% confidence interval) in cases where the population was unknown and it was planned to reach at least 647 female students. Twelve female students who did not fully respond to the survey questions were

not included in the scope of the research and the research was completed with 660 female students. Unhealthy female students who could not read and understand Turkish and who had a disease in their reproductive organs that prevented childbearing were excluded from the scope of the research.

Data collection tools

The data of the study were collected by applying a questionnaire on the WEB page and face-to-face interviews. A questionnaire form consisting of 48 items prepared on the basis of the literature to evaluate the knowledge and attitudes of female university students about ovarian reserve awareness and technologies for ovarian reserve was used for data collection [14-16].

This study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (dated 15.12.2015, number: 60116787-020/4280 and, meeting and numbered 21). After the ethics committee permission was obtained, institutional permissions were obtained for the implementation of the research. After each participant was informed about the purpose of the research, its implementation, and the voluntariness of participating in the research, a voluntary consent form was given to those who wanted to participate and their consent was obtained. In addition, the study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

SPSS programme was used for statistical analysis of the obtained values. Arithmetic mean, standard deviation and percentage distributions were made for descriptive data.

Results

The mean age of 660 female university students was 21.23±8.17 years. 96.1% of the students have undergraduate education and 48.8% of them want to have postgraduate education. 86.2% of the students want to have children in the future and 75.8% of them plan to have children between the ages of 26-30. The mothers of 26.1% of the students entered menopause at the age of 40-49 years. Socio-demographic characteristics of female students are presented in Table 1.

The opinions of the students about the use of medical technologies developed to evaluate the current status of ovarian reserves are given in Table 2. 56.7% of the students were aware of the existence of tests related to ovarian reserve and 86.6% of them demanded the development of new tests. Only 2% of the students had tests for ovarian reserve evaluation (Table 2).

89.8% of the students would like to obtain information about their ovarian reserve in general, 79.1% immediately, 90.5% in five years and 88.2% in 10 years. 90.2% of the students think that the number of eggs is “something that should be known”. 80% of the students stated that they would pay for infertility tests in the future even if they were not covered by insurance (Table 2).

The responses of the students to the questions asked based on the assumption of low ovarian reserves are given in Table 3. In case of low ovarian reserves, 45.2% of the students could get married earlier, 65.6% could have children earlier, 68% could freeze their eggs, 51.2% could freeze their embryos, 34. 4% could benefit from egg donation, 73.8% could adopt, 7.7% could leave their current job/education, 18.2% could postpone their job/education, 84.7% could continue their job/education (Table 3).

The correct answers given by the students to the questions about reproductive health are presented in Table 4. Only 14.5% of the students stated that “The ability of women to have children begins to decrease in the 30-34 age group”. A significant majority of the students (74.4%) stated that women’s reproductive ability decreases in the 35-39 age group. 54.4% of the students stated that “when a girl is born, she is born with the number of eggs she will have all her life”, 89.8% stated that “smoking can reduce the number of eggs in a woman”, 97.1% stated that “the number of eggs in the ovaries of women can vary even if they are at the same age”, 84. 5% correctly answered the statement “a woman who will undergo cancer treatment may decide to freeze her eggs before treatment”, 86.7% correctly answered the statement “women with a family history of early menopause (menstrual cessation) may enter early menopause” (Table 4).

Table 1. Socio-demographic characteristics of female students (n:660)

Variable	mean±SD
Age* (min.-max.=18-30)	21.23±8.17
Marital Status	**n (%)**
Married	10 (1.5)
Single	650 (98.5)
Section	
Economics	283 (42.9)
Nursing	227 (34.3)
Education Sciences	56 (8.5)
Architecture	44 (6.7)
Engineering	38 (5.8)
Faculty of Medicine	12 (1.8)
Current level of education	
Associate degree	10 (1.5)
Undergraduate	634 (96.1)
Master's degree	11 (1.7)
PhD	5 (0.8)
Expectations about educational career	
I want to stay as an associate degree graduate	10 (1.5)
I want to stay as a bachelor's graduate	243 (36.8)
I want to do a master's degree	322 (48.8)
I want to do a PhD	19 (2.9)
I want to continue my academic career after my PhD	66 (10)
Childbearing status	
Yes	3 (0.5)
No	657 (99.5)
Desire to have children	
I want to	569 (86.2)
I don't want to	28 (4.2)
I don't know	63 (9.6)
Age at which she plans to have a child	
18-25 years old	65 (9.8)
26-30 years old	500 (75.8)
31-35 years old	92 (13.9)
36-40 years old	2 (0.3)
41-45 years	-
46-50 years old	1 (0.2)
Age at menopause of their mother	
<40	27 (4.1)
40-49	172 (26.1)
≥50	91 (13.7)
I don't know	353 (53.5)
Not yet menopausal	17 (2.6)

Data are expressed as n (%), * Means ± standard deviations are given

Table 2. Opinions of female students about ovarian reserves (n:660)

Opinions				
Awareness of tests for ovarian reserve				374 (56.7)
Having tests for ovarian reserve assessment				13 (2.0)
Requesting the development of new tests for ovarian reserve evaluation				585 (86.6)
	Strongly Agree	**I agree**	**Disagree**	**Absolutely Disagree**
I would like to know more about the number of eggs in general	276 (41.8)	317 (48.0)	58 (8.8)	9 (1.4)
I would like to know more about my egg count immediately	194 (29.4)	328 (49.7)	120 (18.2)	18 (2.7)
I would like to know more about my egg count in the next 5 years	283 (42.9)	314 (47.6)	52 (7.9)	11 (1.6)
I would like to know more about my egg count in the next 10 years	284 (43.0)	298 (45.2)	64 (9.7)	14 (2.1)
I feel like the number of eggs is something to know	302 (45.8)	293 (44.4)	57 (8.6)	8 (1.2)
I will pay for infertility tests in the future, even if they are not covered by insurance	243 (36.8)	285 (43.2)	94 (14.2)	38 (5.8)

Data were analysed as n (%)

Table 3. Predictions of female students about what they could do in case of low ovarian reserves (n:660)

Predictions	**Strongly Agree**	**I agree**	**Disagree**	**Absolutely Disagree**
I'd have got married earlier	91 (13.8)	207 (31.4)	290 (43.9)	72 (10.9)
I would have had children earlier	145 (22.0)	288 (43.6)	184 (27.9)	43 (6.5)
I would freeze eggs	132 (20.0)	317 (48.0)	161 (24.4)	50 (7.6)
I would freeze embryos	92 (13.9)	246 (37.3)	241 (36.5)	81 (12.3)
I would use egg donation	51 (7.7)	176 (26.7)	303 (45.9)	130 (20.7)
Adopt a child	166 (25.2)	321 (48.6)	138 (20.9)	35 (5.3)
I would give up my current job / education	19 (2.9)	32 (4.8)	276 (41.8)	333 (50.5)
I would postpone my work/training	23 (3.5)	97 (14.7)	261 (39.5)	281 (42.3)
I would continue my work/education related life from where I left off	257 (38.9)	302 (45.8)	74 (11.2)	27 (4.1)

Data were analysed as n (%)

Table 4. Female students' answers to questions on reproductive health (n:660)

Questions	Answers	Number of correct answers (%)
Multiple choice		
At what age women's ability to have children begins to decline	30-34 age	96 (14.5)
That's right / Wrong		
When a girl is born, she is born with the number of eggs she will have all her life	That's right	359 (54.4)
Smoking can reduce a woman's egg count	That's right	593 (89.8)
Women who take the contraceptive pill maintain a healthy egg count	Wrong	388 (58.8)
Regular menstruation while taking the contraceptive pill is an indication of a healthy egg count	Wrong	274 (41.5)
IVF treatment enables even women with a very low egg count to conceive	Wrong	111 (16.8)
Even at the same age, the number of eggs in the ovaries of women can vary	That's right	641 (97.1)
Exercise and a healthy diet help women to maintain the number of eggs	Wrong	43 (6.5)
A woman who will undergo cancer treatment may decide to freeze her eggs before treatment	That's right	558 (84.5)
Women with a family history of early menopause (menstrual cessation) may enter early menopause	That's right	572 (86.7)

Data were analysed as n (%)

58.8% of the students evaluated the statement "the number of eggs of women taking birth control pills is preserved in a healthy way", 41.5% of the students evaluated the statement "regular menstruation while taking birth control pills is an indicator of a healthy number of eggs", 16.8% of the students evaluated the statement "in vitro fertilisation treatment enables even women with a very low number of eggs to become pregnant" and only 6.5% of the students evaluated the statement "exercise and a healthy diet enable women to preserve the number of eggs" as incorrect (Table 4).

Discussion

In this study, although 56.7% of the students were aware of the technologies (tests) developed to assess the current status of ovarian reserves, only 2% stated that they had the relevant tests. 86.6% of the students were interested in the development of new tests to assess the current status of ovarian reserves. A significant majority of the students (90.2%) think that the number of eggs is "something that should be known". Students mostly plan to have children between the ages of 26-30 (75.8%). In other words, although they wanted not to have children in an average of 5-10 years, they did not make any attempt for ovarian reserve evaluation. In general, 89.8%, 79.1%, 90.5%, 90.5% and 88.2% of the students wanted to obtain information about their ovarian reserves immediately, within five years and within 10 years, respectively. In a study conducted by Bavan et al. [14] (2011) on the attitudes of university students towards ovarian reserve technologies, 79% of the participants stated that they were interested in technologies for the evaluation of ovarian reserve, but the rate of those who wanted their ovarian reserves to be evaluated now decreased to 43%, and 70% of the participants wanted to know the status of their ovarian reserves within the first five years and 87% within the first 10 years. In line with these results, the fact that the mean age of the students was 21.23±8.17 years, that they had undergraduate education (96.1%), planned postgraduate education (61.7%), were aware of the tests and wanted to have children (86.2%) may be an indication that they did not attempt to evaluate their ovarian reserves.

In this study, the attitudes of the students in case of decreased ovarian reserves were also evaluated. The responses of the students to the questions asked based on the assumption of low ovarian reserves focused on the options of becoming a younger mother, getting married at an earlier age, egg freezing, embryo freezing and adoption. However, it was concluded that although being fertile is very important for these young women, it is not more important than their work/education life. In the study conducted by Bavan et al. [14] (2011), it was found that two-thirds of young women did not want to interfere with their work/education careers in order to have a child. In the same study, it was found striking that women were open to using assisted reproductive technologies despite this behaviour. In our study, the fact that young women mostly stated that they would pay for infertility tests in the future even if they were not covered by insurance supports this finding.

In line with the answers given by university students regarding reproductive health, it was determined that they lacked knowledge on this subject. For example, 14.5% of them answered correctly that women's ability to have children starts to decrease between the ages of 30-34. In a study conducted similar to this result, it was stated that one third of young women answered this question correctly [14]. Another study shows that although there are many reasons to postpone parenthood, female university students lack knowledge about the decline in fertility with age [17]. In line with the findings of this study, it is important that young women, especially in this age group, need education on fertility and reproductive health and that the gap in this area should be closed by health professionals.

Another important finding regarding reproductive health is that 83.2% of the students think that it is possible to have a child with in vitrofertilisation (IVF) despite decreased ovarian reserve. Similar results were found in a previous study [14]. In order to prevent this important misconception, it is inevitable that young women should be educated about assisted reproductive technologies. In addition, 93.5% of the students in this study believed that it was possible to protect the ovarian reserve of women with exercise and healthy nutrition. Similarly, in the study conducted by Bavan et al. [14] (2011), approximately three-quarters of the women who completed the questionnaire thought that exercise paired with a healthy diet could protect ovarian reserve. Due to all these lack of information, it is surprising for women who have had good health in the past and who do not have any medical problems or symptoms to be diagnosed with infertility when they want to have children [14, 18]. It is seen that the average age at which women give their first birth increases with increasing education level. It is important to detect the decline in ovarian reserves of women in the early period so that they can make more conscious career and family planning.

In conclusion; in this study, as a result of taking the opinions of young university women about ovarian reserve, deficiencies related to this subject were revealed. Especially young women should have an awareness for early evaluation of ovarian reserves. It has been reported that providing fertility information at an early age, such as during college, can help correct common misconceptions about fertility and support realistic family formation planning without negatively affecting educational and career goals (18). Based on the results of this study, it was revealed that health professionals should organise awareness trainings on ovarian reserve and fertility awareness for young women.

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Anatomical evaluation of proximal femur fractures in trauma patients aged 65 or older admitted to the emergency department

Acil servise kabul edilen 65 yaş ve üzeri travma hastalarında proksimal femur kırıklarının anatomik değerlendirmesi

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Abstract

Purpose: This retrospective study aimed to assess the association between classification systems for proximal femur fractures and mid-term mortality in elderly patients, focusing on their clinical and anatomical aspects.

Materials and methods: Radiological images of patients aged 65 years and older who underwent surgical procedures for proximal femur fractures were reviewed. Various classification systems were applied, including Anatomical, Pipkin, Garden, Evans-Jensen, Seinsheimer, and AO/OTA classifications. Electronic hospital records provided patient data, and statistical analyses were performed.

Results: The study included 298 patients, and the mean age was 81.7±7.3 years, and 63.1% were female. Median length of stay in hospital 7 (1-63) days, 19.1% requiring intensive care, and a 13.8% mortality rate within 3 months. Patients were distributed based on anatomical classification, and the distribution of intracapsular and extracapsular fractures according to clinical classifications was detailed. The findings suggest that proximal femur fracture classification systems do not significantly influence mortality rates (p=0.787).

Conclusion: Anatomical classification systems may be favored for their simplicity and potential to establish a common language among healthcare professionals. This study provides valuable insights into proximal femur fractures in elderly patients, informing clinical practice.

Keywords: Anatomy, classification, femur, geriatric, hip fractures.

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Öz

Amaç: Bu retrospektif çalışma, proksimal femur kırıkları için sınıflandırma sistemleri ile yaşlı hastalarda orta vadeli mortalite arasındaki ilişkiyi değerlendirmeyi amaçlamıştır, odaklanılan nokta ise klinik ve anatomik yönleridir.

Gereç ve yöntem: Cerrahi işlem uygulanan proksimal femur kırıklı hastaların radyolojik görüntüleri incelendi. Anatomik, Pipkin, Garden, Evans-Jensen, Seinsheimer ve AO/OTA sınıflandırmaları olmak üzere çeşitli sınıflandırma sistemleri uygulandı. Elektronik hastane kayıtları hastaya ait verileri sağladı ve istatistiksel analizler yapıldı.

Bulgular: Çalışma, 298 hastayı içeriyordu ve ortalama yaş 81,7±7,3 yıl idi, %63,1'i kadındı. Hastanede kalış süresi, ortalama 7 (1-63) gün idi, %19,1'i yoğun bakım gerektiriyordu ve 3 ay içinde %13,8'lik bir mortalite oranı görüldü. Hastalar, anatomik sınıflandırmaya göre dağıtıldı ve klinik sınıflandırmalara göre intrakapsüler ve ekstrakapsüler kırıkların dağılımı detaylandırıldı. Bulgular, proksimal femur kırık sınıflandırma sistemlerinin mortalite oranlarını önemli ölçüde etkilemediğini öne sürmektedir (p=0,787).

Sonuç: Anatomik sınıflandırma sistemleri, basitliği ve sağlık profesyonelleri arasında ortak bir dil oluşturma potansiyeli nedeniyle tercih edilebilir. Bu çalışma, yaşlı hastalarda proksimal femur kırıkları hakkında değerli içgörüler sağlayarak klinik uygulamayı bilgilendirir.

Anahtar kelimeler: Anatomi, sınıflandırma, femur, geriatri, kalça kırıkları.

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Introduction

Hip fractures are one of the most prevalent types of fractures, predominantly afflicting the elderly demographic. A discernible escalation in hip fracture incidence is observed, concomitant with the aging global populace, and is principally attributed to pervasive osteoporosis. Projections delineate a significant amplification in both the incidence and the concomitant medical expenditures associated with hip fractures in the forthcoming decades [1, 2]. In the orchestration of strategic treatment paradigms for elderly individuals besieged with hip fractures, healthcare professionals customarily leverage established fracture classification systems. These classification frameworks wield a consequential influence, dictating the trajectory of treatment modalities and inherently impacting the prospective complications and therapeutic outcomes associated with the elected treatment strategies [3, 4].

Hip fractures are systematically categorized into two predominant groups, delineated based on their relational proximity to the capsular attachment: intracapsular and extracapsular fractures [5]. Intracapsular fractures, situated within the confines of the hip joint capsule, are subject to a multitude of classification paradigms. Prominent among these classification mechanisms are Garden's Classification, Pauwels' Classification, and the Arbeitsgemeinschaft für Osteosynthesefragen/ Orthopaedic Trauma Association (AO/OTA) Classification, each offering a nuanced approach to fracture assessment and categorization [6-8]. The Garden Classification is a system used to radiologically assess femoral neck fractures, categorizing them into four types based on the degree of fracture displacement. The Pauwels Classification, on the other hand, classifies femoral neck fractures according to the angle formed between the fracture line and the horizontal plane. Conversely, extracapsular hip fractures manifest externally to the hip joint capsule and encompass variations such as intertrochanteric and subtrochanteric fractures. These fractures are evaluated and classified utilizing a diverse array of mechanisms, with notable classifications including the Evans Classification, AO/OTA Classification, Jensen Classification, and Seinsheimer Classification. Each classification system provides a structured framework, facilitating a comprehensive and nuanced understanding of the fracture's anatomical and clinical intricacies [2, 9]. The Evans Classification categorizes fractures based on stability, assessing them according to the direction of fracture lines and the degree of displacement. The Jensen Classification evaluates intertrochanteric fractures based on stability and the extent of comminution. The Seinsheimer Classification grades fractures according to the degree and number of fragments, assessing the severity of the fracture. The AO/OTA Classification provides a detailed categorization of bone fractures based on anatomical and biomechanical principles.

Hip fractures are anatomically delineated based on the specific location and nature of the fracture within the hip joint, primarily bifurcating into intracapsular and extracapsular fractures. Extracapsular fractures manifest externally to the hip joint capsule and are further subclassified into intertrochanteric fractures, located between the greater and lesser trochanters, and subtrochanteric fractures, occurring below the lesser trochanter and extending into the femoral shaft [2, 5, 9]. Intracapsular fractures, on the other hand, are localized within the hip joint capsule. These fractures are further categorized into femoral neck fractures, which occur at the juncture of the femoral neck and head, and femoral head fractures, which involve the femoral head directly. Femoral head fractures are relatively rare, predominantly associated with high-energy traumatic incidents [3]. Femoral neck fractures can be classified into subcategories such as subcapital fractures, transcervical fractures, and basicervical fractures [10]. Basicervical fractures typically manifest proximal to or along the intertrochanteric line and are generally categorized as extracapsular, with their treatment protocols aligning closely with those of intertrochanteric fractures [11, 12].

Classification systems for hip fractures play a pivotal role in steering clinical decisions, influencing treatment strategies, forecasting potential complications, and outcomes associated with various fracture types. These systems facilitate informed decision-making regarding surgical interventions, implant selections, and the formulation of robust rehabilitation strategies tailored to individual patient needs [8, 13]. However, the

diversity of classification systems and the varied nomenclature employed within clinical settings often breed confusion and ambiguity, complicating the communication and decision-making processes.

We advocate for the prioritization of anatomical classification systems, as they foster a unified language and enhance clarity among clinicians, thereby streamlining clinical communications and decision-making. In our study, we aimed to assess the efficacy of anatomical classification in predicting mortality outcomes for proximal femur fractures, juxtaposed against other prevalent classification methodologies utilized in clinical settings.

Materials and methods

A retrospective study was meticulously designed and conducted at a single center during the period from January 1, 2019 to December 31, 2020. This study primarily involved the meticulous examination of radiological images belonging to patients aged 65 years and above, who had endured proximal femoral fractures and subsequently underwent surgical procedures. Radiological images utilized in this study were diligently sourced from the Picture Archiving and Communication System (PACS). Ethical clearance for conducting this study was graciously accorded by the “Medical and Health Sciences Ethics Committee – 1” of Muğla Sıtkı Koçman University on May 15, 2023, bearing the reference number 220046-50. Given the retrospective nature of this study, the customary requirement for obtaining informed written consent from participants was judiciously waived.

The criteria for inclusion in this study were meticulously defined to ensure a focused and relevant participant selection. Eligible participants were required to be aged 65 years or older, with a documented history of experiencing a slip, fall, or trivial trauma, and must have undergone a surgical procedure. Additionally, a minimum of 3-months of follow-up data was necessitated for each participant. Exclusion criteria were also carefully delineated to maintain the study’s integrity. Participants who had encountered multiple traumas, those who opted not to receive treatment, and individuals whose radiological images were inaccessible through the PACS were systematically excluded from participation in the study.

Upon the successful identification of eligible participants, a comprehensive assessment of their radiological images was undertaken by expert Emergency Physicians, who conducted the evaluation with an unbiased approach, devoid of prior knowledge regarding the patients’ identities. A multifaceted classification strategy was employed, utilizing a diverse array of classification systems such as the Anatomical, Pipkin, Garden, Evans-Jensen, Seinsheimer, and AO/OTA classifications. In a concerted effort to garner a holistic understanding of the patients’ medical histories and current health statuses, electronic hospital records were meticulously reviewed. This review process aimed to collate essential information, including the patients’ age, gender, the necessity for admission to the Intensive Care Unit (ICU), the duration of their hospital stay, and the incidence of mortality within a 90-day period, encompassing all causative factors.

Statistical analysis

The distribution of the data was rigorously evaluated for normality utilizing the Kolmogorov-Smirnov test. Continuous variables were articulated through two distinctive methods to enhance the precision and clarity of the presentation. For data adhering to a normal distribution, values were depicted as means accompanied by their respective standard deviations (mean±SD). Conversely, for data not conforming to a normal distribution, values were presented as median (min-max). Categorical variables were meticulously represented, employing absolute values and their corresponding percentages to facilitate a comprehensive and nuanced understanding. Comparative analyses between groups were executed utilizing a Chi-square test, fostering a robust comparative evaluation. For all tests, $p<0.05$ (2 sided) was considered statistically significant. All analyses were performed using SPSS version 23.0 statistical software (SPSS, Inc, Chicago, IL).

Results

Over a two-year period, 64,890 patients presented to the ED trauma area, of which 19.955 were aged 65 and over. Among all patients, 1.152 were diagnosed with a femur fracture based on ICD codes. After excluding patients with multiple traumas, the number was reduced to 524. Further exclusions were made

for those with a history of previous surgery, those who refused treatment at our hospital, and those who were not treated surgically, resulting in a study cohort of 326 patients. During the three-month mortality follow-up, data were successfully obtained for 304 patients, while direct radiographs were missing for 6 patients in the PACS system. Ultimately, the final analysis included data from 298 patients (Figure 1).

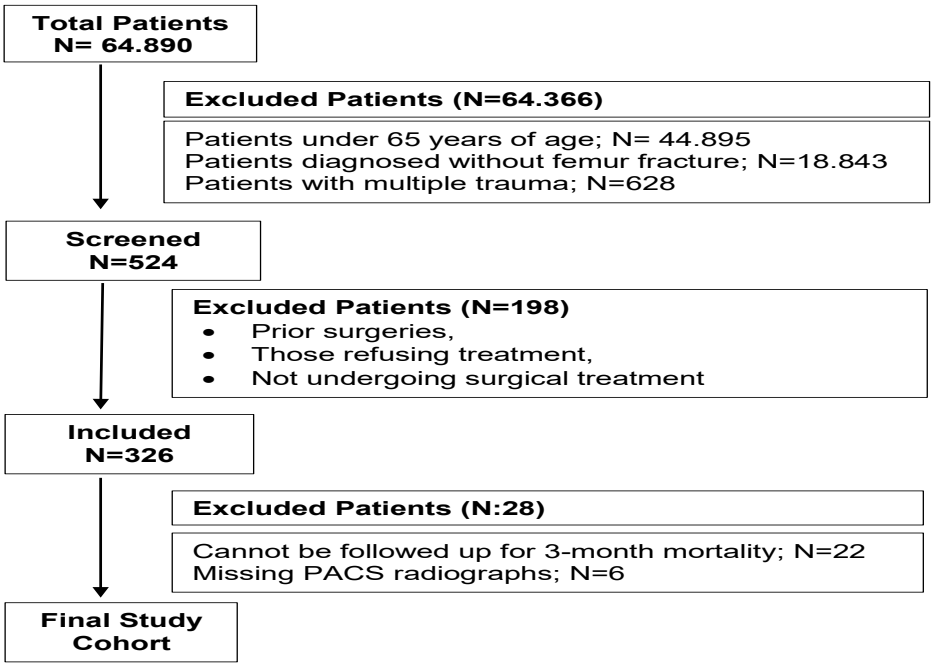

Figure 1. Flow chart

A comprehensive demographic analysis revealed that the participants' ages were distributed with a mean of 81.7±7.3 years, ranging broadly from 65 to 102 years, and a median age manifesting at 83 years. A notable predominance of females was observed within the study population, constituting 63.1% of the total participants, thereby highlighting a gender-based inclination in the occurrence of the fractures. An anatomical perspective of the fractures disclosed that 156 patients, representing 52.3% of the population, sustained fractures in their right femur. Conversely, the left femur was implicated in the fractures sustained by 142 patients, accounting for 47.7% of the participants, thus illustrating a relatively balanced distribution of fractures across the anatomical locations.

The hospitalization period exhibited variability among the patients, with the median duration of stay established at 7 (1-63) days. A segment of the patient population, constituting 19.1% (57 patients), necessitated admission into ICUs as part of their treatment protocol, underscoring the severity and complexity of their clinical presentations. In a pursuit to elucidate the mid-term mortality rates, a survival analysis spanning a 3-month period post-surgery was meticulously conducted. The findings from this analysis unveiled a mortality rate of 13.8%, representing 41 patients who unfortunately succumbed within the initial 3 months subsequent to their surgical procedures.

Table 1 meticulously delineates the distribution of patients who sustained proximal femur fractures, categorized based on anatomical classifications, and correlates these classifications with respective mortality rates. Moving on, Table 2 provides a comprehensive display of the distribution of patients who endured intracapsular fractures, with classifications articulated according to various established criteria such as the Pipkin, Garden, and AO/OTA classifications. Concluding this segment, Table 3 and Table 4 systematically presents the distribution of patients afflicted with extracapsular fractures, classified according to several recognized systems including the Evans-Jensen, Seinsheimer, and AO/OTA classifications.

Table 1. Patient distribution based on anatomical classification and mortality

	Intracapsular, n:100 (100.0)				Extracapsular, n:198 (100.0)		
	Femoral Head (n=2)	**Subcapital (n=12)**	**Transcervical (n=66)**	**Basocervical (n=20)**	**Interthoracanteric (n=185)**	**Subthoracanteric (n=13)**	***P* (test value)**
Survivor, n (%)	2 (2.0)	11 (91.7)	56 (84.8)	18 (90.0)	157 (84.9)	13 (100)	0.776 (2.470)[a]
Mortality, n (%)	0	1 (8.3)	10 (15.2)	2 (10.0)	28 (15.1)	0	

	Intracapsular, n:100 (100.0)	**Extracapsular, n:198 (100.0)**	***P* (test value)**
Survivor, n (%)	87 (87)	170 (85.9)	0.927 (0.008)[b]
Mortality, n (%)	13 (13.0)	28% (14.1)	

Data are expressed as count (n) and %. Abbreviations: AO/OTA, Arbeitsgemeinschaft für Osteosynthesefragen/Orthopaedic Trauma Association

[a] Fisher-Freeman Halton test was used as Chi-square test result to comparise subgroups of femoral fractures in term of mortality

[b] Yates' corrected-Chi-square test was used for Chi-square test result to comparise intracapsular vs extracapsular fractures in term of mortality

Table 2. Distribution of intracapsular fracture patients according to clinical classifications and mortality numbers

-		AO/OTA classification	Survivor, n:87 (%)	Mortality, n:13 (%)
Pipkin classification	**Pipkin 1**	31-C1.1	0	0
		31-C1.2	0	0
		31-C1.3	0	0
	Pipkin 2	31-C2.1	0	0
	Pipkin 3	31-C2.2	1 (1.1)	0
	Pipkin 4	31-C2.3	3 (3.4)	0
Garden classification	**Garden 1**	31-B1.1	1 (1.1)	0
		31-B1.2	5 (5.8)	0
		31-B1.3	2 (2.3)	1 (7.7)
	Garden 2	31-B2.1	11 (12.7)	2 (15.4)
		31-B2.2	14 (16.1)	5 (38.5)
		31-B2.3	9 (10.3)	2 (15.4)
	Garden 3	31-B3	29 (33.3)	2 (15.4)
	Garden 4		12 (13.8)	1 (7.7)

Data are expressed as count (n) and %. AO/OTA, Arbeitsgemeinschaft für Osteosynthesefragen/Orthopaedic Trauma Association

Table 3. Distribution and mortality numbers of patients with extracapsular fractures according to Evans-Jensen classification and OA/ATO classification

-		AO/OTA classification	Survivor, n:157 (%)	Mortality, n:28 (%)
Evans-Jensen classification	**Type 1**	31-A1.1	4 (2.5)	1 (3.6)
	Type 2	31-A1.2	25 (15.9)	1 (3.6)
	Type 3	31-A1.3	106 (67.5)	19 (67.8)
	Type 4	31-A2.1	6 (3.8)	1 (3.6)
		31-A2.2	6 (3.8)	1 (3.6)
		31-A2.3	1 (0.7)	1 (3.6)
	Type 5	31-A3.1	1 (0.7)	0
		31-A3.2	3 (1.9)	4 (14.2)
		31-A3.3	5 (3.1)	0

Data are expressed as count (n) and %. AO/OTA, Arbeitsgemeinschaft für Osteosynthesefragen/Orthopaedic Trauma Association

Table 4. Distribution and mortality numbers of patients with extracapsular fractures according to Seinsheimer classification and OA/ATO classification

-		AO/OTA classification	Survivor, n:16 (%)	Mortality, n:4 (%)
Seinsheimer classification	**Type 1**	-	1 (6.25)	0
	Type 2		6 (37.5)	0
	Type 3		5 (31.25)	0
	Type 4		1 (6.25)	0
	Type 5	31-A3.2	3 (18.75)	4 (100)

Data are expressed as count (n) and %. AO/OTA, Arbeitsgemeinschaft für Osteosynthesefragen/Orthopaedic Trauma Association

Discussion

In our research, we conducted an in-depth analysis of proximal femur fractures, a prevalent medical concern, particularly among the elderly population. We examined these fractures from a multifaceted perspective, considering both their anatomical characteristics and clinical attributes. Our specific focus was on understanding the relationship between these factors and mid-term mortality rates. This detailed investigation revealed an intriguing insight: the classification systems we meticulously examined did not demonstrate a significant advantage over one another. This finding highlights the importance of revisiting the approaches used in clinical settings and potentially shifting the emphasis towards a more cohesive and streamlined method for classifying proximal femur fractures.

In 2018, the AO Foundation and the OTA collaborated to establish a comprehensive classification system. This system was meticulously designed to offer a standardized and logically structured approach for categorizing and documenting bone fractures and dislocations, commonly referred to as the AO/OTA classification [14]. Notably, this classification system employs a sophisticated and highly specific methodology, rendering it particularly well-suited for academic and research purposes [15]. However, it is worth noting that our analysis did not reveal any substantial advantages in terms of mortality. Therefore, it may be more practical to consider utilizing existing clinical or anatomical classifications instead of this particular system.

The femur, known as the body's strongest bone, derives its strength from its unique anatomical features. However, its proximal region, consisting of the neck and trochanteric part, is particularly vulnerable. Proximal femur fractures are predominantly observed in this area and are associated with severe morbidity and mortality. In the 1990s, reports indicated that approximately 1.3 million patients worldwide suffered from femur fractures annually. However, projections suggest a significant increase, ranging from 7.3 to 21.3 million cases by 2050. Notably, a substantial portion of those affected by proximal femur fractures consists of elderly patients [16]. A comprehensive investigation into the factors contributing to frailty in the elderly has highlighted several key elements, including undesirable weight loss, diminished grip strength, self-reported burnout, reduced walking speed, and low levels of physical activity [17]. Furthermore, factors such as alterations in the femoral neck angle and age-related osteoporosis are believed to substantially contribute to the prevalence of these fractures, resulting in a higher incidence of femur fractures [8, 18]. Our research aligns with existing literature, focusing on the elderly population. Our patient demographics closely mirror the characteristics described in the literature, with a higher representation of females, and an equitable distribution of fracture types.

Fractures affecting the femur can be anatomically classified as intracapsular and possess the potential to disrupt blood supply to the femoral head, potentially leading to avascular necrosis after traumatic events. For intracapsular fractures, the Pipkin classification is employed. In Pipkin types 1 and 2, the fracture is associated with the foveal line, and clinical recommendations encompass a conservative approach or surgical intervention following closed reduction. However, in the case of Pipkin types 3 and 4, there is not only a femoral head fracture but also concomitant femoral neck and acetabulum fractures. In these complex scenarios, the blood supply to the femoral head is compromised, necessitating immediate surgical intervention [19]. Within intracapsular fractures, femoral neck fractures can be further subdivided into subcapital, transcervical, and basicervical fractures. These subdivisions are often managed according to the Garden classification. Garden 1 fractures denote non-displaced and stable fractures, typically amenable to conservative management. In contrast, Garden 2 fractures, although not distinctly categorized, are often associated with impaired blood supply. Garden 3 and 4 fractures are characterized by a complete separation of the femoral neck. Garden 2, 3 and 4 fractures necessitate surgical treatment [20]. In summary, the clinical management of these intricate fractures requires meticulous classification and a tailored approach to ensure optimal patient outcomes.

Extracapsular fractures of the femur are subdivided into intertrochanteric and subtrochanteric categories, primarily based on their location relative to the trochanter.

Among these, intertrochanteric fractures are frequently classified using the Evans/Jensen classification system, which comprises five distinct types. Each type reflects various fracture characteristics, including displacement, angulation, comminution, involvement of the greater trochanter, participation of the lesser trochanter, and oblique extension. Notably, except for Type 1, which designates a stable fracture, all other types necessitate surgical intervention. On the other hand, subtrochanteric fractures are often categorized according to the Seinsheimer classification system. This system designates Type 1 as a nondisplaced fracture, whereas Types 2, 3, and 4 represent transverse, oblique, and comminuted fractures, respectively. Type 5 is characterized by a fracture extending into the trochanteric region [21]. Importantly, it's worth noting that this classification system lacks an equivalent representation within the AO/OTA classification.

In a study focused on classification systems, it was determined that there were no significant variations in terms of the effectiveness of all the classification systems considered [22]. When deciding on a classification system, it's advisable to choose systems that facilitate effective communication among clinicians. Furthermore, an ideal classification system should aid in diagnosing the patient, devising a treatment plan, and predicting the likely outcome. With this perspective in mind, it might be worth considering the adoption of an anatomical classification system that is more user-friendly for clinicians, such as the academically established AO/OTA classification system.

The risk of death following a hip fracture in older individuals is significantly elevated, with mortality rates being 5 to 8 times higher than those in the general population [23]. Various studies have reported differing mortality statistics for proximal femur fractures, with annual mortality rates ranging from 14% to 36% [24]. In a research effort that investigated annual mortality based on the anatomical location of the fracture, mortality rates were found to be 26.8% for intracapsular fractures, 28.2% for intertrochanteric fractures, and 24.2% for subtrochanteric fractures. Interestingly, the study did not identify any significant differences in mortality rates based on the location of the fracture [25]. In another study examining short-term mortality, the 30-day mortality rates were reported as 6.5% for intertrochanteric fractures, 17.2% for subtrochanteric fractures, and 7.5% for intracapsular fractures. Similar to the previous study, no significant disparities in mortality rates were observed among different fracture locations. According to the results of the study, patient comorbidities and clinical frailty scores were identified as significant determinants of mortality [26]. In our own study, we focused on mid-term mortality, and the mortality rates for intracapsular (13%) and extracapsular (14.1%) fractures were consistent with findings in the existing literature. Furthermore, our study, like others, did not establish a significant association between the location of the fracture and mortality (p=0.787).

While the findings from our research have broad applicability, there are certain limitations to consider. Our study was conducted at a single center, which may affect the generalizability of the results. Additionally, as it was a retrospective study, there were challenges in accurately identifying and retrieving patient data from their medical records. Only patients who underwent surgical procedures were included, and patients with stable fractures were excluded since they did not require surgery. This exclusion limits our ability to accurately determine the prevalence of lower-level patients in the classifications. Furthermore, our sample size and exclusion criteria may have limited our ability to predict patient mortality outcomes accurately. This represents another constraint in our study. To address these limitations, future research conducted prospectively, involving multiple medical centers, is expected to yield more extensive and thorough results.

In conclusion, individuals afflicted with proximal femur fractures, confronted with a notable 3-month mortality rate of 13.8%, represent a patient cohort marked by a substantially heightened mortality risk. Although various classification methodologies exist for the evaluation of the clinical attributes of these patients, none of these systems manifest a discernible superiority in prognosticating mortality outcomes. Among the accessible classification systems, the adoption of

anatomical classification may be preferable due to its straightforwardness and its capacity to engender a standardized lexicon among healthcare practitioners.

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Research Article

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The influence of anesthesia type on recurrence of the non-muscle invasive bladder tumor according to risk groups: 3 year follow up

Risk gruplarına göre non-muscle invaziv mesane tümörünün nüksüne anestezi türünün etkisi: 3 yıllık takip

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Abstract

Purpose: Many risk factors affecting bladder cancer recurrence, such as genetic and environmental factors, have been previously identified. It has been stated that risk factors that cause immunosuppression play a role in the spread of cancer cells. Anesthetic agent, which is a perioperative factor, may affect the risk of cancer recurrence by disrupting the immune system. The aim of this study was to compare the effect of regional anesthesia (RA) and general anesthesia (GA) on non-muscle invasive bladder cancers (NMIBC) recurrence.

Materials and methods: A total of one hundred seventy-eight patients who underwent transurethral bladder tumor resection (TURBT) for NMIBC and underwent surgery under GA or RA between 2011 and 2016 in the urology departments of Pamukkale University and Uludag University were included in the study. In the first group, 80 patients had RA. In the second group, 98 patients underwent GA during TURBT for NMIBC.

Results: The recurrence time was shorter in the GA group (5.5 months) than in the RA group (11 months) (p=0.015). First-year relapse was higher in the GA group than in the RA group (p=0.048), but there was no difference in third-year relapse between groups (p=0.810). The mean recurrence time was 11 months (95% CI; 9.058-12.942) in the RA group and 5 months (95% CI; 2.090-7.910) in the GA group (p=0.031).

Conclusion: During transurethral resection of the bladder tumor, an increase in the recurrence time was observed in patients with intermediate-risk NMIBC who received RA compared to patients who received GA. RA provided a 7-month benefit in relapse delay.

Keywords: Bladder cancer, bladder tumor, regional anesthesia, general anesthesia.

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Öz

Amaç: Mesane kanseri nüksünü etkileyen genetik ve çevresel faktörler gibi birçok risk faktörü önceden belirlenmiştir. İmmünsüpresyon yaratan risk faktörlerinin, kanser hücrelerinin yayılmasında rol oynadığı belirtilmiştir. Perioperatif bir faktör olan anestezik ajan, bağışıklık sistemini bozarak kanser nüksü riskini etkileyebilir. Bu çalışmanın amacı, bölgesel anestezi (BA) ve genel anestezi (GA) etkilerini özellikle kas invaziv olmayan mesane kanseri (KİOMK) nüksü üzerinde karşılaştırmaktır.

Gereç ve yöntem: Pamukkale Üniversitesi ve Uludağ Üniversitesi Üroloji bölümlerinde 2011-2016 yılları arasında KİOMK için transüretral mesane tümör rezeksiyonu (TURM) geçiren toplamda 178 hasta çalışmaya dahil edildi. Birinci grupta 80 hasta BA aldı. İkinci grupta ise 98 hasta KİOMK için TURM sırasında GA aldı.

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Bulgular: Nüks süresi GA grubunda (5,5 ay) BA grubundan (11 ay) daha kısa idi (p=0,015). İlk yıl nüks, GA grubunda BA grubuna göre daha yüksekti (p=0,048), ancak gruplar arasında üçüncü yıl nüksünde farklılık yoktu (p=0,810). Ortalama nüks süresi BA grubunda 11 ay (%95 CI; 9,058-12,942) ve GA grubunda 5 ay (%95 CI; 2,090-7,910) idi (p=0,031).
Sonuç: Mesane tümörünün transüretral rezeksiyonu sırasında BA alan orta riskli KİOMK hastalarında GA alan hastalara göre nüks süresinde artış gözlendi. BA, nüks gecikmesinde 7 aylık bir fayda sağladı.

Anahtar kelimeler: Mesane kanseri, mesane tümörü, bölgesel anestezi, genel anestezi.

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Introduction

Bladder cancer (BC) is the ninth most common cancer in the world [1]. In total, 70% of bladder cancers are non-muscle invasive bladder cancers (NMIBCs) at the time of diagnosis and are treated with transurethral bladder tumor resection (TURBT) as primary treatment [2]. Many risk factors, such as genetics and environmental determinants, that affect bladder cancer recurrence, have been previously described. However, a limited number of studies focusing on the relation between perioperative factors and NMIBC recurrences were found in the literature [3]. The anesthetic agent, a perioperative factor, may have an influence on the risk of cancer recurrence, which could have a wide-ranging impact on progression by disrupting the balance of cancer immune editing [4].

In recent years, risk factors leading to immunosuppression have been described, and these factors play a role in spreading cancer cells. It has been reported that opioids used in RA have less immunosuppression and fewer negative effects on anti-cancer cells [3, 5]. Similar results have been reported for better oncological outcomes of RA in a meta-analysis [6]. However, the impact of anesthesia type on NMIBC recurrence has not been widely evaluated.

The European Organization for Research and Treatment of Cancer's (EORTC) Genito-Urinary Cancer Group developed a scoring system to predict the risks of disease recurrence [7]. The risk groups differ from each other in accordance with oncological behaviors. Analyzing the recurrence according to the EORTC risk group could prevent the effect of risk groups on the anesthesia type. To the best of our knowledge, there is only one study investigating the effect of anesthesia type on NMIBC recurrence rates with regard to EORTC risk groups [8].

In our study, we hypothesized that regional anesthesia (RA) decreased the recurrence of NMIBC compared to general anesthesia (GA) after TURBT. The aim of this study was to compare the effect of RA and GA on the recurrence of NMIBC in different EORTC risk groups.

Materials and methods

Data source and selection criteria

This retrospective study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee. (Board meeting dated 27.11.2018 and numbered 22). Six hundred and twenty-six patients who had undergone TURBT for NMIBC between 2011 and 2016 in the urology department of the Pamukkale University and Uludag University under GA or RA were evaluated. The patients' medical data were obtained from the electronic health record system. Patients operated on BC previously, those with failed RA, incomplete TURBT, those lost to follow-up or with incomplete data, those with benign pathology or patients with clinical stages 3, 4, or pathological stage 2 cancer, and those with synchronous tumors were excluded (Table 1). In total, one hundred and seventy-eight patients were included in the study. In the first group (Group RA), 80 patients had undergone RA. In the second group (Group GA), 98 patients had undergone GA during TURBT for NMIBC.

Table 1. Exclusion criteria

	General anesthesia	Exclusion criteria	Regional anesthesia
Total	360		266
Excluded n	13	Loss on Follow up	9
	9	Failed RA	6
	49	Clinical stage 3, 4	35
	67	Incomplete TURBT	58
	26	Incomplete data	22
	44	Muscle invasive tumor	18
	31	Reoperation	22
	23	Other tumor or surgery	16
Included n	98		80

RA: regional anesthesia, TURBT: transurethral bladder tumor resection

Demographic information including the American Society of Anesthesiologists (ASA), age, sex, tumor characteristics (e.g., tumor number, size, and pathological characteristics), and intravesical Bacillus Calmette-Guérin (BCG) treatment were collected. The EORTC Genito-Urinary Cancers Group risk table was used as risk categorization to stratify into low, intermediate, or high risks to ignore the heterogeneity. Installation of BCG or mitomycin into the urinary bladder was recorded. The cystoscopic evaluation was performed once every three months during the first and second years, and once every six months in the third year unless recurrence developed. All recurrences were histologically verified with cystoscopy.

Anesthesia techniques

All of the enrolled patients had undergone TURBT under GA or RA. The type of anesthesia administered to the patient was determined according to the preference of the anesthesiologist, depending on the clinical condition of the patient. Administration of GA included propofol (2-3 mg/kg) and 0.5 mcg/kg/min remifentanil infusion for induction and rocuronium (0.5-0.6 mg/kg) for muscle relaxation. GA was performed with 50% oxygen, 50% air, and sevoflurane (1-3 vol%). We had no painful procedure; hence, analgesics or opioids were not used intraoperatively. However, 30 mg intravenous ketorolac was administered for postoperative pain when necessary. For RA, spinal anesthesia (SA) was applied, and 10-12 mg of 0.5% heavy bupivacaine was used. The patient was sedated with midazolam (2-5 mg) intravenously.

The time of the first recurrence was noted. The rates of recurrence during the 1st, 2nd, and 3rd years were compared.

Statistical methods

SPSS version 22 (IBM Corp, Armonk, NY, USA) was used to perform all statistical analyses. The Shapiro-Wilk test was used to assess the normality of the continuous variables. The normally distributed continuous variables were analyzed using the Student's t-test, and the non-normally distributed continuous variables were analyzed using the Mann-Whitney U-test. The nominal data were assessed by the chi-square test. The Kaplan–Meier analysis was used to estimate the recurrence curves after TURBT. The log-rank test was performed to provide a statistical comparison between the groups. A p value of <0.05 was considered statistically significant.

Results

The median age of the patients was 72.5 years in the RA group and 70 years in the GA group. The mean follow-up time was 36.7 (±4.23) months in the RA group and 37.15 (±5.97) months in the GA group (p=0.56). The clinical demographic characteristics of the groups are presented in Table 2. No differences were observed in any parameters between the groups except for tumor stage. Tumor stage T1 was more prevalent in the GA group than in the RA group (42.9% vs. 22.5%, respectively; p=0.004). Hospital stays and operation times were similar between the groups. No patient received blood transfusions during the perioperative period.

Table 2. The clinical and demographic characteristics of the groups

		Group RA n=80	Group GA n=98	*p* value
Age (years) Median (min- max)		72.5 (38-93)	70.0 (32-87)	0.179♦
BMI (kg/m²) (Mean±SD)		27.03±3.73	27.67±5.65	0.383◊
Hospital stay (days) (Mean±SD)		4.34±0.65	4.48±0.63	0.143◊
Operation time (minutes) (Mean±SD)		52.25±8.42	54.78±9.87	0.067◊
ASA physical status	ASA 1 n (%)	16 (20)	10 (10.2)	0.109¥
	ASA 2 n (%)	53 (66.3)	78 (79.6)	
	ASA 3 n (%)	11 (13.8)	10 (10.2)	
Gender	Female n (%)	7 (8.8)	14 (14.3)	0.255¥
	Male n (%)	73 (91.2)	84 (85.7)	
Smoking	No n (%)	47 (59.5)	62 (63.3)	0.608¥
	Yes n (%)	32 (40.5)	36 (36.7)	
Alcohol use	No n (%)	78 (97.5)	96 (98.0)	1.000#
	Yes n (%)	2 (2.5)	2 (2.0)	
Diabetes mellitus comorbidity	No n (%)	65 (81.2)	75 (76.5)	0.445¥
	Yes n (%)	15 (18.8)	23 (23.5)	
Hypertension comorbidity	No n (%)	32 (40.0)	51 (52.0)	0.109¥
	Yes n (%)	48 (60.0)	47 (48.0)	
Heart disease comorbidity	No n (%)	45 (56.3)	62 (63.3)	0.342¥
	Yes n (%)	35 (43.7)	36 (36.7)	
Tumor size	<3 cm n (%)	32 (40.0)	48 (49.0)	0.148¥
	>3 cm n (%)	48 (60.0)	50 (51.0)	
Tumor number	Single n (%)	38 (47.5)	59 (60.2)	0.062¥
	Multiple n (%)	42 (52.5)	39 (38.8)	
Tumor grade	Low grade n (%)	55 (68.8)	64 (65.3)	0.373¥
	High grade n (%)	25 (31.3)	34 (34.7)	
Tumor stage	Ta n (%)	62 (77.5)	56 (57.1)	0.004*¥
	T1 n (%)	18 (22.5)	42 (42.9)	
Post-operative early Mitomycin-C application	No n (%)	65 (81.2)	85 (86.7)	0.317¥
	Yes n (%)	15 (18.8)	13 (13.3)	
Risk category	Low-risk tumours n (%)	10 (12.5)	22 (22.5)	0.161¥
	Intermediate-risk tumours n (%)	13 (13.7)	16 (16.3)	
	High-risk tumours n (%)	59 (73.8)	60 (61.2)	
Carcinoma in situ	No n (%)	72 (90.0)	94 (95.9)	0.117¥
	Yes n (%)	8 (10.0)	4 (4.1)	

♦ Mann-Whitney U test, ◊ Student t test, ¥ Chi-square test, # Fisher's exact test, *: p<0.05 statistically significant
ASA: American Society of Anesthesiologists, GA: general anesthesia, RA: regional anesthesia

The recurrence time was shorter in the GA group (5.5 months) than in the RA group (11 months) (p=0.015). While the recurrence rate in the first year was higher in the GA group compared to the RA group (p=0.048), there was no difference in the third-year recurrence rate between the groups (p=0.810) (Table 3).

The median recurrence time was 11 months (95% CI; 9.058-12.942) in the RA group and 5 months (95% CI; 2.090-7.910) in the GA group (p=0.031).

The results regarding the effect of the EORTC risk category on recurrence were as follows: an earlier recurrence time was observed in high-risk tumors than in low- and intermediate-risk tumors (p=0.010 and p=0.002, respectively). There was no difference between low-risk and intermediate-risk tumors (p=0.460).

The recurrence times according to the risk categories are displayed in Table 4. There was a significant difference between the groups regarding recurrence times, regardless of the risk category (p=0.008). The recurrence times according to the risk categories of the groups are given in Table 5. The recurrence times were similar in low-risk tumors (p=0.489). The recurrence time was significantly longer in the RA group than in the GA group for intermediate-risk tumors (17 and 10 months, respectively; p=0.028). The recurrence time was longer in the RA group than in the GA group for high-risk tumors (9 and 4 months, respectively; p=0.057).

Table 3. Recurrence properties of groups

		Group SA (Spinal Anesthesia) n=80	**Group GA (General Anesthesia) n=98**	***p* value**
Recurrence time (months) Median (Min.- Max.)		11.0 (1-36)	5.5 (1-36)	0.015*♦
1th Year Recurrence	No n (%)	36 (45.0)	30 (30.6)	0.048*¥
	Yes n (%)	44 (55.0)	68 (69.4)	
3th Year Recurrence	No n (%)	13 (36.1)	10 (33.3)	0.810¥
	Yes n (%)	23 (63.9)	20 (66.7)	

♦ Mann-Whitney U test, ¥ Chi-square test, *: p<0.05 statistically significant

Tablo 4. Recurrence properties of risk group stratification

Risk group stratification	**Median time (months)**	**95% Confidence Interval**	
		Lower Bound	**Upper Bound**
Low-risk tumours	13.0	8.574	17.426
Intermediate-risk tumours	12.0	9.819	14.181
High-risk tumours	5.0	3.666	6.334

♦ The Kaplan–Meier analysis

Tablo 5. Recurrence times according to the risk categories of the groups

Risk group stratification		Median time (months)	95% Confidence Interval		*p* value
			Lower Bound	Upper Bound	
Low-risk tumours	Group RA	14.0	10.901	17.099	0.489
	Group GA	9.0	0.956	17.044	
Intermediate-risk tumours	Group RA	17.0	16.740	30.351	0.028*
	Group GA	10.0	8.055	11.945	
High-risk tumours	Group RA	9.0	4.489	13.511	0.057
	Group GA	4.0	2.741	5.259	

♦ The log-rank test ,RA: regional anesthesia, GA: general anesthesia, *: $p<0.05$ statistically significant

Discussion

In the current study, we investigated the relationship between the anesthesia type and NMIBC postoperative recurrence. We found that the recurrence time was longer in the RA group than in the GA group. Many mechanisms have been defined to explain the potential benefit of RA on tumor recurrence. It was reported that RA decreased factor-1 production, resulting in reduced cancer cell proliferation; however, volatile anesthetic agents induced factor-1 production [9]. Wada et al. [10] stated that epidural anesthesia increased the phagocytic function of monocytes during total hip arthroplasty surgery; however, GA decreased this function. Ahlers et al. [11] reported that RA reduced stress and decreased immunosuppression by activating the hypothalamic-pituitary-adrenal system, which reduced the activities of NK cells, T cells, and macrophages. In accordance with the mentioned studies, a recent in vitro study showed that lidocaine and ropivacaine used for RA inhibited the proliferation of gastric cancer cells [12].

Angiogenesis plays an essential role in tumor growth and metastasis [13]. Angiogenic factors are found in NMIBC [14]. Several researchers have agreed that the use of opioids increases pro-angiogenic effects [3, 15-17]. GA induces angiogenesis, mitogenesis, and metastasis in tumors, and opioid analgesics also reduce the response of the immune system [9].

In the literature, conflicting results have been reported on the influence of the anesthesia type on urological cancer recurrence, and most of these studies have evaluated prostate cancer. However, a limited number of studies have investigated the relationship between anesthesia type and bladder cancer recurrence. A meta-analysis on the effect of anesthesia type on prostate cancer outcomes reported no differences between GA and combined RA and GA with regard to biochemical recurrence-free survival or progression-free survival with a median follow-up of 3.2 to 16.2 years [18]. However, the mortality rate was decreased by 19% in patients receiving RA combined with GA, significantly improving overall survival [16]. Doiron et al. [19] reported no differences in the effect of neuroaxial analgesia (single dose intrathecal opioid) on muscle-invasive bladder cancer. However, these studies compared GA with GA combined with RA, which is different from the current study.

In their retrospective study, Jang et al. [20] found no detectable differences in recurrence rates after five years of follow-up between patients who had undergone TURBT under RA and GA, but they reported significant partial correlations between an increased 5-year survival and RA. However, the number of patients receiving GA was far fewer than those receiving RA in that study. On the contrary, in the present study, RA reduced the recurrence of patients with NMIBC compared to GA. Likewise, Choi et al. [21] showed that RA was associated with a lower incidence of recurrence and a longer time (approximately 6 months) to recurrence with a median follow-up of 35 months compared to GA in patients who had undergone TURBT. Different from those studies, we additionally evaluated recurrence according to the EORTC risk group.

The EORTC Genito-Urinary Cancer Group developed a scoring system to predict the risks of disease recurrence and progression based on the number of tumors, tumor diameter, prior recurrence rate, pathology characteristics including stage, concurrent CIS, and grade [7]. The treatment and follow-up protocols are based on the prognosis of patients with NMIBC [22].

NMIBC is classified as low-, intermediate-, and high-risk according to the EORTC classification. We analyzed the patients according to the EORTC classification, as the risk groups differ from each other in accordance with recurrence rate, progression, and oncological behavior. In this way, we could prevent the influence of the differences in the risk groups on recurrence.

In the present study, subgroup analysis revealed that RA reduced cancer recurrence in the intermediate-risk group, but there was no benefit of RA on recurrence in the low-risk group. However, in the high-risk group, RA had a 5-month recurrence benefit over GA (p=0.056). It should be kept in mind that several factors, such as the oncological characteristics of high-risk NMIBC, are dissimilar to those of intermediate- and low-risk NMIBC. High-risk NMIBC has different oncogenic features, and circulating tumor cells are defined in some high-risk patients [23, 24]. In our study, these factors may have limited the effect of RA on the recurrence of high-risk NMIBC. On the contrary, Koumpan et al. [8] stated that GA was associated with a higher incidence of recurrence and an earlier time to recurrence compared to RA in patients with NMIBC who had undergone TURBT. They also reported that RA significantly reduced the rate of recurrence in the high-risk patient group [24]. The different results noted between that study and ours may relate to the variations between the high-risk patients included in the studies.

This study had some limitations. This study includes retrospective data taken from two centers. However, a standard data sheet was used for data extraction to minimize the scope for bias. The number of patients with high-risk NMIBC was higher in the GA group than in the RA group, which was another limitation of this study. To prevent the influence of the differences in the risk groups on recurrence, we compared the anesthesia groups according to EORTC risk groups. Biological and environmental factors can affect cancer progression. It is also important whether patients smoke or not during their follow-up. Despite these limitations, the present findings serve as a recommendation for clinicians when choosing the type of anesthesia during TURBT for NMIBC.

Further prospective multicenter studies are needed to compare the effects of different types of anesthesia on cancer recurrence after TURBT with a larger sample size.

In conclusion, during transurethral resection of the bladder tumor, an increased recurrence time was observed in patients with intermediate-risk NMIBC who received RA compared to those who received GA. RA provided a 7-month benefit in delaying relapse.

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Research Article

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Impact of cisplatin on Kasumi-1 leukemia cell line: gene expression and DNA damage

Sisplatinin Kasumi-1 lösemi hücre hattı üzerindeki etkisi: gen ekspresyonu ve DNA hasarı

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Abstract

Purpose: Leukemia is a type of cancer caused by the uncontrolled proliferation of blood cells. The purpose of this study was to investigate the effects of cisplatin (CIS), a chemotherapeutic agent used in the treatment of leukemia, on the Kasumi-1 leukemia cell line.

Materials and methods: The study measured the effect of CIS on Kasumi-1 cells by calculating IC50 values for cell viability. The mRNA expression levels of apoptosis and cell cycle-related genes were then assessed using Real-Time PCR. In addition, the effects of CIS on DNA damage were investigated using the comet assay.

Results: Significant changes in apoptosis and cell cycle-related genes were observed in CIS-treated groups. These included alterations in the mRNA levels of p53, BCL-2, CHECK 1, CDC25C, CDK 6, URG4/URGCP, GADD45A, CCND1, GADD45G, and ATM genes. Comet analysis confirmed CIS's effects on DNA damage.

Conclusion: This study aimed to better understand how CIS affects genetic mechanisms in leukemia cells and provide new insights into leukemia treatment. The findings will help us better understand the role of CIS in leukemia treatment and will serve as a valuable reference for future research.

Keywords: Leukemia, acute myeloid leukemia, cisplatin, Kasumi-1 cell, DNA damage.

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Öz

Amaç: Lösemi, kan hücrelerinin kontrolsüz çoğalması sonucu ortaya çıkan bir kanser türüdür. Bu çalışmanın amacı, lösemi tedavisinde kullanılan kemoterapötik bir ajan olan sisplatinin (CIS) Kasumi-1 lösemi hücre hattı üzerindeki etkilerini araştırmaktır.

Gereç ve yöntem: Çalışmada, hücre canlılığı için IC50 değerleri hesaplanarak CIS'in Kasumi-1 hücreleri üzerindeki etkisi ölçülmüştür. Apoptoz ve hücre döngüsü ile ilgili genlerin mRNA ekspresyon seviyeleri daha sonra Real-Time PCR kullanılarak değerlendirilmiştir. Ayrıca, CIS'in DNA hasarı üzerindeki etkileri comet testi kullanılarak araştırılmıştır.

Bulgular: CIS ile tedavi edilen gruplarda apoptoz ve hücre döngüsü ile ilgili genlerde önemli değişiklikler gözlendi. Bunlar arasında p53, BCL-2, CHECK 1, CDC25C, CDK 6, URG4/URGCP, GADD45A, CCND1, GADD45G ve ATM genlerinin mRNA seviyelerindeki değişiklikler yer aldı. Comet analizi CIS'in DNA hasarı üzerindeki etkilerini doğrulamıştır.

Sonuç: Bu çalışma, CIS'in lösemi hücrelerindeki genetik mekanizmaları nasıl etkilediğini daha iyi anlamayı ve lösemi tedavisine yeni bakış açıları sağlamayı amaçlamıştır. Bulgular, CIS'in lösemi tedavisindeki rolünü daha iyi anlamamıza yardımcı olacak ve gelecekteki araştırmalar için değerli bir referans görevi görecektir.

Anahtar kelimeler: Lösemi, akut miyeloid lösemi, sisplatin, Kasumi-1 hücresi, DNA hasarı.

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Introduction

Leukemia, also known as blood cancer, is a serious type of cancer that affects millions of people worldwide [1]. The detection of leukemia cancer has been linked to the uncontrolled proliferation of cancer stem cells. These cancer stem cells were first discovered in acute myeloid leukemia (AML) [2]. AML is a type of leukemia distinguished by an interruption or increase in the maturation of myeloid cells in the bone marrow. This can cause granulocytopenia, anemia, and hematopoietic failure with or without leukocytosis [3]. Chemotherapy is one of the most common treatment options for leukemia, as it is for many other cancers [4]. Chemotherapy's primary goal is to prevent cancer cells from spreading uncontrollably or to kill them. However, because this treatment method involves the use of numerous chemotherapeutic drugs, it affects both cancerous and normal healthy cells that proliferate [5]. Cisplatin is one of the most commonly used chemotherapy drugs.

CIS is an inorganic compound that belongs to the group of platinum-based chemotherapeutic drugs (Figure 1). This drug is a coordinate compound that has been widely used since 1960, when it was first developed by a group of Rosenberg University researchers [6]. In 1969, the same researchers investigated CIS's antitumor activity in leukemia and sarcoma cell lines. Furthermore, CIS is currently used as an antitumor drug in a variety of cancers, including head and neck, breast, testicular, colorectal, and bladder cancers [7].

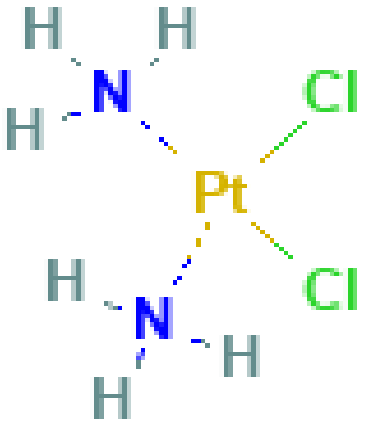

Figure 1. Chemical Structure of Cisplatin [Pubchem]

CIS is one of the most potent chemotherapeutic drug groups. However, as with other chemotherapy drugs, it has been shown to have serious side effects on healthy cells. CIS primarily damages DNA in the cells in which it first acts. It exerts its effect by passing through purine bases in DNA and forming cross-linked covalent bonds [8]. In addition to DNA damage, it inhibits RNA and protein synthesis. This causes disruptions or dysfunctions in the cell cycle and has an antitumor effect by inducing apoptosis [9, 10].

The purpose of this study was to determine the effect of CIS, a chemotherapeutic drug, on one of the AML cell lines, Kasumi-1, on cell proliferation, DNA repair, and cell cycle-related genes, as well as the expression changes of specific oncogene URG4/URGCP and DNA damage formation. In this context, the goal is to gain a better understanding of cisplatin's effects on these cells and shed light on potential therapeutic approaches.

Material and methods

Given that our research was conducted in an in vitro setting, it does not necessitate approval from an ethics committee.

Propagation of the Kasumi-1 cell line

The Kasumi-1 cell line was cultured in RPMI1640 medium. This medium contains 10% fetal bovine serum, 100 mg/ml streptomycin, 25 mM L-glutamine, and 100 IU/ml penicillin. The incubation was carried out in a humidified atmosphere of 95% and 5% CO_2 in an incubator at 37°C.

Cell viability test

To determine the IC50 value in the Kasumi-1 leukemia cell line, doses of CIS ranging from 2.5 µM to 160 µM were applied. The effects in this dose range were assessed depending on time and dose. Cell viability was determined using the CellTiter-Glo method, a sensitive luminometric method based on the measurement of ATP from living cells. This determination was conducted at time intervals of 24, 48, and 72 hours.

Total RNA and cDNA retrieval

To determine gene expression levels, total RNA isolation was performed in all groups, both control and dose, with the Trizol reagent. This isolation procedure was used to extract all of the RNA contained in the cells using the Trizol reagent. The reverse transcription procedure for cDNA synthesis was then performed. The "Transcriptor First Strand cDNA Synthesis Kit" was used to complete the reverse transcription procedure.

Real-time PCR analysis

The changes in mRNA expression levels of the genes p53, ATM, ATR, CHECK1, CHECK2, CDC25A, CDC25C, ERCC1, GADD45A, GADD45G, CCND1, CDK6, BAX, BCL-2, and URG4/URGCP were analyzed using the Real-Time PCR method. In this analysis method, gene-specific primers were used. The primer sequences of the genes examined in the study are shown in Table 1.

Table 1. Primer sequences of genes analyzed in real-time PCR

Gene Name	Base Sequences of Genes
p53	Forward 5′ATCTACAAGCAGTCACAGCACA3′
	Reverse 5′GTGGTACAGTCAGAGCCAACC3′
ATM	Forward 5'TGTTCCAGGACACGAAGGGAGA3'
	Reverse 5'CAGGGTTCTCAGCACTATGGGA3'
ATR	Forward 5'GGAGATTTCCTGAGCATGTTCGG3'
	Reverse 5'GGCTTCTTTACTCCAGACCAATC3'
CHECK1	Forward 5'GTGTCAGAGTCTCCCAGTGGAT3'
	Reverse 5'GTTCTGGCTGAGAACTGGAGTAC3'
CHECK2	Forward 5'GACCAAGAACCTGAGGAGCCTA3'
	Reverse 5'GGATCAGATGACAGCAGGAGTTC3'
CDC25A	Forward 5'TCTGGACAGCTCCTCTCGTCAT3'
	Reverse 5'ACTTCCAGGTGGAGACTCCTCT3'
CDC25C	Forward 5'AGAAGCCCATCGTCCCTTTGGA3'
	Reverse 5'GCAGGATACTGGTTCAGAGACC3'
ERCC1	Forward 5'GCTGGCTAAGATGTGTATCCTGG3'
	Reverse 5'ATCAGGAGGTCCGCTGGTTTCT3'
GADD45A	Forward 5'CTGGAGGAAGTGCTCAGCAAAG3'
	Reverse 5'AGAGCCACATCTCTGTCGTCGT3'
GADD45G	Forward 5'CGTCTACGAGTCAGCCAAAGTC3'
	Reverse 5'CGATGTCGTTCTCGCAGCAGAA3'
CCND1	Forward 5'AGCTCCTGTGCTGCGAAGTGGAAAC3'
	Reverse 5'AGTGTTCAATGAAATCGTGCGGGGT3'
CDK6	Forward 5'AGACCCAAGAAGCAGTGTGG3'
	Reverse 5'AAGGAGCAAGAGCATTCAGC3'
BAX	Forward 5'AGAGGATGATTGCCGCCGT3'
	Reverse 5'CAACCACCCTGGTCTTGGATC3'
BCL-2	Forward 5'TTGGCCCCCGTTGCTT3'
	Reverse 5'CGGTTATCGTACCCCGTTCTC3'
URG4/URGCP	Forward 5′CGGGAGATGGGACAGTTTTA3′
	Reverse 5′CATGGTGTTGAGGAGTGTGG3′

Comet assay method

The Comet Assay method was used to assess the DNA damage caused by CIS in Kasumi-1 cells. After applying the determined IC50 values to the cells, they were washed three times with 0.1M PBS. The cells were then treated with trypsin and removed from the Petri dishes. Three frosted glass slides were prepared for the control and CIS-treated dose groups. These slides were made by adding three layers of low melting point agarose gel at 37°C. After solidifying the first layer of 1.8% low melting point agarose gel, add 25 µL of sample and 1% low melting point agarose gel to the second layer. The third layer was filled with 1% low melting point agarose gel. The slides were incubated for 1 hour at +4°C in a pH-adjusted cold lysis solution with 1% Triton X-100, 100 mM EDTA, 10% DMSO, 2.5 M NaCl, and 10 mM Tris. The electrophoresis was conducted at 25 volts. After electrophoresis, the slides were washed in a solution adjusted to 0.4 M Tris; pH 7.5. They were then immersed in methanol at -20°C for five minutes and left to dry until the slides were dry. The emissions were observed at 590 nm while excitation filters were measured at 510/560 nm using a Nikon fluorescence microscope. Before examining under the microscope, all slides were exposed to ethidium bromide. The images of the comet tails were used at 20X magnification under the microscope [11].

Statistical analysis

The analysis of the data was conducted quantitatively using the ΔΔCT method through computer software. In this analysis, the web-based "RT² Profiler™ PCR Array Data Analysis" program was used, and Volcano Plot analyses were performed. The ΔΔCT method is based on the comparison of two expression results within ±3SD. With this method, the expression values of the relevant genes of the control and dose groups were determined relatively. Additionally, the comparison of the groups was statistically evaluated using the "Student t-test" analysis found in the "RT² Profiler™ PCR Array Data Analysis" program. For the determination of DNA damage, measurements were made using Comet Assay IV Version 4.3.2 for Basler FireWire.

Results

Cell viability

As a result of the conducted study, cell viability was initially determined by applying CIS to Kasumi-1 cells, one of the AML cell lines. The CellTiter-Glo kit, a luminometric method, was used to determine cell viability. Measurements were performed at 24, 48, and 72 hours, based on the application made to the cells used in the study. The effect of CIS on the Kasumi-1 cell line varied in a time- and dose-dependent manner. The IC50 value for the groups treated with CIS was determined to be 160 µM at 48 hours for the Kasumi-1 cell line. According to the results obtained, the distribution of CIS in Kasumi-1 cells depending on dose and time is shown in the graph below, Figure 2.

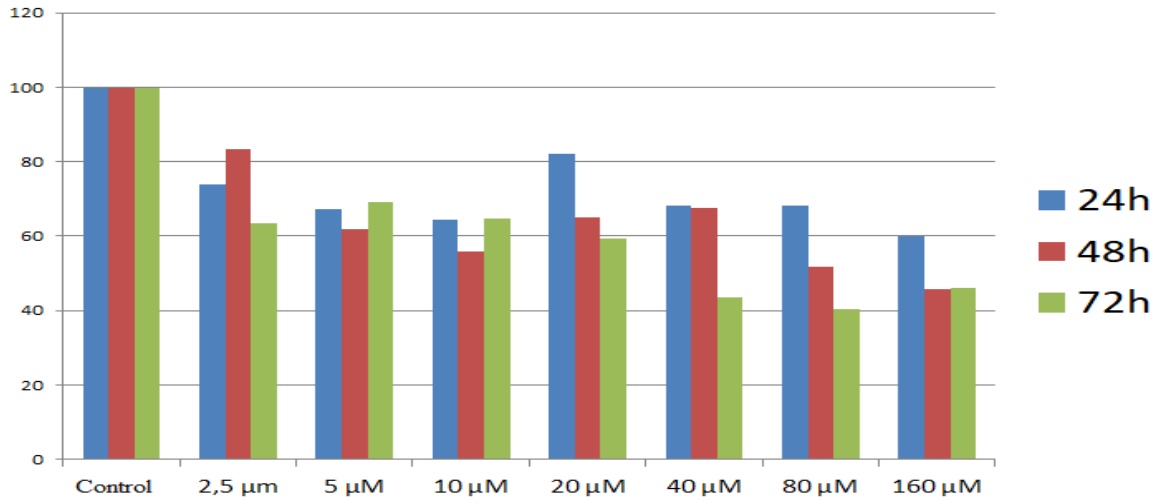

Figure 2. Percentages of cell viability in Kasumi-1 cells after CIS treatment based on time and dose

Real-time PCR analysis

cDNA synthesis was performed using the Transcriptor First Strand cDNA Synthesis Kit from the total RNAs obtained from the groups treated with CIS and the control group. Subsequently, the Real-Time PCR method was used to investigate the mRNA expression levels of the genes commonly known to be associated with apoptosis and the cell cycle, including p53, ATM, ATR, CHECK1, CHECK2, CDC25A, CDC25C, ERCC1, GADD45A, GADD45G, CCND1, CDK6, BAX, BCL-2, and URG4/URGCP. Based on the results obtained, significant results were found in the expression levels of the genes p53, BCL-2, CHECK 1, CDC25C, CDK 6, URG4/URGCP, GADD45A, CCND1, GADD45G, and ATM. As no statistically significant difference was found in the results obtained for ATR, CHECK2, CDC25A, ERCC1, and BAX genes, they were not included in the graph (Figure 3).

Comet assay

The Comet assay was conducted to detect DNA damage in cells after treating the Kasumi-1 cell line with CIS for 48 hours at a dose of 160 µM. In the control group, it was observed that there was no DNA damage in Kasumi-1 cells, which appeared with a round shape (Figure 4). However, in Kasumi-1 cells treated with CIS, both the tail length and the percentage of DNA in the tail significantly increased in the dose group, as analyzed (Figure 5).

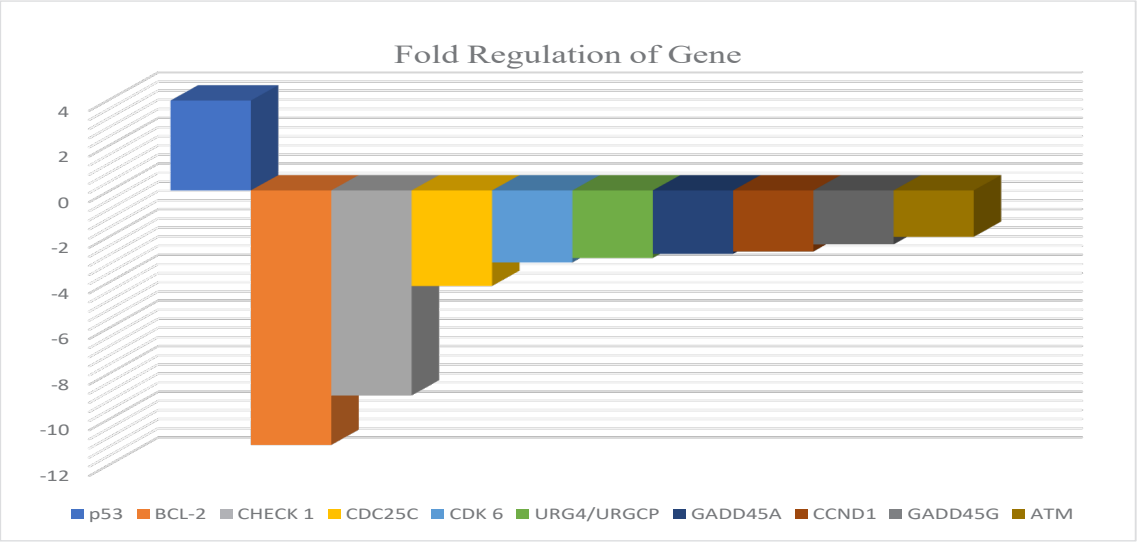

Figure 3. Fold Changes in the expression of p53, BCL-2, CHECK 1, CDC25C, CDK 6, URG4/URGCP, GADD45A, CCND1, GADD45G, and ATM genes at the mRNA level in Kasumi-1 cell lines after CIS treatment

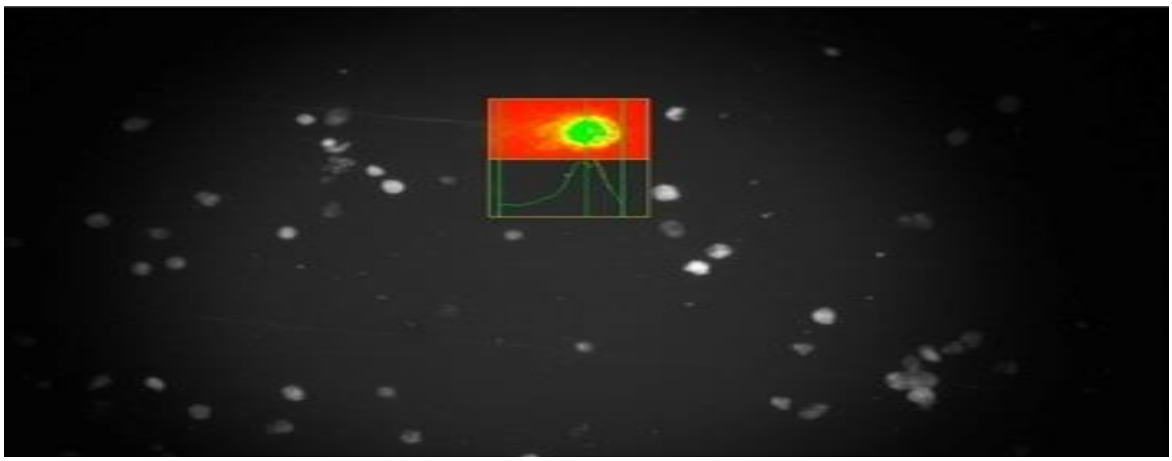

Figure 4. Comet assay result obtained from the control group

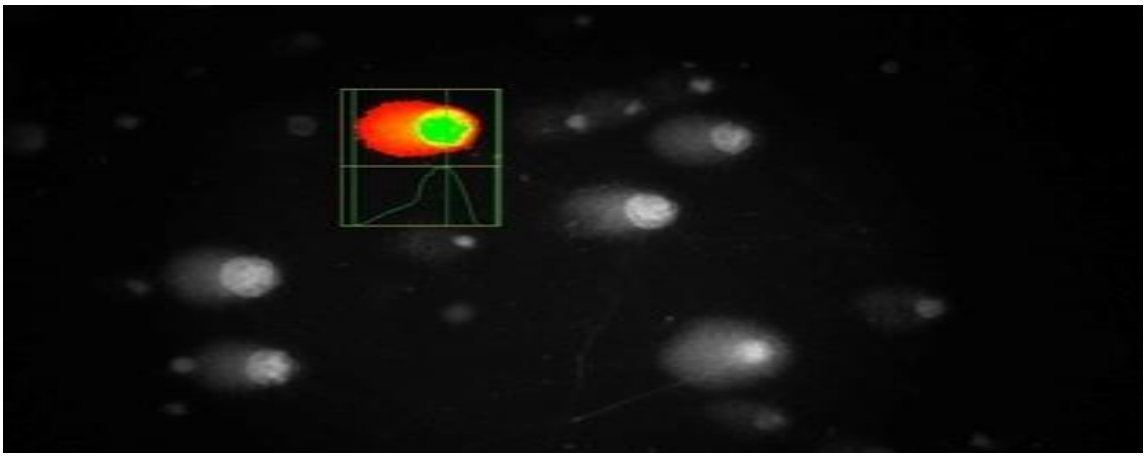

Figure 5. Comet assay result showing

Discussion

Leukemia is a type of cancer characterized by the abnormal and uncontrolled proliferation of blood cells [12, 13]. Another well-known form of this cancer type is AML cells. AML is a type of blood cancer that results from the uncontrolled proliferation of myeloid stem cells located in the bone marrow [14, 15]. This condition leads to genetic changes that cause the abnormal maturation of myeloid stem cells. AML cells can lead to the accumulation of abnormal cells in the blood, bone marrow, or sometimes other body tissues, which can cause various symptoms to appear [16, 17]. As with many cancer treatments, this cancer treatment usually involves processes with chemotherapy, radiotherapy, cell transplantation, or the use of different drugs [18]. The most commonly used treatment method is chemotherapy. In patients undergoing chemotherapy, the main goal is actually to heal the cancerous tissue and improve patient quality of life [19]. However, various chemotherapeutic drugs are used during the treatment process, and these drugs actually affect healthy proliferating cells outside the target tissue. So, as much as we want to achieve positive results with this method, we may also encounter more negative outcomes [20]. One of the chemotherapeutic agents that have been widely known and used for years in chemotherapy is CIS.

CIS, which goes by the chemical name cis/diamine/dichloro/platinum (II), is known as a platinum compound that hosts amine and chlorine atoms [21]. It was first found to have an antibacterial effect with the growth of Escherichia coli bacteria. Later, it was discovered to create an anti-neoplastic effect on cancer cells and entered the class of strong chemotherapeutic drugs used in cancer treatment [22]. This platinum-based drug is still widely used in the treatment of various cancers such as lymphoma, testicular, head-neck, ovarian, and cervical cancers [23]. When cisplatin enters the cells, it loses the chloride ligand and becomes activated. Once active, it forms covalent bonds with guanine bases in DNA [24]. These bonds cross-link the two strands of DNA, thereby inhibiting the normal function of DNA. This process, especially during cell division, inhibits DNA replication and transcription, stopping the proliferation of cancer cells. However, the DNA damage caused by cisplatin triggers the cell's repair mechanisms [25]. One of these repair mechanisms is the ATM pathway. The ATM pathway detects DNA damage, then stops the cell cycle and initiates DNA repair. This allows cells to recover from DNA damage, thereby ensuring cell survival. When the ATM pathway is activated, many genes responsible for stopping cell cycle growth such as the p21 gene, DNA damage-inducing gene 45 (GADD45) involved in DNA repair, and Bax/BCL-2 involved in apoptosis are activated [21, 26]

The aim of this study was to investigate the effect of CIS, a frequently used chemotherapeutic agent in chemotherapy, on the Kasumi-1 cell, one of the AML cells, on cell proliferation, cell cycle, and apoptotic genes, as well as the expression changes of the oncogene URG4/URGCP, and additionally, to examine its effects on DNA damage with comet analysis to show more clearly the role of CIS in DNA damage. Firstly, a cell viability test was conducted using the Celltiter-Glo kit.

Measurements were taken at 24, 48, and 72 hours following the application. According to the data obtained from the measurements in the study, the IC50 value in the groups where CIS was applied was determined as 160 μM at 48 hours in the Kasumi-1 cell line. Then, Real-Time PCR was performed to examine the mRNA expression levels of the p53, ATM, ATR, CHECK1, CHECK2, CDC25A, CDC25C, ERCC1, GADD45A, GADD45G, CCND1, CDK6, BAX, BCL-2, and URG4/URGCP genes. As a result of the PCR, significant results were obtained in the expression levels of the p53, BCL-2, CHECK 1, CDC25C, CDK 6, URG4/URGCP, GADD45A, CCND1, GADD45G, and ATM genes. In the groups treated with CIS, a decrease in the mRNA expression level of the BCL-2 gene, which is effective in the apoptosis pathway, was detected. At the same time, an increase in the expression level of another apoptotic gene, p53, was observed. Many studies on CIS have found that the expression levels of the BCL-2 gene lead to different results in different cell lines [27, 28]. On the other hand, increases in the expression level of the p53 gene have been shown in different studies where CIS was applied [28-30]. In this regard, cell line in this study show similarities with the literature. Additionally, in the study, the expression levels of the CHECK 1, CDC25C, CDK 6, URG4/URGCP, GADD45A, CCND1, GADD45G, and ATM genes, which are involved in the cell cycle, were examined. According to the results obtained, it was determined that the expression levels of these genes decreased. The low expression of these genes indicates that there are malfunctions in the cell cycle or even that the cell cycle is stopped. The effect of CIS has reduced the expression of these genes, and most of the studies in the literature show evidence that CIS is effective in the cell cycle [31-33]. Finally, comet analysis was conducted to detect DNA damage in the Kasumi-1 cell line with CIS. According to the data obtained from this analysis, no deformation was observed in the control group. However, in the cells treated with CIS, it was observed that the tail length and the percentage of DNA in the tail increased compared to the control group. Based on these results, we can say that DNA breaks occurred in the groups treated with CIS [34-37]. By investigating the mechanisms through which CIS induces changes in gene expression and DNA integrity, this study adds valuable insights into the broader understanding of chemotherapy's impacts, particularly in the treatment of leukemia with AML characteristics.

This study evaluated the mRNA levels of genes such as p53, BCL-2, CHECK 1, CDC25C, CDK 6, URG4/URGCP, GADD45A, CCND1, GADD45G, and ATM by examining the effects on DNA repair mechanisms, cell cycle mechanisms, and apoptotic genes in the Kasumi-1 cell line. Additionally, we identified the significant effects of CIS on the cell cycle and DNA repair mechanisms through analytical methods used to detect DNA breaks. The results of the comet assay also confirmed that CIS causes DNA breaks. The findings demonstrate that this study carries unique value and will serve as an important reference for future research. We hope that these findings will illuminate studies on the treatment and prevention of AML, a type of leukemia cancer, and will occupy a significant place in the literature.

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Authors contributions: Y.D. have constructed the main idea and hypothesis of the study. Y.D., M.S., L.E. and N.D. conducted experiments. Y.D., M.S., S.S., and U.P. analyzed data. Y.D., S.S. and Z.A. wrote the manuscript. 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.

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Research Article

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Relationship of anxiety-related symptoms with pain, disability and postoperative period after degenerative lumbar spinal stenosis surgery

Dejeneratif lomber spinal stenoz cerrahisi sonrası anksiyete ilişkili semptomlar ile ağrı, engellilik ve postoperatif süre arasındaki ilişki

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Abstract

Purpose: This study investigates the anxiety symptom levels of patients who underwent surgery due to degenerative lumbar spinal stenosis (DLSS), and the relationship between anxiety symptoms and postoperative pain, disability, and time elapsed after surgery was investigated.

Materials and methods: The research study group comprises 71 patients, and the control group comprises 65 healthy individuals. In the study, the pain, disability, and anxiety symptom levels of the patients were evaluated. Postoperative State Anxiety Inventory (SAI) and Trait Anxiety Inventory (TAI) scores of DLSS patients and healthy individuals were compared. The variability of the anxiety scores of the individuals in the study group was analyzed in separate groups divided according to gender, postoperative period, pain, and disability scores. Patients were divided into two groups: painful (VAS >6) and pain-free (VAS <7), according to Visual Analogue Scale (VAS) scores representing pain levels.

Results: The results show that the patient's anxiety symptom levels are higher than the healthy controls and that anxiety scores increase even more in patients with high pain and disability scores. Higher pain and disability scores were associated with higher SAI scores. Moreover, it was found that both the painful early postoperative and painful late postoperative groups had higher SAI scores compared to the pain-free early postoperative and pain-free late postoperative groups.

Conclusion: These findings emphasize the importance of considering pain, physical disabilities, and anxiety symptoms together in supporting the postoperative recovery process. It can be said that holistic approaches focusing on pain, physical disabilities and anxiety can positively affect recovery. More extensive and prospective studies are needed to elucidate the causal relationship between these concepts.

Keywords: Anxiety, disability, pain, spinal stenosis, surgery.

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Öz

Amaç: Bu çalışmada dejeneratif lomber spinal stenoz (DLSS) nedeniyle ameliyat geçiren hastaların anksiyete ilişkili semptom düzeyleri ve bu durumun ameliyat sonrası ağrı, engellilik ve ameliyattan sonra geçen süre ile ilişkisi araştırılmıştır.

Gereç ve yöntem: Araştırma çalışma grubu 71 hastadan, kontrol grubu ise 65 sağlıklı bireyden oluşmaktadır. Çalışmada hastaların ağrı, sakatlık ve anksiyete ilişkili semptom düzeyleri değerlendirilmiştir. DLSS hastalarının ve sağlıklı bireylerin Postoperatif Durumluk Anksiyete Envanteri (DAE) ve Süreklilik Anksiyete Envanteri (SAE) puanları karşılaştırılmıştır. Çalışma grubundaki bireylerin anksiyete puanlarının değişkenliği cinsiyete, ameliyat sonrası geçmiş olan süreye, ağrıya ve sakatlık puanlarına göre ayrı gruplar halinde analiz edilmiştir. Hastalar ağrı düzeylerini temsil eden Görsel Analog Skala (VAS) puanlarına göre ağrılı (VAS >6) ve ağrısız (VAS <7) olmak üzere iki gruba ayrılmıştır.

Bulgular: Sonuçlar hastaların anksiyete semptom düzeylerinin sağlıklı kontrollerden daha yüksek olduğunu ve yüksek ağrı ve sakatlık puanına sahip hastalarda anksiyete puanlarının daha da arttığını göstermektedir.

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Daha yüksek ağrı ve sakatlık puanları daha yüksek DAE puanlarıyla ilişkilendirildi. Dahası hem ağrılı erken postoperatif hem de ağrılı geç postoperatif gruplarının ağrısız erken postoperatif ve ağrısız geç postoperatif gruplarına kıyasla daha yüksek DAE puanlarına sahip olduğu bulundu.
Sonuç: Bu bulgular, postoperatif iyileşme sürecini desteklemede ağrı, fiziksel sakatlıklar ve anksiyete semptomlarının birlikte ele alınmasının önemini vurgulamaktadır. Ağrıya, fiziksel sakatlıklara ve anksiyeteye odaklanan bütünsel yaklaşımların iyileşmeyi olumlu yönde etkileyebileceği söylenebilir. Bu kavramlar arasındaki nedensel ilişkiyi açıklamak için daha kapsamlı ve prospektif çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Anksiyete, engellilik, ağrı, spinal stenoz, ameliyat.

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Introduction

Degenerative lumbar spinal stenosis (DLSS) is a chronic pathology that causes axial low back pain and neurological deficits due to the narrowing of the spinal canal and compression of neural structures resulting from spinal degeneration [1]. DLSS is a common indication for spine surgery today, where the aging population and physical workload are increasing [2]. Hughey et al. [3], in their study on the prevalence of spine surgeries, showed that there were 54,000 spine surgeries in 1993, and this number increased dramatically to 350,000 per year in 2007. According to current epidemiological data, there are 103 million people with symptomatic DLSS in the US, and lumbar fusion and decompression surgeries for its treatment are rapidly increasing [4].

Surgical interventions often evoke intense emotional reactions in patients due to their high medical risk, and these emotional reactions have adverse effects on the course of the disease [5]. Studies report that the impact of psychological factors is more remarkable in musculoskeletal system surgeries than in other surgeries [6]. Furthermore, it has been shown that depression and anxiety are the most common psychiatric disorders in patients with musculoskeletal system disorders [7, 8]. Patients suffering from chronic lumbar spine disease are at serious risk for anxiety due to their long-standing severe pain and consequent functional losses. Those candidates for surgical repair are particularly at risk due to the severity of their symptoms. In addition, patients who undergo lumbar spine surgery are exposed to many stressors that trigger anxiety in the postoperative period [9].

Numerous studies investigating preoperative anxiety in patients undergoing lumbar spine surgery and its effects on surgical outcomes have drawn attention [10-19]. However, the concept of postoperative anxiety in surgically treated patients has gained popularity, especially in recent years. Surgery is a stressful experience associated with a variety of psychological and physiological consequences. Therefore, psychiatric conditions such as anxiety can indeed arise from various factors, such as chronic pain syndromes, loss of ability, predisposing personality traits, unmet expectations, and other stress sources. A significant subgroup of surgical patients develops clinically meaningful new-onset anxiety and depression symptoms. However, the incidence, risk factors, and outcomes of new-onset anxiety after spine surgery have not been fully defined [20].

This study is designed to investigate postoperative anxiety in patients undergoing surgery for DLSS and its relationship with postoperative pain and disability levels and the time elapsed since surgery.

Material and methods

Sample: The required sample size for the results to be obtained before the research to have sufficient power was determined by power analysis. Accordingly, it was determined that the required number of people for two-way hypothesis, medium effect size, 95% confidence level (α=0.05), 80% power level (1-β=0.20) should be 47 for each group, a total of 94 people. Power analysis was performed using the Gpower (version 3.1) package program.

The study group of this research consists of 71 patients aged 18 years and over who applied to Etlik City Hospital Brain and Nerve Surgery outpatient clinics in September 2023 and underwent surgery for DLSS in our clinic in

the last six months. The control group consists of 65 individuals, similar in age and gender to the study group, who were examined for various reasons in the clinic and were found to be healthy in terms of spine. These people were selected among patients who applied for non-spine reasons, whose neurological examinations were normal, and who were not diagnosed with any disease in their examinations. Additionally, those who had previously been diagnosed with psychiatric illnesses such as depressive disorders, dementia, anxiety disorders, bipolar disorder, schizophrenia, and obsessive-compulsive disorder were excluded from both the study group and the control group. Patients with trauma and malignancy among surgical indications were excluded due to the presence of secondary factors affecting mental health.

In addition, four patients from the study group were excluded because they could not participate in the data collection processes. Two of the four excluded patients were illiterate, and the other two could not comply with the tests due to language differences.

Surgical technique: In the patients included in the study, the surgical procedure was performed by neurosurgery specialists with the necessary competence and experience in spine surgery. After general anesthesia, a midline lumbar incision was applied to the patients, and the paravertebral muscles were subperiosteally dissected. Instrumentation was applied with transpedicular corpus screws under fluoroscopy guidance. Laminectomy, foraminotomy, and discectomy, if necessary, were performed. Bilateral rods and transverse connectors provided stabilization. Autologous bone grafts were placed posterolaterally for fusion purposes. After the surgical drain placement, the layers were sutured in the anatomical plane.

Data collection tools: The State Anxiety Inventory (SAI) and the Trait Anxiety Inventory (TAI) were administered to the individuals in the study and control groups to measure their state and trait anxiety levels. The Visual Analog Scale (VAS) scores were recorded to calculate the postoperative pain levels of patients who underwent lumbar spinal surgery. The Oswestry Disability Index (ODI) scores were also assessed to measure the physical disability levels of the study group.

State and trait anxiety ınventory: It was developed by Spielberger et al. [21] in 1970. Öner and Lecompte conducted the Turkish adaptation and validity-reliability study in the Turkish population. The SAI consists of 20 items that need to be answered as (1) none, (2) a little, (3) quite, and (4) entirely to express the anxiety level of the individual at a specific time and in a specific situation. The TAI consists of 20 items that need to be answered as (1) rarely, (2) sometimes, (3) much of the time, and (4) almost always, to express the general anxiety level of the individual within the scope of personality traits. There are direct and reverse statements in the scales. Direct statements represent negative emotions, while reverse statements represent positive emotions. There are ten reverse statements in the SAI (1., 2., 5., 8., 10., 11., 15., 16., 19., 20. items) and 7 in the TAI (21., 26., 27., 30., 33., 36., and 39. items). The minimum score of both inventories is 20, while the maximum score is 80. Many studies have found this inventory useful in assessing clinical populations' state and trait anxiety symptom levels [22, 23].

Visual analog scale: It is a subjective expression of pain, a Likert-type scale scored between 0 and 10, with 0=no pain, 5=Moderate pain, and 10=the most severe pain experienced or unbearable pain level [24].

Oswestry disability index: The scale is designed to express the levels of disability in daily physical activities due to low back pain and consists of 10 items with six options ranging from "0" to "5". The first item evaluates the pain level, while the others consider the level of performing daily activities (e.g., dressing, bathing, walking...). The total scale score is divided by 50 if the person answers all the questions and multiplied by 100. The result is expressed as a percentage. It ranges from 0% for patients with no disability to 100% for patients with complete disabilities. The values are classified as minimal disability (0-20%), moderate disability (21-40%), severe disability (41-60%), complete disability (61-80%), and bedridden or exaggerated disability (81-100) [25]. Yakut et al. [26] conducted the Turkish adaptation and validity-reliability study in 2004.

Data collection process: The data for the study were obtained from the self-report scales applied to patients who had undergone surgery for DLSS in the last six months and came to the outpatient clinic for control and individuals who applied to the outpatient clinic and were not diagnosed with any pathological condition (similar to the study group in terms of age and gender) between them, who were healthy in terms of mental and spine health. Neurosurgery resident doctors accompanied the individuals during the process of answering the scales. While expressing VAS scores, the study group individuals were asked to consider the pain level they frequently experienced actively during the day.

Analysis of data: In the study, patients were classified as “early postoperative” (those who underwent surgery within the last three months) and “late postoperative” (those who underwent surgery within the previous six months but more than three months ago) in terms of the postoperative period from surgery to evaluation. VAS scores were considered “painful” for patients with scores of “7” and above and “pain-free” for those with scores of “6” and below. Previous studies have shown that a VAS score above 6 points predicts severe pain [27-29]. In our study, we classified patients who described severe pain as “painful.” Thus, a classification was made for the patients in the study group as “painful early postoperative,” “pain-free early postoperative,” “painful late postoperative,” and “pain-free late postoperative.” The ODI values of the patients in the study group were grouped as 1-minimal disability (0-20%), 2-moderate disability (21-40%), 3-severe disability (41-60%), 4-complete disability (61-80%), and 5-bedridden or exaggerated disability (81-100%). Patients with ODI values of 1 and 2 were considered as “no disability,” and those with 3, 4, and 5 were considered “ disability present.”

Firstly, the frequency and percentages of the sociodemographic characteristics of the study and control groups included in the research were calculated and compared. Especially the gender was considered here. Additionally, the frequency and percentages of the study group in terms of postoperative pain and postoperative period, as well as both groups evaluated together were determined. After the normality analysis of the data obtained from the measurement tools, the SAI and TAI scores of the study group and control group were compared to compare the anxiety levels of patients who underwent surgery due to DLSS with normal healthy individuals. Furthermore, the gender difference between SAI and TAI scores within the study group was evaluated.

To examine the relationship between the anxiety levels of patients who underwent lumbar surgery and their postoperative pain levels, the SAI and TAI scores of the group considered painful were compared with the pain-free group. To evaluate the relationship between patients’ anxiety levels and their physical disability status, the anxiety scores of the group considered to have physical disability were compared with the group evaluated as having no disability. Finally, the SAI and TAI scores of the early postoperative group and late postoperative group were compared to test the relationship between the anxiety levels of patients who underwent surgery and the time elapsed after surgery. In addition, patients were divided into four separate groups based on pain and postoperative period as described above, and the anxiety scores of each group were compared with each other.

Statistical analysis

Data analysis was performed using the SPSS (“Statistical Package for the Social Sciences”) (Version 25) package program and Microsoft Office Excel program. An alpha level of 0.05 was considered for statistical significance. Descriptive statistics measures (frequencies and percentages) were used for summarizing the population and sample, normality tests (Komogorov-Smirnov and Shapiro-Wilk) for the distribution of measurements obtained from measurement tools, the Mann-Whitney U test for variables with two groups, and the Kruskal Wallis H test for variables with more than two groups. The Mann-Whitney U test (with Bonferroni correction) was used to determine the source of the significant difference in the variables as a result of the Kruskal-Wallis test.

The measurements obtained from the measurement tools used in the research show a normal distribution for the control group;

however, they do not show a normal distribution for the patient group (p<0.05). So, due to the measurements obtained from the measurement tools, which mainly do not show a normal distribution for the patient group, non-parametric methods have been used to test the hypotheses within the scope of the research.

This study was conducted according to the principles stated in the Declaration of Helsinki and approved by the human research ethics committee of Etlik City Hospital (Registration No:AEŞH-EK1-2023-439 / 09.08.2023).

Results

The study group comprises 71 patients who underwent surgery due to DLSS, while the control group comprises 65 healthy individuals. Table 1 summarizes the frequency and percentages of the sociodemographic characteristics of the study and control groups included in the research.

Upon examining Table 1, it has been determined that the patient and control groups are similar regarding gender and age variables. The average age of the study group is 57.7 (±8.1), while the average age of the control group is 56.6 (±6.9). Among the patients who underwent surgery, 64.8% are in the pain-free group, and 35.2% are in the painful group. Additionally, the numbers of patients in the early and late postoperative periods are similar.

The normality test results of the measurement tools are summarized in Table 2. According to the results of the Mann Whitney U test for comparing SAI and TAI scores, the difference between both SAI and TAI scores of the patient and control groups was found to be statistically significant (SAI p=0.000, TAI p=0.000) (Table 3). To determine which group this difference favors, rank averages have been examined, and it has been found that the patient group has higher SAI and TAI scores. When discussing the effect size calculated for the practical significance of this statistically significant difference, it is observed to have a medium effect.

After comparing the anxiety scores of the patient and control groups, the anxiety scores of patients who underwent surgery due to DLSS have been compared in terms of gender, pain, disability, and postoperative period.

Table 1. Frequency and percentages of sociodemographic information of the patient and control groups

Variables	Categories	Patients (n=71)		Controls (n=65)		t/χ^2	p
		f/mean	%/SD	f/mean	%/SD		
Sex	Female	21	29.6	24	36.9	0.83	0.466
	Male	50	70.4	41	63.1		
Age		57.66	12.59	56.57	12.84	0.50	0.617
Postoperative Pain	Pain-free	46	64.8	--	--	--	--
	Painful	25	35.2				
Postoperative Period	Early periods	37	52.1	--	--	--	--
	Late periods	34	47.9				
Pain and Period	Early – Pain-free	23	32.4	--	--	--	--
	Late – Pain-free	23	32.4				
	Early – Painful	14	19.7				
	Late – Painful	11	15.5				

SD: Standart Deviation, t:independent t-test, χ^2 : Chi-square test

Table 2. Results of normality tests of measuring instruments

Measuring tools	Group	K-S Test		S-W Test	
		Statistics	*p*	Statistics	*p*
SAI	Patient	0.218	0.000*	0.853	0.000*
	Control	0.100	0.173	0.977	0.257
TAI	Patient	0.171	0.000*	0.851	0.000*
	Control	0.110	0.048	0.974	0.1

*: $p<0.05$, K-S Test: Kolmogorov-Smirnov test, S-W Test: Shaphiro-Wilk test, SAI: State Anxiety Inventory, TAI: Trait Anxiety Inventory

Table 3. Mann Whitney U test results regarding the comparison of SAI and TAI scores of the patient and control groups

Variables	Group	N	Mean	SD	Z	*p*	*d*
SAI	Patient	71	37.28	12.10	-4.45	0.000*	0.382
	Control	65	28.35	3.06			
TAI	Patient	71	30.73	4.43	-3.58	0.000*	0.307
	Control	65	28.17	3.43			

*: $p<0.05$, SD: Standart Deviation, d: Cohen's effect size, SAI: State Anxiety Inventory, TAI: Trait Anxiety Inventory

Firstly, the anxiety scores of the patient group were compared according to gender, and it was found that SAI and TAI scores in the patient group did not differ (SAI $p=0.553$, TAI $p=0.371$) (Table 4).

The results of the examination of the patient group's anxiety scores based on the postoperative pain status are summarized in Table 5. In the patient group, the SAI scores are statistically significantly higher in the painful group ($p=0.000$). Considering the rank means, it has been determined that this difference has a significant practical effect. On the other hand, it is found that the TAI scores of the patients do not show a statistically significant difference according to the pain status ($p=0.147$). When rank means are examined, it is understood that the TAI scores are higher in the group with pain, but this difference is not significant.

Table 4. Mann Whitney U test results regarding the comparison of SAI and TAI scores of the patient group by gender

Variable	Gender	N	Mean	SD	Z	*p*	*d*
SAI	Male	21	39.19	13.79	-0.59	0.553	--
	Female	50	36.48	11.38			
TAI	Male	21	30.76	5.72	-0.90	0.371	--
	Female	50	30.72	3.83			

SD: Standart Deviation, d: Cohen's effect size, SAI: State Anxiety Inventory, TAI: Trait Anxiety Inventory

Table 5. Mann Whitney U test results regarding the comparison of SAI and TAI scores of the patient group according to pain status

Variable	Postoperative Pain	N	mean	SD	Z	*p*	*d*
SAI	Pain-free	46	30.02	4.95	-6.62	0.000*	0.786
	Painful	25	50.64	9.72			
TAI	Pain-free	46	30.41	4.88	-1.45	0.147	--
	Painful	25	31.32	3.45			

*: $p<0.05$, SD: Standart Deviation, d: Cohen's effect size, SAI: State Anxiety Inventory, TAI: Trait Anxiety Inventory

When the disability levels of the patients are compared with the SAI and TAI scores, it is seen that the SAI scores of the patients are statistically significantly different according to their physical disability status (p=0.000). The Mann-Whitney U test was performed to determine which group or groups caused the statistical difference, and according to the study design, the SAI scores of patients with disability (Group 3 and above) were higher than those without disability (Groups 1 and 2). According to our statistical analysis, the TAI scores of the patients did not differ significantly according to the physical disability of the patient group (p=0.261) (Table 6).

In another part of the analysis, the anxiety scores of the patient group were compared in terms of the time elapsed after surgery; it was found that there was no statistically significant difference between the SAI and TAI scores of the patients in the early postoperative and late postoperative period (SAI p=0.584, TAI p=0.441) (Table 7).

Table 6. Kruskal Wallis H test results regarding the comparison of SAI and TAI scores of the patient group according to their disability levels

Variable	Disability (ODI)	N	Mean	SD	H	sd	*p*	Difference (Mann-Whitney)
SAI	1-Minimal disability	32	29.81	3.89	43.25	3	0.000*	3>1 4>2 3>2 4>1
	2-Moderate disability	14	31.50	10.34				
	3-Severe disability	15	47.07	7.82				
	4-Crippled	10	54.60	9.72				
	5-Bed bound / Exaggerating	0						
TAI	1-Minimal disability	32	30.94	5.51	4.00	3	0.261	
	2-Moderate disability	14	29.21	2.81				
	3-Severe disability	15	31.60	2.92				
	4-Crippled	10	30.90	4.25				
	5-Bed bound / Exaggerating	0						

*: $p<0.05$, SD: Standart Deviation, ODI: Oswestry Disability Index, SAI: State Anxiety Inventory, TAI: Trait Anxiety Inventory

Table 7. Mann Whitney U test results for the comparison of SAI and TAI scores of the patient group according to the time elapsed after surgery

Variable	Postoperative Period	N	Mean	SD	Z	*p*	*d*
SAI	Early	37	36.38	11.15	-0.55	0.584	--
	Late	34	38.26	13.16			
TAI	Early	37	30.32	4.12	-0.77	0.441	--
	Late	34	31.18	4.76			

SD: Standart Deviation, d: Cohen's effect size, SAI: State Anxiety Inventory, TAI: Trait Anxiety Inventory

In the last stage of the analyses, the patient group was grouped in terms of postoperative pain and period, and these groups were compared in terms of SAI and TAI scores (Table 8). According to Table 8, it is revealed that patients' SAI scores differ between the groups determined when postoperative pain and period are evaluated together (p=0.000). Pairwise groups were formed to determine which group this difference originated from, and Mann-Whitney U tests were performed. As a result of the pairwise comparisons, it was found that the painful early postoperative and painful late postoperative groups had higher SAI scores compared to both pain-free early postoperative and pain-free late postoperative groups. Within the groups with only painful or pain-free patients, SAI scores did not differ in terms of postoperative period. Patients' TAI scores did not show a statistically significant difference between the groups (p=0.346).

Table 8. Kruskal Wallis H test results regarding the comparison of SAI and TAI scores of the patient group by considering postoperative pain and period together

Variable	Pain and Period	N	Mean	SD	H	sd	*p*	Diffrence (Mann-Whitney)
SAI	Pain-free and early (1)	23	29.48	4.60	44.75	3	0.000*	1>3 1>4 2>3 2>4
	Pain-free and late (2)	23	30.57	5.32				
	Painful and early (3)	14	47.71	9.28				
	Painful and late (4)	11	54.36	9.35				
TAI	Pain-free and early (1)	23	29.70	4.31	3.31	3	0.346	--
	Pain-free and late (2)	23	31.13	5.40				
	Painful and early (3)	14	31.36	3.71				
	Painful and late (4)	11	31.27	3.26				

*p<0.05, SD: Standart Deviation, SAI: State Anxiety Inventory, TAI: Trait Anxiety Inventory, 1: Painful and early, 2: Painful and late
3: Pain-free and early, 4: Pain-free and late

Discussion

In this study, we found that SAI and TAI scores were significantly higher in the patient group who underwent surgery for DLSS compared to healthy controls; within the study group, SAI and TAI scores were not affected by gender and postoperative period, but higher SAI scores were observed in patients with tremendous postoperative pain and disability, while TAI scores remained unchanged. We also designed a unique model in which postoperative pain and duration were evaluated together to prevent bias arising from the pain factor when analyzing the change in anxiety scores over time after surgery. In this model, we confirmed that the difference between the groups originated from the pain variable; when the pain status was not a variable, SAI, and TAI scores did not differ in terms of postoperative duration. In this respect, our study has the feature of being the first in the literature.

Spine surgeries generally play a role in alleviating or exacerbating depressive and anxiety symptoms in patients with spine problems. Although the words depression and anxiety are uttered in this and similar studies, what is meant is depressive and/or anxiety-related symptoms. The scales were used to measure the levels of depressive and anxiety symptoms, not the levels of depression or anxiety. These depressive and anxiety symptoms decrease in patients as symptoms due to the spine improve after surgery. At the same time, new psychiatric problems are encountered in patients who experience negativity during the postoperative recovery process or suffer from physical disabilities [30]. Previous studies have focused mainly on psychiatric issues in the preoperative period, often conflating anxiety and depression. However, it is a known fact that anxiety and depression represent different clinical conditions. The earliest study on postoperative anxiety, conducted by Surman in 1987 [31], reported more postoperative complications in patients with high levels of anxiety. Falavigna et al. [32] have recently shown that postoperative psychiatric conditions are more critical than preoperative psychiatric conditions in predicting poor quality of life and disability after surgery. Subsequently, Angelini et al. [33] found that the incidence of postoperative anxiety in elective lumbar surgeries was high and affected clinical recovery negatively. We believe that this study, which addresses the anxiety status of patients who have undergone spine surgery in the postoperative period without clustering with depression, is valuable in terms of the information it provides.

The results of this study showing higher levels of state and trait anxiety in patients who underwent surgery for DLSS compared to the healthy controls are consistent with similar studies in the literature. Zieger et al. [15] reported that patients who underwent surgery for lumbar disc herniation had increased levels of anxiety and depression compared to the average population, which negatively affected postoperative pain and returned to work. Another study with 495 spinal surgery patients reported new-onset depression and anxiety rates of 6% and 11.2%, respectively [20]. While the increase in state anxiety levels in the patient group compared to the healthy population can be seen as an understandable result, the high score in trait anxiety may have two reasons. First, this result may be due to the nature of the measurement tool we preferred to use in our study. It has been shown in several studies that the SAI and TAI scales can successfully measure anxiety in populations and represent the theoretical distinction between state and trait anxiety [23, 34, 35]. However, in some exceptional cases and selected populations, it has been observed that the increase in state anxiety levels affects the participants' trait anxiety scores as well [36]. Second, due to DLSS and symptoms such as pain and disability, patients may have developed generalized anxiety in the preoperative period. In addition to these, interpreting the high level of trait anxiety in the patient group as an independent psychiatric condition rather than a disease state would be too theoretical and not consistent with the fact that randomly selected patients with anxiety disorders are grouped in the study population.

Effective management of postoperative anxiety, pain, and physical disability is essential for reducing complications, accelerating recovery, and enhancing patient satisfaction [37]. It has been shown that postoperative pain and physical disability trigger anxiety and depressive symptoms in patients undergoing spinal surgery and generally decrease their quality of life [38-40]. The consensus is that there

is a bidirectional and cumulative relationship between back pain and anxiety after spinal surgery. In addition to pain triggering anxiety and depression, the increase in these psychiatric disorders also worsens pain symptoms [41-44]. Jimenez Almonte et al. [45] found that there was no relationship between psychiatric disorders and physical disability in patients undergoing spinal surgery. Still, there was a significant relationship between postoperative pain and anxiety. In another study, Inci and Senol [46] reported a relationship between postoperative pain and anxiety in patients with DLSS. In the same study, they showed that anxiety and depression scores were also high in patients with high levels of disability but not statistically significant. Our study found that patients with higher levels of pain and physical disability had increased state anxiety-related symptom levels. Still, there was no difference in terms of continuous anxiety levels compared to other patients. This result highlights the coexistence of pain, disability, and anxiety but does not clarify the causal mechanisms. But, it is valuable in demonstrating the coexistence of pain, disability, and anxiety in patients who have undergone spinal surgery.

There was no difference in SAI and TAI scores between patients who had undergone surgery for DLSS and had passed three months or less and those who had passed more than three months but less than six months after surgery. Additionally, patients were divided into four groups based on their pain and time variables: early postoperative with pain, late postoperative with pain, early postoperative without pain, and late postoperative without pain, to prevent bias on the results caused by postoperative pain as a variable. These groups were compared in terms of SAI and TAI scores. In this model, it was observed that postoperative duration was not related to either state or trait anxiety and that the pain variable was related to state anxiety but not to trait anxiety score level.

The sample size is the most significant limitation of our study. More extensive sample studies are needed to test the changes in SAI and TAI scores by age and gender more accurately. Another limitation is that patient data are based on self-reports. Structured clinical interview-based studies will provide more accurate results. The standardization of the surgical technique applied seems to be an advantage in terms of the power of the study. However, posterior stabilization surgeries have become quite diverse today. For example, dynamic stabilization systems are thought to provide patients a more comfortable postoperative recovery process [47]. In this context, our study reflects the results of classical rigid stabilization surgeries. On the other hand, we did not take an active role in the postoperative rehabilitation processes of the patients we included in our study. We do not know how they continued these rehabilitation processes. However, studies show that standardized physical rehabilitation processes positively affect patients' physical and mental well-being [48]. Additionally, more extended follow-up periods may be required to understand the relationships between symptoms related to the spine and mental health.

According to the results of our study, the anxiety levels of patients who underwent surgery due to DLSS are generally higher than those of the healthy controls. Additionally, individuals with more pain and physical disability in these patients have higher anxiety symptom levels specific to their situation compared to other patients. We believe that a comprehensive approach focusing on pain, physical disabilities, and anxiety is necessary to support the postoperative recovery process of patients who have undergone surgery due to a degenerative spine. Comprehensive approaches focusing on pain, disability, and anxiety symptoms may positively affect the recovery process. More extensive and prospective studies are needed to reveal the causal relationship between these concepts.

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Research Article

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Use of albumin and CRP related immuno-nutritional markers for prediction of locoregional response to treatment in unresectable hepatocellular carcinoma

İnoperable Hepatosellüler karsinom'da lokorejyonel tedavi yanıtını öngörmek için albümin ve CRP ilişkili immüno-nutrisyon belirteçlerin kullanımı

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Abstract

Purpose: We examined the relationship between albumin and C reactive protein (CRP)-related inflammation markers such as Controlling nutritional status (Conut) score, lymphocyte-albumin factor (LA), albumin-bilirubin score (ALBI), highly sensitive modified Glasgow prognostic score (Hs-mGPS), Glasgow prognostic score (GPS) and locoregional treatment response in HCC.

Materials and methods: One hundred and eighty HCC patients and 63 patients who underwent locoregional therapy were included in this study. Routine laboratory tests between the fourth and eighth week after treatment were recorded and albumin and CRP-related immuno-nutrition scores were calculated. Cut-off values from the literature were used. The predictive and prognostic value of these markers for overall survival (OS) and disease-free survival (DFS) after treatment were analyzed.

Results: The mean age was 63 years (min-max:26-87) and 59 (93.7%) of the patients were male. The mean follow-up period was 25 months and 53 patients were deceased (84.1%). Median overall survival (mOS):18.56 months (min-max:13.13-23.99); median disease-free survival (mDFS):7 months (min-max:3.63-10.37) after locoregional treatment. Age (p=0.019), Conut (p=0.001), GPS (p=0.028), Hs-mGPS (p=0.012), LA (p=0.017) and ALBI (p=0.002) were significantly correlated with mOS. Conut (p=0.002), GPS (p<0.001), Hs-mGPS (p=0.002), LA (p=0.002) and ALBI (p=0.001) were significantly correlated with mDFS. Multivariate analysis revealed that those aged ≥65 years (HR:2.10; 95% CI:1.02-4.30; p=0.042) and those who received no systemic therapy (HR:4.11; 95% CI:1.35-12.56; p=0.013) had an increased risk of death (p<0.001). Another significant result was that a GPS of '2' (HR:6.62; 95% CI:1.13-38.62; p=0.036) predicted a higher risk of progression (p<0.001).

Conclusion: In this study, we found that age, Conut score, GPS, HsmGPS, LA and ALBI score significantly predicted mOS and mDFS in locoregionally treated HCC patients. All these results suggest that our prognostic modelling may be useful in clinical practice.

Keywords: HCC, TACE, Conut score, GPS, HsmGPS.

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Öz

Amaç: Controlling nutritional status (Conut) skor, lenfosit-albümin faktörü (LA), Albümin-bilirubin skor (ALBİ), high sensitif modifiye Glasgow prognostik skor (Hs-mGPS), Glasgow prognostik skoru (GPS) gibi albümin ve C-reaktive protein (CRP) ilişkili inflamasyon belirteçleri ile HCC'de lokorejyonel tedavi yanıtı arasındaki ilişkiyi inceledik.

Gereç ve yöntem: Yüz seksen HCC hastasından lokorejyonel tedavi uygulanan 63 hasta bu çalışmaya dahil edildi. Tedavi sonrası dördüncü-sekizinci hafta aralığındaki rutin laboratuvar testleri kaydedilerek albümin ve CRP ilişkili immüno-nutrisyon skorları hesaplandı. Literatürde yer alan cut-off değerleri kullanıldı. Bu belirteçlerin genel sağkalım (OS) ve tedavi sonrası hastalıksız sağkalım (DFS) için prediktif ve prognostik değeri analiz edildi.

Bulgular: Yaş ortalaması 63 (min-max:26-87) olan hastaların 59'u (%93,7) erkekti. Ortalama takip süresi 25 ay olup 53 hasta merhumdu (%84,1). Median genel sağkalım (mOS):18,56 ay (min-max:13,13-23,99); lokorejyonel tedavi sonrası mDFS:7ay (min-max:3,63-10,37) olarak belirlendi. Yaş (p=0,019), Conut (p=0,001), GPS (p=0,028), Hs-mGPS (p=0,012), LA (p=0,017) ve ALBI (p=0,002) ile mOS arasında istatistiksel anlamlı ilişki bulundu. Conut (p=0,002), GPS (p<0,001), Hs-mGPS (p=0,002), LA (p=0,002) ve ALBI (p=0,001) ile median hastalıksız sağkalım (mDFS) arasındaki ilişki istatistiksel olarak anlamlı bulundu. Multivariate analiz sonucunda; ≥65 yaş olanların (HR:2,10; %95 CI:1,02-4,30; p=0,042) ve hiç sistemik tedavi almayanların (HR:4,11; %95 CI:1,35-12,56; p=0,013) ölüm riskinin arttığı belirlendi (p<0,001). Bir diğer anlamlı sonuç ise GPS'nin '2' olmasının (HR:6,62; %95 CI:1,13-38,62; p=0,036) yüksek progresyon riskini öngördüğü idi (p<0,001).

Sonuç: Lokorejyonel tedavi uygulanan HCC'li hastalarda; yaş, Conut skor, GPS, HsmGPS, LA ve ALBI skorun, hastaların mOS ve mDFS için önemli prediktif faktörler olduğunu saptadık. Tüm bu sonuçlar oluşturduğumuz prognostik modellemenin klinik pratikte kullanımının yararlı olabileceğini düşündürmektedir.

Anahtar kelimeler: HCC, TACE, Conut skor, GPS, HsmGPS.

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Introduction

Hepatocellular carcinoma (HCC) typically develops in the context of chronic liver disease and cirrhosis, making it the third leading cause of cancer-related mortality. Screening at-risk patients using alpha-feto protein (AFP) and abdominal ultrasonography (USG) allows for early diagnosis, which enables curative treatments such as surgery or local therapies. For patients ineligible for liver transplantation, systemic and/or local therapies are used based on the stage of the disease, tumour size, location, and liver reserve. Treatment options include surgery (resection or transplantation), radiofrequency ablation (RFA) for smaller tumours, transarterial chemoembolization (TACE) for patients with adequate liver reserve, and systemic therapy (atezolizumab +bevacizumab, sorafenib, lenvatinib, cabozantinib, regorafenib) for patients with good performance status. In cases of poor Eastern Cooperative Oncology Group performance score (ECOG performance), best supportive care is recommended [1-3].

The five-year survival rate for advanced HCC is around 18%, but early diagnosis can raise this to 70%. Delayed diagnosis leads to a significant loss in survival, but this can be mitigated through early detection and the use of prognostic markers during treatment [4-6]. Prognosis varies depending on symptoms and tumour burden, and the Child-Pugh classification may not fully capture disease progression in all patients. Some symptoms, such as ascites or portal hypertension, can elevate Child scores, yet patients may still respond to symptomatic treatment. Consequently, there is ongoing research into more precise prognostic markers for guiding treatment decisions. A review of the literature suggests that combining different parameters with a single marker could enhance the accuracy of the Child score [7-11].

Treatment response for HCC is often assessed using radiological tests, such as contrast-enhanced abdominal magnetic resonance (MRI), which is performed one month after treatment and then every three months. The MRI should be dynamic, covering

the late arterial, portal venous, and late venous phases post-contrast. Lack of contrast uptake in the tumour center after local treatment suggests necrosis, whereas contrast uptake indicates residual tumour [12, 13]. However, coagulative hemorrhagic necrosis following RFA or TACE may cause bright images that can complicate the interpretation of residual tumours, particularly in the first month. Specialized radiologists and technicians are needed to minimize these issues [14].

Due to the heterogeneity of advanced HCC patients, MRI alone may not provide a full evaluation of all cases, and clinician experience can affect the objectivity of radiological results. Additionally, two-dimensional tumour size measurements are insufficient for fully evaluating treatment response. The clinician must assess viable tumour tissue, vascularity, margins, and any residual or recurrent tumour using imaging, though this can be limited by access and expertise [12, 13].

Locoregional treatment response is typically assessed between four and eight weeks post-treatment. Several prognostic markers derived from routine blood and biochemical tests have been identified in solid tumours, which could allow for quicker treatment adjustments in patients with disease progression.

In this study, we retrospectively evaluated inoperable HCC patients who underwent locoregional therapy. Haemogram and biochemistry values were recorded between the fourth and eighth week post-treatment. While albumin and CRP alone are markers of inflammation, we analyzed the relationship between albumin and CRP-related inflammation markers such as Controlling nutritional status (Conut) score, Glasgow prognostic score (GPS), highly sensitive modified Glasgow prognostic score (Hs-mGPS), Albumin-bilirubin score (ALBI) and lymphocyte-albumin factor (LA) and treatment response in HCC. To the best of our knowledge, no previous study has systematically modeled these markers in HCC patients receiving locoregional therapy, and we believe our findings will contribute to the literature.

Materials and methods

Patient characteristics and data collection

Locoregional therapies are powerful treatments frequently used in the treatment of patients who are not suitable for operation in HCC treatment. Different local treatment options are offered according to the size, number and location of the tumour. Radiofrequency ablation and transarterial treatments were applied in our patients. Between January 2011 and December 2023, 180 HCC patients who were followed up in the Medical Oncology Clinic of Pamukkale University were reviewed. Among all patients, 63 patients who underwent locoregional treatment during follow-up were included in the study. Clinical and demographic characteristics of the patients such as complaints at presentation, diagnostic features, exposure to etiological risk factors, performance status, tumour size, stage at presentation were evaluated. In studies evaluating locoregional treatments, the timing of treatment response has been suggested as the fourth to eighth week interval on average, and in this study, routine laboratory tests and clinical data requested in the fourth to eighth week interval were evaluated. Haemogram, biochemistry and hepatic viral serology results were recorded. Albumin, CRP, haemoglobin, lymphocyte, platelet, bilirubin, cholesterol, lactate dehydrogenase (LDH) and body mass index (BMI) values were taken from these routine blood values and albumin and CRP-related inflammation scores were calculated. These scores are Conut score, LA factor, ALBI score, Hs-mGPS, GPS.

Inflammatory response and nutrition are important in cancer pathology [10, 15-17]. There is no prognostic scoring that can predict DFS and OS after locoregional treatment in patients with HCC. Child score which is frequently used in the follow-up of HCC, does not adequately cover all of these patients with a heterogeneous group. Of the two parameters included in the Child score, the degree of encephalopathy and ascites are left to the clinician's interpretation, which limits objective scoring and is insufficient to sensitively predict response after local treatment in HCC [15]. Therefore, a prognostic marker is needed.

To the best of our knowledge, there is no clinical study in the literature evaluating whether the albumin and CRP-related inflammatory scores investigated in this study predict treatment response, early recurrence due to residual tumour, DFS and OS in locoregionally treated HCC cases. This study was planned considering that prognostic markers that are easily accessible to clinicians and can be easily repeated at each visit will be guiding.

Purposes of use and calculationof albumin and CRP related prognostic markers

Conut score: Is an immunonutrition score calculated on the basis of albumin, cholesterol and lymphocyte count in peripheral blood. The score obtained by adding the scores obtained from these three parameters constitutes the Conut score. '0-1' indicates normal malnutrition, '2-4' indicates light malnutrition and '5-8' indicates moderate malnutrition (Table 1) [10].

Table 1. Conut score calculation undernutrition status

	Albumin (g/dL)	Albumin Score	Total lymphocytes (/mm^3)	Total lymphocytes score	Total cholesterol (mg/dL)	Total cholesterol score	Conut Score
Normal	≥3.5	0	>1600	0	>180	0	0-1
Light	3.0-3.49	2	1200-1599	1	140-180	1	2-4
Moderate	2.5-2.9	4	800-1199	2	100-139	2	5-8

ALBI score: A simple and objective liver function test using only serum albumin and bilirubin levels. Unlike Child scoring, which is limited to use in patients with cirrhosis, this score can be used in all stages of liver disease. It was calculated with the formula "($_{log10}$ bilirubin (micromol/L)x0.66)+(albumin (g/L)x-0.085)" obtained from data analysis of more than 6000 HCC patients in the literature. ALBI score was grouped as I (score ≤-2.60), II (score >-2.60 with ≤-1.39) and III (>-1.39) [15].

GPS: This prognostic marker including albumin and CRP predicts inflammation and nutrition. The scoring system is shown in Table 2 [16].

Hs-mGPS: A more sensitive prognostic immuno-nutrition score was obtained by changing the cut-off values of CRP and albumin used in GPS. The scoring system is given in Table 2 [16].

Table 2. The Glasgow Prognostic Score and High sensitivity modified Glasgow prognostic score systems

The Glasgow Prognostic Score (GPS)	
Scoring systems	**Score**
CRP (≤10 mg/L) and albumin (≥35 g/L)	0
CRP (≤10 mg/L) and albumin (<35 g/L)	1
CRP (>10 mg/L) and albumin (≥35 g/L)	1
CRP (>10 mg/L) and albumin (<35 g/L)	2
High sensitive modified Glasgow prognostic score (Hs-mGPS)	
Scoring systems	**Score**
CRP (≤0.3 mg/L) and albumin (≥3.5 mg/L)	0
CRP (>0.3 mg/L) and albumin (≥3.5 mg/L)	1
CRP (>0.3 mg/L) and albumin (<3.5 mg/L)	2

CRP: C-reaktive protein

LA factor: This marker, which predicts inflammation, is calculated as "lymphocyte × albumin" by multiplying lymphocyte count and albumin concentration. It has been defined as a prognostic marker in different solid tumours in the literature [17].

Statistics analysis

Statistical analyses were performed using "IBM SPSS Statistics for Windows. Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)". Descriptive statistics are presented as Median±SD for continuous variables, n and % for categorical variables. Kaplan Meier method was used for survival (OS, DFS) analyses. Univariate analysis was performed. Finally, Multivariate Cox Regression results were given for the evaluation of statistically significant parameters in survival analysis. Statistically significant results (p<0.05) are indicated with a (*) sign next to the p value.

We evaluated which of these markers were predictive and prognostic for DFS and OS. DFS was defined as the time from the date of locoregional treatment to the first progression of disease. OS was defined as the time from the date of diagnosis to the time of death or last follow-up. Cut-off values of prognostic markers in the literature were used.

Pamukkale University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee approval was obtained (number: E-60116787-020-507040, board meeting dated 20.03.2024 and numbered E.507040).

Results

Clinical and demographic characteristics of HCC patients who underwent locoregional therapy are shown in Table 3. The mean age was 63 years (min-max: 26-87) and 59 (93.7%) of the patients were male. The etiology of HCC was hepatitis B virus (HBV) in 25 (39.7%), hepatitis C virus (HCV) in 5 (7.9%), non-alcoholic steatohepatitis (NASH) on the background of diabetes mellitus (DM) in 12 (19%) and alcohol use in 11 (17.5%). The most common presenting complaint was abdominal pain (27 patients (42.9%). The mean follow up period was 25 months and 53 patients were deceased (84.1%) (Table 3). Cut-off values of prognostic markers in the literature were used. The power of LA factor (p=0.045) and ALBI score (p=0.014) to predict mortality was statistically significant. Clinical and demographic data of the patients who underwent locoregional treatment are presented in Table 3.

In this study, age, Conut score, GPS, HsGPS, HsGPS, LA factor and ALBI score were included in the model created from prognostic markers. The power of these markers to predict two and five year OS and DFS after locoregional therapy in inoperable HCC was evaluated. In the whole patient group, mOS:18.56 months (min-max:13.13-23.99); mDFS:7 months (min-max:3.63-10.37) after locoregional therapy. Age (p=0.019), Conut score (p=0.001), GPS (p=0.028), Hs-mGPS (p=0.012), LA (p=0.017) and ALBI score (p=0.002) were significantly correlated with mOS. The relationship between Conut (p=0.002), GPS (p<0.001), Hs-mGPS (p=0.002), LA (p=0.002) and ALBI (p=0.001) and mDFS was statistically significant (Table 4).

In patients under 65 years of age, the two and five year OS rates were 70.4% and 48.6%, respectively; in patients over 65 years of age, the two and five year OS rates were 46.3% and 8.6%, respectively (p=0.019). In patients with normal Conut score, two and five year OS rates were 80% and 40%, respectively; in patients with mild and moderate malnutrition according to Conut score, two year OS rates were 49.7% and 26.1% and five year OS rates were 26.1% and 8.7%, respectively (p=0.001). In patients with GPS '0', two and five year OS rates were respectively 63.2% and 29.2%; in patients with GPS '1' and '2', two year OS rates were 32% and 8%, respectively; five year OS rates were 13.7% and 8%, respectively (p=0.028). In patients with Hs-mGPS '0', the two and five year OS rates were 68.8% and 31.3%, respectively, while in patients with Hs-mGPS '1' and '2', the two year OS rates were 45% and 20.6%, respectively (p=0.012).

Table 3. Clinical and demographic characteristics of HCC patients who underwent locoregional therapy

Variables	Total n=63 (%)
Age, Median (min-max)	63 (26-87)
≤65	34 (54%)
>65	29 (46%)
Gender, n (%)	
Male	59 (93.7%)
Female	4 (6.3%)
Etiology, n (%)	
HBV (Hepatitis B virüs)	25 (39.7%)
HCV (Hepatitis C virüs)	5 (7.9%)
Diabetes Mellitus	12 (19%)
Alcohol, n (%)	
-	52 (82.5%)
+	11 (17.5%)
Ecog, n (%)	
0-1	59 (93.6%)
≥2	4 (6.3%)
Location, n (%)	
Right	40 (63.5%)
Left	6 (9.5%)
Multifocal	17 (27.0%)
Diagnostic sympton, n (%)	
Asymptomatic	20 (31.7%)
Abdominal swelling	4 (6.3%)
Abdominal pain	27 (42.9%)
Fatigue	10 (15.9%)
Jaundice and Nause	2 (3.2%)
TM size, n (%)	
<50 mm	27 (42.9%)
≥50 mm	36 (57.1%)
Number of TMs, n (%)	
Multiple	28 (44.4%)
1	28 (44.4%)
2	7 (11.1%)
Stage, n (%)	
1	22 (34.9%)
2	7 (11.1%)
3	15 (23.8%)
4	19 (30.2%)
1. line treatment, n (%)	
-	9 (14.3%)
Sorafenib	51 (81.0%)
Other treatments (atezolizumab+bevacizumab/nivolumab)	3 (4.8%)
Mortality, n (%)	
Live	10 (15.9%)
Deceased	53 (84.1%)
Follow up period, Mean±SD	24.96±27.37

Descriptive statistics are presented as Median±SD for continuous variables, n and % for categorical variables

Table 4. Predictive effect of prognostic markers on two year and five year os and dfs data in locoregionally treated HCC Patients

OS (months)	2 years (%)	5 years (%)	Median (95% CI)	*p*
Overall	59.5	36.6	18.56 (13.13-23.99)	
Age				
<65	70.4	48.6	22.20 (6.93-37.47)	0.019*
≥65	46.3	8.6	10.76 (2.60-18.93)	
CONUT score				
Normal	80.0	40.0	47.33 (26.65-68.01)	
Mild	49.7	28.4	20.80 (2.88-38.72)	0.001*
Moderate	26.1	8.7	10.76 (0.00-22.61)	
GPS				
0	63.2	29.6	37.70 (18.90-58.49)	
1	32.0	13.7	15.56 (6.14-24.98)	0.028*
2	8.0	8.0	4.60 (0.39-8.80)	
Hs-mGPS				
0	68.8	31.3	37.70 (14.70-60.69)	
1	45.5	-	15.56 (-)	0.012*
2	20.6	8.8	7.10 (0.00-15.85)	
LA				
>4.08	57.7	32.4	34.73 (9.21-60.25)	0.017*
≤4.08	23.6	8.8	11.76 (4.82-18.70)	
ALBI				
≥-2.6 (score-2)	38.9	9.0	7.10 (0.00-16.11)	0.002*
<-2.6 (score-1)	60.6	31.1	37.70 (15.86-59.53)	
DFS (months)	**2 years (%)**	**5 years (%)**	**Median (95% CI)**	***p***
Genel	19.0	4.8	7.00 (3.63-10.37)	
Age				
<65	26.5	2.9	9.00 (4.42-13.57)	0.191
≥65	10.3	6.9	3.00 (1.02-4.97)	
CONUT				
Normal	60.0	20.0	27.00 (0.00-54.91)	
Mild	29.6	7.4	10.00 (4.91-15.08)	0.002*
Moderate	4.3	-	3.00 (2.54-3.45)	
GPS				
0	52.6	10.5	27.00 (12.84-41.15)	
1	6.9	3.4	6.00 (2.17-9.82)	<0.001*
2	-	-	1.00 (0.01-1.99)	
Hs-mGPS				
0	56.3	12.5	27.00 (11.44-42.55)	
1	9.0	-	7.00 (4.84-9.15)	0.002*
2	5.6	2.8	3.00 (2.67-3.23)	
LA				
>4.08	40.0	8.0	14.00 (2.31-25.68)	0.002*
≤4.08	5.3	2.6	3.00 (2.66-3.34)	
ALBI				
≥-2.6 (score-2)	5.3	2.6	3.00 (2.66-3.33)	0.001*
<-2.6 (score-1)	40.0	8.0	16.93 (8.77-25.09)	

95% CI: Confidence interval, Conut: nutritional score, GPS: Glasgow score, HsmGPS: high sensitive modified Glasgow score LA: lymphocyte-albumin factor, ALBI: albumin-bilirubin score, $p<0.05$ was considered statistically significant, Kaplan Meier curve, Long rank test, Statistically significant results ($p<0.05$) are indicated with a (*) sign next to the *p* value

In patients with LA >4.08, two and five year OS rates were 57.7% and 32.4%, respectively; in patients with LA ≤4.08, two and five year OS rates were 23.6% and 8.8%, respectively (p=0.017). In patients with ALBI score-1, two and five year OS rates were 60.6% and 31.1%, respectively; in patients with ALBI score-2, two and five year OS rates were 38.9% and 9%, respectively (p=0.002). In this study, Conut, GPS, HsmGPS, HsmGPS, LA, ALBI were found to statistically significantly predict mOS and mDFS after local treatment in HCC patients treated locoregionally. All these results suggest that the use of our prognostic modelling in clinical practice is useful (Table 4).

As a result of univariate analysis, age, CONUT, Glasgow, Hs-mGPS, LA and ALBI variables were found statistically significant in terms of predicting the risk of death and DFS after locoregional treatment (p<0.05). These variables found to be significant in the univariate analysis were included in the multivariate Cox regression model. According to the results of multivariate analysis, it was determined that being over 65 years of age (HR:2.10; 95% CI:1.02-4.30; p=0.042) and not receiving systemic treatment before or after locoregional treatment (HR:4.11; 95% CI:1.35-12.56; p=0.013) increased the risk of death (p<0.001). Another significant result obtained in the multivariate analysis showed that GPS of '2' (HR:6.62; 95% CI:1.13-38.62; p=0.036) and no systemic treatment before or after locoregional treatment (HR:7.00; 95% CI:2.22-22.09; p=0.001) predicted a higher risk of progression (p<0.001) (Table 5). There were no statistically significant results in the multivariate analysis of other markers in the prognostic model (age, Conut score, HsGPS, LA factor and ALBI score) (p>0.05).

Table 5. OS and DFS after locoregional therapy in HCC with prognostic markers multivariate cox regression results of their power to predict data

Variables	OS Multivariate HR (95% CI)	*p*	DFS Multivariate HR (95% CI)	*p*
Age (Ref:≤65)	2.10 (1.02-4.30)	0.042	-	
Conut (Ref:normal)		0.057		0.821
Mild	1.23 (0.36-4.17)	0.737	1.07 (0.35-3.24)	0.892
Moderate	1.96 (0.23-16.20)	0.532	1.84 (0.31-10.64)	0.494
Severe	7.23 (0.71-73.74)	0.095	1.52 (0.21-10.86)	0.673
GPS (Ref:0)		0.141		0.024*
1	0.58 (0.82-4.18)	0.595	2.47 (0.48-12.57)	0.276
2	1.31 (0.16-10.57)	0.797	6.62 (1.13-38.62)	0.036*
Hs-mGPS (Ref:0)		0.875		0.422
1	0.76 (0.11-5.02)	0.777	0.64 (0.12-3.33)	0.596
2	0.45 (0.01-11.55)	0.633	0.23 (0.02-2.83)	0.257
LA (Ref:>4.08)	0.61 (0.21-1.75)	0.365	1.14 (0.50-2.62)	0.742
ALBI (Ref:<-2.6)	0.22 (0.04-1.02)	0.054	0.36 (0.11-1.17)	0.092
	p<0.001; -2 Log Likelihood=313.10		p<0.001; -2 Log Likelihood=367.37	

Nutritional score, GPS: Glasgow score, Hs-mGPS: high sensitive modified Glasgow score, LA: lymphocyte-albumin factor
ALBI: albumin-bilirubin score, p<0.05 was considered statistically significant, Multivariate Cox Regression analysis
Statistically significant results (p<0.05) are indicated with a (*) sign next to the p value

Discussion

The prognostic model created in this clinical study included patient age, Conut score, GPS, Hs-mGPS, LA, and ALBI score. These markers' ability to predict two- and five-year OS and DFS after locoregional therapy in inoperable HCC patients was evaluated. In the entire patient group, the mOS was 18.56 months (min-max:13.13-23.99) and the mDFS was 7 months (min-max:3.63-10.37) after locoregional therapy. Age (p=0.019), Conut (p=0.001), GPS (p=0.028), Hs-mGPS (p=0.012), LA (p=0.017), and ALBI (p=0.002) were significantly correlated with mOS. Similarly, Conut (p=0.002), GPS (p<0.001), Hs-mGPS (p=0.002), LA (p=0.002), and ALBI (p=0.001) were significantly correlated with mDFS. In patients under 65 years, the two- and five-year OS rates were 70.4% and 48.6%, respectively, while for those over 65 years, the rates were 46.3% and 8.6% (p=0.019). Age over 65 was identified as an independent parameter for increased risk of recurrence and death after treatment (HR:2.10; 95% CI:1.02-4.30; p=0.042).

In univariate analysis, age, Conut, GPS, Hs-mGPS, LA, and ALBI were significant predictors of death and DFS after locoregional therapy (p<0.05). Multivariate analysis showed that increased age (HR:2.10; 95% CI:1.02-4.30; p=0.042) and absence of systemic therapy before locoregional therapy (HR:4.11; 95% CI:1.35-12.56; p=0.013) were independent predictors of increased mortality. GPS score of '2' was also significantly associated with increased risk of progression (HR:6.62; 95% CI:1.13-38.62; p=0.036). However, other markers in the prognostic model (age, Conut score, Hs-mGPS, LA ratio, and ALBI score) did not yield statistically significant results for OS and DFS (p>0.05).

Despite improvements in survival following locoregional therapy for HCC, the five-year recurrence rate remains high, at 70-80%, even in patients who undergo curative surgical resection or locoregional treatment. Due to the lack of strong post-treatment prognostic markers, tumour number, size, and pathological differentiation are currently used as indicators [18]. Necrosis and fibrosis, common in tumours treated with RFA, microwave ablation, TACE, and transarterial radioembolisation (TARE), also pose challenges in follow-up. While imaging modalities can distinguish treatment responders from non-responders, the correlation between necrosis extent and treatment outcomes is unclear, as imaging may overestimate necrosis [19, 20].

Inflammation plays a key role in the carcinogenesis of fatty liver, steatohepatitis, and HCC. Chronic inflammation creates an immunosuppressive microenvironment, promoting tumour formation and metastasis, and accelerating recurrence and metastasis [21]. This process informs the selection of prognostic markers. Previous clinical studies have aimed to predict locoregional treatment response in HCC patients based on various markers. For instance, Schobert et al. [22] included 46 HCC patients undergoing TACE and found that high pre-treatment neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were associated with poor tumour response and shorter PFS. The LA ratio, derived from lymphocytes and albumin, was also evaluated, with higher LA values predicting better outcomes, though these findings were not statistically significant in multivariate analysis.

Young et al. [23] used NLR, PLR, and aspartate aminotransferase to lymphocyte ratio index (ALRI) scores in a study of 167 HCC patients treated with TARE. The ALRI score, which combines AST and lymphocytes, was significantly associated with local recurrence and PFS. These markers, along with the Conut score, have been shown to predict malnutrition and have been validated as prognostic in studies involving liver resection or transplantation for HCC [24, 25].

Harimoto et al. [25] found that a higher Conut score was an independent risk factor for HCC recurrence and poor OS in 2461 HCC patients. However, in another study of 280 patients undergoing liver transplantation, while NLR and PLR were independent variables for predicting death and tumour recurrence, the Conut score was not predictive [26].

In this study, patients with low Conut scores had two- and five-year OS rates of 80% and 40%, respectively, compared to 49.7% and 26.1% for patients with mild malnutrition, and 26.1% and 8.7% for those with moderate malnutrition (p=0.001). Although the Conut score did not predict death and recurrence risk

after treatment, it remains a useful guide for the early detection and treatment of malnutrition. Other studies have demonstrated the prognostic value of markers such as GPS, tumour size, and PLR for PFS [27].

A retrospective study of 1625 HCC patients reported median OS of 15.7 months and a significant predictive value for GPS and Hs-mGPS, though multivariate analysis yielded no significant results [16]. Zhao et al. [28] found that the ALBI score was an independent predictor of progression after hepatic arterial infusion chemotherapy. Although ALBI scores were statistically significant as indicators of relapse and death, multivariate analysis did not find them to be independent variables for OS and DFS.

This study has limitations, including its single-centre design, small sample size, and retrospective data collection. Prospective studies with larger patient cohorts are necessary to confirm these findings. Similar limitations exist in studies evaluating inflammatory markers in cancer survival. However, inflammation and nutritional markers have shown significant associations with survival and recurrence risk, supporting their use in clinical practice.

In conclusion, this study hypothesizes that a prognostic marker could predict post-treatment outcomes in locoregionally treated HCC patients. We evaluated the predictive value of albumin and CRP-related inflammation and nutritional scores on survival and DFS. Our findings suggest that age, Conut score, GPS, Hs-mGPS, ALBI scores, and decreased LA ratio may help predict recurrence and death after locoregional therapy, providing valuable insight for clinical practice.

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Authors contributions: M.O. and G.G.D. constructed the main idea and hypothesis of the study. M.O. and G.G.D. developed the theory and arranged/edited the material and method section. M.O. and G.G.D. has done the evaluation of the data in the results section. The discussion section of the article was written by M.O. and G.G.D. and reviewed, corrected, and approved by S.D., B.Y.T., A.G.D., and A.Y. In addition, all authors discussed the entire study and approved the final version.

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Research Article

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Investigation of the effects of biotinidase deficiency on plasma cholinesterase activity

Biyotinidaz eksikliğinin plazma kolinesteraz aktivitesi üzerindeki etkilerinin araştırılması

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Abstract

Purpose: Biotinidase deficiency (BD) is a rare autosomal recessive metabolic disorder that impairs the body's ability to recycle biotin, a crucial coenzyme for carboxylase enzymes involved in various metabolic processes. This study aims to evaluate the effects of biotinidase deficiency on cholinesterase activity in plasma, hypothesizing that the metabolic disruptions caused by inadequate biotin recycling may lead to alterations in cholinesterase function.

Materials and methods: Plasma samples were collected from 73 individuals categorized into four genetic groups: wild type (n=12), heterozygous (n=30), homozygous (n=19), and compound heterozygous (n=12). Cholinesterase activity was measured using a colorimetric method.

Results: The study discovered that the cholinesterase activity of the Heterozygous group was higher than the homozygous group (p=0.0356). Additionally, cholinesterase activity was significantly lower in homozygous and compound heterozygous people than in wild and heterozygous groups (p=0.0272). The statistically significant changes suggested a relationship between biotinidase deficiency and altered cholinergic activity.

Conclusion: The findings indicate that biotinidase deficiency, particularly in its severe variants, may cause considerable reductions in cholinesterase activity, contributing to the neurological symptoms found in affected patients. More studies are needed to investigate the processes behind this association and develop strategies for reducing the effects of BD on cholinesterase activity and neurological health.

Keywords: Biotinidase deficiency, cholinesterase, metabolic disorder.

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Öz

Amaç: Biotinidaz eksikliği (BD), vücudun çeşitli metabolik süreçlerde yer alan karboksilaz enzimleri için kritik bir koenzim olan biotini geri dönüştürme yeteneğini bozan nadir bir otozomal resesif metabolik bozukluktur. Bu çalışma, yetersiz biotin geri dönüşümünün neden olduğu metabolik bozulmaların kolinesteraz fonksiyonunda değişikliklere yol açabileceği hipotezini test ederek, biotinidaz eksikliğinin plazmadaki kolinesteraz aktivitesi üzerindeki etkilerini değerlendirmeyi amaçlamaktadır.

Gereç ve yöntem: Plazma örnekleri, yabanıl tip (n=12), heterozigot (n=30), homozigot (n=19) ve bileşik heterozigot (n=12) olarak kategorize edilen 73 bireyden toplandı. Kolinesteraz aktivitesi kolorimetrik bir yöntem kullanılarak ölçüldü.

Bulgular: Bu çalışmada, heterozigot grubun kolinesteraz aktivitesinin homozigot gruptan daha yüksek olduğu bulundu (p=0,0356). Ek olarak, yabanıl tip ve heterozigot gruplarına kıyasla homozigot ve bileşik heterozigot bireylerde kolinesteraz aktivitesinin önemli ölçüde azaldığı bulundu (p=0,0272). Farklılıklar istatistiksel olarak anlamlıydı ve bu durum, biotinidaz eksikliği ile değişmiş kolinerjik fonksiyon arasında potansiyel bir bağlantıyı işaret etmektedir.

Sonuç: Bulgular, özellikle ciddi formlarında biotinidaz eksikliğinin, kolinesteraz aktivitesinde önemli azalmalarla sonuçlanabileceğini ve bu durumun etkilenen bireylerde gözlemlenen nörolojik semptomlara katkıda bulunabileceğini düşündürmektedir. Bu ilişkiyi açıklamak ve BD'nin kolinesteraz aktivitesi ve nörolojik sağlık üzerindeki etkilerini hafifletmeye yönelik terapötik stratejiler geliştirmek için daha fazla araştırmaya ihtiyaç vardır.

Anahtar kelimeler: Biyotinidaz eksikliği, kolinesteraz, metabolik bozukluk.

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Introduction

Biotinidase deficiency (BD) is a rare autosomal recessive metabolic disorder that impairs the body's ability to recycle the vitamin biotin, a critical coenzyme for carboxylase enzymes involved in various metabolic processes, including fatty acid synthesis, amino acid catabolism, and gluconeogenesis. The deficiency of biotinidase, an enzyme responsible for the cleavage of biotin from biocytin and other biotinyl-peptides, leads to reduced availability of free biotin, ultimately affecting the function of biotin-dependent carboxylases. If left untreated, BD can result in a range of neurological and dermatological symptoms, such as seizures, hypotonia, ataxia, alopecia, and skin rashes, which can be severe and irreversible in some cases [1].

Cholinesterase enzymes, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), play a crucial role in the nervous system by hydrolyzing the neurotransmitter acetylcholine, thereby terminating synaptic transmission [2, 3]. These enzymes are not only vital for cholinergic signaling but also have been implicated in various non-neuronal processes, such as lipid metabolism, immune responses, and inflammation [2-4]. Alterations in cholinesterase activity have been associated with several neurodegenerative diseases, liver dysfunction, and metabolic disorders [5-7].

Recent studies have begun to explore the potential interactions between metabolic disorders and cholinergic function, particularly focusing on how metabolic imbalances might influence cholinesterase activity. Given the critical role of biotin in cellular metabolism and the potential consequences of its deficiency on overall metabolic homeostasis, it is plausible that biotinidase deficiency may also impact cholinesterase activity in the plasma [7-11]. Understanding this relationship could provide new insights into the broader metabolic implications of BD and its potential role in neurodevelopmental disorders.

This study aims to evaluate the effects of biotinidase deficiency on cholinesterase activity in plasma, hypothesizing that the metabolic disruptions caused by inadequate biotin recycling may lead to alterations in cholinesterase function. By analyzing cholinesterase activity in plasma samples from individuals with biotinidase deficiency, this research seeks to contribute to the growing body of knowledge on the systemic effects of metabolic disorders and their potential links to neurological function.

Materials and methods

Study population, sample collection and BTD gene analysis

In this study, plasma samples were collected from 73 patients diagnosed with biotinidase deficiency at the Department of Medical Genetics, Harran University Faculty of Medicine. Exclusion criteria included patients with other metabolic disorders, neurological conditions unrelated to BD, or those receiving treatment that could affect cholinesterase levels (e.g., cholinesterase inhibitors). Peripheral blood samples (2 cc) were drawn into tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) for DNA isolation. Genomic DNA was then extracted, and sequence analysis was performed using primers that target the exon regions of the BTD gene. The resulting data were analyzed with the Mutation Surveyor program. The patients were classified into four genetic categories: Wild type, Heterozygous, Homozygous, and Compound Heterozygous. Each group was further stratified by gender, and age-related statistical parameters were calculated (Table 1).

Permission was obtained from the Harran University Faculty of Medicine Clinical Research Ethics Committee for the study (approval date: December 11, 2023, and number: HRÜ/23.23.26).

Table 1. Gender and age distribution of ındividuals across different genetic groups

Group	Gender	Patient Count	Mean Age	Age Std Dev	Median Age	Age Range
Wild type	Male	9	1.30	0.67	1	1-3
	Female	3	3.00	2.83	2	0-8
Heterozygous	Male	16	2.06	1.06	2	1-6
	Female	14	2.14	0.86	2	1-3
Homozygous	Male	11	2.82	1.34	3	1-6
	Female	8	2.20	0.79	2	1-3
Compound Heterozygous	Male	5	2.40	1.14	3	1-3
	Female	7	3.43	0.79	3	2-5

Measurement of cholinesterase activity

Cholinesterase activity in human-plasma was quantitatively determined using the Cholinesterase Gen.2 kit (Roche Diagnostics, Mannheim, Germany) on the Roche Cobas c, c 502. The test is based on a colorimetric method, where cholinesterase catalyzes the hydrolysis of butyrylthiocholine to thiocholine and butyrate. Thiocholine reduces hexacyanoferrate (III) to hexacyanoferrate (II), and the resulting decrease in absorbance at 415 nm is measured spectrophotometrically. The measurement range for cholinesterase activity was 100–14.000 U/L, with a lower detection limit of 100 U/L. The precision of the method was evaluated based on within-run and between-run coefficients of variation (CV). Within-run CVs were 0.5% for samples with mean activities of 4.887 U/L (Precinorm U) and 5.331 U/L (Precipath U). Between-run CVs were 1.0% for Precinorm U and 0.9% for Precipath U.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.4.2. The Shapiro-Wilk test was chosen to assess data normality due to its suitability for small sample sizes [12]. Normally distributed data were analyzed using the Student's t-test, while non-normally distributed data were evaluated with the Mann-Whitney U test. Results are reported as mean ± standard deviation, with a *p*-value of ≤0.05 indicating statistical significance.

Results

Cholinesterase activities were measured in individuals with biotinidase deficiency, categorized into four groups: Wild type, Heterozygous, Homozygous, and Compound Heterozygous. The gender and age distributions of individuals in the groups are shown in Table 1. The cholinesterase activities for each group were as follows: Wild type: 9419±2302 U/L, Heterozygous: 9379±1561 U/L, Homozygous: 8437±1326 U/L, and Compound: 8648±1540 U/L (Figure 1A). A statistically significant difference was observed between the Heterozygous and Homozygous groups (p=0.0356, t=2.166). Cholinesterase activities were further analyzed by grouping individuals into two main categories: Wild type + Heterozygous and Homozygous + Compound Heterozygous. The cholinesterase activities for each group were as follows: Wild type +Heterozygous: 9391±1778 U/L and Homozygous + Compound Heterozygous: 8519±1391 U/L (Figure 1B). The difference between these two groups was statistically significant (p=0.0272, t=2.255).

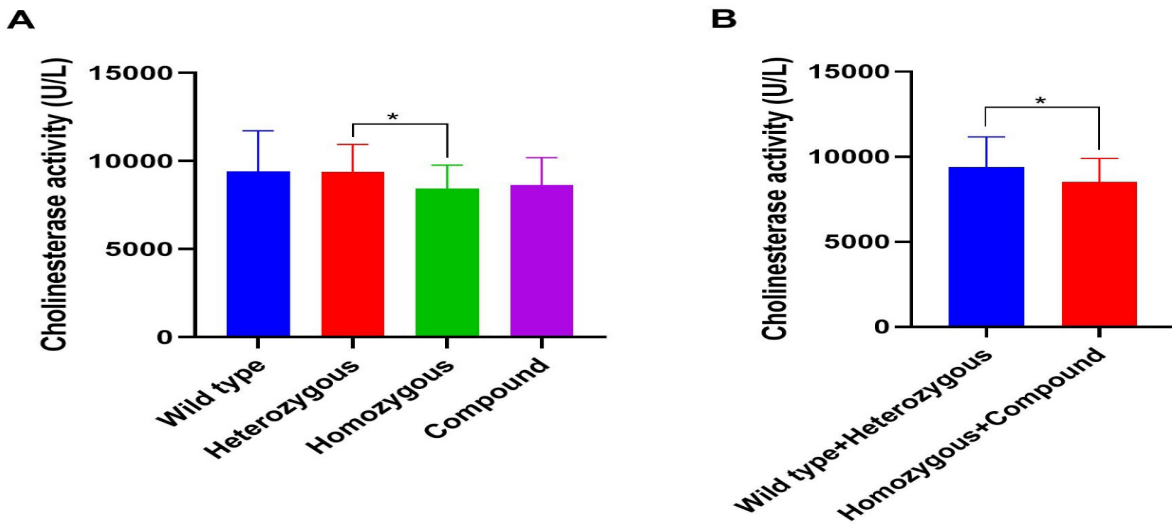

Figure 1. Effects of biotinidase deficiency on cholinesterase activity

(A) Results from four distinct genetic groups (Wild type, Heterozygous, Homozygous, and Compound Heterozygous)
(B) Results from grouping individuals into two main categories: Wild type + Heterozygous and Homozygous + Compound Heterozygous
All results are presented as mean ± standard deviation. * indicates $p<0.05$

Discussion

This study aimed to explore the potential impact of Biotinidase Deficiency (BD) on cholinesterase activity in plasma, an area that has received limited attention in previous research. Our findings indicate that individuals with BD, particularly those classified as Homozygous or Compound Heterozygous, exhibit significantly reduced cholinesterase activity compared to their Wild type and Heterozygous counterparts. These results provide valuable insights into the broader metabolic implications of BD, highlighting its potential effects on cholinergic function and, by extension, neurological health.

The observed reduction in cholinesterase activity among Homozygous and Compound Heterozygous individuals may be attributed to the metabolic disruptions caused by inadequate biotin recycling. Biotin, a crucial coenzyme for carboxylase enzymes, plays an essential role in various metabolic processes, including fatty acid synthesis, amino acid catabolism, and gluconeogenesis [13]. A deficiency in biotinidase impairs the recycling of biotin from biocytin and other biotinyl-peptides, leading to a reduced availability of free biotin and subsequent dysregulation of biotin-dependent metabolic pathways [11].

Previous studies have suggested that alterations in cholinesterase activity are associated with several neurodegenerative diseases and metabolic disorders [14, 15]. The reduced cholinesterase activity observed in our study may reflect a broader metabolic imbalance resulting from BD, potentially contributing to the neurological symptoms commonly seen in affected individuals, such as seizures, hypotonia, and ataxia [16, 17]. Our findings are consistent with the literature which reported that metabolic disorders could influence cholinergic function, thereby affecting neurological outcomes [16, 17].

The statistically significant difference in cholinesterase activity between the Wild type + Heterozygous and Homozygous + Compound Heterozygous groups (p=0.0272, t=2.255) further underscores the potential link between BD and cholinergic dysfunction. This result suggests that even partial impairment of biotin recycling, as seen in heterozygous individuals, may not significantly disrupt cholinesterase activity, whereas more severe forms of the deficiency (i.e., Homozygous and Compound Heterozygous) lead to notable alterations in enzyme function. This study adds to the growing body of evidence suggesting that BD has systemic effects beyond the well-characterized neurological and dermatological symptoms, potentially influencing broader metabolic and enzymatic processes [10, 18].

Given the critical role of cholinesterase enzymes in terminating synaptic transmission and their involvement in various non-neuronal processes, such as lipid metabolism and inflammation [4, 19, 20], the implications of reduced cholinesterase activity in BD are significant. The potential link between BD and cholinergic dysfunction could open new avenues for understanding the pathophysiology of the neurological symptoms associated with this condition. Further research is warranted to explore the mechanisms underlying this relationship and determine whether therapeutic interventions to restore biotin levels can normalize cholinesterase activity and improve neurological outcomes in affected individuals.

A limitation of this study is the relatively small sample size, which may affect the generalizability of the results. Additionally, other unaccounted factors, such as patients' nutritional status or the use of medications that could affect cholinesterase activity, were not controlled for. Future studies with larger cohorts and broader inclusion criteria are recommended.

In conclusion, our study demonstrates a significant reduction in cholinesterase activity in individuals with Biotinidase Deficiency, particularly those with Homozygous or Compound Heterozygous mutations. These findings highlight the potential for BD to impact cholinergic function, thereby contributing to the neurological symptoms observed in this condition. Future research should focus on elucidating the mechanisms driving this relationship and exploring potential therapeutic strategies to mitigate the effects of BD on cholinesterase activity and neurological health.

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Authors contributions: M.O. and M.E. have constructed the main idea and hypothesis of the study. M.O., O.O., and M.E. developed the theory and edited the material and method section. M.O. and M.E. have evaluated the data in the Results section. The discussion section of the article was written by M.O. and corrected and approved by O.O. and M.E. 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.

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Research Article
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Nutrition literacy status of university students, influencing factors, and its relationship with healthy nutrition attitudes

Üniversite öğrencilerinin beslenme okuryazarlığı durumu, etkileyen faktörler ve sağlıklı beslenme tutumu ile ilişkisi

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Abstract

Purpose: This study aims to evaluate the nutrition literacy status of university students, the factors influencing it, and its relationship with healthy nutrition attitudes.

Materials and methods: This is a descriptive and cross-sectional study. A total of 317 students aged 18-24 studying at Pamukkale University were included in the study. A questionnaire was administered to the students, which included questions about sociodemographic characteristics, the Adolescent Nutrition Literacy Scale (ANLS), and the Attitude Scale for Healthy Nutrition (ASHN) assessing nutrition attitudes.

Results: Of the 317 students who participated in the study, 48.9% (n=155) were female, and 17.4% (n=55) were studying in health-related departments. The mean nutrition literacy score of the students was 70.85±9.69. Nutrition literacy scores were found to be significantly higher in those studying in health-related departments compared to other departments, in those who received nutritional education compared to those who did not, and in those who exercised regularly compared to those who did not (p=0.001; p=0.000; p=0.000). Among the students, 56.8% had a high level, 10.1% had an ideal level, and 32.8% had a moderate level of healthy nutrition attitudes. A significant relationship was found between nutrition literacy and healthy nutrition attitudes (r=0.404; p=0.000).

Conclusion: The study found that the mean nutrition literacy score of the students was 70.85±9.69, and 56.8% of them had a high level of healthy nutrition attitudes. It was determined that nutrition literacy was higher among students who exercised regularly, received nutritional education/knowledge, and studied in health-related departments. Additionally, an increase in students' nutrition literacy positively influenced their healthy nutrition attitudes.

Keywords: Nutrition literacy, healthy nutrition, attitude, university students.

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Öz

Amaç: Bu çalışmada; üniversite öğrencilerinin beslenme okuryazarlığı durumlarını, etkileyen faktörleri değerlendirmek ve sağlıklı beslenme tutumu ile ilişkisini belirlemektir.

Gereç ve yöntem: Tanımlayıcı ve kesitsel tipte bir çalışmadır. Çalışmaya Pamukkale Üniversitesinde öğrenim gören 18-24 yaş arası 317 öğrenci dahil edildi. Öğrencilere sosyodemografik özellikler, adolesan beslenme okuryazarlığı ölçeği (ABOÖ) ve beslenme tutumunu değerlendiren sağlıklı beslenmeye ilişkin tutum ölçeği (SBİTÖ)'ni içeren anket uygulandı.

Bulgular: Çalışmaya katılan 317 öğrencinin %48,9'u (n=155) kadın, %17,4'ü (n=55) sağlık ile ilgili bölümlerde öğrenim görmekteydi. Öğrencilerin beslenme okuryazarlığı puan ortalaması 70,85±9,69 idi. Beslenme okuryazarlığı puanının, sağlıkla ilgili bölümlerde okuyanların diğer bölümlere göre, beslenme hakkında eğitim alanların almayanlara göre, düzenli egzersiz yapanların yapmayanlara göre istatistiksel olarak anlamlı yüksek bulundu (p=0,001; p=0,000; p=0,000). Öğrencilerin %56,8'in yüksek düzeyde, %10,1'i ideal düzeyde ve %32,8'i orta düzeyde sağlıklı beslenme tutumuna sahipti. Beslenme okuryazarlığı ile sağlıklı beslenme tutumu arasında istatiksel olarak anlamlı bir ilişki saptandı (r=0,404; p=0,000).

Sonuç: Çalışmada öğrencilerin beslenme okuryazarlığı puanının ortalaması 70,85±9,69 olduğu ve beslenme tutumunun %56,8'inde yüksek düzeyde olduğu saptandı. Beslenme okuryazarlığının düzenli egzersiz yapan öğrencilerde, beslenme eğitimi/bilgisi alanlarda ve sağlık alanı bölümlerinde okuyanlarda daha yüksek olduğu tespit edildi. Ayrıca öğrencilerin beslenme okuryazarlığının artması sağlıklı beslenme tutumunu olumlu yönde etkilemekteydi.

Anahtar kelimeler: Beslenme okuryazarlığı, sağlıklı beslenme, tutum, üniversite öğrencileri.

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Introduction

Beginning from the earliest stages of fetal development to old age, nutrition is fundamental in human life, health, and development [1]. Healthy nutrition is the basis of optimal health and quality of life. Adequate and balanced nutrition both protects the health of individuals and prevents diseases. In a globalizing world, achieving quality of life is only possible by increasing the nutritional awareness of the society and making healthy nutrition a lifestyle. Therefore, improving and developing healthy lifestyles of individuals is of great importance for public health [2]. Nutrition literacy plays a crucial role in developing behaviors oriented towards healthy nutrition [3]. Zoellner et al. [4] define nutrition literacy as the level at which individuals have the ability to obtain, process, and understand basic information about nutrition. Poor nutrition literacy impedes healthy nutrition [5].

Nutrition literacy (NL) is classified into three dimensions, i.e. functional, interactive, and critical nutrition literacy [6, 7]. Functional nutrition literacy refers to the basic reading and writing skills necessary to access information about nutrition [7, 8]. Interactive nutrition literacy encompasses advanced literacy skills, including cognitive and interpersonal communication abilities, which are required to effectively interact with healthcare professionals regarding nutrition [9].

At a basic level, interactive nutrition literacy includes the ability to translate information into healthy nutrition choices [6, 7]. Critical nutrition literacy can be defined as the ability to critically analyze information and recommendations about nutrition, increase awareness, and strive against barriers to healthy nutrition [9].

Although nutritional problems are prevalent in society, one of the groups with insufficient and unbalanced nutrition is university students. This is because the university period represents a transition to a new phase of life for young individuals. When students move away from home and start living independently for the first time, they reshape their health and eating habits [10, 11].

As students enter a new life-routine, they become more sensitive to external influences and, due to the accelerated pace of life, may experience an increase in inadequate, unbalanced, and unhealthy eating habits. These unhealthy eating habits can negatively affect students' mental and physical health, which in turn can lower their academic performance [10].

Research indicates that many university students avoid healthy food choices due to inadequate nutritional knowledge, negative attitudes, and practices, often skipping meals and generally distancing themselves from a healthy lifestyle [12, 13]. It is crucial to identify and regulate students' eating habits to prevent the problems that unhealthy nutrition can cause [10]. In this context, the aim of this study is to determine the nutrition literacy status of university students, the factors influencing it, and to evaluate its relationship with healthy nutrition attitudes.

Materials and method

This is a descriptive and cross-sectional study. Approval for the research was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (date: 17.05.2022- number: E-60116787-020-208345).

Population and sample

The study population consisted of 31.417 students enrolled at the central campus of Pamukkale University during the 2022-2023 academic year. The sample size was calculated to represent the population with a 95% confidence level, ±5% margin of error, and using the prevalence rate of a reference study [14], which reported that 76.4% of students had a high level of attitude towards healthy nutrition. Based on these parameters, the required sample size was calculated to be at least 277 students. A total of 317 students were included in the study. Surveys were administered to students who agreed to participate between 01/09/2022 and 31/10/2022.

Data collection and data collection tools

The survey consists of three main sections: a socio-demographic descriptive section, the Adolescent Nutrition Literacy Scale (ANLS), and the Attitude Scale for Healthy Nutrition (ASHN). The first section of the survey is the socio-demographic descriptive section, which inquires about age, gender, department of study, mother's educational level, father's educational level, family's monthly income level, height, weight, smoking status, regular exercise status, frequency and duration of exercise, meal skipping status, skipped meals and reasons for skipping, nutritional education/knowledge level, and sources of nutritional education/knowledge.

Adolescent nutrition literacy scale: Developed by Bari in 2012 [15] and validated in Turkish by Sonay Türkmen et al. [16] in 2017, this scale consists of 22 questions and is in the form of a five-point Likert scale. The scale includes three sub-dimensions: critical, interactive, and functional nutrition literacy. The highest possible score on the scale is 110, and the lowest is 22. A higher scale score indicates a higher level of nutrition literacy.

Attitude scale for healthy nutrition: Created and validated by Tekkurşun Demir et al. [17] in 2019, this scale consists of 21 items in a five-point Likert format. The scale is divided into four factors: Malnutrition (MP), Emotion for Nutrition (EN), Information on Nutrition (IN), and Positive Nutrition (PN). The highest possible score on this scale is 105, and the lowest is 21. When interpreting the scores, 21 points indicate a very low level, 23-42 points indicate a low level, 43-63 points indicate a moderate level, 64-84 points indicate a high level, and 85-110 points indicate an ideal level of healthy nutrition attitude.

Data analysis

The data were analyzed using IBM SPSS Statistics 25 (Armonk, NY: IBM Corp.) software. Descriptive statistics were presented as numbers and percentages for categorical variables, and as arithmetic means and standard deviations for continuous variables. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normal distribution. To evaluate the differences between groups, One-Way ANOVA, independent samples t test, Kruskal-Wallis Variance Analysis, Mann-Whitney U tests were applied. Additionally, Spearman's correlation analysis was used to assess the relationships between continuous variables. A *p*-value of less than 0.05 was considered statistically significant.

Results

The age range of the 317 students included in the study was 18-24 years, with a mean age of 20.75±1.65 years, and 48.9% (n=155) were female students. Of the students, 17.4% (n=55) were enrolled in health-related departments (such as medicine, dentistry, physiotherapy and rehabilitation, the faculty of health sciences, and the vocational school of health services), while 82.6% (n=262) were studying in other departments. The sociodemographic variables of the students are presented in Table 1.

Among the students, 55.5% (n=176) reported performing regular exercise. Of those who exercised regularly, 52.8% (n=93) exercised 1-2 days a week, and 43.7% (n=76) exercised for 30-60 minutes. It was found that 74.8% (n=237) of the students skipped meals, with lunch being the most frequently skipped meal at 50.2% (n=119). When asked about the reasons for skipping meals, 30.8% (n=73) stated "I don't feel like eating/I have no appetite," and 22.8% (n=54) reported "I don't have time." It was found that 53.3% (n=169) of the students had not received any nutritional education or knowledge. Among those who had received nutritional education or knowledge, 17.7% (n=56) stated they received it as a course at school (Table 2).

The overall Nutrition Literacy (NL) score among the students ranged from 37 to 105, with a mean score of 70.85±9.69. The mean score on the Attitude Scale for Healthy Nutrition (ASHN) was 69.01±11.29 (min:38, max:102), with 10.1% (n=32) of the students having an ideal level and 56.8% (n=180) having a high level of healthy nutrition attitude (Table 3).

Table 4 presents the comparison of overall NL and subscale scores with certain student variables. The overall NL, interactive NL, and functional NL mean scores were found to be significantly higher among students enrolled in health-related departments compared to those in other departments (p=0.001; p=0.015; p=0.011, respectively).

Table 1. The sociodemographic features of the students

	n	%
Gender		
Female	155	48.9
Male	162	51.1
Department		
Health-related departments	55	17.4
Others	262	82.6
Mother's level of education		
Primary school graduate and below	132	41.6
Secondary school graduate	65	20.5
High school graduate	82	25.9
University graduate	38	12.0
Father's level of education		
Primary school graduate and below	104	32.8
Secondary school graduate	50	15.8
High school graduate	107	33.7
University graduate	56	17.7
Family Income		
Income is less than expenditure	88	27.8
Income is equal to expenditure	155	48.9
Income is more than expenditure	74	23.3
Body mass index		
Underweight	33	10.4
Normal weight	213	67.2
Overweight and obesity	71	22.4
Smoking status		
Yes	143	45.1
No	174	54.9
Total	317	100

Table 2. Students' exercise status, eating habits, nutritional education/information status and source

	n	%
Regular exercise status		
Yes	176	55.5
No	141	44.5
Frequency of exercise (n=176)		
1-2 day	93	52.8
3-4 day	47	26.7
5-6 day	24	13.6
Every day	12	6.8
Daily exercise duration (n=176)		
<30 minute	37	21.3
30-60 minute	75	42.6
1-2 hours	48	27.6
>2 hours	13	7.5
No time specified	3	1.7
Meal skipping status		
Yes	237	74.8
No	80	25.2
Skipped meals		
Breakfast meal	104	43.9
Luch meal	119	50.2
Dinner meal	14	5.9
Reasons for skipping meal		
I don't feel like eating/I have no appetite	73	30.8
I can't wake up in the morning	24	10.1
I don't have time	54	22.8
I'm on a diet	8	3.4
I do not have a habit	24	10.1
No one prepares it	23	9.7
My economic opportinities are insufficient	27	11.4
Others (living in a dormitory)	4	1.7
Nutrition education/information status		
Yes	148	46.7
No	169	53.3
Source*		
School lessons	56	17.7
Physician/Healthcare provider	43	13.6
Books/Newspapers/Magazines	20	6.3
Media (Tv/internet)	53	16.7
Family/Friends	18	5.7
Other (Sports trainer)	2	0.6

* More than one option has been marked

Table 3. Students' nutritional literacy total and sub-dimension, attitude scale for healthy nutrition total score and level distribution

	Min-Max	Mean±SD
Total NL scores	37-105	70.85±9.69
Functional NL	8-35	24.53±5.09
İnteractive NL	6-30	18.07±4.95
Critical NL	16-41	28.25±4.10
ASHN score	38-102	69.01±11.29
ASHN level	**n**	**%**
Mild	1	0.3
Moderate	104	32.8
High	180	56.8
İdeal	32	10.1

NL: Nutrition Literacy, ASHN: Attitude Scale for Healthy Nutrition, SD: Standard deviation

Table 4. Comparison of some independent variables with total NL and sub-dimension scores

	Functional NL	Interactive NL	Critical NL	Total NL
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**
Gender				
Female (n=155)	24.65±4.92	18.54±4.95	27.91±4.27	71.10±9.47
Male (n=162)	24.42±5.26	17.62±4.91	28.58±3.93	70.62±9.92
Test value	Z=-0.395	Z=-1.781	Z=-1.045	Z=-0.321
p value	0.693	0.075	0.296	0.748
Department				
Health-related departments (n=55)	26.36±4.69	19.64±4.77	29.31±3.57	75.31±10.25
Others (n=262)	24.15±5.09	17.74±4.93	28.03±4.18	69.92±9.32
Test value	Z=-2.531	Z=-2.423	Z=-1.825	Z=-3.434
p value	0.011*	0.015*	0.068	0.001*
Body mass index				
Underweight (n=33) (a)	24.24±4.34	16.33±4.57	28.00±3.61	68.58±8.22
Normal weight (n=213) (b)	24.48±4.9	17.96±4.86	28.32±4.27	70.76±9.44
Overweight and obesity (n=71) (c)	24.82±5.93	19.21±5.14	28.17±3.85	72.20±10.91
Test value	F =0.174	Kwh=8.338	Kwh=0.150	Kwh =4.005
p value	0.841	0.015 (a-c)*	0.928	0.135
Mother's level of education				
Primary school graduate and below (n=132)	24.05±4.80	17.76±4.97	28.46±4.46	70.27±9.37
Secondary school graduate (n=65)	24.00±5.59	18.45±5.15	28.11±3.70	70.55±11.05
High school graduate (n=82)	25.40±5.05	18.54±4.59	28.33±3.81	72.27±9.31
University graduate (n=38)	25.24±5.10	17.50±5.30	27.61±4.19	70.34±9.17
Test value	Kwh=3.893	F=0.711	F=0.464	Kwh=2.406
p value	0.273	0.546	0.708	0.493

Table 4. Comparison of some independent variables with total NL and sub-dimension scores (continued)

	Functional NL	Interactive NL	Critical NL	Total NL
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**
Father's level of education				
Primary school graduate and below (n=104)	23.68±4.51	17.48±4.50	28.21±4.58	69.38±8.48
Secondary school graduate (n=50)	24.88±5.68	18.98±5.41	28.56±4.38	72.42±11.91
High school graduate (n=107)	24.79±5.32	18.38±5.13	28.06±3.44	71.23±9.34
University graduate (n=56)	25.30±5.01	17.75±4.90	28.43±4.18	71.48±10.16
Test value	F=1.578	F=1.281	Kwh=2.399	F=1.381
p value	0.195	0.281	0.494	0.248
Family income				
Income is less than expenditure (n=88)	24.50±4.98	17.82±4.98	28.48±3.88	70.80±9.31
Income is equal to expenditure (n=155)	24.88±5.07	18.47±4.88	28.45±4.15	71.80±9.51
Income is more than expenditure (n=74)	23.84±5.25	17.53±5.03	27.58±4.25	68.95±10.33
Test value	Kwh=1.389	Kwh=2.223	Kwh=3.195	Kwh=3.822
p value	0.499	0.329	0.202	0.148
Smoking status				
Yes (n=143)	24.34±5.19	17.73±4.96	28.01±4.50	70.08±10.32
No (n=174)	24.69±5.01	18.35±4.93	28.45±3.75	71.49±9.12
Test value	Z=-0.194	T=-1.117	Z=-0.814	Z=-1.370
p value	0.846	0.265	0.416	0.171
Regular exercise status				
Yes (n=176)	25.28±5.19	18.86±5.01	28.66±4.20	72.81±9.98
No (n=141)	23.60±4.81	17.09±4.7	27.74±3.94	68.42±8.75
Test value	Z=-2.763	Z=-3.002	Z=-1.987	Z=-3.963
p value	0.006*	0.003*	0.047*	0.000*
Meal skipping status				
Yes (n=237)	24.44±5.06	17.84±5.04	28.15±4.14	70.43±9.46
No (n=80)	24.81±5.17	18.76±4.61	28.55±4.01	72.13±10.31
Test value	Z=-0.470	Z=-1.547	Z=-0.901	Z=-1.541
p value	0.638	0.122	0.368	0.123
Nutrition education/information status				
Yes (n=148)	26.09±4.48	19.39±4.96	29.13±3.99	74.61±9.85
No (n=169)	23.17±5.21	16.91±4.65	27.49±4.06	67.56±8.27
Test value	Z=-5.305	Z=-4.237	Z=-3.470	T=6.851
p value	0.000*	0.000*	0.000*	0.000*

Mann Whitney U (Z), Kruskal Wallis (Kwh), One-Way Anova (F), independent samples t test (T), SD: Standard deviation, *: $p<0.05$ statistically significant

An examination of the relationship between age and overall NL and its subscales revealed a weak but significant positive correlation with functional NL, while no significant relationship was observed with interactive NL, critical NL, and overall NL (r=0.131 *p*=0.020; r=0.040 *p*=0.477; r=0.011 *p*=0.840; r=0.090 *p*=0.110, respectively).

There was a statistically significant, moderately positive correlation between the Nutrition Literacy (NL) score and Attitude Scale for Healthy Nutrition (ASHN) scores (r=0.404 *p*=0.000) (Table 5).

Table 5. The relationship between students' total NL and sub-dimension scores and their ASHN scores

		Functional NL	İnteractive NL	Critical NL	Total NL	ASHN
Functional NL	r	1.000	0.256**	0.098	0.680**	0.276**
	p		0.000	0.081	0.000	0.000
İnteractive NL	r		1.000	0.248**	0.730**	0.267**
	p			0.000	0.000	0.000
Critical NL	r			1.000	0.582**	0.299**
	p				0.000	0.000
Total NL	r				1.000	0.404**
	p					0.000
ASHN	r					1.000
	p					

*$p<0.05$, **$p<0.01$, NL: Nutrition Literacy, ASHN: Attitude Scale for Healthy Nutrition

Discussion

The increase in risky eating habits during university years is concerning as it can lead to adverse health-related outcomes, as well as reduced physical and academic performance, both in the present and later stages of life [18, 19]. Nutrition literacy is considered an important indicator of dietary characteristics [20-22]. The present study indicated that 56.8% of the students had a high level of healthy nutrition attitudes and that there was a relationship between nutrition literacy and healthy nutrition attitudes. It was concluded that by increasing nutritional literacy, having basic knowledge about nutrition, accessing the right nutritional information and using them effectively, instead of choosing unhealthy foods, healthy eating attitudes can be increased with positive nutritional behaviors. It was shown that especially students studying in the field of health, who received nutrition education and exercised regularly, had better nutritional literacy and higher healthy eating attitudes. It is important to eliminate the knowledge gap about healthy nutrition, especially in students who study outside the field of health, who have not received nutrition education and who do not have healthy lifestyle behaviors.

Studies have shown that health education is effective in adopting healthy lifestyle behavior, including nutrition [23]. In the present study, it was observed that students enrolled in health-related departments had higher nutrition literacy compared to students in other departments, and they were more competent in possessing basic nutritional knowledge and translating it into healthy eating choices. Another study also reported that students in the faculty of health sciences had higher nutrition literacy compared to students in other departments [24].

One of the primary goals of nutritional education is to increase nutrition literacy [3]. In the study by Hassani et al. [25], it was found that following the nutrition education provided to

participants, their nutritional knowledge scores increased, and positive changes occurred in their eating habits. A study conducted with students from a faculty of health sciences reported that the nutrition education provided as part of their curriculum improved their nutritional knowledge scores [26]. In the present study, it was also observed that students who received nutritional education or knowledge had higher nutrition literacy. Additionally, these students demonstrated higher levels of basic nutritional knowledge, more advanced communication skills with healthcare professionals, family, and friends, and the ability to critically evaluate nutrition advice.

The level of nutrition literacy is influenced by physical activity [27]. Moreover, it has been shown that an increase in physical activity leads to positive developments in eating habits [28]. According to the World Health Organization, at least 150 minutes of moderate-intensity aerobic exercise per week or 75 minutes of high-intensity aerobic exercise per week is recommended for individuals aged 18 to 64 years [29]. The present study found that students who engaged in regular exercise had higher overall NL scores, better comprehension of basic nutritional knowledge, easier communication about nutrition with healthcare professionals and their relatives, and greater ability to critically interpret and evaluate nutrition recommendations. Similar to this study, the research by Koca and Arkan [30] found that young people who engaged in sports had higher critical NL, interactive NL, and overall NL scores compared to those who did not engage in sports. In a study conducted in Norway with university students and staff, it was observed that individuals who were more physically active had higher nutrition literacy [24].

Obesity, considered the pandemic of this age, poses a significant risk for university students who frequently consume low-nutrient, high-calorie foods. One potential cause of this issue is low levels of nutrition literacy [31]. Increasing nutrition literacy is regarded as an effective and preventive measure for achieving an ideal BMI in adolescents and reducing overweight and obesity in adulthood [32]. A study conducted by Mearns et al. [33] with nursing students reported a negative relationship between nutrition literacy and anthropometric measurements such as BMI and body fat percentage, indicating that knowledge about nutrition had positive effects on maintaining a healthy weight.

Although it is hoped that high nutrition literacy would positively impact BMI, this study found no significant difference in BMI levels and overall nutrition literacy scores among students. However, it was observed that overweight and obese students had higher interactive NL scores compared to their thinner counterparts, indicating a greater interest in seeking and applying nutritional knowledge to improve their eating habits. A study conducted with educational faculty students found that students who were underweight had lower overall and interactive NL scores compared to other students [34]. On the other hand, a study conducted by Koca and Arkan [30] with adolescents established no relationship between BMI and nutrition literacy.

While it is generally expected that women would have higher nutrition literacy, given their greater interest in nutrition and concern for body image, this study did not find any significant impact of gender on NL [35]. In a study conducted in Denizli province, similar to our study, no relationship was found between students' gender and nutrition literacy [36]. Contrary to the present study, research conducted with adolescents in Uganda found that female students had higher levels of interactive and critical nutrition literacy compared to male students [15]. Another study conducted with university students found that female students scored higher than male students in all three sub-dimensions of nutrition literacy [37]. We believe that the difference in the sample groups may have affected the results. In addition, the fact that the students in our study had similar levels of education may be the reason why there was no difference in terms of nutrition literacy in terms of gender.

Michou et al. [38] reported that socioeconomic status affects health and nutrition literacy, with lower income levels being associated with lower nutrition literacy. Similarly, Gibbs et al. [39] found that a family's income level influences the dietary quality of children. However, in this study, it was observed that the nutrition literacy of students was not influenced by their families' monthly income. A similar finding was reported in a study conducted with adolescents, which showed that nutritional knowledge levels were independent of the family's income level [40].

Skipping meals is a common habit among university students [10, 26]. Often living away from their families, students face challenges related to nutrition and may skip meals to suppress hunger rather than maintain a balanced diet [10]. A study with adolescents found that those who skipped meals had significantly lower functional NL compared to those who did not skip meals [30]. Another study conducted with high school students revealed that despite having high food literacy, students commonly engaged in negative eating habits such as skipping meals, increasing the consumption of unhealthy snacks, and reducing fruit intake. The study also found that students were inadequate in translating their nutritional knowledge into positive eating behavior [41]. In the present study, meal skipping was found to be common among students, regardless of their level of nutrition literacy. The most frequent reasons cited for skipping meals included lack of appetite, lack of habit, financial constraints, and lack of time.

The goal of nutrition literacy is to access accurate nutritional knowledge and use it effectively to develop healthy eating habits [42]. A study conducted by Al Tell et al. [43] found that nutrition literacy is linked to eating habits. Another study conducted among university students identified that eating habits influence nutrition literacy [37]. This study also demonstrated that nutrition literacy positively supports healthy nutrition attitudes. Possessing basic knowledge about nutrition, obtaining useful information from various sources, gaining the ability to critically evaluate such information, and increasing the willingness to participate actively in healthy nutrition studies can enhance healthy nutrition attitudes by encouraging positive dietary habits instead of preferring unhealthy foods due to emotional and hedonic wants.

In conclusion, nutrition literacy was found to be higher among students who had nutritional education/knowledge, were studying in health-related fields, and engaged in regular physical exercise. Additionally, an increase in students' nutrition literacy positively influenced their healthy nutrition attitudes. In order to sustain healthy nutrition, it is necessary to increase the nutritional literacy levels of individuals and societies. For this purpose, it is important to develop health policies that include university students. Furthermore, it would be beneficial to include nutrition-related courses in the curriculum of all university students, regardless of the department of study, and to organize periodic seminars and conferences by health professionals to reduce information pollution on healthy eating and nutrition. It is also recommended to offer healthy food options in areas such as cafeterias and canteens on campus and to create favorable conditions to increase students' physical activity.

The limitations of the study are that the results obtained cannot be generalized due to the small sample size, the fact that it was conducted in only one university and that it was conducted on a voluntary basis. Further research with different sample groups and other factors affecting nutrition will contribute more to the literature.

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Research Article

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Effects of quercetin on adipokine profile in fructose-induced metabolic syndrome

Fruktoz ile indüklenen metabolik sendromda quercetinin adipokin profili üzerine etkileri

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Abstract

Purpose: Metabolic syndrome (MetS) is a health condition characterized by obesity, insulin resistance, dyslipidemia and type 2 diabetes (T2DM). This study aimed to assess the effects of quercetin, a natural flavonoid on MetS induced by fructose in Sprague Dawley rats.

Materials and methods: The rats, aged 8-10 weeks, were divided into 4 groups: control (C) group, metabolic syndrome (MetS) group, control+quercetin (C+Q) group, and metabolic syndrome+quercetin (MetS+Q) group. The MetS groups received a 20% fructose solution in drinking water for a duration of 10 weeks. For the last 4 weeks of the study, rats in the Q groups were administered 50 mg/kg/body weight quercetin. After 10 weeks, serum samples were tested using ELISA for Triglycerides (TG), High-Density Lipoprotein (HDL), fasting insulin, resistin, (Interleukin 6) IL6, Tumor Necrosis Factor-alpha (TNF-α), leptin, C-Reactive Protein (CRP) and, adiponectin (ADP). The body weights, Lee index and HOMA-IR scores were also measured.

Results: Fructose-fed rats showed significant increases in body weight, Lee index, HOMA-IR scores and, fasting insulin with significant decrease in HDL compared to controls. In MetS group, ADP levels were significantly lower compared to control group. In MetS+Q group, there was a tendency for reduced levels of resistin, IL-6, and leptin compared to the untreated MetS group.

Conclusion: These findings suggest that quercetin may be beneficial in managing MetS, though further research is needed to explore its mechanisms and effectiveness.

Keywords: Adipokine, fructose, metabolic syndrome, quercetin.

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Öz

Amaç: Metabolik sendrom (MetS) obezite, insülin direnci, dislipidemi ve tip 2 diyabet (T2DM) ile karakterize bir sağlık durumudur. Bu çalışmada, Sprague Dawley sıçanlarında fruktoz ile indüklenen MetS üzerinde doğal bir flavonoid olan quercetinin etkilerinin değerlendirilmesi amaçlandı.

Gereç ve yöntem: 8-10 haftalık sıçanlar 4 gruba ayrıldı: kontrol (C) grubu, metabolik sendrom (MetS) grubu, kontrol+quercetin (C+Q) grubu ve metabolik sendrom+quercetin (MetS+Q) grubu. MetS gruplarına 10 hafta boyunca içme suyunda %20 fruktoz çözeltisi verildi. Çalışmanın son 4 haftasında Q verilen gruplardaki sıçanlara 50 mg/kg/vücut ağırlığı quercetin uygulandı. 10 hafta sonra, serum örnekleri Trigliserid (TG), Yüksek Yoğunluklu Lipoprotein (HDL), açlık insülini, resistin, (Interleukin 6) IL6, Tümör Nekroz Faktörü-alfa (TNF-α), leptin, C-Reaktif Protein (CRP) ve adiponektin (ADP) için ELISA kullanılarak test edildi. Vücut ağırlıkları, Lee indeksi ve HOMA-IR skorları da ölçüldü.

Bulgular: Fruktozla beslenen sıçanların vücut ağırlığında, Lee indeksinde, HOMA-IR skorlarında ve açlık insülininde kontrol grubuna kıyasla anlamlı artışlar, HDL'de ise anlamlı düşüşler görüldü. MetS grubunda ADP seviyeleri kontrol grubuna kıyasla anlamlı derecede düşüktü. MetS+Q grubunda, tedavi edilmeyen MetS grubuna kıyasla resistin, IL-6 ve leptin seviyelerinde azalma eğilimi vardı.

Sonuç: Bu bulgular, quercetinin MetS yönetiminde faydalı olabileceğini, ancak mekanizmalarını ve etkinliğini keşfetmek için daha fazla araştırmaya ihtiyaç olduğunu göstermektedir.

Anahtar kelimeler: Adipokin, fruktoz, metabolik sendrom, quercetin.

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Introduction

Metabolic syndrome (MetS) is an illness that is characterized by the following clinical manifestations: hypertension, insulin resistance, dyslipidemia, abdominal obesity, and hyperglycemia [1]. The development of MetS is greatly affected by factors caused by increased adipose tissue and visceral obesity [2]. Hyperplasia and hypertrophy are the processes that contribute to adipose tissue growth [3]. Insulin resistance develops as a result of the production of many cytokines by hypertrophied adipocytes. Adipose tissue releases a wide range of biomolecules, many of which have been identified and the metabolic impacts and role they play in the development of disease have been thoroughly studied. Adipose tissue releases chemicals known as adipocytokines, which cross the bloodstream and help control how fat and carbohydrate metabolism are regulated [4]. For example, various bioactive molecules secreted by adipocytes, such as visfatin, resistin, adiponectin (ADP), leptin and tumor necrosis factor alpha (TNF-α), are involved in the regulation of many physiological processes, including inflammation, insulin sensitivity and energy metabolism [5, 6]. ADP is an adipokine negatively correlated with visceral adipose tissue mass and exerts anti-inflammatory effects by suppressing pro-inflammatory factors such as TNF-α, interleukin 6 (IL6) and C reactive protein (CRP). Conversely, pro-inflammatory factors are also known to suppress ADP production [7]. Disruption of adipose tissue-derived adipokine secretion in favor of pro-inflammatory cytokines may be one of the causes of systemic inflammation and ultimately the development of metabolic diseases. Limited results are obtained with treatments such as lifestyle modification (diet and exercise), pharmacotherapy, bariatric and metabolic surgical interventions for the control of MetS [8]. Plant metabolites, known as flavonoids and phytochemicals, possess antioxidant, anti-inflammatory, and anti-diabetic properties that are recognized for their protective benefits on MetS-related illnesses [9]. Quercetin is a significant member of the flavonoid family. Quercetin has been found to have several pharmacological effects in both animal and human research. These effects include reducing blood pressure [10], protecting the cardiovascular system [11], promoting weight loss, and improving hyperglycemia [12]. As far as we know, no research has been conducted to examine the impact of quercetin on the release of cytokines from adipose tissue and its potential beneficial effects on different pathophysiological processes. We hypothesized that quercetin affects the inflammatory process in metabolic syndrome. The objective of this study was to examine the impact of quercetin on the levels of pro-inflammatory cytokines, including IL-6, TNF-α, resistin, and CRP, as well as anti-inflammatory cytokines such as leptin and ADP, in a MetS model caused by high fructose.

Materials and methods

The Pamukkale University Animal Experiments Ethics Committee for authorized all experimental protocols utilized in this study (PAUHDEK-2023/32-19.10.2023, 2023/06). The animals were kept in stainless-steel cages under controlled conditions, with a temperature of 24±2°C and 50±5% humidity. They were exposed to a 12-hour cycle of light and darkness.

Experimental design

In this investigation, 28 Sprague-Dawley male rats (8-10 weeks old, 130-200 grams) were utilized. The rats were randomly assigned to one of two groups: control (C) (n=14) and metabolic syndrome (MetS) (n=14). MetS group had a D-fructose-enriched drink (D-fructose 20% (20 g/ml) for 6 weeks (Biomatik, CAS:57-48-7, MW: 180.16), while the other group C had tap water. In this study, the experimental period is 10 weeks in total. At the end of 6 weeks, the MetS and C rats were assigned to two experimental groups. C groups: control (C) (n=7) and control+quercetin (C+Q) (n=7), MetS groups: metabolic syndrome (MetS) and metabolic syndrome +quercetin (MetS+Q) groups. In the last 4 weeks of the study; the C group had tap water, the C+Q group: had tap water+50 mg/kg/day quercetin was administered by oral gavage, the MetS group: had a fructose-enriched drink

(D-fructose 20%), MetS+Q: fructose-enriched drink (D-fructose 20%) +50 mg/kg/day Q (Lot: SLCC9071, 10G SIGMA) was administered by oral gavage. Details of the experimental timeline are shown in Figure 1. It was observed that the effect size obtained in the reference study was quite strong (F=11.06). As a result of the power analysis performed considering that a lower effect size (F=0.9) could be obtained; it was calculated that 90% power could be obtained at 95% confidence level when at least 24 rats (at least 6 rats for each group) were included in the study. Considering the possibility of subject loss, it was considered to include 1 more rat in each group.

Preparation of fructose

In this investigation, a 20% solution of D-Fructose (Biomatik 99%, CAS:57-48-7, MW: 180.16) was used to create a rat model of MetS. Fructose-enriched beverages were freshly prepared on alternate days. In order to create a mixture consisting of 20% fructose, 20 grams of fructose were mixed with 100 milliliters of tap water [13]. The rats were provided with no limit to the HF drinks.

Application of quercetin

A solution of quercetin (Lot: SLCC9071, 10G SIGMA) powder (1 ml ethanol + 4 ml 0.9% saline) was given orally via gastric gavage to rats at a dose of 50 mg/kg/day [14].

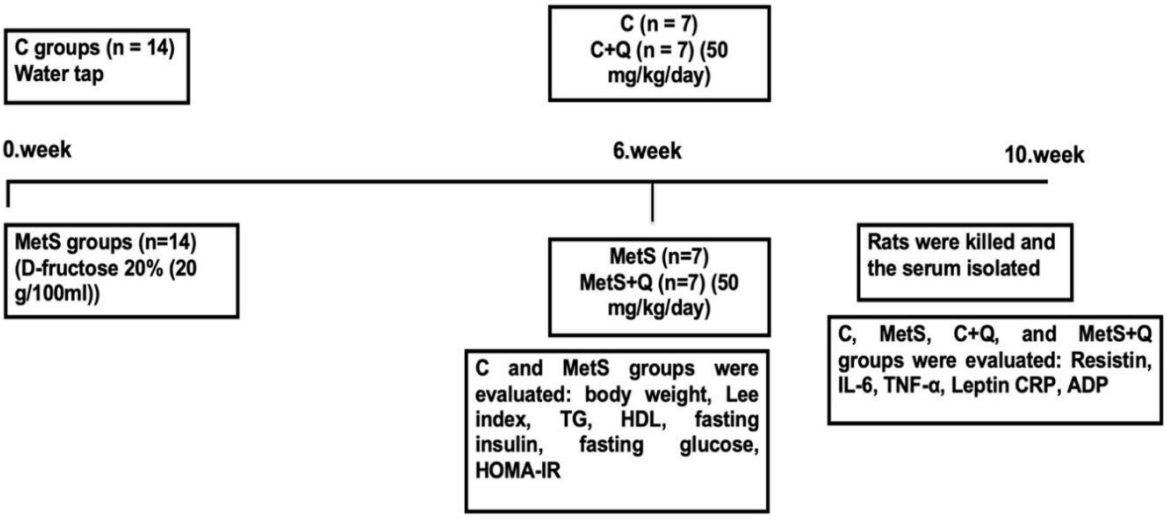

Figure 1. Illustration of experimental design

C rats that received water tap during the experimental period, MetS rats received D-fructose (20% (20 g/ml)), Q was administered 50 mg/kg/day, C: control, MetS: metabolic syndrome, Q: quercetin

Serum biochemical parameters

Following the completion of the experiment, the animals were starved for 8 hours. Serum was obtained by centrifuging blood samples extracted from the abdominal aorta of rats that had been given anesthesia. Samples were kept frozen at -80°C. Commercial kits were used for evaluating the levels of fasting TG (ELISA, BT Lab, E0249Ra, China), HDL (ELISA, Andy gene, AD1756Ra, USA), insulin (ELISA, Elabscience, E-EL-R3034, USA), resistin (ELISA, Andy gene, AD3196Ra, USA), interleukin (IL6) (ELISA, Andy gene, AD2567Ra, USA), TNF-α (ELISA, Andy gene, AD3238Ra, USA), leptin (ELISA, BT Lab, E0561Ra, China), CRP (ELISA, BT Lab, E0053Ra, China) and, ADP (ELISA, Andy gene, AD3187Ra, USA) on the experimental day.

Body weight, Lee index and HOMA-IR measurement

Body weight (BW) was measured and the results were recorded. Lee index was calculated [body weight 1/3 (g) / nasoanal length (cm)] to evaluate the growth performance of the rats

and the development of obesity. Blood samples were collected from the tail of each animal after 8 h of fasting and glucose was measured using a handheld glucometer (ACCU-CHEK Performa Nano). The following formula was used to determine the Homeostatic Model Assessment of Insulin Resistance. (HOMA-IR) index: Fasting glucose (mmol/L) × fasting insulin (mIU/L) / 22.5 is the formula for HOMA-IR.

Statistical analysis

The data were analyzed with the software package SPSS 25.0. Continuous variables are expressed as the mean ± standard error (SEM) and median (minimum- maximum values). The Shapiro-Wilk test was used to determine whether the data had a normal distribution. For parametric tests, we used an independent-sample t-test and one-way analysis of variance (Tukey test for pairwise examinations). Non-parametric testing were performed using the Mann-Whitney U test and the Kruskal-Wallis analysis of variance (Mann-Whitney U test with Bonferroni adjustment for paired analyses). In all analyses, $p \leq 0.05$ was considered statistically significant.

Results

Results of feeding fructose to rats

The development of MetS in rats fed with fructose was verified by assessing MetS indicators including Lee index, lipid profile, fasting glucose and insulin levels, and HOMA-IR score. The rats in the fructose-treated group exhibited an important rise in body weights and Lee indices, which are used as a measure of obesity, as comparison to the animals in the control group. Based on the lipid profile data, there was no significant difference in the TG level between the two groups. However, the fructose-treated group exhibited a substantial reduction in HDL level compared to the control group. The findings of the insulin metabolism study revealed that the administration of fructose to rats for a duration of 6 weeks caused elevated levels of fasting glucose and insulin, as well as the development of insulin resistance, as indicated in Table 1. The study's findings revealed that the injection of MSG to neonatal rats had a substantial impact on the advancement of MetS throughout their adult lives.

Effect of quercetin on adipocytokine levels

Upon evaluating the impact of orally administering quercetin to rats for a duration of 4 weeks, no notable disparity was found in the levels of adipocytokines (resistin, IL6, TNF-α, leptin, and ADP) and the inflammation marker CRP among the four groups (Figure 2-6). The group in which MetS was generated by fructose administration showed a significant decrease in ADP level compared to the control group. However, the administration of quercetin had no effect on ADP level (Figure 7).

Table 1. Arithmetic mean and the standard error (A.O±S.E) is used to express the results, (n=7)

Parameters	C	MetS	*p* / Test value
Body weight (g)	347±37.18	400±29.55	0.012* (t=-2.953)
Lee index (g/cm)	0.0297±0.0009	0.0317±0.00065	0.008* (t=-2.138)
TGs (mmol/L)	4979.22 (4942.05-6718.86)	5826.74 (4890.01-5945.68)	1 (z=-0.218)
HDL (pg/mL)	234.05±17.14	117.7±26.81	0.0001* (t=-7.313)
Fasting insülin (ng/mL)	43.59±11.48	75.07±14.91	0.016* (t=-1.253)
Fasting glucose (mg/dL)	125.14±24.3	255.14±46.19	0.0001* (t=-6.59)
HOMA-IR	516.06±139.79	1132.95±276	0.011* (t=-3.932)

$p \leq 0.05$ is considered statistically significant
Results for parametric data are expressed as Mean ± Standard error of the mean (SEM)
while for non-parametric data are expressed as median (minimum and maximum values)
Results having statistical significance are represented by *, t: Independent sample, t-test, z: Mann-Whitney U test, C: Control group
MetS: Metabolic Syndrome group, TGs: Triglyceride, HDL: high-density lipoprotein
HOMA-IR: Homeostatic model assessment of insulin resistance

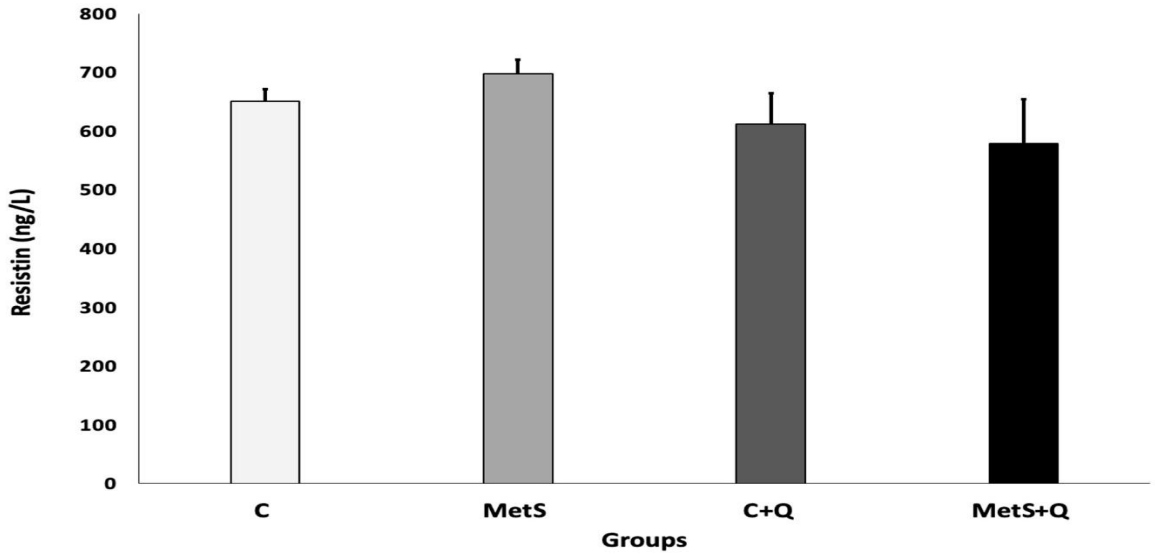

Figure 2. Analysis of resistin following a 10-week experiment, (n=7)

Arithmetic mean and the standard error is used to express the results, $p \leq 0.05$ is considered statistically significant
One-way Analysis of Variance (ANOVA) was used to analyze the data (p=0.416; F=1.026, C: 650.94±19.71, MetS: 697.16±23.92
C+Q: 612.03±52.47, MetS+Q: 579.19±75.37). C: Control, MetS: Metabolic Syndrome, C+Q: Control+Quercetin group
MetS+Q: Metabolic Syndrome+Quercetin group

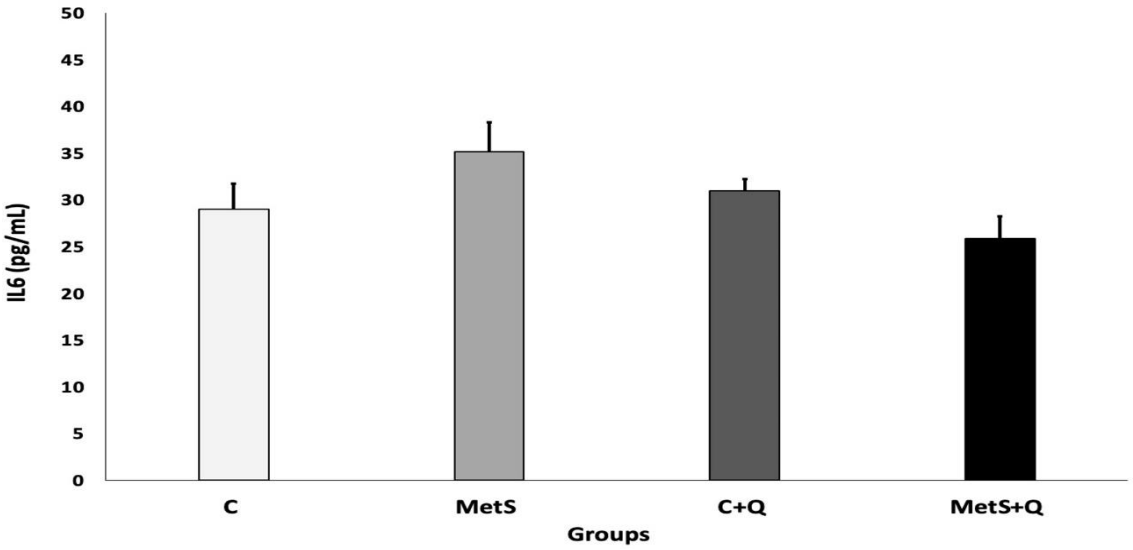

Figure 3. Analysis of IL6 following a 10-week experiment, (n=7)

Arithmetic mean and the standard error is used to express the results, $p \leq 0.05$ is considered statistically significant. Kruskal-Wallis Variance Analysis was used to analyze the data (p=0.09; kw=6.442, C: 29.03±2.71, MetS: 35.20±3.12, C+Q: 30.94±1.30, MetS+Q: 25.86±2.41)
C: Control, MetS: Metabolic Syndrome, C+Q: Control+Quercetin group, MetS+Q: Metabolic Syndrome+Quercetin group

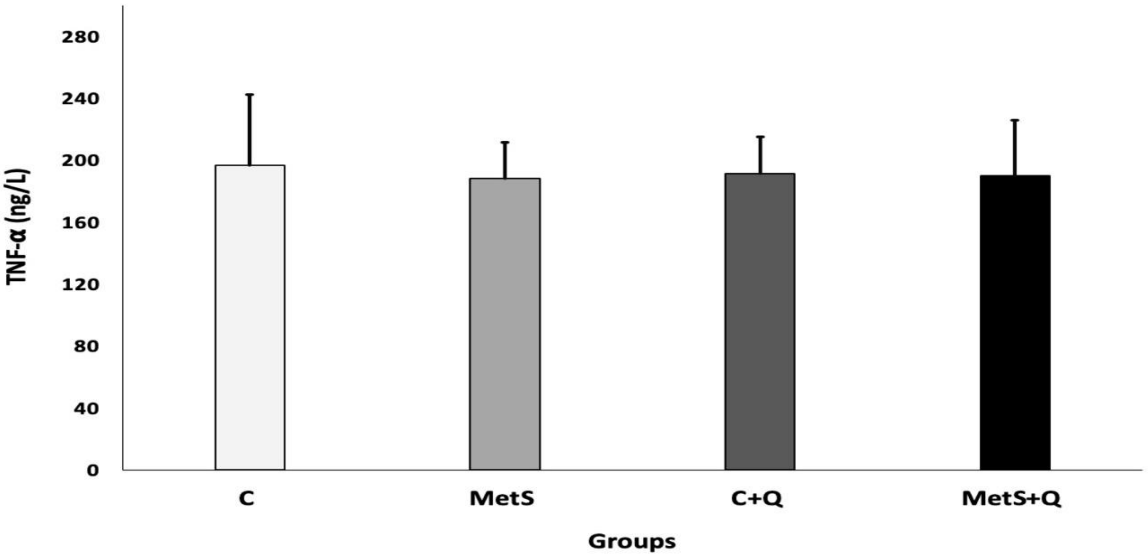

Figure 4. Analysis of TNF-α following a 10-week experiment, (n=7)

Arithmetic mean and the standard error is used to express the results, $p \leq 0.05$ is considered statistically significant. One-way Analysis of Variance (ANOVA) was used to analyze the data (p=0.998, F=0.012, C: 196.89±45.69, MetS: 188.50±23.08, C+Q: 191.42±23.78 MetS+Q: 189.9±35.99). C: Control, MetS: Metabolic Syndrome, C+Q: Control+Quercetin group, MetS+Q: Metabolic Syndrome+Quercetin group

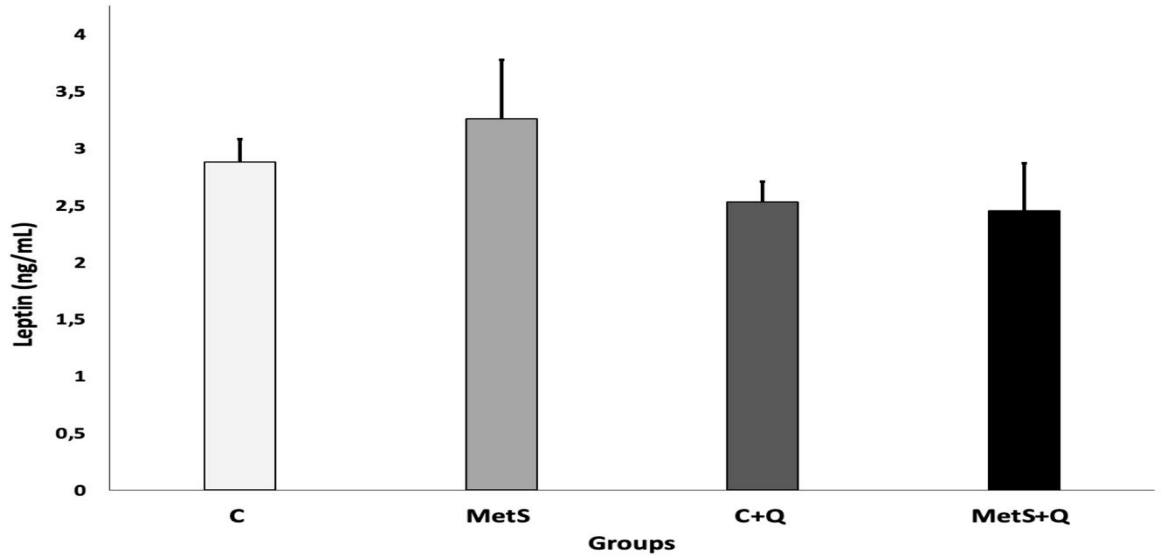

Figure 5. Analysis of leptin following a 10-week experiment, (n=7)

Arithmetic mean and the standard error is used to express the results, $p \leq 0.05$ is considered statistically significant. One-way Analysis of Variance (ANOVA) was used to analyze the data (p=0.494, F=0.841, C: 2.88±0.2, MetS: 3.26±0.515, C+Q: 2.53±0.174413 MetS+Q: 2.45±0.42). C: Control, MetS: Metabolic Syndrome, C+Q: Control+Quercetin group, MetS+Q: Metabolic, Syndrome+Quercetin group

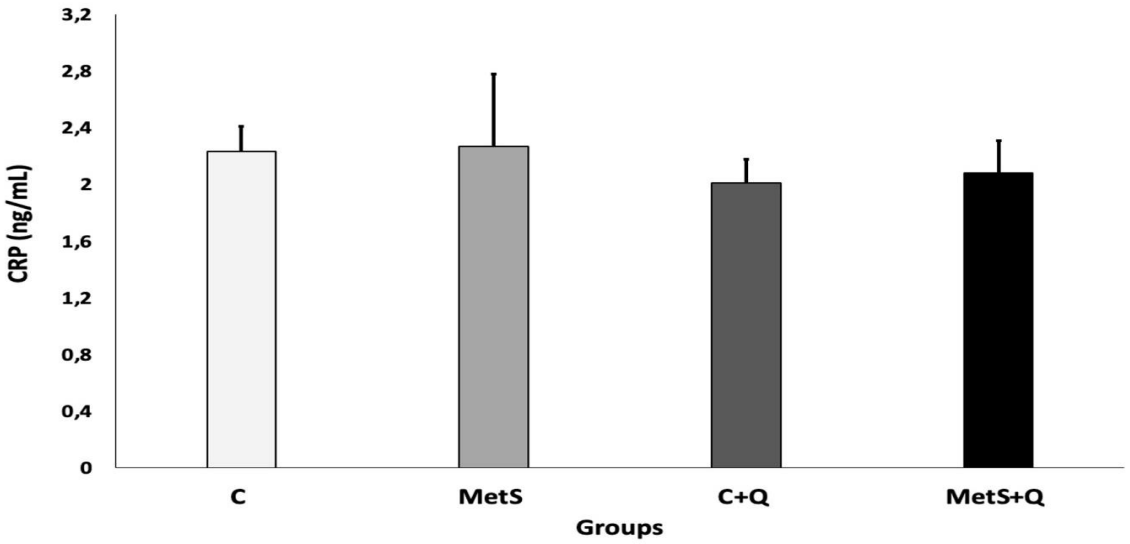

Figure 6. Analysis of CRP following a 10-week experiment, (n=7)

Arithmetic mean and the standard error is used to express the results, $p \leq 0.05$ is considered statistically significant. Kruskal-Wallis Variance Analysis was used to analyze the data (p=0.871, kw=0.708, C: 2.23±0.18, MetS: 2.27±0.51, C+Q: 2.01±0.17, MetS+Q: 2.08±0.23). C: Control MetS: Metabolic Syndrome, C+Q: Control+Quercetin group, MetS+Q: Metabolic Syndrome+Quercetin group

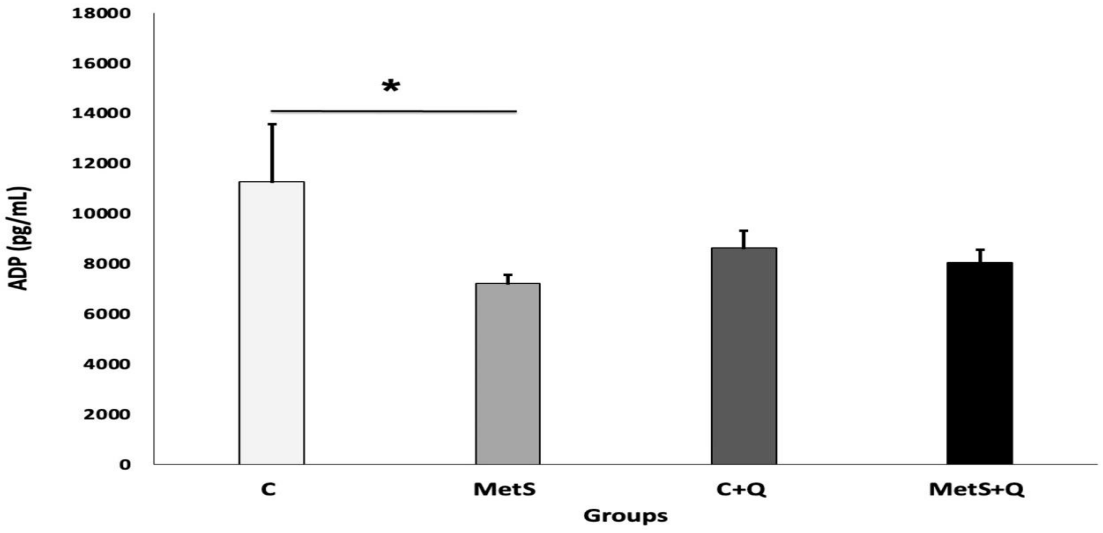

Figure 7. Analysis of ADP following a 10-week experiment, (n=7)

Arithmetic mean and the standard error is used to express the results, $p \leq 0.05$ is considered statistically significant. Kruskal-Wallis Variance Analysis was used to analyze the data (p=0.021, kw=9.725, C: 11272.50±2293.39, MetS: 7202.20±354.41, C+Q: 8629.60±687.67 MetS+Q: 8043.5±521.11). * indicates groups that differ from C group C: Control, MetS: Metabolic Syndrome, C+Q: Control+Quercetin group MetS+Q: Metabolic Syndrome+Quercetin group

Discussion

This study aimed to examine the possible impact of quercetin on adipokine levels in an animal model of MetS induced by fructose. The findings of our study demonstrated that the Lee index, fasting insulin, fasting glucose, and HOMA-IR score exhibited an increase, whereas HDL levels observed a significant reduction in the group treated with fructose, as compared to the control group. The data demonstrated that MetS was successfully induced in these rats 6 weeks following fructose administration. Within this investigation, the fructose-induced MetS model animals exhibited elevated levels of resistin, IL6, and leptin. However, these increases were not statistically significant. The animals exhibited a significant decrease in ADP levels. Furthermore, although not of significant importance, the administration of quercetin to rats with MetS resulted in a reduction in resistin, IL6, and leptin levels.

The development of MetS is influenced by both genetic and environmental variables, which are equally significant. Studies have indicated that dietary fructose is associated with a rise in visceral fat tissue. Unlike glucose, fructose does not quickly induce the release of leptin or insulin, which means it does not activate the typical feeling of satiety (satiety) [15]. Research findings from both randomized clinical trials and observational studies show that consuming high amounts of fructose is associated with an increase in energy intake, weight gain, and a higher risk of obesity [16]. Research indicates that these illnesses may be associated with oxidative stress and endoplasmic reticulum stress [17, 18]. Kumar et al. [19] discovered that introducing a 20% fructose solution into the rats' drinking water for a duration of 12 weeks resulted in elevated body weight, increased body fat, and negatively affected their lipid profile. Sánchez Lozada et al. [20] identified a metabolic abnormality in Sprague-Dawley rats after two weeks of consuming drinking water containing 10% fructose. According to these findings, a diet high in fructose was found to be efficient in creating a MetS model in rats.

In obesity, adipose tissues become dysfunctional, pro-inflammatory molecules increase and anti-inflammatory adipokines decrease. The disorder is marked by reduced levels of ADP and leptin, elevated levels of inflammatory adipo/cytokines, increased digestion of fats, and a low response to insulin. Clinical study has demonstrated that reduced levels of ADP contribute to insulin resistance, while elevated levels of resistin in obesity are associated with insulin resistance and the development of type 2 diabetes in mice [21]. Researchers discovered that individuals who are morbidly obese have elevated levels of resistin compared to individuals of normal weight. While there may be inconsistencies in certain animal models indicating that resistin levels are low in obesity, it is widely accepted that resistin levels are up in obesity. Several human studies failed to demonstrate a significant correlation between resistin levels and obesity or insulin resistance [22, 23]. There is a direct relationship between the amount of IL6 produced in adipose tissue and its presence in the bloodstream, and both of these factors are associated with obesity and insulin resistance. Our investigation did not find a statistically significant rise in IL6 levels, although observing a gradual increase in relation to MetS. The level of leptin is directly correlated with the quantity of adipose tissue [24]. The study demonstrated a substantial elevation in leptin levels in obese rats as compared to control animals [25]. The observed elevation in IL6 and leptin levels in the MetS group in our study may be attributed to the increase in adipose tissue mass in these animals.

Various investigations have indicated that some medicinal plants and active compounds can potentially regulate MetS by decreasing blood glucose levels, blood pressure, and fat buildup [26]. Quercetin, a significant flavonoid, is present in the human diet and is a constituent of other flavonoids such hesperidin, naringenin, and rutin. Quercetin has been found to have several pharmacological effects in both animals and humans. These effects include reducing blood pressure [10], protecting the cardiovascular system [11], promoting weight reduction, improving high blood sugar levels [12], and reducing lipid levels [27]. Quercetin supplementation is believed to possess antidiabetic characteristics by promoting glucose uptake through a process involving mitogen-activated protein kinase (MAPK), which is dependent on insulin. It also decreases the activity of enzymes involved in gluconeogenesis and reduces the generation of glucose in liver cells (hepatocytes) [28]. MAPK has been

identified as a stimulator of adipogenesis through the activation of adipogenic and inflammatory factors such as Nuclear Factor kappa B (NF-κB), TNF-α, IL-1β, and IL-6. Research shown that quercetin effectively suppressed the activity of MAPK in adipocytes and decreased the production of adipogenic and inflammatory cytokines in a cell line model using 3T3-L1 cells [29]. Quercetin treatment at a dosage of 10 mg/kg in obese Zucker rats effectively decreased inflammation by inhibiting TNF-α and ADP stimulation [30]. The observed decline in resistin, IL6, and leptin levels in MetS mice following quercetin administration may be attributed to the actions of quercetin. When TNF-α was present, the administration of 1 and 10 micromolar quercetin to 3T3-L1 adipocytes resulted in an increase in ADP levels and a decrease in resistin levels. A study demonstrated that giving 20 mg/kg of quercetin three times a week to male Wistar rats aged 30 days, after six weeks of consuming drinking water with 10% fructose, resulted in an increase in ADP levels and a decrease in TNF-α and resistin levels [31]. After consuming 10% fructose drinking water for 45 days, 5-week-old Wistar albino rats were given a daily intraperitoneal dose of 15 mg/kg quercetin for 10 days. This resulted in a notable variation in resistin levels compared to the MetS group [32]. Based on this data, we believe that the outcomes of our study may be influenced by factors such as the initial age of the rats, the species, and the number of subjects involved. This study attempted to explain the effects of quercetin on adipokine levels in MetS. This study has some limitations. Confirmation of the obtained adipokine levels using different molecular assays would have increased the reliability of the results. The study of the effect of quercetin on the parameters of MetS could have contributed to the evaluation of this polyphenol in MetS as a whole.

In conclusion, although the mechanisms of action of polyphenols remain unclear, it is very important to adjust the duration and dose of these flavonoids in order to obtain beneficial effects. Our findings indicate that quercetin, when given to animals with MetS, may have the ability to control the levels of adipokines. However, additional research is necessary to determine the specific signaling pathways of adipokines originating from adipose tissue.

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

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Research Article

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Investigation of erythrocyte membrane lipid profile, oxidative stress and DNA damage parameters in patients with chronic lymphoid leukemia

Kronik lenfoid lösemili hastalarda eritrosit membran lipid profili, oksidatif stres ve DNA hasarı parametrelerinin araştırılması

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Abstract

Purpose: Chronic lymphocytic leukemia (CLL) is the most seen type of leukemia in adults. There are few biomarkes that are used for better understanding how oxidative stress is involved in the pathophysiology of hematologic malignency. We aimed to evaulate oxidative stress, DNA damage and erythrocyte membrane lipid profile in CLL patients in this study. The study is included 38 CLL patients and 38 age-sex matched controls.

Materials and method: Total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), DNA damage examination with Comet assay, serum 8-OHdG measurement and gas chromatographic analysis were performed between the case and control groups.

Results: It was observed that TOS and OSI values were higher in the case group than in the control group (p=0.014 and p=0.022, respectively). DNA damage measured by Comet method was found to be increased in the case group (p<0.05). Although erythrocyte membrane fatty acid levels were found to be decreased in the case group compared to the control group, no statistically significant difference was found (p=0.641).

Conclusion: It has been shown that CLL patients had higher oxidant capasity as consequence. Oxidative stress and DNA damage are increased in CLL patients in this study. It is early to evaulate on erythrocyte membrane lipid profile in CLL patients. However, the study can be ligthed the way future studies on the subject.

Keywords: Oxidative stress, DNA damage, erythrocyte membrane lipid profile, chronic lymphocytic leukemia.

Karakula B, Akgun Cagliyan G, Unver Koluman B, Baser MN, Tunc Ata M, Altintas F, Kilic Toprak E, Cort A. Investigation of erythrocyte membrane lipid profile, oxidative stress and DNA damage parameters in patients with chronic lymphoid leukemia. Pam Med J 2025;18:129-136.

Öz

Amaç: Kronik lenfositik lösemi (KLL), erişkinlerde en sık görülen lösemi türüdür. Hematolojik malignite patofizyolojisinde oksidatif stresin nasıl rol oynadığını daha iyi anlamak için kullanılan az sayıda biyobelirteç vardır. Bu çalışmada KLL hastalarında oksidatif stres, DNA hasarı ve eritrosit membran lipit profilini değerlendirmeyi amaçladık. Çalışmaya 38 KLL hastası ve yaş-cinsiyet uyumlu 38 kontrol dahil edildi.

Gereç ve yöntem: Olgu ve kontrol grupları arasında total oksidan durum (TOS), toplam antioksidan durum (TAS), oksidatif stres indeksi (OSI), Comet assay ile DNA hasarı incelemesi, serum 8-OHdG ölçümü ve gaz kromatografik analizi yapıldı.

Bulgular: TOS ve OSI değerlerinin olgu grubunda kontrol grubuna göre daha yüksek olduğu görüldü (sırasıyla p=0,014 ve p=0,022). Comet yöntemiyle ölçülen DNA hasarının olgu grubunda arttığı belirlendi (p<0,05). Olgu grubunda eritrosit membran yağ asidi düzeylerinde kontrol grubuna göre azalma saptanmasına rağmen istatistiksel olarak anlamlı fark bulunamadı (p=0.641).

Sonuç: Sonuç olarak KLL hastalarının oksidan kapasitesinin daha yüksek olduğu gösterilmiştir. Çalışmamızda KLL hastalarında oksidatif stres ve DNA hasarı artmıştır. KLL hastalarında eritrosit membran lipid profilini değerlendirmek için henüz erkendir. Ancak çalışmamız konuyla ilgili gelecekte yapılacak çalışmalara ışık tutabilir.

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Anahtar kelimeler: Oksidatif stress, DNA hasarı, eritrosit membran lipid profili, kronik lenfositik lösemi.

Karakula B, Akgün Çağlıyan G, Ünver Koluman B, Başer MN, Tunç Ata M, Altıntaş F, Kılıç Toprak E, Çört A. Kronik lenfoid lösemili hastalarda eritrosit membran lipid profili, oksidatif stres ve DNA hasarı parametrelerinin araştırılması. Pam Tıp Derg 2025;18:129-136.

Introduction

CLL is a slowly progressive, monoclonal lymphoproliferative disorder characterized by increased production and accumulation of mature but immunologically dysfunctional B lymphocytes [1]. Oxidative stress can be defined as too much oxidant exposure or inadequate antioxidant systems. Oxidative stress causes many structural and functional changes in DNA, proteins and lipids [2]. Irreversible accumulation of oxidative damage leads to structural and functional disorders at cell, tissue and organ levels. It is known to tumor cells have altered antioxidant systems and increased formation of reactive oxygen radicals. In general, high lipid peroxidation and various DNA lesions have been detected in most neoplastic tissues [3]. Therefore, in our study, we investigated to the oxidative status, DNA damage and erythrocyte membrane lipid profile in newly diagnosed CLL patients.

Materials and methods

We informed all participants about the study processes and the consent form. Our study was performed with 38 newly diagnosed CLL patients and 38 age-sex matched controls. The effect size obtained in the reference study was strong (d=1.96). As a result of the power analysis we conducted by assuming that we could obtain a lower effect size (d=0.6) based on the results in the reference study, it was calculated that 80% power could be obtained at 95% confidence level when at least 64 people (at least 32 people for each group) were included in the study. For CLL diagnosis, the 2018 International Workshop on Chronic Lymphocytic Leukemia (iwCLL) update was used. The patient group was included participants without a history of comorbidities such as diabetes, hypertension, renal failure, cerebrovascular disease, gastrointestinal disease, coronary artery disease, chronic liver disease, hematologic or solid organ malignancy. The control group was selected from participants who were age-sex-matched and had similar characteristics to the patient group. The control group had no known history of disease and drug use. A commercial kit was used for TOS, TAS measurement. The Comet assay was performed after leukocyte isolation from venous blood to measure DNA damage. Head length (HL), tail length (TL), head density (HI) and tail density (TI) were used for statistical analysis as parameters of DNA damage. Details of the methods used are explained below.

Leukocyte isolation from blood samples

Peripheral venous blood was drawn in the morning on an empty stomach. We collected blood samples in 10 mL vacuum tubes containing K3EDTA (Vacusera, Türkiye) and separated lymphocytes using Histopaque-1077 (Sigma Aldrich, Inc., St Louis, Mo, USA). We diluted the blood 1:1 with phosphate buffered saline (PBS, Life Technologies, Rockville, MD, USA) and transferred directly into a Leucosep tube (Greiner Bio-One, Austria). We then centrifuged at 800 g and room temperature for 15 minutes. We removed the Buffy coatings and washed them twice with PBS.

Serum isolation from blood samples

In the morning, 8 milliliters of peripheral venous blood were collected on an empty stomach. Blood samples were collected in vacuum gel tubes (Vacusera, Türkiye). We separated the samples from cellular fragments by centrifugation at 7260 rpm for 6 minutes at room temperature. We aliquoted the serum samples and stored them at -80°C until analysis.

Total oxidant level measurement

The principle of measurement is based on the conversion of the ferrous ion chelator complex formed from oxidants in the sample to ferric ion, which reacts with the chromogen in an acidic environment, causing an increase in absorbance. The increase in absorbance seen spectrophotometrically is directly proportional to the oxidant molecules in the sample. TOS was determined in homogenates of serum samples obtained at the end of the experiment using a commercial kit (Rel Assay Diagnostic, Gaziantep, Türkiye). The intensity of the color,

which is related to the amount of oxidants (lipids, proteins, etc.) present in the sample, was measured spectrophotometrically with an ELISA reader at a wavelength of 492 nm. Results were expressed per µmol H_2O_2 equivalent/L.

Total antioxidant level measurement

The measurement principle is based on the fact that antioxidants in the sample convert the blue-green 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical into colorless reduced ABTS. The change in absorbance is directly proportional to the level of antioxidants. TAS was measured using a commercial kit (Rel Assay Diagnostic, Gaziantep, Türkiye). 405 nm wavelength was evaluated spectrophotometrically with an ELISA reader. Results were expressed per mmol Trolox equivalent/L.

Oxidative stress index measurement

Another parameter indicating the level of oxidative stress is the Oxidative Stress Index (OSI), which is obtained by calculation. This index was calculated with the following formula using TAS and TOS values:

OSI= TOS (µmol H_2O_2 Eqv/L) / TAS (mmol Trolox Eqv/L) X 100

DNA damage analysis with comet assay

Five ml of anticoagulated blood from the experimental groups was taken into a tube and diluted 1:1 with PBS. This 10 ml of diluted blood was transferred to another tube containing 3 ml of Ficol-1077 and centrifuged at 400 g for 20 minutes. The pellet obtained by centrifugation was washed 2-3 times with 1 ml RPMI and counted with a hemocytometer and adjusted as ^{2x104} cells per 100 µL. From the lymphocyte suspension prepared in this way, 80 µL was taken and resuspended with 100 µl of "Low melting" agarose (LMA, BioShop, Canada) prepared with 0.5% $^{Ca+2}$ and $^{Mg+2-free}$ PBS at 37°C. This LMA + cell mixture was poured in a thin layer onto a slide previously coated with 1% "normal melting" agarose (NMA, Sigma, USA) and kept on ice for 30 minutes, followed by a third layer of 70 µl of 0.5% LMA and kept on ice for 10 minutes. The slide was then treated with cold lysis binding buffer with a pH of 10 at 40°C for 60 minutes to remove cellular proteins. After lysis, the slides were transferred to horizontal gel electrophoresis (BIO-RAD, California, USA) and incubated in freshly prepared alkaline electrophoresis buffer for 30 minutes. At the end of this period, electrophoresis was performed at 25 V, 300 mA for 30 minutes at the same temperature. After electrophoresis, the slides were washed 3 times for 5 minutes at 4°C with neutralization buffer (0.4M Tris-HCl, pH 7.5) to remove alkaline and detergents. Following neutralization, the slides were stained with 60 µl ethidium bromide (2µl/ml) and examined under fluorescence microscopy and possible DNA damage was assessed using the «Comet assay IV System (AutoComet)» software (Perceptive Instruments, United Kingdom). Damage assessment was expressed as HL (µm), TL (µm), HI (percentage of DNA in head, % H-DNA) and TI (percentage of DNA in tail, % T-DNA) using a software.

Serum 8-OHdG measurement

Reactive oxygen radicals produce more than 20 oxidative base damage products in DNA. Among the damaged bases, 8-OHdG is the most sensitive and most common marker of oxidative DNA damage. Its level can be measured in leukocytes or urine. Serum 8-OHdG level was measured by ELISA method using Elabscience 8-OHdG (8-Hydroxydeoxyguanosine) ELISA Kit (E-EL-0028) at Pamukkale University Faculty of Medicine Physiology Laboratory.

Gas chromatographic analysis

This analysis was conducted at Pamukkale University Advanced Technology Application and Research Center Laboratories. The instrument used for Gas Chromatographic (GC) analysis was equipped with a flame ionization detector and aRtx-2330 column (90% biscyanopropyl-10% phenylcyanopropyl polysiloxane capillary column; 60 m, 0.25 mm i.d., 0.20-mm film thickness). The heat application was started at 160°C for 55 minutes, increased by 5°C per minute and kept at 195°C for 10 minutes and then increased by 10°C per minute to 250°C. The constant pressure was applied at 29 psi and the temperature was started at 150°C and increased by 8°C per minute to 250°C after 1 minute. The constant pressure mode was selected as 13 psi and all fatty acids and isomers were determined by GC analysis. Validation was done by comparison with references. Fatty acid content of cell membranes was given as percentages.

This study was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee (date: 31.05.2021, issue: 60116787-020-56364). We conducted all procedures involving human participants in accordance with the ethical standards of institutional and/or national research committees and the Declaration of Helsinki.

Statistical analysis

Mean, standard deviation, median, minimum, maximum, frequency and ratio values were used in descriptive statistics of the data. The distribution of variables was measured using the Kolmogorov-Smirnov test. Independent sample t test and Mann-Whitney U test were used to analyze quantitative independent data. Chi-square test was used in the analysis of qualitative independent data. Spearman correlation analysis was used for correlation analysis. Statistical Package for Social Sciences (SPSS) version 28.0 (IBM SPSS Statistics Inc., Chicago, IL) software was used in the analysis. In all analyses, $p<0.05$ was considered statistically significant at 95% confidence interval in each analysis.

Results

Age and gender distribution were not significantly different between the case and control groups ($p=0.680$ *and* $p=0.488$, respectively) (Table 1). TOS and OSI values were significantly higher in the case group than in the control group ($p=0.014$ and $p=0.022$, respectively). TAS value between case and control group did not show a significant difference ($p=0.798$) (Table 2). Tail length, tail intensity, tail moment and tail migration value in case group was significantly higher than the control group ($p=0.002$, $p=0.003$, $p=0.003$, $p=0.005$ and $p=0.000$, respectively). Head intensity value in the case group was significantly lower than the control group ($p=0.003$). Head length value between case and control group did not show a significant difference ($p=0.670$) (Table 3). 8-OhdG value between case and control group did not show a significant difference ($p=0.809$) (Table 4). Hexadecanoate, Nonadecanoate, Octadecanoate, Tetradecanoate values, which are monounsaturated fatty acids in erythrocyte membrane, did not show a significant difference between the case and control groups ($p=0.733$) (Table 5).

Table 1. Intergroup analysis of age and gender data

		Control Group			Case Group			*p* value	
		Mean±ss n - %		Median	Mean±ss n - %		Median		
Age		66.9±10.9		66.5	65.9±11.3		66.0	0.680	t (t=0.414)
Gender	**Woman**	18	47.4%		15	39.5%		0.488	X^2 (x^2=0.482)
	Male	20	52.6%		23	60.5%			

t independent sample t test, X^2 Chi-square test, Mean: Mean, ss: Standard Deviation
Age and gender distribution were not significantly different between the case and control groups ($p>0.05$)

Table 2. Intergroup analysis of oxidative stress parameters

	Control Group		Case Group		*p* value	
	Mean±ss	Median	Mean±ss	Median		
TOS (µmol H2O2 equivalent/l)	6.3±1.7	6.3	8.5±3.6	8.5	0.014*	m (z=-2.462)
TAS (mmol Trolox equivalent/l)	1.07±0.30	1.05	1.05±0.33	1.06	0.798	t (t=0.257)
OSI (arbitrary unit, A.U)	648.3±311.2	583.9	970.6±888.1	802.0	0.022*	m (z=-2.296)

t independent sample t test, m Mann-whitney u test, Mean: Mean, ss: Standard Deviation
TOS: Total Oxidant Level, TAS: Total Antioxidant Level, OSI: Oxidative Stress Index
TOS and OSI values were significantly higher in the case group than in the control group ($p=0.014$ and $p=0.022$, respectively)
TAS between case and control group value did not show a significant difference ($p>0.05$)

Table 3. Intergroup analysis of DNA damage parameters by comet method

	Control Group		Case Group		*p* value	
	Mean±ss	**Median**	**Mean±ss**	**Median**		
Head Length (µm)	29.3±2.3	29.0	29.0±2.0	28.8	0.670	m (z=-0.426)
Head Intensity (%)	83.5±9.1	85.9	76.4±11.3	76.2	0.003*	m (z=-2.997)
Tail Length (µm)	24.4±6.0	22.9	28.3±5.8	27.9	0.002*	m (z=-3.132)
Tail Intensity (%)	16.5±9.1	14.1	23.6±11.3	23.8	0.003*	m (z=-2.997)
Tail Moment	2.6±2.0	1.9	3.9±2.3	3.5	0.005*	m (z=-2.826)
Tail Migration	9.7±5.9	7.4	13.9±5.5	13.6	0.000*	m (z=-3.766)

m Mann-whitney u test Mean: Mean ss: Standard Deviation
Tail length, tail intensity, tail moment and tail migration in case group value was significantly higher than the control group (p=0.002, p=0.003, p=0.003, p=0.005 and p=0.000, respectively) Head intensity in the case group value was significantly lower than the control group (p=0.003) Head length between case and control group value did not show a significant difference (p>0.05)

Table 4. Intergroup Analysis of 8-OHdG levels

	Control Group		Case Group		*p* value	
	Mean±ss	**Median**	**Mean±ss**	**Median**		
8-OHdG (ng/ml)	10.0±8.4	7.7	11.3±15.5	6.3	0.809	m (z=-0.242)

m Mann-whitney u test Mean: Mean ss: Standard Deviation,
8-OHdG between case and control group value did not show a significant difference (p>0.05)

Table 5. Intergroup analysis of erythrocyte membrane fatty acids

Monounsaturated Fatty Acids	**Control Group**		**Case Group**		*p* value	
Hekzadekanoat	6	30.0%	6	27.3%	0.733	X^2 (x^2=1.284)
Nonadecanoate	2	10.0%	5	22.7%		
Octadecanoate	6	30.0%	6	27.3%		
Tetradecanoate	6	30.0%	5	22.7%		
	Control Group		**Case Group**			
	Mean±ss / n-%	**Median**	**Mean±ss / n-%**	**Median**		
Percentage of Area (%) - FAME	30.0±27.9	30.0	27.3±30.0	15.9	0.641	m (z=-0.466)

m Mann-whitney u test Mean: Mean ss: Standard Deviation
Hexadecanoate, Nonadecanoate, Octadecanoate, Tetradecanoate values, which are monounsaturated fatty acids in erythrocyte membrane, did not show a significant difference between the case and control groups (p>0.05)

Discussion

CLL is the most common adult leukemia in western countries [4]. The incidence increases with age, it is about 2 times more common in men than in women [5]. In etiologic studies, the relationship between environmental, chemical and radiation exposure, diet, virus infection and autoimmune diseases and CLL development has not been proven [6]. In a study comparing the first-degree relatives of the case and control groups, it was shown that the risk was 8.5 times higher in the relatives of the case group, suggesting a genetic tendency. It has been shown in previous studies that CLL is a disease susceptible to changes in antioxidant enzymes and oxidative stress, and that there is a dominant oxidative stress in these patients [7, 8]. In this study, we investigated the presence of DNA damage and erythrocyte membrane lipid profile in lymphocytes isolated by comet

method in addition to systemic oxidative stress parameters in CLL patients. With the data obtained, we aimed to make a scientific contribution to the pathogenesis of CLL and future treatments.

We used some biomarkers in our study. Total oxidative status and total antioxidant status were measured and information was provided about the total status rather than individual parameters. TOS and OSI values of the case group in our study were significantly higher than the control group (p=0.014 and p=0.022, respectively). TAS value was not significantly different between the case and control groups (p=0.798). When similar studies in the literature are evaluated together, there are some limitations to the comparison such as evaluation of oxidative stress with different parameters, different oxidative stress measurement kits, differences in age and gender distribution, variability of disease stages and heterogeneity between groups. However, as a common result; oxidative stress parameters are increased and antioxidant parameters are decreased in CLL patients [9-13]. We think that the different results obtained in oxidative level measurements in the body may be due to the difference in the measurement kit and the oxidative parameter measured and the measurement method. Because methods including colorimetric, fluorescence or chemiluminescence can be used to measure oxidative status [14, 15]. In our study, the oxidative status caused by CLL was evaluated and the parameter differences between TAS, TOS, OSI, serum 8-OHdG level and the level of DNA damage by comet method were also revealed. The markers reflecting the oxidant status are also contradictory among themselves. The fact that it is not known which method of oxidative stress measurement is more specific for CLL and the lack of superiority studies of the methods can be considered among the limiting reasons of our study. In this respect, it is seen that there is a need for further research and the establishment of measurement parameters to be established with international standards in determining the indicators of oxidant level.

Like the oxidative processes that proteins, carbohydrates and lipids undergo, DNA is also affected despite its stable structure. Due to DNA repair processes, oxidative damage can be detected even in healthy individuals. In DNA, 8-OHdG, the most well-known of the base mutations, is formed by the interaction of the hydroxy radical at the 8th position of the guanine base. 8-OHdG is one of the oxidative base damage products of ROS on DNA. 8-OHdG level in serum and urine is evaluated as a marker of oxidative stress. ROS have short half-lives and therefore direct in vivo measurement of ROS is difficult. Currently, 8-OHdG is one of the few ROS-mediated products used to assess oxidative stress on DNA instead of ROS itself. In this study, in addition to the total oxidative stress in the body, we aimed to show the effects of CLL on DNA with ROS products and to contribute to the studies on DNA damage repair mechanism by measuring 8-OHdG level. In our study, there was no difference in 8-OHdG levels between the case and control groups (6.3 vs. 7.7 ng/ml, p=0.809). When other studies on the same subject in the literature are examined, it is observed that 8-OHdG levels in blood and urine are increased in case groups [15, 16]. In the results of our study, the lack of difference in the comparison of 8-OHdG levels, which is an indicator of the total oxidant status in the body and the effect of oxidant levels on DNA, between the groups may have been due to the uncertainty of the amount of oxidant that may cause damage to DNA, the lack of a certain measurement standardization, the experimental conditions and the differences in the commercial kits used.

Comet assay, also known as single cell gel electrophoresis, is a simple method for measuring DNA damage and repair in eukaryotic cells. Damage to DNA can be assessed using the comet assay, which combines the effects of genotoxic, cytotoxic, and oxidative stress. It is an economical, reliable, and fast technique. The shape, size, and amount of DNA in the ‘comet’ play an important role in determining the level of damage. In this assay, DNA damage parameters are measured by measuring tail length, density and momentum, and head length and density. The study of these parameters is done using the relevant software. Depending on the degree of damage, DNA breaks move from the nucleus to the periphery and elongate from the center to the edge. As a result, cells with increased damage develop a comet appearance [17]. In our study, DNA damage was measured by comet

method and values were recorded as head length, tail length, head intensity, tail intensity, tail moment, tail migration. Tail length, tail intensity, tail moment and tail migration values were significantly higher in the case group than in the control group (28.3±5.8 vs. 24.4±6.0 μm, p=0.002; 23.6±11.3 vs. 16.5±9.1%, p=0.003; 3.9±2.3 vs. 2.6±2.0, p=0.005; and 13.9±5.5 vs. 9.7±5.9, p=0.000). The head intensity value was significantly lower in the case group than in the control group (76.4±11.3 vs. 83.5±9.1%, p=0.003). There was no significant difference in head length between the case and control groups (29.0±2.0 vs. 29.3±2.3 μm, p=0.670). Therefore, DNA damage is increased in CLL patients compared to the control group. Similar results have been obtained in other studies on CLL patients using the comet method [18].

In our study, four different monounsaturated fatty acids (MUFA) in erythrocyte membrane were examined and the percentages of each fatty acid were given. Among the erythrocyte membrane lipids analyzed, nonadecanoate was found to be higher in the case group (22.7%) compared to the control group (10.0%) (p=0.733). Hexadecanoate was found to be high in the control group (30.0%) and decreased in the case group (27.3%) (p=0.733). Octadecanoate was also found to be high in the control group (30.0%) and low in the case group (27.3%) (p=0.733). Tetradecanoate levels were found to be high in the control group (30.0%) and decreased in the case group (22.7%) (p=0.733). When the MUFAs included in the study were compared between the case and control groups, a decrease was observed in the case group (15.9%) compared to the control group (30%) (p=0.641). When the literature was reviewed, no other study investigating erythrocyte membrane lipid profile in CLL patients was found. Our study is the first to examine this issue. The lack of statistically significant difference between our results may be related to the small number of samples in which membrane lipids were evaluated, and the length of the disease process in the case group may also affect this situation. For these reasons, we believe that further research in larger sample groups is needed.

In conclusion, despite the limitations of our study, we demonstrated that oxidative stress and DNA damage are increased in CLL patients. We believe that this study will be instructive for future studies on erythrocyte membrane lipid profile in CLL patients with a larger sample size.

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The effect of endobronchial coil treatment (EBCT) on hemorheological parameters and oxidative stress: a pilot study

Endobronşiyal koil tedavisinin (EBCT) hemoreolojik parametreler ve oksidatif stres üzerine etkisi: pilot çalışma

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Abstract

Purpose: Chronic Obstructive Pulmonary Disease (COPD) is a common, preventable, curable disease characterized by persistent airflow limitation, respiratory symptoms due to airway and/or alveolar abnormalities caused by severe exposure to harmful particles, gases. During the endobronchial coil treatment (EBCT) process, the volume of the lung parenchyma is reduced by shrinking the elastic recoil. Although there are studies showing worsening of hemorheological parameters in COPD exacerbations, no study investigated whether hemorheological parameters are improved after coil. The aim of this study was to assess the effects of coil therapy on erythrocyte deformability, whole blood viscosity (WBV) measured at autologous, standard (40%) hematocrit and plasma viscosity (PV) in COPD patients.

Material and methods: Venous blood samples were taken once from the healthy control group (n=17) and before and 1 month after the treatment from the COPD patients who had been indicated for coil according to GOLD guidelines (n=20). To assess erythrocyte deformability, shear-dependent erythrocyte elongation was measured at 0.3-3.0 Pa by an ektacytometer (LORCA), while WBV, PV were measured using a rotational viscometer.

Results: Erythrocyte deformability measured at shear stresses between 0.3-5.33 Pa were found to be higher following treatment compared to pre-coil values. EBCT did not have a statistically significant effect on WBV measured at autologous, 40% hematocrit, PV and oxidative stress indices.

Conclusion: Increased erythrocyte deformability determined following EBCT at the shear stresses observed at the pulmonary level is a favourable finding, showing that the procedure may positively affect the hemodynamics of COPD patients as well as causing clinical improvement.

Keywords: Chronic obstructive pulmonary disease, endobronchial coil therapy, erythrocyte deformability, hemorheology, oxidative stress.

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Öz

Amaç: Kronik Obstrüktif Akciğer Hastalığı (KOAH), zararlı partiküllere, gazlara şiddetli maruziyetin neden olduğu hava yolu ve/veya alveolar anormalliklere bağlı kalıcı hava akımı kısıtlılığı, solunum semptomları ile karakterize yaygın, önlenebilir, tedavi edilebilir bir hastalıktır. Endobronşiyal koil tedavisi (EBCT) işlemi sırasında elastik geri tepme küçültülerek akciğer parankiminin hacmi azaltılır. KOAH alevlenmelerinde hemoreolojik parametrelerin kötüleştiğini gösteren çalışmalar olmasına rağmen, koil sonrası hemoreolojik parametrelerin iyileşip iyileşmediğini araştıran bir çalışma yoktur. Bu çalışmanın amacı, KOAH hastalarında koil tedavisinin eritrosit deformabilitesi, otolog, standart (%40) hematokritte ölçülen tam kan viskozitesi (WBV) ve plazma viskozitesi (PV) üzerindeki etkilerini değerlendirmektir.

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Gereç ve yöntem: Sağlıklı kontrol grubundan (n=17) ve GOLD yönergelerine göre coil için endikasyon konulmuş olan KOAH hastalarından (n=20) tedaviden önce ve 1 ay sonra venöz kan örnekleri alındı. Eritrosit deformabilitesini değerlendirmek için, kaymaya bağlı eritrosit uzaması 0,3-3,0 Pa'da bir ektasitometre (LORCA) ile ölçülürken, WBV, PV rotasyonel bir viskozimetre kullanılarak ölçüldü.
Bulgular: Eritrosit deformabilitesi 0,3-5,33 Pa arasındaki kayma streslerinde ölçülmüş ve tedavi sonrasında coil öncesi değerlere kıyasla daha yüksek bulunmuştur. EBCT'nin otolog, %40 hematokrit, PV ve oksidatif stres indekslerinde ölçülen WBV üzerinde istatistiksel olarak anlamlı bir etkisi olmamıştır.
Sonuç: EBCT sonrasında pulmoner düzeyde gözlenen kayma gerilimlerinde belirlenen artmış eritrosit deformabilitesi, işlemin KOAH hastalarının hemodinamiğini olumlu yönde etkileyebileceğini ve klinik iyileşmeye neden olabileceğini gösteren olumlu bir bulgudur.

Anahtar kelimeler: Kronik obstrüktif akciğer hastalığı, endobronşiyal koil tedavisi, eritrosit deformabilitesi, hemoreoloji, oksidatif stress.

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Introduction

Chronic Obstructive Pulmonary Disease (COPD) is an important cause of morbidity and mortality worldwide [1]. COPD has two primary types as chronic bronchitis and emphysema [2]. Emphysema is a destructive process of the pulmonary parenchyma characterized by the permanent expansion of distal airways [3]. This results in dynamic hyperinflation, loss of elastic recoil, air trapping and decreased exercise capacity, shortness of breath and increased mortality [4].

Smoking cessation, pharmacological treatments, rehabilition, education & self management, oxygen support, vaccination programs are among the primary treatment options for COPD [3, 5, 6]. However, their effects in patients with emphysema are limited. Since the main pathology in emphysema is hyperinflation due to permanent elastic tissue damage, the search for novel treatment has come to the fore. Endoscopic volume reduction treatments can be considered as important alternatives in certain emphysema patients that are dyspneic despite optimal medical treatment [6]. There are many studies which report that endobronchial coil treatment (EBCT) which is one of the endoscopic volume reduction treatments, reduces hyperinflation while improving the quality of life of patients [7, 8].

Hemorheology is the scientific field interested in blood flow properties and deformability of its cellular components [9]. Its compnents may be summarized as red Blood Cell (RBC) deformability, viscosity of blood and hematocrit (Hct) [10]. Many studies suggest that flow behaviors of blood are essential for maintaining proper tissue perfusion [10].

It is known that, oxidative stress is effective in various physiological conditions and in many diseases including COPD pathogenesis [11]. Although enhanced oxidative stress and / or decreased antioxidant status were suggested to be involved in in the pathogenesis of COPD, the precise mechanism was not yet revealed [12]. Oxidative stress is the result of increased formation of reactive oxygen species and/or decreased antioxidant capacity [13, 14].

Oxidative stress is closely associated with hemorheological alterations [15]. Increment in oxidative stress was demonstrated to be responsible for certain hemorheological changes [16]. Oxidative stress was determined in order to explain the possible alterations in hemorheological parameters in the current study.

A limited number of studies have been carried out until now on the hemorheological parameters and oxidative stress in chronic pulmonary diseases [6, 15, 17, 18]. The aim of this study was to examine whether EBCT has an effect on hemorheological and oxidative parameters in patients with emphysema. As far as we have researched and found, no study has been found examining the effects of interventional treatment method on hemorheological and oxidative stress parameters.

Materials and method

Study population

All patients who underwent coil treatment between July 2019 and February 2020 and agreed to participate were included in the study. A total of 24 patients who were followed up at the Pulmonary Diseases clinic and diagnosed with stage 3 or stage 4 COPD according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) diagnosis criteria were involved [5]. These patients were also emphysematous and suitable for EBCT. The inclusion and exclusion criteria of the patients are given in Table 1 [19]. Cardiopulmonary rehabilitation programs of all patients were completed prior to the procedure.

Age and sex matched healthy voulnteers consisting the same number of individuals without chronic disease or smoking history were involved as the control group.

Table 1. Patient inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Patients undergoing optimal medical treatment (quitting smoking, maximum pharmacological treatment, pulmonary rehabilitation)	Severe PHT (PAP >50 mmHg in ECO)
GOLD Stage 3 or 4	Clinically severe bronchiectasis
CAT score ≥10, mMRC ≥2	Suspected pulmonary module
FEV_1 20-45%	Diagnosed lung cancer or suspicion
$RV_{expected}$ ≥175% or RV/TLC ≥58%	Interstitial fibrosis
6-minute walk test 100-500 m	Severe tracheobronchomalacia

GOLD: Global Initiative for Chronic Obstructive Lung Disease, CAT: COPD Assessment Test
mMRC Modified Medical Research Council Dyspnea Scale, FEV_1: forced expiratory volume in first second
RV: residual volume, TLC: total lung capacity, PHT: pulmonary hypertension, sPAP: systolic pulmonary arterial pressure

Procedure

Application of EBCT (PneumRx, Inc., MountainView, Calif., USA)

The procedure was carried out at the operating room under general anesthesia with the accompaniment of fluoroscopy. The airway in the selected segment was first determined bronchoscopically and measured using a guide wire. The coil wire of suitable length (generally 100 mm, 125 mm or 150 mm) was left at the targeted segment using a carrier catheter which then takes on the shape of a coil. Airway shrinks as the coil wire pulls on the lobe, thus the lung collapses and shrinks. The targeted lobe was systematically treated with 10-14 coil wires on average. Initially one lobe was treated with the other targeted lobe in the opposite lung treated 4-8 weeks later [8, 19]. Postero-anterior radiography of the patient with bilateral coil procedure is shown in Figure 1.

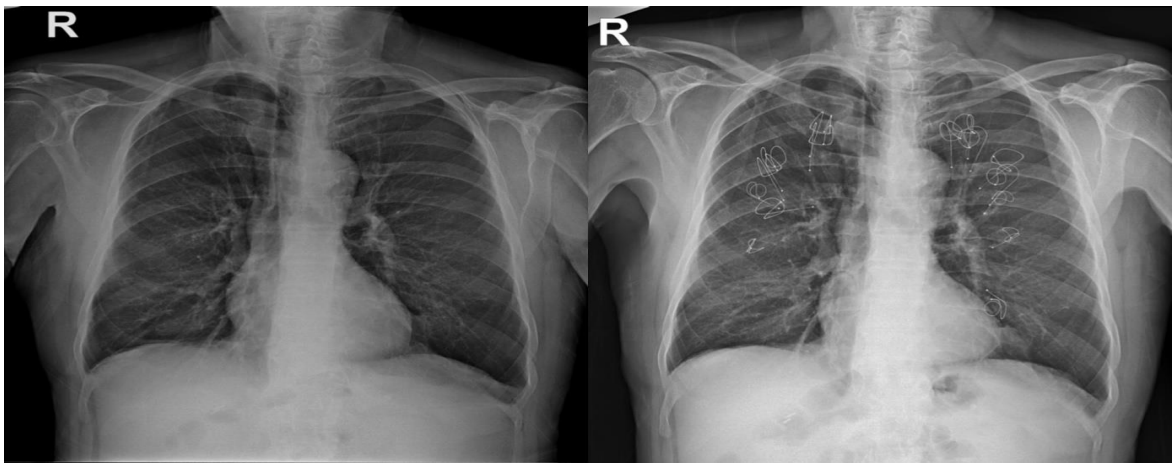

Figure 1. Postero-anterior radiography of the patient with bilateral coil

Samples and measurements

Blood samples were taken from the antecubital vein by venipuncture into standard tubes containing EDTA (1.5 mg/ml) for hemorheological measurements. Samples were taken after 12 hours of overnight fasting on the morning of the day before the coil treatment and in the first month after coil tratment. In the same way, blood was taken from healthy volunteers in the morning after fasting. After the samples were properly transferred to the Physiology Laboratory, hemorheological tests were performed within 3 hours according to the "new guidelines for hemorheological laboratory techniques" [20]. Hematological parameters were determined by an electronic hematology analyzer (Siemens ADVIA® 2120i System, Siemens Healthcare Diagnostics, Japan). For the determination of oxidative stress parameters, blood samples collected into yellow top blood collection tubes, were centrifuged at 5000 g for 6 min. The serum layer was separated and stored at -80°C until being used for the analysis.

Determination of erythrocyte deformability

RBC deformability was measured by laser diffraction analysis with an ektacytometer (Laser assisted optical rotational cell analyzer (LORCA), RR Mechatronics, Hoorn, The Netherlands) at various shear stresses between 0.3-30 Pa at 37°C as previously described [21]. RBC were suspended in isotonic 4% polyvinylpyrrolidone 360 solution (MW 360 kD; Sigma P 5288; St. Louis, MI). According to the LORCA instrument measuring principle, a laser beam was directed through the sample and the diffraction pattern produced by the shape-shifting erythrocytes was analyzed by a microcomputer. Results were given as elongation index (EI). EI=(L−W)/(L+W). L is the length and W is the width of the diffraction pattern.

Measurement of the whole blood and plasma viscosity

A cone-plate rotational viscometer (model DV-II+Pro, Brookfield engineering Labs, Middleboro, MA) was used to determine whole blood viscosity (WBV) and plasma viscosity (PV) at 37°C. WBV was measured at both native and standard (40%) Hct at shear rates of 38, 76 and 190 s^{-1}, whereas PV was measured at 190 s^{-1}.

2.6 Determination of total oxidant status (TOS) and total antioxidant status (TAS)

TOS and TAS were measured by commercial kits (Rel Assay Diagnostics, Turkey) according to the manufacturer's instructions [22, 23].

Calculation of oxidative stress index (OSI)

OSI was calculated using the following Formula;

OSI (arbitrary unit)=TOS (μmolH_2O_2 Equiv./L)/TAS (mmol Trolox Equiv./L)X100 [24].

Statistical analyses

All the statistical analyses of the obtained clinical and demographic data were carried out using Statistical Package for the Social Sciences (SPSS) v.25 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean (standard deviation (SD)), median (minimum and maximum values), while categorical variables as number and percentage. The suitability of the data for normal distribution was examined by the Shapiro-Wilk test. When parametric test conditions were satisfied Independent samples t test was used for comparisons among groups. If parametric test conditions were not satisfied, Mann Whitney U test was used for comparisons among groups. For pairwise comparisons; if parametric test conditions were satisfied Paired Samples t test; and if parametric test conditions were not satisfied Wilcoxon signed rank test was used. $P<0.05$ was considered statistically significant.

The present study was carried out in accordance with the Helsinki Declaration and was approved by the Pamukkale University Non-Interventional Clinical Research Ethics Committee.

Results

Since 1 patient died during the follow-up after the procedure, 2 people did not come to

the follow-ups, and blood samples of 1 patient could not be analyzed due to technical issues, evaluations were carried out on 20 patients, although it was started with 24 patients. All patients were male and mean (SD) age was 66.75 (6.98). All of the 17 age- and sex-matched healthy control group were male and the mean (SD) age was 62.59 (6.66) (p>0.073). Table 2 presents the sociodemographic and clinical data of the patients.

Table 2. Sociodemographic characteristics and some clinical parameters

	Number (n)	**Percentage (100%)**
Procedure		
Unilaterale	11	55
Bilateral	9	45
Emphysema distribution		
Homogeneous	11	55
Heterogeneous	9	45
GOLD spirometric stage		
Stage 3	11	55
Stage 4	9	45
USOT use		
Yes	16	80
No	4	20
	Mean (SD)	**Median (min-max)**
Age	66.75 (6.98)	67.5 (49-76)
Used coil (qty.)	14.1 (5.12)	14.5 (6-22)
Respiratory Function Test		
FVC (%)	57.19 (15.76)	51.75 (34.6-94.3)
FEV_1 (%)	30.21 (8.01)	29.65 (20.2-44.6)
FEV_1/FVC (%)	41.43 (4.56)	40.90 (33.3-51.1)
RV (%)	378.05 (191.35)	312 (180-916)

Abbreviations: SD, Standard Deviation; min-max, minimum-maximum values; FVC, forced vital capacity; FEV_1, forced expiratory volume in first second; RV, residual volume

RDW (red blood cell distribution width) of COPD patients was higher compared to control group (p_1=0.012) whereas MCHC (mean corpuscular hemoglobin concentration) of COPD patients was lower than control group (p_1=0.001). Similarly, after the coil, RDW was higher and MCHC was lower in the COPD group compared to the control group (p_2). The RBC count, hemoglobin, Hct, RDW, MCV (mean corpuscular volume) and MCHC of each subject, before and after the procedure were similar (p_3) (Table 3).

Table 4 demonstrates erythrocyte deformability (given as EI) values of the subjects. RBC deformability of COPD patients was lower than control group (p_1). After EBCT, a statistically significant increase was observed in erythrocyte deformability at 0.30-5.33 Pa (p_3). Consistent with these findings, RBC deformability measured at shear stresses between 0.30 and 1.69 Pa in the COPD group after EBCT was not different from that of the control group (p_2).

It was observed that the effect size of the RBC deformability results obtained from 20 patients was at a strong level (dz=0.626) (for the pre-post treatment alteration obtained at 0.3 Pa). For this effect size, our study reached 85% power at 95% confidence level.

Table 3. Comparison of RBC, hemoglobin, Hct, RDW, MCV, MCHC control group and before and after EBCT

	Control group (n=17) Mean (SD)	Patient group (n=20)		P_1	P_2	P_3
		Before EBCT Mean (SD)	After EBCT Mean (SD)			
RBC (M/uL)	4.96 (0.51)	4.97 (0.59)	4.97 (0.5)	0.971 (t=-0.037) a	0.965 (t=-0.044) a	0.996 (t=-0.005) c
Hemoglobin (g/dL)	14.68 (1.22)	14.2 (1.73)	14.05 (1.83)	0.337 (t=0.973) a	0.23 (t=1.222) a	0.555 (t=0.601) c
Hct (%)	43.78 (3.5)	43.22 (5.96)	43.52 (5.25)	0.734 (t=0.342) a	0.859 (t=0.179) a	0.750 (t=-0.323) c
RDW (fL)	13.55 (1.38)	15.03 (2.02)	15.23 (1.64)	0.012* (z=-2.485) b	0.001* (z=-3.541) b	0.282 (t=-1.108) c
MCV (fL)	88.69 (6.25)	88.4 (6.55)	87.75 (6.93)	0.619 (z=-0.518) b	0.94 (z=-0.091) b	0.322 (t=1.017) c
MCHC (g(/dL)	33.56 (0.87)	32.37 (1.15)	32.27 (1.17)	0.001* (t=3.503) a	0.0001* (z=3.218) b	0.706 (t=0.383) c

Values are expressed as means±SD. Abbreviations: EBCT, endobronchial coil therapy;
SD, standard deviation; RBC, red blood cell; Hct, hemotocrit; RDW, red blood cell distribution width; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; P_1, difference between COPD patient group and control group before EBCT; P_2, difference between COPD patient group and control group after EBCT; P_3, difference before and after EBCT in COPD patients; a: Independent samples t test; b: Mann Whitney U test; c: Paired Samples t test

Table 4. Comparison of red blood cell deformability control group and before and after EBCT

Shear stress (Pa)	Control group (n=17) Mean (SD)	Patient group (n=20)		P_1	P_2	P_3
		Before EBCT Mean (SD)	After EBCT Mean (SD)			
0.3	0.05 (0.01)	0.03 (0.02)	0.04 (0.01)	0.005* (t=3.035) a	0.189 (t=1.341) a	0.029* (t=-2.37) c
0.53	0.1 (0.02)	0.08 (0.03)	0.09 (0.02)	0.004* (t=3.055) a	0.17 (t=1.401) a	0.030* (t=-2.348) c
0.95	0.19 (0.02)	0.17 (0.03)	0.18 (0.03)	0.005* (t=2.968) a	0.188 (t=1.342) a	0.034* (t=-2.28) c
1.69	0.3 (0.02)	0.27 (0.04)	0.29 (0.03)	0.003* (t=3.191) a	0.103 (t=1.676) a	0.045* (t=-2.146) c
3	0.41 (0.02)	0.37 (0.03)	0.39 (0.03)	0.0001* (t=4.210) a	0.012* (t=2.635) a	0.032* (t=-2.317) c
5.33	0.49 (0.01)	0.46 (0.03)	0.47 (0.02)	0.0001* (t=4.834) a	0.001* (t=3.729) a	0.05* (t=-2.059) c
9.49	0.55 (0.01)	0.52 (0.02)	0.53 (0.02)	0.0001* (t=4.444) a	0.0001* (t=4.133) a	0.17 (t=-1.426) c
16.87	0.59 (0.01)	0.57 (0.02)	0.58 (0.02)	0.0001* (z=-3.508) b	0.0001* (z=-3.646) b	0.603 (t=-0.529) c
30	0.62 (0.01)	0.61 (0.02)	0.61 (0.02)	0.0001* (z=-3.783) b	0.0001* (z=-3.663) b	0.982 (t=-0.023) c

Values are expressed as means±SD. Abbreviations: EBCT, endobronchial coil therapy;
SD, standard deviation; P_1, difference between COPD patient group and control group before EBCT; P_2, difference between COPD patient group and control group after EBCT; P_3, difference before and after EBCT in COPD patients; a: Independent samples t test; b: Mann Whitney U test; c: Paired Samples t test

Oxidative stress parameters (TAS, TOS, OSI) were also evaluated, and it was found that the oxidative stress index was higher in the COPD group than the control group (p_1=.0001). However, no statistically significant change in TAS, TOS, and OSI values following coil treatment in the COPD group were observed (Table 5).

Viscosity could not be studied in the control group due to technical problems. Statistically significant alterations were not observed in WBV measured at both autologous and standard (40%) Hct and PV values in the COPD group (Table 6).

Table 5. Comparison of oxidative stress parameters before and after EBCT

Oxidative stress parameters	Control group (n=17) Mean (SD)	Patient group (n=20) Before EBCT Mean (SD)	Patient group (n=20) After EBCT Mean (SD)	P_1	P_2	P_3
TOS ($\mu molH_2O_2$ Equiv. /L)	2.86 (1.48)	6.38 (4.28)	6.31 (2.32)	0.0001* (z=-4.426) b	0.0001* (z=-4.815) b	0.341 (z=-0.953) d
TAS (mmol Trolox Equiv./L)	2.67 (0.31)	1.07 (1.09)	1.08 (0.43)	0.0001* (z=-4.664) b	0.0001* (t=-12.689) a	0.126 (z=-1.531) d
OSI (arbitrary unit)	0.11 (0.05)	0.79 (0.48)	0.68 (0.37)	0.0001* (z=-4.345) b	0.0001* (z=-5.181) b	0.361 (t=0.936) c

Values are expressed as means±SD. Abbreviations: EBCT, endobronchial coil therapy; SD, standard deviation; TOS, total oxidant status; TAS, total antioxidant status; OSI, oxidative stress index; P_1, difference between COPD patient group and control group before EBCT; P_2, difference between COPD patient group and control group after EBCT; P_3, difference before and after EBCT in COPD patients; a: Independent samples t test; b: Mann Whitney U test; c: Paired Samples t test; d: Wilcoxon Signed Rank test

Table 6. Comparison of whole blood viscosity (WBV) at native, standard (40%) hematocrit and plasma viscosity (PV) before and after EBCT

	Before EBCT Mean (SD)	After EBCT Mean (SD)	*P* value
WBV at native Hct (38 s^{-1})	5.835 (0.832)	5.718 (1.73)	0.715 (z=-0.365) d
WBV at native Hct (76 s^{-1})	4.626 (0.866)	5.751 (1.144)	0.401 (z=-0.840) d
WBV at native Hct (190 s^{-1})	3.928 (0.687)	4.803 (1.167)	0.282 (t=-1.127) c
WBV at standard (40%) Hct (38 s^{-1})	5.363 (0.599)	5.028 (1.063)	0.465 (z=-0.730) d
WBV at standard (40%) Hct (76 s^{-1})	4.184 (0.972)	4.73 (0.591)	0.225 (z=-1.214) d
WBV at standard (40%) Hct (190 s^{-1})	3.93 (0.541)	4.231 (1.045)	0.760 (t=-0.312) c
PV (190 s^{-1})	1.943 (0.838)	1.953 (1.415)	0.333 (z=-0.968) d
Hct (%)	44 (4.46)	44.421 (4.776)	0.633 (t=-0.486) c

Values are expressed as means±SD. Abbreviations: EBCT, endobronchial coil therapy;
SD, standard deviation; WBV, whole blood viscosity; PV, plasma viscosity; Hct, hematocrit
c: Paired Samples t test; d: Wilcoxon Signed Rank test

Discussion

The results of the current study show significant changes in oxidative stress and hemorheological parameters, particularly erythrocyte deformability, whole blood viscosity (WBV), and plasma viscosity (PV), in patients with Stage 3 and 4 COPD following EBCT. To our knowledge, no other study in the literature has reported findings that overlap with these results. RBC deformability measured at share stresses of 0.3-5.33 Pa was increased following EBCT. The treatment applied did not affect WBV determined at either native, or standard (40%) Hct, PV and oxidative stress indices.

COPD is characterized by airflow obstruction and an abnormal inflammatory response of the lungs to noxious particles or toxic gases. Since considerable evidence supports the hypothesis that oxidative stress plays an important role in the development of COPD [25], we aimed to demonstrate oxidative response to EBCT in grade 3 and 4 COPD patients. Previous studies on COPD and oxidative stress indicate that especially TAS was reduced in patients with COPD, TOS was increased thus leading to increased OSI [15, 26, 27]. Similarly, in our study, oxidative stress parameters were found to be statistically significantly increased in the COPD group compared to the healthy control group. The alterations in oxidative stress markers in patients with COPD were shown to be correlated with the progression of the disease [28, 29]. Inflammatory cells also play a pivotal role in COPD as they are involved in the release of a variety of mediators, such as proteases, oxidants, and cytokines [30]. RDW is another parameter which may be associated with inflammation is. Although RDW is often used for the differential diagnosis of anemia, it was also demonstrated to increase in cardiovascular diseases, cancer, and diabetes. RDW has been reported to be related to inflammation. There are also studies demonstrating its correlation with severity of the disease and exacerbations in patients with COPD [6, 31, 32]. In our study, RDW was significantly increased in COPD patients compared to the healthy control group. Few studies showing the relationship between COPD and MCHC have reported that MCHC is associated with prognosis in COPD exacerbations. Although the precise mechanisms underlying the association cannot be clearly elucidated, it has been reported that the decrease in MCHC may be related to the intensity of inflammation [33, 34]. In the current study, MCHC was found to be lower in the COPD group compared to the control group.

Although it is possible to measure components of oxidative pathways separately from biological samples in humans, it could be time-consuming and expensive. Instead, determining TOS and TAS, reflecting synergistic and cumulative action of oxidant and antioxidants, is a more practical method to examine oxidant/antioxidant balance [22, 35]. For these reasons TOS and TAS were determined in the current study [22, 35]. Similarly, OSI was calculated to determine the overall oxidative stress in the organism. As far as we know, no report exists in literature demonstrating oxidative stress response to EBCT in COPD. Our results demonstrate that, TOS, TAS and OSI were not altered in COPD patients 1 month after EBCT. The limited patient number and post-procedure follow-up period may be among the causes of these results. Since the patients were severe, frequently experienced attacks and the mortality rate was high, we concluded the study in the 1st month following EBCT.

Blood rheology plays an important role in maintaining the microcirculation properly and impaired hemorrheological parameters are associated with many diseases [17, 36, 37]. Hemorrheology is interested in flow properties of blood and the blood - vessel relationship. Erythrocyte deformability, RBC aggregation, hematocrit, WBV and PV are among the main components of blood rheology [10, 38]. Erythrocyte deformability may be defined as the ability of the RBC to adopt blood flow properties by changing its shape under shear stress, and enhanced elongation index (EI) is associated with increased erythrocyte deformability [10]. The ability of RBC to change its shape is especially important for microcirculation, where erythrocytes have to pass through vessels smaller than their own diameter. Erythrocyte deformability is also an important parameter determining blood flow resistance and plays an important role in the pathogenesis of ischaemia [39, 40]. Rheological properties of blood are affected by a number of pathophysiological processes, including a variety of pulmonary diseases, leading to an increase in the clinical

importance of hemorrheological field [36, 41]. It may be suggested that, impaired RBC deformability and aggregation may be realted with COPD pathogenesis [6]. A decrement in RBC deformability may diminish lung oxygenation and also pulmonary functions. Hypoxia is one of the prognostic factors in COPD [42].

Findings of our study demonstrate that, EBCT results in increment of RBC deformability measured 1 month after the procedure at shear stresses between 0.3-5.33 Pa. The shear stress level of normal pulmonary circulation was demonstrated to be around 2-3 Pa [43]. Thus, the finding that RBC deformability determined at 1.69 and 3 Pa increases after EBCT gains more impartance. Although tissue oxygenation primarily depends on alterations in perfusion-ventilation matching after the treatment, the rise in erythrocyte deformability may also be evaluated as a favorable alteration in terms of oxygenation. We observed that erythrocyte deformability was higher in the healthy control group of similar age and gender compared to COPD patients. Our results may demonstrate that EBCT may not only be beneficial by reducing hyperinflation through volume reduction, it may also contribute to the improvement of the patient's life quality by positively affecting perfusion through an enhancement in erythrocyte deformability. The increase in RBC deformability following EBCT in our study may indicate that the pulmonary functions and oxygenation may improve. The mechanism by which the EBCT causes increment of RBC deformability is unknown. One of the reasons for evaluating oxidative stress in this study was to contribute to the explanation of the mechanisms of the alterations in hemorheological parameters. The increase in oxidative stress in COPD patients reduces erythrocyte deformability and leads to hypoxia resulting in reduced life expectancy [15]. Since no statistically significant alteration in TOS, TAS and OSI following EBCT was observed and RDW, MCV and MCHC of each subject, before and after the procedure were similar, the rise in erythrocyte deformability cannot be explained by altered oxidative stress and hematological parameters mentioned above. 9.49-30 Pa shear stresses at which we did not find a statistically significant alteration in RBC deformability are quite high shear stresses that are not observed at the pulmonary level.

Other hemorheological parameters determined in the current study are the whole blood viscsity (WBV) and PV. Decreased RBC deformability was shown to lead increment of apparent blood viscosity and hence flow resistance in larger vessels [44]. Sınce plasma is the component of blood which is in contact with the vessle wall due to the axial migration, PV is an important parameter of the flow regulation [10].

Properties of plasma and the cellular components of blood as well as shear rate determine blood fluidity. Erythrocyte deformability, PV and Hct are important determinants of viscosity at physiological shear rates [45]. For these reasons, WBV was determined at both native and standard (40%) Hct and under shear rates of 38, 76 and 190 s^{-1} in our study. High Htc value may be considered as one of the factors enhancing blood viscosity in COPD [46]. However, our results demonstrate that, Hct value of Grade 3 and 4 COPD patients was unaltered 1 month after the treatment.

Cheng et al. [47] demonstrated that viscosity of blood has an important association with pulmonary blood flow and pulmonary vascular resistance in univentricular circulations where low-shear non-pulsatile blood flow is present in the pulmonary arterial tree. Almarshad and Hassan showed that smoking alters the rheological properties by increasing WBW and PV levels [48]. Moreover, Lowe and coworkers confirmed a significant reduced blood flow after smoking resulted from high blood viscosity and PV [49]. Our results demonstrate that PV and WBV determined at both native and standard Hct and under shear rates of 38, 76 and 190 s^{-1} were not affected following 1 month of EBCT in COPD patients. In our study, we did not observe significant changes in TOS, TAS, or OSI levels in COPD patients at the 1st month following EBCT. The lack of improvement in oxidative stress parameters suggests that the observed increase in erythrocyte deformability cannot be attributed solely to changes in oxidative stress, contrary to our expectations. Although there is strong evidence in the literature supporting the role of oxidative stress in the pathogenesis of COPD [25], we were unable to corroborate this improvement through oxidative stress parameters. This finding raises the possibility that the improvement in deformability could

be related to mechanisms other than oxidative stress, or that the blood samples taken at the 1st month might have been assessed at an early time point. Considering that the oxidative stress response may emerge over a longer period, improvements in TAS, TOS, and OSI could potentially be observed at later stages. However, due to the unstable nature of COPD patients, with frequent exacerbations and hospitalizations, we opted for a shorter follow-up period to minimize the risk of additional complications that could alter the parameters. To clarify these findings and better understand the relationship between oxidative stress and hemorheological parameters, further studies with larger patient cohorts and longer follow-up periods are needed.

The most important limitation of our study was the relatively smaller number of patients. The fact that we could not determine RBC aggregation, one of the hemorheological parameters due to technical problems can be considered as a second limitation. Additionally, this study does not reveal the effects of EBCT longer than 1 month. Even though interventional treatment options are included in the guidelines for COPD treatment, controversy over EBCT continues. The results of this pilot study suggest for the first time that EBCT may not only reduce hyperinflation but also potentially increase erythrocyte deformability, which could improve tissue perfusion in COPD patients under shear stresses observed at the pulmonary level. To our knowledge, there are no similar findings reported in the literature. Despite limitations, our results provide supportive evidence on the benefit of EBCT in the treatment of COPD.

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EU.: Conception, Design, Supervision, Materials, Literature Review, Writing.

E.K.T: Conception, Data Collection and/or Processing, Analysis and/or Interpretation, Literature Review, Writing

N.C.: Design, Fundings, Materials, Data Collection and/or Processing, Literature Review, Writing

O.K.E.: Fundings, Data Collection and/or Processing, Analysis and/or Interpretation, Writing

H.P.: Fundings, Data Collection and/or Processing, Analysis and/or Interpretation, Writing G.A.: Design, Supervision, Writing, Critical Review

M.B.K.: Conception, Design, Supervision, Analysis and/or Interpretation, Literature Review, Writing, Critical Review

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Research Article

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Outcomes of patients with complement-mediated thrombotic microangiopathy

Kompleman aracılı trombotik mikroanjiyopatili hastaların sonuçları

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Abstract

Purpose: Complement-mediated thrombotic microangiopathy (CM-TMA) is a rare, progressive and life-threatening type of thrombotic microangiopathy (TMA) characterized by microangiopathic hemolytic anemia, thrombocytopenia and associated acute kidney disease (AKI) caused by dysregulation of the alternative complement pathway. The aim of this study was to retrospectively analyze the clinical features, follow-up, treatment and mortality of patients with CM-TMA.

Materials and methods: This was a retrospective study evaluating 13 patients diagnosed with CM-TMA who were followed retrospectively from 2024. Data were collected through a comprehensive review of electronic medical records of patients diagnosed with CM-TMA and receiving Eculizumab in the Department of Hematology and Nephrology.

Results: Thirteen patients with a mean age at diagnosis of 36.0±17.8 years were included. Age at disease onset ranged from 17 to 66 years. Only 3 (23.1%) patients were over 50 years of age. All patients were female. The mean follow-up period was 78.6±34.6 months. After an increase in GFR with eculizumab treatment, 76.9% of patients were withdrawn from dialysis.

Conclusion: CM-TMA was found to be predominant in young women. Eculizumab treatment provided significant improvements in clinical and laboratory values of the patients.

Keywords: CM-TMA, aHUS, C5 inhibitorleri, eculizumab, complement-mediated thrombotic microangiopathy.

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Öz

Amaç: Kompleman aracılı trombotik mikroanjiyopatili (CM-TMA), alternatif kompleman yolunun düzensizliğinden kaynaklanan mikroanjiyopatik hemolitik anemi, trombositopeni ve eşlik edebilen akut böbrek hastalığı (ABH) ile karakterize nadir, ilerleyici ve yaşamı tehdit eden bir trombotik mikroanjiyopati (TMA) türüdür. Bu çalışmada retrospektif olarak izlenilen CM-TMA hastaların klinik özellikleri, takibi tedavisi ve mortalitesinin incelemesi amaçlanmıştır.

Gereç ve yöntem: Bu çalışma, 2024 yılından itibaren geriye dönük olarak izlenilen 13 CM-TMA tanılı hastaları değerlendiren retrospektif bir çalışma olarak yapıldı. Veriler, Hematoloji ve Nefroloji Bölümü'nde CM-TMA tanısı konulan ve Eculizumab alan hastaların elektronik tıbbi kayıtlarının kapsamlı bir incelemesiyle toplanmıştır.

Bulgular: Tanı anındaki yaş ortalamaları 36,0±17,8 olan 13 hasta alındı. Hastalık başlangıç yaşı 17 ile 66 arasında değişmekteydi. 50 yaş üzerinde olan sadece 3 (%23,1) hasta vardı. Hastaların tümü kadındı. Hastaların ortalama takip süresi 78,6±34,6 aydı. Eculizumab tedavisi ile GFR de artma sonrası hastaların %76,9 diyalizden çıkarılarak iyileşti.

Sonuç: CM-TMA genç kadınlarda baskın olduğu görüldü. Eculizumab tedavisi ile hastaların klinik ve laboratuvar değerlerinde önemli düzelmeler sağladı.

Anahtar kelimeler: CM-TMA, aHUS, C5 inhibitörleri, eculizumab, kompleman aracılı trombotik mikroanjiyopati.

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Introduction

Complement-mediated thrombotic microangiopathy (CM-TMA), also called atypical hemolytic uremic syndrome (aHUS), is a rare, difficult-to-diagnose disease with a serious risk of morbidity and mortality. Clinical criteria include thrombocytopenia, microangiopathic hemolytic anemia and threshold renal dysfunction. Often there may be no evidence of other TMA syndromes such as TTP or Shiga toxin hemolytic uremic syndrome. These criteria are not specific for CM-TMA and the diagnosis of CM-TMA can be challenging due to the lack of specific criteria [1].

In the last decade, great progress has been made in understanding the etiology and pathophysiology of CM-TMA. The role of complement regulation has emerged. The traditional classification of diarrhea-positive HUS (D+HUS) and diarrhea-negative HUS (D-HUS) was replaced by a new classification of HUS based on pathogenic mechanisms. This classification is organized considering the etiology of HUS: 1) HUS caused by infection (Shiga toxin-producing Escherichia coli, Streptococcus pneumoniae, Influenza A, human immunodeficiency virus); 2) HUS with coexisting diseases or conditions (bone marrow or solid organ transplantation, systemic malignancies, autoimmune conditions, drugs, malignant hypertension); 3) HUS due to cobalamin C deficiency; and 4) HUS due to alternative complement pathway dysregulation and mutation in the diacylglycerol kinase ε (DGKE) gene [2-5].

CM-TMA is relatively rare, with an estimated incidence of 0.23 to 1.9 per million population per year. Data are limited due to inconsistencies in definitions between studies and lack of general epidemiologic studies [6].

Anti-C5 monoclonal antibodies developed against complement subsequently became standard treatments. The introduction of Eculizumab ravulizumab and Iptacopa, which effectively block complement activation, drastically changed the treatment and outcomes of patients with CM-TMA due to alternative complement pathway dysregulation.

The aim of this study was to retrospectively analyze the clinical characteristics, follow-up, treatment and mortality of patients with CM-TMA followed up in the nephrology department.

Materials and methods

This was a retrospective study evaluating 13 patients with CM-TMA diagnosed and followed up between 2010 and 2024. Data were collected through a comprehensive review of the electronic medical records of patients diagnosed with CM-TMA and receiving Eculizumab in the Department of Hematology and Nephrology. We were able to identify 13 patients who met the inclusion criteria. All included patients met the CM-TMA criteria, including age older than 18 years, thrombocytopenia, signs of hemolysis and sudden deterioration in renal function.

The study was conducted in accordance with the Declaration of Helsinki and approval was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee (approval number: E-60116787-020-569349, dated 20.08.2024 and numbered 15).

Exclusion criteria were ADAMTS13 deficiency (less than 10% activity, Shiga toxin-associated CM-TMA, Direct Coombs test positive, Patients on chronic hemodialysis or peritoneal dialysis. Demographic data, including age, gender, clinical information, admission characteristics, laboratory results, and treatment administered were collected.

In this study, AKI was defined as an increase in serum creatinine of ≥0.3 mg/dL or ≥1.5 times baseline in the last 48 hours (known or assumed to have occurred in the last 7 days) or an increase in urine volume of <0.5 mL/kg per hour for 6 hours according to the Kidney Disease: Improving Global Outcomes guidelines defined as a urine volume <0.5 mL/kg per hour for 6 hours. Chronic kidney disease (CKD) was defined as the presence of kidney damage (typically urinary albumin excretion ≥30 mg/day or equivalent) or reduced renal function (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2) and persisting for 3 or more months. End-stage renal disease (ESRD) was defined as an eGFR <15 mL/min/1.73 m^2 with signs and symptoms of uremia such as nausea, vomiting and pericarditis with the need for dialysis.

Statistical analysis

Data were analyzed with SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.). Continuous variables were expressed as mean ± standard deviation and categorical variables as number and percentage.

Results

Thirteen patients with a mean age at diagnosis of 36.0±17.8 years were included. The age at disease onset ranged between 17 and 66 years. Only 3 (23.1%) patients were over 50 years of age. All patients were female.

İn total of 2 (15.4%) of the patients were transferred while being monitored in the neurology ward for altered consciousness. Four patients (30.8%) presented with diarrhea, two (15.4%) developed symptoms after pregnancy, and the remaining patients presented with gastrointestinal symptoms (38.4%).

At the time of diagnosis, all patients had anemia 13 (100%) and kidney disease (100%), and all but one patient had thrombocytopenia 12 (92.3%) (Table 1). C3 deficiency with hypocomplementemia was detected in 9 patients (69.2%) (Table 2).

Table 1. Mean values of patients with complement-mediated thrombotic microangiopathy at the time of diagnosis, at discharge after treatment and at the last follow-up visit

	At the time of Diagnosis mean at presentetation		While being discharged mean at presentetation		The last follow-up visit mean at presentetation	
		n (%)		n (%)		n (%)
WBC <4 K/uL	15.4±8.2					
Hb <12 g/dL	7.3±1.6	13 (100)	10.6±1.3	11 (84.6)	13.1±1.7	3 (23.1)
PLT <150 K/uL	80.3±57.7	12 (92.3)	280.2±112	1 (7.7)	250.5±58	0 (0)
Urea >48.5 mg/dL	110.2±46.0	12 (92.3)	50.3±32.3	6 (46.2)	45.46±42.8	3 (23.1)
Creatinine >0.95 mg/dL	3.6±1.5	13 (100)	2.0±1.7	8 (61.5)	2.2±2.2	6 (46.2)
LDH >214 U/L	1263.7±853	3 (100)	255.1±118.1	9 (69.2)	180.3±38.0	2 (15.4)

Anemia: Hb <12 g/dL in women, 13 g/dL in men, Hb: Hemoglobin, Plt: A platelet count, LDH: Lactate dehydrogenase

Table 2. Test values of patients with complement-mediated thrombotic microangiopathy at diagnosis

Laboratory parameters	Patient n (%)
Elevated ALT (>41 IU/L)	6 (46.2)
Elevated AST (>40 IU/L	7 (53.8)
Elevated T. Bil (>1.2 mg/dL)	6 (46.2)
Elevated I. Bil (>0.9 mg/dL)	5 (38.5)
Elevated CRP (<0.5 mg/dL)	10 (76.9)
Low C3 (90-180 mg/dL)	9 (69.2)
Low C4 (10-40 mg/dL)	2 (15.4)

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TBil: Total bilirubin, IBil: Indirect bilirubin, CRP: C-reactive protein

The mean follow-up period was 78.6±34.6 months. Supportive therapies such as erythrocyte suspension, platelet suspension, plasmapheresis and hemodialysis were provided. Eculizumab treatment was started within 1 month. 8 (61.5%) of the patients are still continuing Eculizumab treatment. The 3 patients who did not continue Eculizumab treatment were patients who developed CM-TMA while being followed up with a diagnosis of kidney transplantation. All 3 renal transplant patients were receiving classical triple immunosuppression with mycophenolate mofetil and tacrolimus and methylprednisolone. 3 patients continued with dialysis. One of the patients died in the follow-up. Since the social security institution did not cover the treatment of 2 patients, treatment could not be continued (Table 3).

Table 3. Treated patients

	n (%)
Receiving Eculizumab	13 (100)
Eculizumab ongoing	8 (61.5)
Receiving dialysis treatment	13 (100)
Removed from dialysis	10 (76.9)

Discussion

All patients in the study had anemia, thrombocytopenia and ABH at the time of diagnosis. The median age at presentation was 35 years and all patients were female. The number of females was similar to the studies by Sperati et al. [7] and Amisha et al. [8] 82.4% and 75%, respectively.

Neurologic involvement is the most important mortality and morbidity of non-renal involvement which can be seen in 20-50% of CM-TMA patients [9]. Neurologic symptoms ranging from irritability to coma may occur due to cerebral microangiopathy, cerebral edema or delay in treatment. Brocklebank et al. [10] found the rate of patients presenting with neurologic symptoms to be 22%. In this study, the proportion of patients presenting with neurologic symptoms was 15.4%.

Low C3 level indicates dysregulation in the complement cascade of CM-TMA cases but is not necessary for the diagnosis of aHUS. Different rates have been reported in the literature regarding the presence of low C3 levels in atypical hemolytic uremic syndrome. In a study of 19 patients by Conkar et al. [11] low C3 level was found to be 10.5%, while in a study of 15 patients by Baskin et al. [12] low C3 level was found to be 50%. Štolbová et al. [13] reported low C3 levels in 71% of 21 pediatric aHUS patients. Kara and Kılıç et al. [14] found 64.3%. Similar to this study, we found low C3 levels in 69.2% of 9 patients.

Before eculizumab treatment, CM-TMA was treated with plasma exchanges or infusions. However, plasma exchanges did not affect the underlying problem and only maintained hematologic parameters. CM-TMA was therefore associated with high morbidity and mortality. In 2011, eculizumab was approved by the FDA and EMA for the treatment of CM-TMA, which significantly improved patient lives by inhibiting the underlying mechanism of CM-TMA. Open-label studies in adult patients showed that after 26 weeks, eGFR improved significantly and 79% of patients were taken off dialysis [12]. In this study, all patients were dialyzed. After a fall in GFR with eculizumab treatment, 76.9% of patients were weaned from dialysis.

In conclusion, the clinical diagnosis of CM-TMA can be challenging, especially when associated with non-renal manifestations. CM-TMA appeared to predominate in young women. The decision to discontinue eculizumab treatment or change treatment to ravulizumab or Iptacopa is likely to reduce health care costs and change patient compliance and safety. Larger multicenter studies or trials are needed to further confirm the findings of this study.

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Limitation: The small sample size of the study was an important limitation.

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

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Can cognitive impairment be observed independently of neurological symptoms in Behçet's disease?

Behçet hastalığında nörolojik semptomlardan bağımsız olarak bilişsel bozukluk mevcut olabilir mi?

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Abstract

Purpose: Behçet's disease (BD) is a chronic, multisystem inflammatory disorder that causes mortality and morbidity. Despite data indicating cognitive impairment in patients without neurological involvement, there is currently no consensus on how to screen patients. The Montreal Cognitive Assessment (MOCA) is a practical, easy-to-use screening scale that can detect mild cognitive impairment. We aimed to detect cognitive dysfunction with MOCA in BD without neurological findings.

Materials and methods: This prospective study included patients diagnosed with BD without neurological findings, and healthy individuals matched for age, gender, and education. Behçet's Disease Current Activity Form (BDCAF) was applied to determine disease activity, and MOCA was applied to all participants.

Results: The total score of the MOCA scale was significantly lower in Behçet's patients than in the control group (p=0.001). While no difference was found between BD and controls in terms of MOCA subtests ‘‘Orientation’’ and ‘‘Abstraction’’ (p=0.667, p=0.077, respectively), scores in other subtests were significantly lower in patients. A negative correlation was found between BDCAF scores and total MOCA scores (r=-0.454, p=0.000). A positive correlation was found between total MOCA score and years of education (r=0.345, p=0.000).

Conclusion: In BD, a decrease in cognitive functions may exist without neurological involvement. Cognitive screening of patients with BD is crucial for detecting subclinical inflammation and improving quality of life. Our results demonstrate that the MOCA is an effective tool for detecting cognitive function decline. However, further large-scale, multi-center studies are needed to establish its routine use.

Keywords: Behçet disease, cognitive impairment, montreal cognitive assessment.

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Öz

Amaç: Behçet hastalığı (BH) birçok sistemin etkilendiği mortalite ve morbiteye yol açan kronik inflamatuvar bir hastalıktır. BH’de nörolojik tutulum bulguları olmaksızın hastalarda bilişsel fonksiyonların etkilendiğine dair veriler olsa da bu hastaların taramasına yönelik br fikir birliğine henüz ulaşılamamıştır. Montreal Bilişsel Değerlendirme (MOBİD), hafif düzeyde bilişsel fonksiyon bozuklukları tespit edebilen pratik, kolay erişilebilir bir tarama ölçeğidir. Çalışmamızda nörolojik bulguları olmayan BH’de MOBİD ile bilişsel fonksiyon bozuklukları tespit edebilmeyi amaçladık.

Gereç ve yöntem: Bu prospektif çalışmaya BH tanısı alan ve nörolojik bulgusu olmayan hastalar ile yaş, cinsiyet ve eğitim açısından eşleştirilmiş sağlıklı bireyler dahil edildi. Hastalık aktitesini belirlemek için Behçet Hastalığı Güncel Aktivite Formu (BDCAF) uygulanırken tüm katılımcılara MOBİD uygulandı.

Bulgular: MOBİD ölçeğinin toplam puanı Behçet hastalarında kontrol grubuna göre belirgin olarak düşüktü (p=0,001). MOBİD alt testlerinden ‘‘Yönelim’’ ve ‘‘Soyutlama’’ açısından BH ve kontroller arasında fark saptanmamışken (sırasıyla, p=0,667, p=0,077) diğer alt testlerde puan hastalarda anlamlı olarak düşüktü. BDCAF skorlarıyla toplam MOBİD puanı arasında negatif korelasyon saptandı (r=-0,43, p=0,000). Total MOBİD puanı ile eğitim yılı arasında pozitif korelasyon saptandı (r=0,35, p=0,000).

Sonuç: BH’de nörolojik tutulum olmaksızın bilişsel fonksiyonlarda azalma mümkündür. BH’de hastaların bilişsel açıdan taranması subklinik inflmasyonun tespit edilmesi ve yaşam kalitesinin iyileştirilmesi için önemlidir. Sonuçlarımız, MOBİD tarama ölçeği ile bilişsel fonksiyonlardaki azalma pratik bir şekilde saptanabileceğini göstermiş olsa da rutin kullanıma girmesi için geniş çaplı, çok merkezli çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Behçet hastalığı, bilişsel bozukluk, montreal bilişsel değerlendirme.

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Introduction

Behçet's disease (BD) is a chronic inflammatory disease that presents with recurrent oral and genital ulcers and ocular, vascular and neurological involvement [1-3]. The prevalence of BD varies widely according to geographical location and ethnic group. The highest prevalence was reported in Türkiye with 420 cases per 100,000 people [1, 4].

Neurological involvement in BD is one of the main causes of long-term morbidity and mortality [5]. Neurological findings associated with this condition include brainstem syndrome, a syndrome resembling multiple sclerosis, movement disorders, meningoencephalitis syndrome, myelopathic syndrome, cerebral venous sinus thrombosis, and intracranial hypertension [6]. Neurocognitive functions are a set of skills localized within the brain that include attention, information processing, memory, language, visual-perceptual processing, reasoning, impulse control, planning, and organization. These functions can be affected by acquired neurological damage [7]. Cognitive impairment is common in Behçet syndrome; working memory, recall, frontal executive functions and attention are primarily affected [8]. Cognitive dysfunction is more severe in individuals with neurological involvement but is also seen in patients without abnormal imaging findings and without other neurological symptoms [7].

It seems probable that impaired neurocognitive functioning in both BD and neuro-Behçet's disease (NBD) is the result of a number of different factors. It is evident that patients diagnosed with both BD and NBD are more prone to experiencing elevated rates of depression and anxiety disorders in comparison to individuals who are free of such ailments. It is established that these psychological disorders have an impact on neurocognition [9]. Furthermore, evidence indicates that pharmacological agents employed in therapeutic regimens, such as corticosteroids, may influence cognitive functions in BD [10].

It is also the case that neurocognitive disorders affecting memory, visuospatial awareness, attention, and frontal-executive functions can occur in patients without neurological involvement. Such cases are sometimes referred to as "subclinical NBD" [10]. It is therefore imperative to enhance our comprehension of cognitive functioning in BD patients who do not exhibit overt neurological impairment, given that the majority of existing research has concentrated on individuals presenting with neurological symptoms.

There is a paucity of studies that evaluate cognitive functions in patients with BD. Furthermore, different assessment scales have been employed in these studies [10-13]. While it is challenging to identify an optimal method for evaluating cognitive functions in patients, there is a necessity for screening tests that can be readily administered by patients and are not time-consuming, even for mild cognitive disorders.

The Montreal Cognitive Assessment (MOCA) is a freely accessible, brief screening tool initially designed in 1996 to identify early cognitive impairment in dementia [14]. Özdilek et al. [15] demonstrated the validity and reliability of the Turkish version of the Montreal Cognitive Assessment Scale for screening cognitive dysfunction in patients with Parkinson's disease. The MOCA is a single-page, straightforward, and brief scale that can be administered in approximately 10 minutes. The scale comprises items that assess various cognitive domains, including attention, concentration, executive functions, memory, language, visual-spatial abilities, abstract thinking, and calculation.

The aim of this study was to detect neurocognitive impairment using the MOBID screening tool in patients with BD without significant neurological symptoms or history, and to investigate its association with depression, anxiety, prednisone use, neuroimaging, human leukocyte antigen (HLA-B51) and disease activity.

Materials and methods

Patients and controls

Patients over 18 with BD diagnosed at Pamukkale University Dermatology and Denizli State Hospital Rheumatology were included in the study. The study population comprised patients with BD who met the International Criteria for Behçet's Disease

(ICBD) and had no significant neurological findings, including aseptic meningitis, brainstem or spinal cord involvement, optic neuritis, epileptic seizures, peripheral neuropathy, demyelinating syndromes, stroke, and cerebral venous thrombosis. Additionally, individuals without chronic rheumatologic or dermatologic diseases, matched for age, gender, and education, were included. The following criteria were used to exclude patients from the study: a history of psychiatric disease, age below 18 years, vision loss, mental retardation, history of malignancy and substance abuse. Patients were subjected to testing for HLA-B51. The Non-Interventional Clinical Research Ethics Committee of Pamukkale University approved the study (date: 2024, number: E-60116787). All patients and control subjects provided informed consent by the Declaration of Helsinki before they participated in this study.

Clinical assessment and scales

The Beck Depression Inventory (BDI) and the BD Current Activity Form (TR-BDCAF) were administered to all subjects. The BDI is one of the most widely used self-report measures of depression in both research and clinical practice [16]. The BDCAF assesses all types of involvement and disease activity in BD [17]. This form is filled out by the clinician and is evaluated considering the day the patient arrives and the last 4 weeks. It is used for new attacks in the last 4 weeks rather than ongoing chronic inflammation. It evaluates clinical findings such as oral and genital ulcers, skin lesions, fatigue, headache, gastrointestinal lesions and joint pain or arthritis. Active findings seen in the last 4 weeks in all systems are scored and a score between 0 and 12 is obtained. Patients with more than four points are considered to have active disease. Montreal Cognitive Assessment scale was used to test cognitive functions in people with BD and healthy controls with similar educational levels. The lowest score is 0 and the highest is 30. The Turkish version has a threshold score of 21 [18].

Statistical analysis

The statistical calculations were done using the SPSS 26.0 program. Shapiro-Wilk test was used to evaluate the normality assumption. The chi-square test was used to compare categories. Student t-test for parametric data and Mann-Whitney U test for non-parametric data. Categorical variables were expressed as a number and percentage. Continuous variables were expressed as a mean and standard deviation. Pearson correlation was used to test the linear correlation between two numerical variables when the parametric test assumptions were met. Spearman's correlation was used when the parametric test assumptions were not met. $p<0.05$ was considered statistically significant.

Results

A total of 61 patients were included in the Behçet patient group, while the control group comprised 49 patients. The mean disease duration was 11.7±6.6 years, with a BDCAF score of 5.1±2.5. No statistically significant difference was observed between the control and patient groups with regard to age, gender, educational status, and Beck Depression Scale scores *(*$p=0.536$*,* $p=0.876$*,* $p=0.586$*,* $p=0.734$*, respectively*). The total score of the MOCA scale, which is used to evaluate cognitive functions, was found to be significantly lower in patients with BD ($p=0.001$). No significant difference was observed between the Behçet patient and control groups in terms of the MOCA subtests "Orientation" and "Abstraction" ($p=0.667$, $p=0.077$, respectively). However, the scores of the Behçet patients in the remaining subtests were found to be significantly lower (Table 1). When the patients were evaluated according to gender, the mean age in male patients was significantly lower than in female patients ($p=0.001$). Although BDCAF scores were higher in males, the difference was not significant. ESR levels were significantly higher in female patients ($p=0.036$). BDI scores were significantly higher in female patients than in male patients ($p=0.033$). Although the total score of the MOCA scale and the scores of its subtests except for "Abstraction" were higher in male patients, there was no significant difference according to gender (Table 2). 21 patients with BD were using steroids. Patients using SCS had higher CRP, ESR and BDCAF scores than those not using SCS. The total MOCA scale score was higher in patients using SCS, but the difference was not statistically significant.

Table 1. Demographic, laboratory and clinical characteristics of Behçet's patients and the control group

	Behçet's patients (n=61)	Control group (n=49)	Test value	*p* value
Age (years)	42.9±9.1	44.4±9.0	z=-0.619	*p=0.536*
Gender (Female/Male)	44/17	36/12	cs:0.025	*p*=0.876
Average years of schooling	11.2±3.2	10.8±3.8	z=-0.545	*p*=0.586
ESR	19.3±17.4	13.3±12.9	z=-1.900	*p*=0.057
CRP	7.3±11.4	4.1±9.1	z=-3.355	*p*=0.001*
BDI	17.3±8.9	17.8±9.3	z=-0.274	*p*=0.734
MOCA-Visuospatial / Executive	3.0±1.5	4.1±1.1	z=-3.715	*p*=0.000*
MOCA-Naming	2.4±0.5	2.8±0.3	z=-4.500	*p*=0.000*
MOCA-Delayed recall	1.3±1.2	3.0±1.3	z=-2.271	*p*=0.023*
MOCA-Attention	4.0±1.5	4.8±1.3	z=-3.109	*p*=0.002*
MOCA-Language	2.6±0.7	2.6±0.6	z=-3.255	*p*=0.001*
MOCA-Abstraction	0.9±0.7	1.2±0.7	z=-1.771	*p*=0.077
MOCA- Orientation	5.5±0.9	5.7±0.5	z=-0.417	*p*=0.667
Total MoCA Score	20.6±4.1	24.5±3.3	z=-4.730	*p*=0.001*

*$p<0.05$, Categorical data were evaluated using chi-square test, ^z: Mann–Whitney's U test were used, Φcs: chi-square
CRP: C reactive protein, ESR: erythrocyte sedimentation rate, BDI: Beck Depression Inventory, MOCA: Montreal Cognitive Assessment

Table 2. MOCA scores, demographic and laboratory characteristics of Behçet's disease patients according to gender

	Female (n=44)	Male (n=49)	Test value	*p* value
Age (years)	45.5±7.5	36.4±9.8	z=-3.434	*p*=0.001*
Average years of schooling	10.6±2.9	12.86±3.2	z=-2.228	*p*=0.026*
ESR	22.1±18.7	12.3±11.1	z=-2.029	*p=0.036**
BDCAF	4.7±2.2	6.1±3.1	z=-1.594	*p*=0.111
BDI	19.3±9.4	13.9±6.0	z=-2.129	*p*=0.033*
MOCA-Visuospatial / Executive	2.9±1.6	3.2±1.2	z=-0.645	*p*=0.519
MOCA-Naming	2.4±0.5	2.6±0.4	z=-1.534	p=0.124
MOCA-Attention	3.9±1.6	4.1±1.2	z=-0.58	*p*=0.954
MOCA-Language	2.1±0.7	2.2±0.7	z=-0.305	*p*=0.761
MOCA-Abstraction	0.9±0.7	0.8±0.7	z=-0.647	*p*=0.518
MOCA- Orientation	5.5±0.9	5.7±0.4	z=-0.212	*p*=0.832
Total MOCA score	20.4±4.3	21.2±3.4	z=-0.323	*p*=0.747

*$p<0.05$, ^z: Mann–Whitney's U test was used. ESR erythrocyte sedimentation rate, BDCAF Behçet's Disease Current Activity Form
BDI Beck Depression Inventory, MOCA Montreal Cognitive Assessment

There was no significant difference in MOCA subtest scores between patients using SCS and those not using SCS (Table 3). There was a link between BDCAF scores and ESR (r=0.303, *p*=0.001) and CRP (r=0.359, *p*=0.000) levels. BDCAF scores also correlated with duration of disease (r=0.798 *p*=0.000). However, BDCAF scores were negatively correlated with total MOCA score (r=-0.454, *p*=0.000). There was a positive link between the total MOCA score and the number of years of education (r=0.345, *p*=0.000). Correlations of total MOCA score with clinical and demographic characteristics are summarized in Table 4. In 41 (67.2%) of the patients with BD, HLA-B51 was positive. HLA-B51 negative patients scored higher on the MOCA scale than HLA-B51 positive patients, but the difference was not statistically significant. There was no significant difference in MOCA subtest scores between HLA-B51 positive and negative patients (Table 5).

Table 3. Comparison of laboratory and assessment scale scores of Behçet's patients according to systemic corticosteroid use

	SCS used (n=21)	SCS non-used (n=40)	Test value	*P* value
Duration of disease (years)	11.1±6.7	12.1±6.6	z=-0.282	*p*=0.545
BDCAF	6.9±2.5	4.1±2.0	z=-3.929	*p*=0.000*
ESR	25.3±19.9	16.1±15.1	z=-2.172	*p*=0.030*
CRP	12.5±17.4	4.5±4.4	z=-2.099	*p*=0.036*
BDI	21.0±8.7	16.1±8.6	z=-2.716	*p*=0.007*
MOCA-Visuospatial /Executive	3.1±1.4	3.0±1.4	z=-0.351	*p*=0.725
MOCA-Naming	2.6±0.4	2.4±0.5	z=-4.500	*p*=0.137
MOCA-Attention	4.2±1.3	3.8±1.6	z=-0.776	*p*=0.438
MOCA-Language	2.3±0.4	2.1±0.7	z=-1.007	*p*=0.314
MOCA-Abstraction	0.8±0.6	0.9±.0.7	z=-0.842	*p*=0.400
MOCA- Orientation	5.5±0.4	5.6±0.9	z=-0.200	*p*=0.842
Total MOCA score	21.4±3.4	20.2±4.3	z=-0.899	*p*=0.318

*$p<0.05$, ^z: Mann-Whitney's U test was used. SCS systemic corticosteroid, BDCAF Behçet's Disease Current Activity Form BDI Beck Depression Inventory, MOCA Montreal Cognitive Assessment

Table 4. Correlation of total MOCA score with clinical and demographic characteristics

	Total MOCA Score	
	***Rho* value**	***p* value**
Duration of disease (years)	r=-0.450	*p*=0.000*
BDCAF	r=-0.454	*p*=0.000*
Average years of schooling	r=0.345	*p*=0.000*
BDI	r=-0.125	*p*=0.192
Age	r=-0.238	*p*=0.013*

*$p<0.05$, BDCAF Behçet's Disease Current Activity Form, BDI Beck Depression Inventory, MOCA Montreal Cognitive Assessment

Table 5. Clinical characteristics, MOCA scores, and laboratory features of Behçet according to HLA-B51 positivity

	HLA-B51 positive (n=21)	HLA-B negative (n=40)	Test value	*p* value
Duration of disease (years)	11.1±6.7	12.1±6.6	z=-0.804	*p*=0.421
BDCAF	5.7±2.6	3.8±1.9	z=-3.339	*p*=0.001*
BDI	18.3±9.0	16.8±8.7	z=-0.445	*p*=0.656
MOCA-Visuospatial/ Executive	2.9±1.6	3.3±1.0	z=-0.441	*p*=0.659
MOCA-Naming	2.5±0.5	2.4±0.5	z=-0.180	*p*=0.986
MOCA-Delayed recall	2.4±1.1	2.6±1.2	z=-0.932	*p*=0.351
MOCA-Attention	4.0±1.5	3.9±1.3	z=-0.963	*p*=0.335
MOCA-Language	2.1±0.6	2.1±0.8	z=-0.671	*p*=0.502
MOCA-Abstraction	0.8±0.6	1.0±0.7	z=1.851	*p*=0.064
MOCA- Orientation	5.5±0.9	5.7±0.5	z=-0.399	*p*=0.690
Total MOCA score	20.2±4.3	21.3±3.6	z=-0.556	*p*=0.578

*$p<0.05$, z^: Mann–Whitney's U test were used, BDCAF Behçet's Disease Current Activity Form, BDI Beck Depression Inventory MOCA Montreal Cognitive Assessment

Discussion

In 27-75% of patients diagnosed with BD, subclinical neurological abnormalities were detected in neuroradiological, neurophysiological or neuropsychological examinations despite the absence of obvious neurological findings [19-22]. Concurrently, patients afflicted with BD who have not been diagnosed with NPH also exhibit elevated rates of cognitive impairment when compared to the general population [10-13]. Prior research has indicated that between 40% and 46% of individuals diagnosed with bipolar disorder who do not present with neurological symptoms exhibit deficits in memory and visuospatial abilities [10, 23]. In another study, 41% of patients diagnosed with bipolar disorder who did not present overt neurological symptoms were observed to exhibit deficits in executive functions, language abilities, and visual-constructional skills [13]. The present study revealed significant dysfunction in memory, language, attention, and visual-constructional abilities among patients compared to the control group.

Depression and anxiety are thought to affect cognitive functions, and there is some evidence to suggest that the neurocognitive impairment observed in BD may be attributable to this factor [24-26]. The current study revealed no statistically significant differences between the patient and control groups with regard to anxiety and depression. Similarly, the study by Özen et al. [11] revealed no correlation between depression levels and cognitive dysfunctions. This indicates that cognitive dysfunctions cannot be attributed to psychological comorbidities in isolation.

It has been put forth that another factor influencing cognitive functions is corticosteroids, which are among the most commonly utilized treatments [27, 28]. Monastero et al. [10] reported that cognitive impairment in BD patients may occur independently of significant neurological involvement and is more common in patients receiving prednisone. Conversely, an alternative study indicated that prednisone administration had a beneficial impact on cognitive performance [22]. The findings of our study indicate that patients who were using corticosteroids exhibited a greater degree of disease activity. Nevertheless, no significant discrepancy was observed in MOCA scores between patients undergoing steroid therapy and those not receiving such treatment. These contradictory results suggest that steroids may have protective effects by suppressing inflammation, in addition to their adverse effects on the central nervous system.

The relationship between BDCAF scores in BD was previously investigated, with BDCAF scores being found to be significantly elevated in patients with cognitive dysfunction [10]. Similarly, our study revealed a negative correlation between the BDCAF score and the total MOBID score. This indicates that the disease may exert an influence on cognitive functions, in addition to other contributing factors.

HLA-B51 is a genetic marker that is frequently associated with BD. The presence of HLA-B51 may affect the clinical manifestations of the disease. Although there is no direct evidence that HLA-B51 is associated with cognitive impairment, its effects on BD, including chronic inflammation and psychological stress, have led to the assumption that it may indirectly affect cognitive dysfunction [8, 29]. However, Cavaco et al. [23] reported that they did not find an association between HLA-B51 and cognitive function in their study. Similarly, our study found no significant difference in cognitive function between patients with and without HLA-B51. Concurrently, Cavaco et al. [23] and colleagues reported that they were unable to identify a correlation between HLA-B51 and cognitive functions in their study. Similarly, our study found no significant difference in cognitive function between patients with and without HLA-B51. The findings of our study corroborate the data indicating that cognitive functions are affected despite the absence of overt neurological involvement in BD [10, 12]. It has been proposed that cognitive impairment is linked to both cerebral parenchymal lesions and brainstem lesions in NBD, while in neurologically silent BD, it is associated with white matter lesions in the frontal lobes [30]. Cognitive impairments in patients with BD have a markedly deleterious impact on quality of life and employment prospects. Early detection of cognitive dysfunction in patients with BD is the first step to improving outcomes. Given the inherent challenges associated with the administration of comprehensive neuropsychological tests in routine clinical practice, only patients exhibiting overt disease manifestations can be tested. This may result in an inadequate diagnosis and subsequent inadequate treatment. It is therefore evident that there is a need for the development of simple, inexpensive, and sensitive brief cognitive screening tools for clinical use [13, 30].

The MOCA test has been successfully employed in the diagnosis of cognitive impairment in numerous connective tissue diseases, including Sjögren syndrome, rheumatoid arthritis, and systemic lupus erythematosus (SLE) [31-34]. In a previous study, the effectiveness of three screening tests, MOCA, Mini-Mental State Examination (MMSE), and Cognitive Symptom Inventory (CSI), was compared with the gold standard neuropsychological battery to determine the most effective screening test for cognitive impairment in patients with SLE. The MOCA test was reported to have the highest concordance with the gold standard test in terms of sensitivity (84%) and specificity (100%), and to be more effective than the MMSE (AUC=92.6%; sensitivity, 54.8%; specificity, 100%) and CSI (AUC=30.6%; sensitivity, 54.8%; specificity, 30.76%) screening tools [32]. Similarly, another study demonstrated that MOCA is a more effective screening test than MMSE in determining CD in SLE patients [33]. This study demonstrated that neurocognitive functions in patients with BD were significantly affected by the MOCA scale in comparison to the control group.

The limitations of the study are the relatively low sample size and the fact that a different screening questionnaire was not used for comparison. However, the study's key strengths lie in its comprehensive approach to the clinical characteristics of the patients, its prospective design, and the inclusion of a control group.

In conclusion, cognitive impairment may occur in patients with BD in the absence of overt neurological symptoms. In order to utilise the MOCA screening scale, which is a practical assessment tool, for the early detection of subclinical neurological involvement and cognitive impairment, further studies with larger patient groups in the BD patient group are required.

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

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Research Article

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Alternative day fasting protocol attenuates high fructose-induced activation of the TGF-beta/Smad signaling pathway

Alternatif günlerde açlık yüksek fruktoz kaynaklı TGF-beta/Smad sinyal yolağının aktivasyonunu azaltır

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Abstract

Purpose: This study aims to explore the protective effects of alternate-day fasting (ADF) against metabolic disturbances induced by high fructose (HF) intake, with a particular focus on modulating the transforming growth factor-beta1 (TGF-β1) / mother against decapentaplegic homolog 2 (Smad2) signaling pathway.

Materials and methods: Four groups of rats (n=7 per group) were included: Control, ADF, HF (20% fructose in drinking water), and HF+ADF. The ADF protocol was applied with 24 hours of ad libitum feeding followed by 24 hours of fasting over a 5-week period. After five weeks, body weight (BW), muscle, and fat mass were measured. Serum samples were analyzed using ELISA to assess levels of TGF-β1, Smad2, connective tissue growth factor (CTGF), and total oxidant-antioxidant status (TOS-TAS).

Results: Results indicated that HF significantly increased final BW, and ADF reduced this weight gain (p=0.001). ADF also led to lower gastrocnemius-soleus muscle weights compared to controls (p=0.001), but mitigated fructose-induced retroperitoneal fat accumulation. TAS levels were higher (ADF vs control (p=0.01); HF vs HF+ADF (p=0.001)), and TOS levels were lower (ADF vs control (p=0.022); HF vs HF+ADF (p=0.001)) in the ADF groups, showing an antioxidant shift. Moreover, ADF significantly attenuated the TGF-β1/Smad2 pathway activation by decreasing serum TGF-β1 (ADF vs control (p=0.011); HF vs HF+ADF (p=0.008)), Smad2 (ADF vs control (p=0.001); HF vs HF+ADF (p=0.001)), and CTGF (ADF vs control (p=0.018); HF vs HF+ADF (p=0.001)) levels, suggesting a protective role against fructose-induced metabolic dysregulation.

Conclusions: These findings suggest that ADF could be an effective dietary intervention for mitigating the metabolic impact of excessive fructose intake, particularly by regulating oxidative stress and the TGF-β1/Smad2 pathway.

Keywords: Alternate-day fasting, high-fructose, TGF-β1/Smad2 pathway, oxidative stress, metabolic health.

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Öz

Amaç: Bu çalışma, yüksek fruktoz (HF) alımının yol açtığı metabolik bozukluklara karşı alternatif gün orucu (ADF) uygulamasının koruyucu etkilerini incelemeyi amaçlamakta olup, özellikle transformasyon büyüme faktörü-beta1 (TGF-β1) / dekapentaplejik homolog 2'ye karşı ana protein (Smad2) sinyal yolunun modülasyonuna odaklanmaktadır.

Gereç ve yöntem: Sıçanlar dört grupta (her grup için n=7) çalışmaya dahil edilmiştir: Kontrol, ADF, HF (içme suyunda %20 fruktoz), ve HF+ADF. ADF protokolü, 5 hafta boyunca 24 saat serbest yem tüketimi ve onu takiben 24 saat açlık olarak gerçekleştirilmiştir. Beş hafta sonunda vücut ağırlığı (VA), kas ve yağ kütlesi ölçülmüştür. Serum örneklerinde TGF-β1, Smad2, bağ dokusu büyüme faktörü (CTGF) ve total oksidan-antioksidan (TOS-TAS) düzeyleri ELISA yöntemi ile analiz edilmiştir.

Bulgular: Sonuçlar, HF'nin final VA'yı anlamlı derecede artırdığını ve ADF'nin bu kilo alımını azalttığını göstermiştir (p=0,001). ADF, kontrol grubuna kıyasla gastroknemius-soleus kas ağırlıklarını azaltmış (p=0,001), ancak fruktoz kaynaklı retroperitoneal yağ birikimini hafifletmiştir. ADF gruplarında TAS düzeyleri daha yüksek (ADF vs kontrol (p=0,01); HF vs HF+ADF (p=0,001)), TOS düzeyleri ise daha düşük (ADF vs kontrol (p=0,022); HF vs HF+ADF (p=0,001)) bulunmuş olup, bu da bir antioksidan kaymasına işaret etmiştir. Ayrıca, ADF, serum TGF-β1 (ADF vs kontrol (p=0,011); HF vs HF+ADF (p=0,008)), Smad2 (ADF vs kontrol (p=0,001); HF vs HF+ADF (p=0,001)) ve CTGF (ADF vs control (p=0.018); HF vs HF+ADF (p=0.001)) seviyelerini düşürerek TGF-β1/Smad2 yolunun aktivasyonunu önemli ölçüde azaltmış olup fruktoz kaynaklı metabolik düzensizliklere karşı koruyucu bir role işaret etmektedir.

Sonuç: Elde edilen veriler ADF'nin, özellikle oksidatif stres ve TGF-β1/Smad2 yolağını düzenleyerek, aşırı fruktoz alımı ile gerçekleşen metabolik etkileri hafifletmek için etkili bir diyet müdahalesi olabileceğini düşündürmektedir.

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Anahtar kelimeler: Alternatif günlerde açlık, yüksek fruktoz, TGF-β1/Smad2 yolağı, oksidatif stres, metabolik sağlık.

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Introduction

Fructose consumption has risen significantly over the past three to four decades, mainly due to its use as a food additive. It is commonly found in dietary sugars like sucrose and high fructose (HF) corn syrup, with excessive intake strongly linked to metabolic diseases and obesity [1, 2]. Studies in rats show that HF intake leads to weight gain and tissue alterations, disrupting cartilage function [3]. Unlike glucose, fructose undergoes a less restrictive metabolism, quickly converting to triglycerides and contributing to cellular ATP depletion [4]. This rapid conversion has been associated with increased visceral adipose tissue and total abdominal fat [5], as well as abnormal collagen formation [6]. However, the precise cellular and molecular mechanisms by which HF intake causes toxicity remain unclear.

Obesity and HF consumption are known to increase oxidative stress, particularly within tissue-specific contexts that regulate metabolic inflammation [7]. Excessive fructose intake can initiate a damaging cycle, where oxidative stress promotes inflammation, cellular damage, and organ dysfunction, especially in the liver, kidneys, and cardiovascular systems [8]. Increased systemic fatty acids and proinflammatory cytokines due to HF intake elevate reactive oxygen species (ROS), thereby contributing to oxidative stress and affecting peripheral tissues [9].

Transforming Growth Factor-Beta (TGF-β) is a potent fibrogenic factor with essential roles in cellular processes, including proliferation, differentiation, apoptosis, migration, and extracellular matrix (ECM) synthesis [10]. TGF-β binds to type-1 and type-2 receptors on cell surfaces, inducing phosphorylation of mother against decapentaplegic homolog 2/3 (Smad2/3) proteins and initiating intracellular signaling. Smad proteins are necessary to transmit signals from active TGF-β1 receptor complexes to the nucleus, with Smad/connective tissue growth factor (CTGF) signaling being critical for TGF-β-induced fibrogenesis [11]. This pathway plays a vital role in various fibrotic disorders with CTGF amplifying the pro-fibrogenic effects of TGF-β1 and modulating TGF-β1/Smad signaling in mesenchymal cells and fibroblasts [12]. The expressions of TGF-β, CTGF, and Smad2 have been shown to increase with HF intake, contributing to metabolic disorders in rodents [13, 14]. Moreover, high glucose intake also induces ROS production and enhances TGF-β activation, which contributes to fibrotic and inflammatory diseases [15].

Dietary strategies are promising for intervention, as reducing energy intake can create a negative energy balance and lead to weight loss [16]. Intermittent fasting (IF) protocols, including alternate-day fasting (ADF), the 5:2 diet, and time-restricted feeding (TRF), are among the most studied [17, 18]. ADF typically involves alternating feast and fast days, with food available ad libitum on feast days and restricted on fast days, typically in 24-hour intervals [19]. IF has been proposed as a strategy to improve health, potentially reducing obesity and metabolic disorders, especially those associated with aging [16]. While ADF can decrease body weight (BW) and fat levels and may affect gastrocnemius-soleus muscle weights [20], more research is needed to determine whether ADF has lasting health benefits or could pose risks over the long term [18].

IF is widely recommended due to its potential to support weight control, reduce inflammatory cytokines, and lower oxidative stress, making it an attractive option for metabolic health [17]. These benefits are largely attributed to physiological adaptations triggered by fasting periods, where short-term fasting may induce mild oxidative stress, while longer-term fasting enhances antioxidant defenses, balancing ROS levels and reducing oxidative stress [21]. Additionally, IF has been reported to increase TGF-β levels, potentially ameliorating inflammatory immune disorders caused by obesity. Although previous studies have

extensively explored the effects of HF intake on oxidative stress and fibrotic pathways, research investigating the specific interaction between HF and ADF on the TGF-β/Smad signaling pathway remains limited. To date, there has been no comprehensive study analyzing the combined effects of HF consumption and ADF on TGF-β activation, oxidative balance, and related fibrogenic markers, particularly in experimental models.

While previous studies have examined the effects of HF intake on oxidative stress and fibrotic pathways, the role of ADF in mitigating these effects remains largely unexplored. Considering this point in the literature, our study aimed to be the first to investigate the effects of ADF on oxidative stress markers and the TGF-β1/Smad signaling pathway in rats exposed to HF intake. Our study aimed to investigate the impact of ADF on serum oxidative stress and TGF-β1/Smad pathway activation.

Materials and method

Ethics Committee approval, dated 27 June 2024 and numbered PAUHADY EK-2024/60758568-020-544586 was received from the local ethics council of Animal Experiments, Pamukkale University and conducted in accordance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals (USA). For this study, twenty-eight male Wistar rats, aged between 10 and 12 weeks, were obtained from the Pamukkale University Medical Experimental Research and Practice Center. The animals were housed under controlled conditions, with the temperature set at 23±2°C and humidity at 60±5%. They were exposed to a 12-hour light/dark cycle, with lights on from 7:00 A.M. to 7:00 P.M. No mortality or adverse effects were occurred during the study, and all animals remained healthy throughout the experimental period.

Experimental and study design

Twenty-eight Wistar male rats were randomly divided into four groups as shown in Table 1.

All groups were fed a standard rodent laboratory chow (Optima, Türkiye) based on the NRC-Requirement of Compounded Feed for Laboratory Mice and Rats (BIS) and the Nutrient Requirement for Maintenance, Growth, and Reproduction of Rats (NRC, 1995). The diet provided 300 kcal per 100 g of feed, consisting of 60% carbohydrates, 20% proteins, and 20% lipids.

Table 1. The experimental groups are described

Groups	Description
Control Group (n=7)	Rats fed ad libitum
ADF Group (n=7)	Rats subjected to ADF (24-hour fasting, 24-hour ad libitum feding/ 5 weeks)
HF Group (n=7)	Rats were fed ad libitum with 20% fructose added to their drinking water for 5 weeks
HF+ADF Group (n=7)	20% fructose was added to the drinking water of rats for 5 weeks + Rats subjected to ADF (24-hour fasting, 24-hour ad libitum feding / 5 weeks)

The IF protocol used was an ADF regimen, where the rats underwent a total fasting period of 24 hours, followed by 24 hours of ad libitum feeding for 5 weeks [22]. Throughout the experiment, all rats had free access to water. This ADF protocol was chosen based on rodent studies [22] and its widespread use in clinical practice, particularly for preventing metabolic diseases [23]. HF diet was created by adding 20% fructose to the drinking water of rats. The survival rate of all groups was 100%, with no observed mortality.

At the end of the experiment, the researchers sacrificed the rats under general anesthesia (using 10 mg/kg of 2% xylazine hydrochloride and 90 mg/kg of ketamine hydrochloride) after fasting them the previous night with free access to water. Blood samples were collected from the abdominal aorta into the tubes without EDTA, and then after the samples were centrifuged for 15 min at 3500 rpm for enzyme-linked immunosorbent assay (ELISA) analysis. The samples were stored at -80°C until the experimental analysis.

Measurement of Body Weight, Gastrocnemius Weight, and Visceral Fat Pad

The rats' BW was measured and recorded using a digital weighing scale at the beginning of the study and the fifth week. BW measurements were taken on non-fasting days to avoid fluctuations due to feed restriction. After sacrifice, retroperitoneal fat tissue and gastrocnemius were dissected and weighed.

Biochemical analysis

ELISA method was used to measure the serum levels TGF-β1 (Elabscience, E-EL0162, Texas/USA), Smad2 (Elabscience, E-EL-R2582, Texas/USA), CTGF (Elabscience, E-EL-R0259, Texas/USA), total antioxidant status (TAS), and total oxidant status (TOS) (BT Lab, E1512Ra, E1710Ra, Zhejiang/ China) using ready-to-use measurement kits according to the manufacturer's instructions

Statistical analysis

All calculations and power analysis were conducted using the G-power program (version 3.1.9.2. Heinrich-Heine-Universitat, Duesseldorf, Germany). The effect size reported in the reference study was substantial (d=1.22). Based on the assumption that a similar effect size (f=0.8) could be achieved in this study, which included 4 groups, a power analysis determined that a sample size of at least 28 rats (7 per group) would provide 80% power at a 95% confidence level.

Data were analyzed using IBM SPSS Statistics 23 software. Continuous variables are expressed as mean ± standard deviation. If the parametric test assumptions were met, a one-way analysis of variance (ANOVA) was performed for group comparisons, followed by the Tukey post hoc test. When the parametric test assumptions were not met, the Kruskal-Wallis variance analysis was used, with subsequent comparisons between independent groups performed using the Mann-Whitney U test with Bonferroni correction. $p<0.05$ threshold was used for statistical significance.

Results

Initial and final BW comparisons between the four groups are shown in Figure 1. At the beginning of the study, all groups had similar starting weights, with no significant differences in initial BW observed (Figure 1A). By the end of the 5-week period, the HF group showed the highest final BW, significantly exceeding the other groups. ADF reduced final BW in the HF+ADF group (240±11.59) compared to the HF group (296.25±9.91) (p=0.001), and the ADF group (237±10.73) had a significantly lower final BW than the control group (260±20.83) (p=0.031). No significant difference was observed between the ADF and HF+ADF groups (Figure 1B). These findings suggest that HF intake promotes weight gain, while ADF counteracts this effect.

Comparisons of muscle weight also showed significant differences between groups. Control group (2.51±0.56) exhibited significantly higher muscle weights than ADF group (1.67±0.23) (p=0.001). Similarly, HF group (2.54±0.46) had greater muscle weight than HF+ADF group (1.87±0.19) (p=0.005), although no significant difference was observed between control and HF groups. These findings indicate that ADF affects muscle mass, leading to reduced muscle weight (Figure 2A), potentially due to metabolic adaptations associated with fasting.

Additionally, the HF group exhibited significantly higher retroperitoneal fat weights than control group (p=0.005). The HF+ADF group (1.90±0.54) had a significantly decreased muscle weight compared to HF group (2.15±0.51) (p=0.041). Similarly, ADF group (0.86±0.15) showed a significantly lower muscle weight than control group (1.70±0.32) (p=0.001). The HF+ADF group also exhibited significantly higher retroperitoneal fat weights than ADF group (p=0.005). These results suggest that fructose consumption leads to fat accumulation, and that ADF may partially modulate this accumulation (Figure 2B).

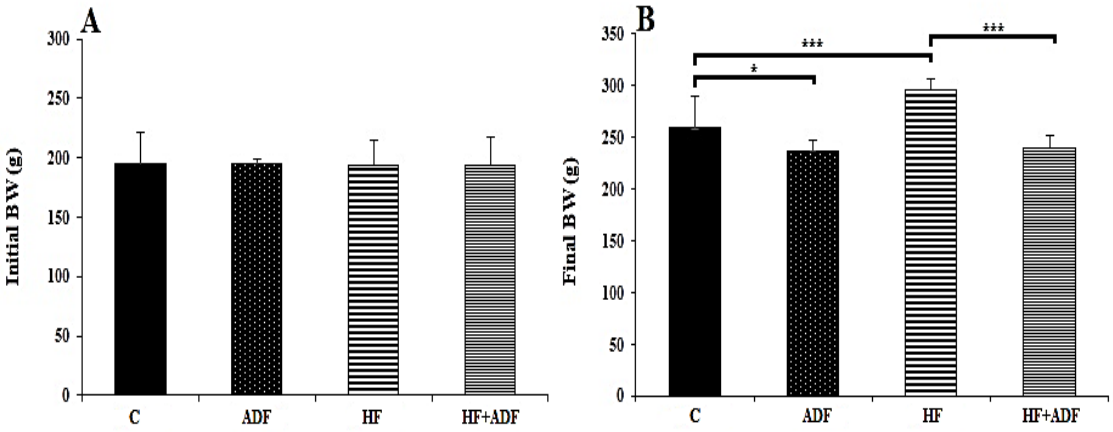

Figure 1. Initial and final BW comparisons between groups

(A) Initial BW (g) of the groups before the intervention, (B) Final BW (g) of the groups after 5 weeks of dietary and fasting interventions.Results are expressed as mean ±SD, with n=7 rats per group. Statistically significant differences are indicated as *p<0.05; ***p<0.001. The groups include C: control group, ADF: alternate-day fasting group, HF: high fructose group, and HF+ADF: high fructose and alternate-day fasting group. (BW: body weight)

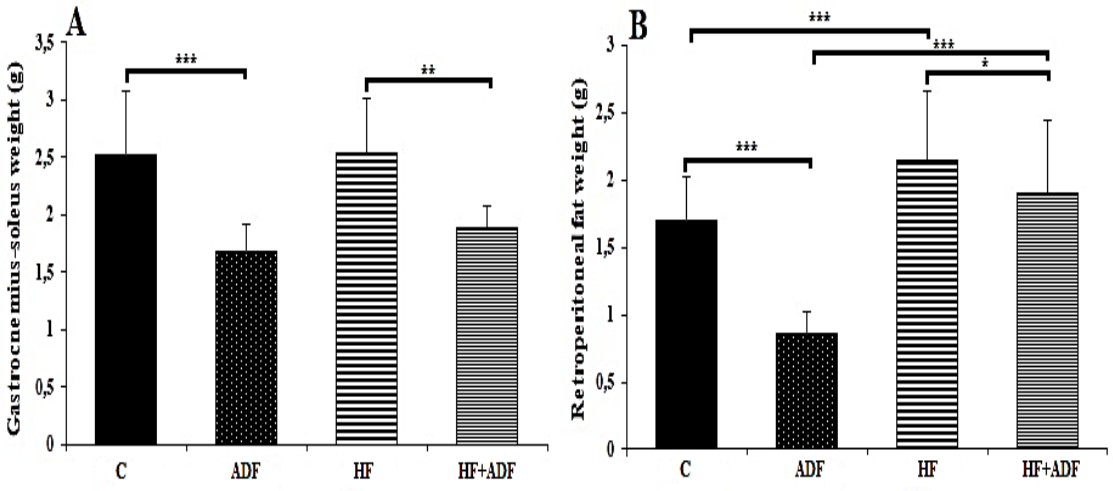

Figure 2. Comparisons of gastrocnemius-soleus weight and retroperitoneal fat weight between groups

(A) Gastrocnemius-soleus weight (g) comparisons and (B) Retroperitoneal fat weight (g) comparisons across the experimental groups. Results are expressed as mean ± SD, with n=7 rats per group. Statistically significant differences are indicated as *p<0.05, **p<0.01, and ***p<0.001. The groups include C: control group, ADF: alternate-day fasting group, HF: high fructose group, and HF+ADF: high fructose and alternate-day fasting group

ADF group (7.73±0.80) had significantly higher TAS levels than control group (6.53±0.37) (p=0.01) and in HF+ADF group (7.53±0.90) compared to HF group (5.72±0.33) (p=0.001, f=10.178) (Figure 3A). In contrast, TOS levels were significantly lower in ADF group (5.52±0.60) compared to control group (7.42±0.24) (p=0.022), and in HF+ADF group (6.55±0.56) compared to HF group (8.91±0.68) (p=0.001). HF group also had significantly higher TOS levels than control group (p=0.006, f=11.695) (Figure 3B). These findings indicate that while fructose intake shifts the oxidant balance towards pro-oxidant status, ADF counteracts this effect, shifting the balance in favor of antioxidants.

Serum TGF-β1, Smad2, and CTGF levels across the four groups are shown in Figures 4. HF group (3.17±0.42) exhibited borderline significantly higher serum TGF-β1 levels compared to control group (2.38±0.40) (p=0.066). ADF significantly reduced TGF-β1 levels in HF+ADF group (2.07±0.41) compared to HF group (p=0.008), and in ADF group (1.19±0.55) compared to control group (p=0.011, f=13.777) (Figure 4A).

Similarly, serum Smad2 levels were significantly higher in HF group (2.07±0.23) compared to control group (1.37±0.27) (p=0.003). ADF significantly reduced Smad2 levels in HF+ADF group (0.69±0.32) compared to HF group (p=0.001), and similarly, in ADF group (0.34±0.16) compared to control group (p=0.001, f=43.786) (Figure 4B).

Additionally, serum CTGF levels were significantly higher in HF group (204.71±19.67) compared to control group (121.53±34.87) (p=0.001). ADF significantly decrased CTGF levels in HF+ADF group (102.03±25.97) compared to HF group (p=0.001), and similarly, in ADF group (66.49±19.42) compared to control group (p=0.018, f=25.887) (Figure 4C).

These results indicate significant modulation of the TGF-β1/Smad pathway by both ADF and fructose consumption, suggesting a potential role in tissue remodeling and metabolic regulation.

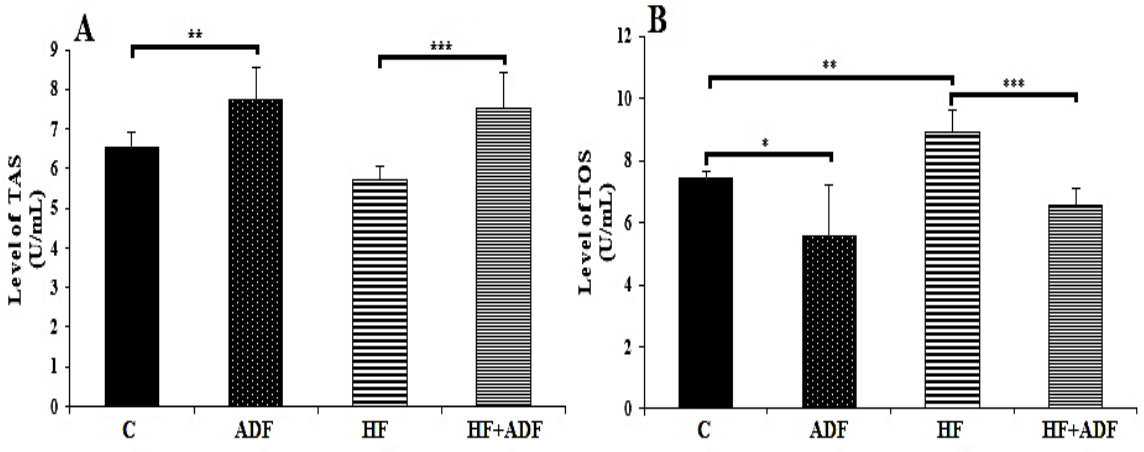

Figure 3. Serum levels of total antioxidant status (TAS) and total oxidant status (TOS) in experimental groups

(A)TAS levels, (B) TOS levels in the experimental groups. Results are expressed as mean ±SD, with n=7 rats per group. Statistically significant differences are indicated as *p<0.05, **p<0.01, and ***p<0.001. The groups include C: control group, ADF: alternate-day fasting group, HF: high fructose group, and HF+ADF: high fructose and alternate-day fasting group

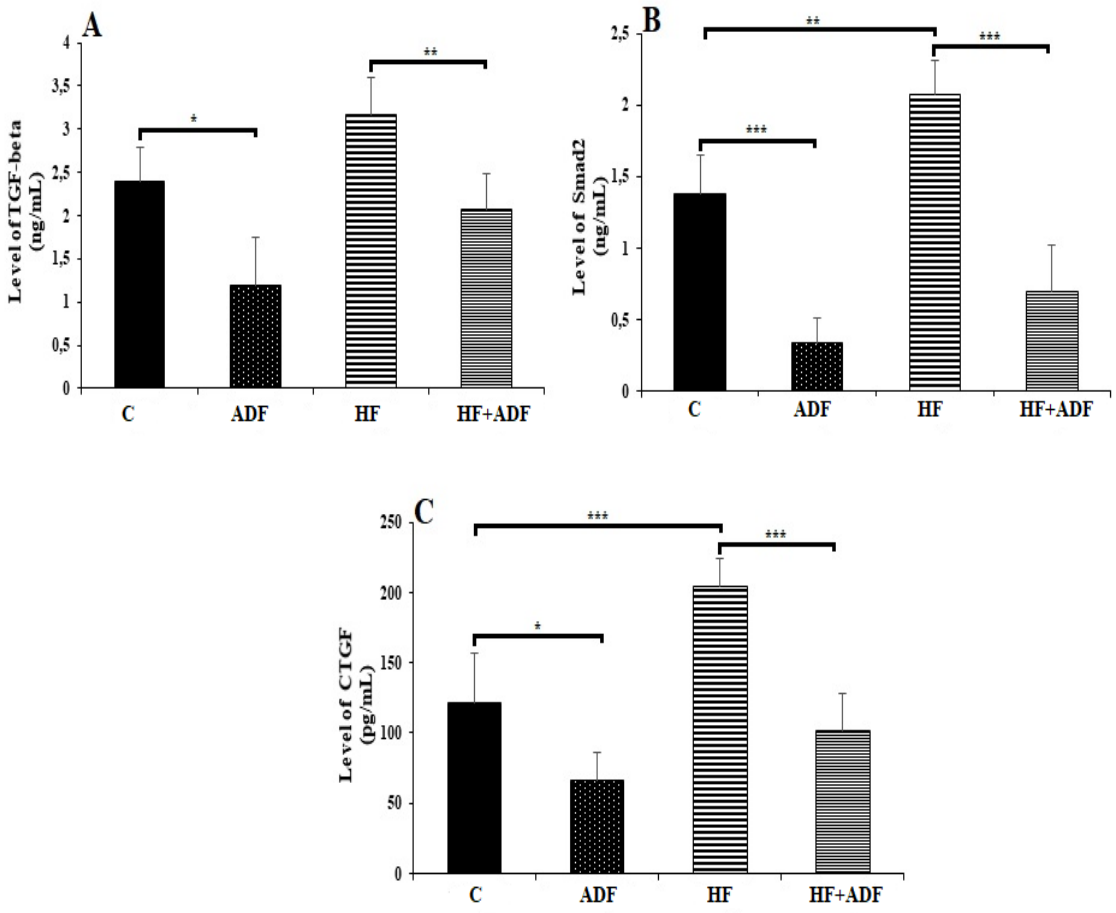

Figure 4. Serum levels of TGF-β1, Smad2, and CTGF in experimental groups.

(A) TGF-β1 levels, (B) Smad2 levels, and (C) CTGF levels in the experimental groups. Results are expressed as mean±SD, with n=7 rats per group. Statistically significant differences are indicated as *p<0.05, **p<0.01, and ***p<0.001. The groups include C: control group, ADF: alternate-day fasting group, HF: high fructose group, and HF+ADF: high fructose and alternate-day fasting group. (ERS: endoplasmic reticulum stress, TGF-β1: transforming growth factor-Beta, Smad2: mother against decapentaplegic homolog 2, CTGF: connective tissue growth factor)

Discussion

Our study revealed that HF intake and ADF had distinct effects on BW, muscle mass, adipose tissue, oxidative stress parameters, and the TGF-β1/Smad signaling pathway. Initial findings showed that HF intake significantly increased final BW compared to other groups, while ADF application lowered this weight gain. Additionally, HF intake led to increased retroperitoneal fat accumulation and decreased muscle mass, effects that were partially mitigated by ADF. The oxidative stress parameters, TAS and TOS, demonstrated a shift towards antioxidant balance with ADF, in contrast to the pro-oxidant imbalance observed with HF intake. Finally, the TGF-β1, Smad2, and CTGF levels were elevated in the HF group, while ADF reduced these fibrosis markers, particularly in the HF+ADF group.

Excessive fructose intake is linked to obesity and metabolic disturbances affecting multiple organs [1]. HF consumption through sweetened drinks and processed foods has become a public health concern, leading to increased morbidity and mortality [24]. Studies have demonstrated that prolonged intake of a 20% fructose solution significantly increases BW in animal models. For instance, Feyisa et al. [25] observed a marked weight gain after 6 weeks of fructose intake in rats, while Tanaka et al. [26] reported similar results with 8 weeks of 20% fructose consumption, showing statistically significant weight gain in both male and female rats. Additionally, Batista et al.

[27] found that rats consuming a 20% purified fructose solution over 8 weeks regulated their energy intake by reducing food consumption but compensating with the fructose solution, suggesting a direct role of fructose in promoting weight gain. Consistently, our study found that 5 weeks of 20% fructose intake led to weight gain. IF protocols, including ADF, are effective for weight loss and may offer health benefits [18, 23]. Fernandez et al. [19] reported that ADF reduced body mass gain in rats, and our findings similarly showed that ADF lowered BW in both healthy and HF rats.

Increased adipose tissue, particularly visceral fat, is closely linked to metabolic disorders. Prior studies indicate that ADF can effectively reduce visceral fat, likely by creating a moderate energy deficit that decreases overall adiposity [28, 29]. For instance, Catenacci et al. [29] observed that ADF significantly reduced visceral and truncal fat, highlighting its potential in managing adiposity-related metabolic risks. In our study, HF consumption led to an increase in retroperitoneal fat accumulation, underscoring the lipogenic effects of excessive fructose intake. However, when combined with ADF, both healthy and HF groups exhibited a notable reduction in retroperitoneal fat, suggesting that ADF may counteract fructose-induced adipose tissue expansion. These findings align with the literature, supporting ADF as an intervention to limit fat accumulation, particularly in the visceral fat.

ROS are partially reduced oxygen metabolites with strong oxidizing capabilities. At high concentrations, ROS are harmful to cells, causing oxidative damage; however, at lower concentrations, they play complex roles in cell signaling. Obesity is closely associated with elevated oxidative stress, a condition exacerbated by excess ROS [30]. Although HF intake has been shown to have detrimental metabolic effects in both humans and rodents, particularly through mechanisms such as hepatic de novo lipogenesis, lipotoxicity, oxidative stress, and hyperuricemia, it remains a significant factor in the growing incidence of metabolic disorders [31]. Chronic ROS production, in particular, plays a central role in the progression of inflammatory diseases. In response to oxidative stress, immune cells release cytokines and chemokines to recruit other immune cells, leading to further ROS generation and tissue damage at the inflammation site [32]. HF consumption caused oxidative stress, consistent with previous studies [30]. This increase in oxidative stress is consistent with literature linking excessive fructose intake to ROS accumulation and inflammation [30]. IF has shown promise in reducing oxidative stress, primarily through physiological adaptations triggered by food deprivation. Studies indicate that ROS levels in liver mitochondria increase after 36 and 72 hours of fasting in rats, resulting in lipid peroxidation and oxidative stress in the liver [33, 34]. Our previous study reported that ADF interventions over 1 to 2 months significantly reduced oxidative stress in both the liver and serum [35]. This suggests that different durations of ADF may influence oxidative stress responses: shorter fasting periods may trigger cellular resistance through low-intensity oxidative stress, while longer durations can enhance antioxidant defenses, balancing ROS production and reducing overall oxidative stress [36]. In our study, a 5-week ADF regimen increased TAS levels and decreased TOS levels in both healthy and HF rats, indicating a beneficial shift toward antioxidant balance. This finding supports the idea that ADF can mitigate oxidative stress, potentially protecting against metabolic disruptions associated with HF intake.

Fibrosis, characterized by excessive tissue growth and ECM accumulation, is largely mediated by the TGF-β/Smad signaling pathway [37, 38]. Upon activation, TGF-β phosphorylates Smad2 and Smad3, which form a complex with Smad4 to regulate fibrogenic gene expression. This process plays a central role in the development of fibrotic conditions, particularly in organs such as the liver and heart [39].

HF intake has been linked to fibrosis in both human and animal models, acting as a risk factor particularly in the liver. Studies have shown that HF consumption activates TGF-β1 and Smad3 expression, contributing to obesity, metabolic disorders, and fibrosis [40, 41]. In a previous study, HF feeding for 24 weeks induced a significant up-regulation of TGF-β1 and Smad3 mRNA levels in the renal tissue of rats [14]. Notably, Smad3 knockout models exhibit protection against HF-induced obesity and insulin resistance, highlighting Smad3's role in

fibrotic and metabolic processes [42]. Our study supports these findings, as 20% HF intake led to significant increases in TGF-β1, CTGF, and Smad3 levels, indicating the activation of the fibrotic pathway.

High concentrations of fructose have also been shown to increase TGF-β and α-SMA gene expression in hepatic stellate cells (HSCs), indicating that fructose can activate HSCs and promote liver fibrosis [13]. TGF-β1, a potent pro-fibrogenic cytokine, binds and activates its receptors, leading to the phosphorylation of Smad3, which then promotes the transcription of fibrogenic genes. Activated Smad3 is known to drive the deposition of ECM components, contributing to tissue fibrosis [43]. In line with these mechanisms, our findings revealed increased TGF-β1 and Smad3 levels in HF rats, reflecting fibrotic changes consistent with prolonged fructose exposure. Further, studies suggest that the JAK2/STAT3 pathway is also activated by HF intake and may interact with TGF-β1/Smad signaling in promoting fibrosis. Yang et al. [44] demonstrated that inhibition of JAK2 prevented the fructose-induced activation of TGF-β1/Smad signaling in liver cells, suggesting a synergistic effect between the JAK2/STAT3 and TGF-β/Smad pathways in HF-induced fibrosis. The potential interaction between these pathways warrants further investigation to better understand their roles in fructose-induced liver fibrosis. ROS production due to HF intake further influences TGF-β1 and CTGF expression, exacerbating fibrotic processes. Fructose has been shown to elevate TGF-β1 expression through ROS accumulation in cardiac cells, leading to myocardial fibrosis [45]. ROS-dependent CTGF overexpression has also been observed in myocardial fibrosis caused by hemodynamic stress, suggesting that CTGF could serve as a diagnostic marker for fructose-induced myocardial hypertrophy and fibrosis [46]. Nagayama et al. [6] found that fructose, unlike glucose, suppressed fibroblast growth and CTGF expression in vitro, which they attributed to decreased cellular viability. This finding was significant as it highlighted a unique effect of fructose on CTGF expression in fibroblasts. Other studies have observed elevated CTGF and TGF-β1 levels in myocardial tissue with chronic fructose feeding, linking these markers to increased fibrosis [47]. Additionally, Liu et al. [48] reported that high glucose levels promoted CTGF expression in vascular smooth muscle cells, implicating dietary sugars in the regulation of fibrosis-related markers at the cellular level. In our study, chronic consumption of 20% HF significantly increased serum levels of TGF-β1, CTGF, and Smad3, supporting the literature on HF's role in driving fibrotic changes through the TGF-β/Smad pathway. These findings underscore the need for further research into dietary sugars' impact on fibrosis and metabolic health, as well as potential interventions to mitigate these effects.

IF protocols, including ADF, have demonstrated favorable effects on fibrosis by reducing TGF-β1 levels and enhancing antioxidant defenses. For example, Han et al. [49] found that IF enhanced the TGF-β-producing capacity of M2 macrophages, potentially reducing inflammation related to obesity. Additionally, Raji Amirhasani et al. [50] showed that fasting regimens lowered TGF-β1 expression and improved kidney function, likely by increasing SIRT1 and reducing oxidative stress. In our study, ADF applied for 5 weeks significantly decreased TGF-β1, CTGF, and Smad3 levels in both healthy and HF rats, suggesting that ADF may effectively downregulate fibrotic signaling pathways and mitigate HF-induced fibrosis.

In conclusion, ADF effectively attenuates HF-induced activation of the TGF-β1/Smad2 signaling pathway, thereby alleviating associated metabolic disturbances. Our results demonstrate that ADF reduces weight gain, controls fat accumulation, and improves oxidative balance in rats subjected to a HF diet. The downregulation of key components of the TGF-beta/Smad pathway by ADF suggests a potential mechanism through which it promotes tissue remodeling and metabolic balance. These findings suggest the therapeutic potential of ADF in counteracting the harmful effects of HF consumption, particularly in preclinical models, supporting its role as a promising dietary approach for managing metabolic disorders. Future studies are needed to determine whether these effects can be replicated in humans and to explore the long-term implications of ADF in managing metabolic disorders.

Limitations of the study: Further research is needed to elucidate the molecular mechanisms underlying these effects and assess their long-term clinical implications.

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Investigation of the effect of quercetin on the accumulation of lipids and the level of adiponectin in 3T3-L1 adipocytes

Quercetin'in 3T3-L1 adipositlerinde lipid birikimi ve adiponektin düzeyi üzerindeki etkisinin araştırılması

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Abstract

Purpose: Obesity disrupts the homeostasis of adipose tissue, leading to an increase in the number and size of adipose cells. Many positive effects of quercetin polyphenol on health through various mechanisms are known in the literature. The aim of our study was to investigate the effects of quercetin on lipid accumulation and adiponectin (ADP) levels in mature and hypertrophic adipocytes.

Materials and methods: In this study, 3T3-L1 differentiated preadipocyte cells were exposed to high glucose media for 8 days to generate mature adipocytes, then for 18 days to produce hypertrophic adipocytes. Quercetin (40 and 80 µM) was administered to mature adipocytes for 24 hours and to hypertrophic adipocytes for 48 hours. Lipid content was visualized by Oil-Red-O staining method. Lipid accumulation and ADP level were measured by ELISA method. For statistical analysis, one-way analysis of variance (post hoc: Tukey test) was used. $p \leq 0.05$ was considered statistically significant.

Results: The administration of both quercetin doses to mature adipocytes significantly reduced lipid accumulation ($p=0.0001$). The administration of 80 µM quercetin in mature adipocytes caused a significant increase in ADP levels ($p=0.0001$). Administration of quercetin to hypertrophic adipocytes caused a significant decrease in ADP levels ($p=0.0001$).

Conclusion: Our study revealed that quercetin decreased lipid accumulation and increased ADP levels in mature adipocytes. However, in hypertrophic adipocytes, quercetin had no significant effect on lipid accumulation and decreased ADP levels. These findings suggest that quercetin has protective effects on health in the early stages of obesity, but its efficacy is limited in the later stages of obesity.

Keywords: Adiponectin, adipocyte, lipid accumulation, obesity, quercetin.

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Öz

Amaç: Obezite, adipoz dokunun homeostazını bozarak adipoz hücrelerinin sayısında ve büyüklüğünde artışa neden olur. Literatürde quercetin polifenolünün çeşitli mekanizmalar aracılığıyla sağlık üzerinde pek çok olumlu etkileri bilinmektedir. Araştırmamızın amacı, quercetinin olgun ve hipertrofik adipositlerde lipit birikimi ve adiponektin (ADP) seviyeleri üzerindeki etkilerini araştırmaktır.

Gereç ve yöntem: Çalışmada, 3T3-L1 preadiposit hücreler farklılaştırıldıktan sonra yüksek glikoz içeren ortamda 8 gün muamele edilerek olgun adipositler, 18 gün muamele edilerek ise hipertrofik adipositler elde edildi. Quercetin (40 ve 80 µM), olgun adipositlere 24 saat, hipertrofik adipositlere ise 48 saat boyunca uygulandı. Oil-Red-O boyama yöntemiyle lipit miktarı görüntülendi. ELISA yöntemi aracılığıyla lipit birikimi ve ADP seviyesi ölçüldü. İstatistiksel analiz için; tek yönlü varyans analizi (post hoc: Tukey testi) kullanıldı. $p \leq 0,05$ değeri istatistiksel olarak anlamlı kabul edildi.

Bulgular: Olgun adipositlere her iki quercetin dozunun uygulaması lipit birikimini anlamlı olarak azalttı ($p=0,0001$). Olgun adipositlerde 80 µM quercetin uygulaması, ADP seviyelerinde anlamlı bir artışa yol açtı ($p=0,0001$). Hipertrofik adipositlere quercetin uygulaması ise, ADP seviyelerinde anlamlı bir azalmaya yol açtı ($p=0,0001$).

Sonuç: Çalışmamız, quercetinin olgun adipositlerde lipit birikimini azaltıp ADP seviyesini artırdığını ortaya koydu. Ancak, hipertrofik adipositlerde ise quercetinin lipit birikiminde anlamlı bir etkisi olmayıp ADP seviyesini azalttığı gözlendi. Bu bulgular, quercetinin obezitenin erken döneminde sağlık üzerindeki koruyucu etkilerini ortaya koymakla birlikte, obezitenin daha ileri döneminde etkinliğinin sınırlı kaldığını göstermektedir.

Anahtar kelimeler: Adiponektin, adiposit, lipit birikimi, obezite, quercetin.

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Introduction

Obesity is defined by a confluence of genetic susceptibility, the intake of high-calorie meals, and diminished physical activity. Currently, it is regarded as the main cause for numerous chronic conditions, including cardiovascular diseases, type 2 diabetes, and specific forms of cancer. It is regarded as both a personal issue and an epidemic that threatens global well-being [1]. Obesity, characterized by an excessive accumulation of adipose tissue, disrupts energy balance at a pathological level. Adipocytes in adipose tissue accumulate excess nutrients through hyperplasia, hypertrophy or a combination of both. During increased energy demand, they release energy substrates via lipolysis. Thus, adipose tissue plays a crucial part in the regulation of energy balance [2]. Adipose tissue not only serves as a store of energy and regulates energy homeostasis but also performs significant endocrine roles by secreting adipokines, including resistin, leptin, and adiponectin (ADP). Furthermore, these hormones are crucial in various physiological processes within the body, including insulin sensitivity, inflammation, and lipid metabolism. In particular, ADP produced in adipocytes is a hormone involved in various metabolic processes and ADP levels are closely related to the size and composition of adipose tissue [3].

Current obesity interventions focus on either diminishing caloric consumption (diets, pharmacotherapy, bariatric surgery) or enhancing energy expenditure via physical exercise. Moreover, the processes by which polyphenols operate in the treatment of obesity have been extensively studied in recent years. Polyphenols are significant bioactive chemicals that modulate lipid metabolism and energy balance in adipocytes. Polyphenols, particularly catechins, resveratrol, and quercetin, inhibit adipogenesis and restrict the development of adipocytes. These polyphenols enhance lipolysis in adipocytes, diminish triglyceride accumulation, and stimulate energy expenditure [4-6]. Moreover, the anti-inflammatory properties of polyphenols protect metabolic health by reducing inflammation linked to obesity [7]. Consequently, polyphenols restrict the proliferation of adipose tissue and contribute to preventing obesity-related problems. The literature has a limited number of studies investigating the effects of quercetin polyphenol on mature adipocytes [8, 9]. No research were identified in the literature analysis that investigated the impact of quercetin on ADP levels in hypertrophic adipocytes. Mature adipocytes represent obesity and hypertrophic adipocytes represent insulin resistant/advanced obesity model [10, 11]. Consequently, our work aimed to explore the effects of quercetin administration on lipid accumulation and ADP levels in both mature and hypertrophic adipocytes.

Materials and methods

Cell culture

Differentiation and establishment of the 3T3-L1 hypertrophic adipocyte model

In this study, we used the 3T3-L1 cell line (ATCC® CL173™), a fibroblast cell line derived from a mouse (Mus musculus) embryo known as a preadipocyte. The cells were cultured in a Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA, Waltham) containing 25 mM glucose supplemented with 10% heat inactivated calf serum (Cegrogen, Germany), and antibiotics (penicillin and streptomycin; Wisent, Saint-Jean-Baptiste, Canada) at 37°C in a humidified atmosphere of 5% CO_2 to create experimental groups. When the cells reached approximately 80% density in the culture dishes, after cell counting, 10^5 cells were seeded in 6-well plate. The 2 ml of medium was added to each well and the growth of the cells. The cells were cultured in the same medium until they reached confluence. After that a medium containing a differentiation cocktail 0.5 Mm 3-Isobutyl-1-methylxanthine (IBMX, Sigma I5879, USA), 1 µM dexamethasone (DEX, Sigma D4902, USA), 10 µg/mL insulin (INS) (Sigma I6634, USA), 10% fetal bovine serum (FBS, Biowest, South America) was added to the medium to induce the differentiation of preadipocytes into adipocytes and the cells were incubated in this medium (MD1) for 48 hours [12]. After incubation, the medium containing

high glucose, FBS and INS (MD2) (Gibco, Waltham, MA, USA) was changed every 2 days. After this stage, the cells will become mature adipocytes after 8 to 10 days and hypertrophic adipocytes after 18 to 21 days [11]. Adipocyte differentiation was evaluated by Oil Red O staining. Our experiments were performed on adipocytes that completed differentiation on day 10 and 18 became mature and hypertrophic.

Administration of quercetin

Quercetin 40 and 80 μM [13] dose was applied to mature 3T3-L1 cells at day 10 post-differentiation for 24 hours and hypertrophic 3T3-L1 cells at day 18 post-differentiation for 48 hours. Details of the experimental timeline are shown in Figure 1.

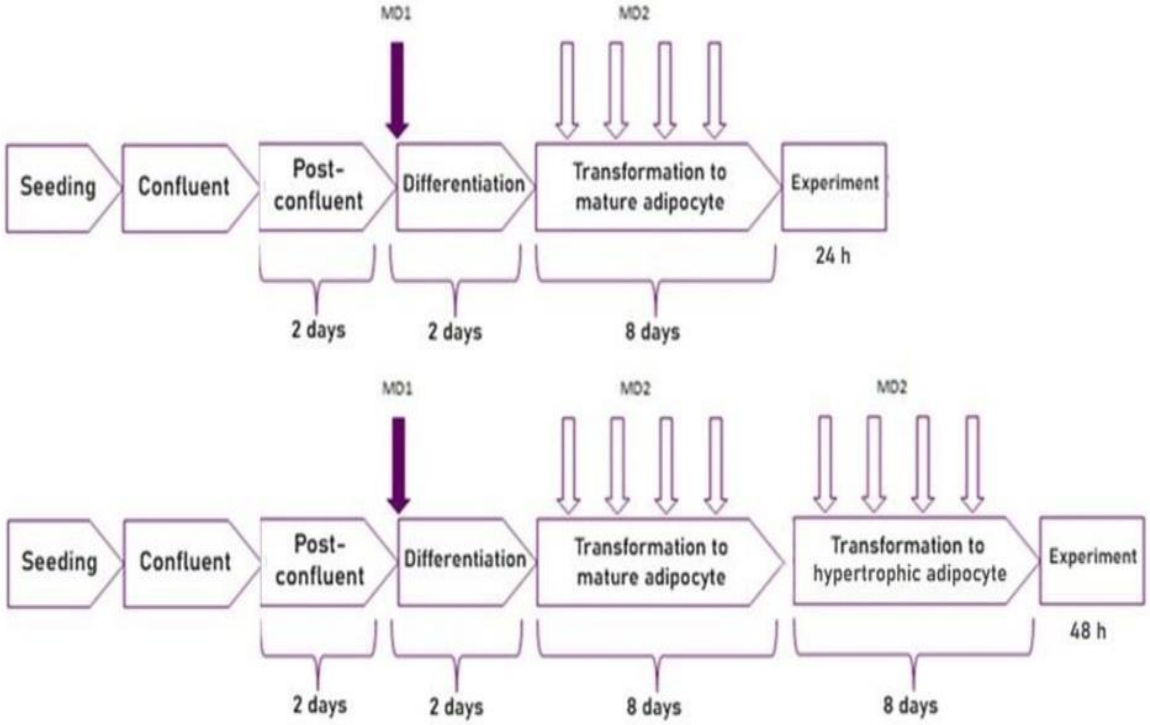

Figure 1. Demonstration of experimental design

Quercetin was applied at 40 and 80 μM dose. MD1: medium 1, MD2: medium 2

Oil red o staining and measurements of adiponectin level

To confirm the transformation of preadipocytes into mature (day 10) and hypertrophic (day 18), they were analysed microscopically using the Oil Red O staining kit [Biovision Lipid (Oil Red O) (Catalog #K580-24)]. Differentiated 3T3-L1 adipocytes were fixed with 10% formalin in Phosphate Buffered Saline (PBS, Wisent, Saint-Jean-Baptiste, Canada) for 1 h and washed twice with 60% isopropanol. The fixed cells were then stained using Oil Red O solution for 30 min and washed with distilled water. After drying, the cells were imaged by scanner. The Oil Red O solution taken up by the cells was then extracted using 100% isopropanol and its optical intensity was measured at 490 nm. A microscope was used to see the triglyserid accumulation at 40X magnifications. ADP levels were measured in 3T3-L1 mature and hypertrophic cell lysates using an ELISA kit according to the manufacturer's instructions (BTLAB E0246Mo, China).

Statistical analysis

The data were analyzed with the software package SPSS 25.0. Continuous variables are expressed as the mean±standard deviation. One-way analysis of variance (post hoc: Tukey test) was used to compare group differences. $p \leq 0.05$ was considered statistically significant.

The study is a cell culture study that does not require ethics committee approval.

Results

Images taken from mature and hypertrophic cells at different stages after cultivation of 3T3-L1 preadipocyte cells showed that these cells had different morphologies in terms of lipid accumulation (Figure 2). Administration of 40 and 80 μM doses of quercetin to mature adipocytes significantly reduced lipid accumulation compared to mature control (C:0.21±0.01; Q40:0.14±0.01; Q80:0.12±0.01; *p*=0.0001) (Figure 3). Although 40 and 80 μM doses of quercetin administered to hypertrophic adipocytes demonstrated a tendency to decrease lipid accumulation compared to hypertrophic control, this difference was not statistically significant (C:0.49±0.07; Q40:0.42±0.05; Q80:0.41±0.07) (Figure 4). Administration of 80 μM quercetin to mature adipocytes significantly increased ADP levels compared to both mature control and 40 μM quercetin groups (C:1.22±0.15; Q40:1.37±0.15; Q80:2.18±0.15; *p*=0.0001) (Figure 5). Administration of 40 and 80 μM quercetin to hypertrophic adipocytes significantly decreased ADP level compared to hypertrophic control (C:2.08±0.15, Q40:1.19±0.01, Q80:0.91±0.01, *p*=0.0001). In hypertrophic adipocytes, 80 μM quercetin significantly decreased ADP levels compared to 40 μM quercetin (Q40:1.19±0.01, Q80:0.91±0.01, *p*=0.001) (Figure 6).

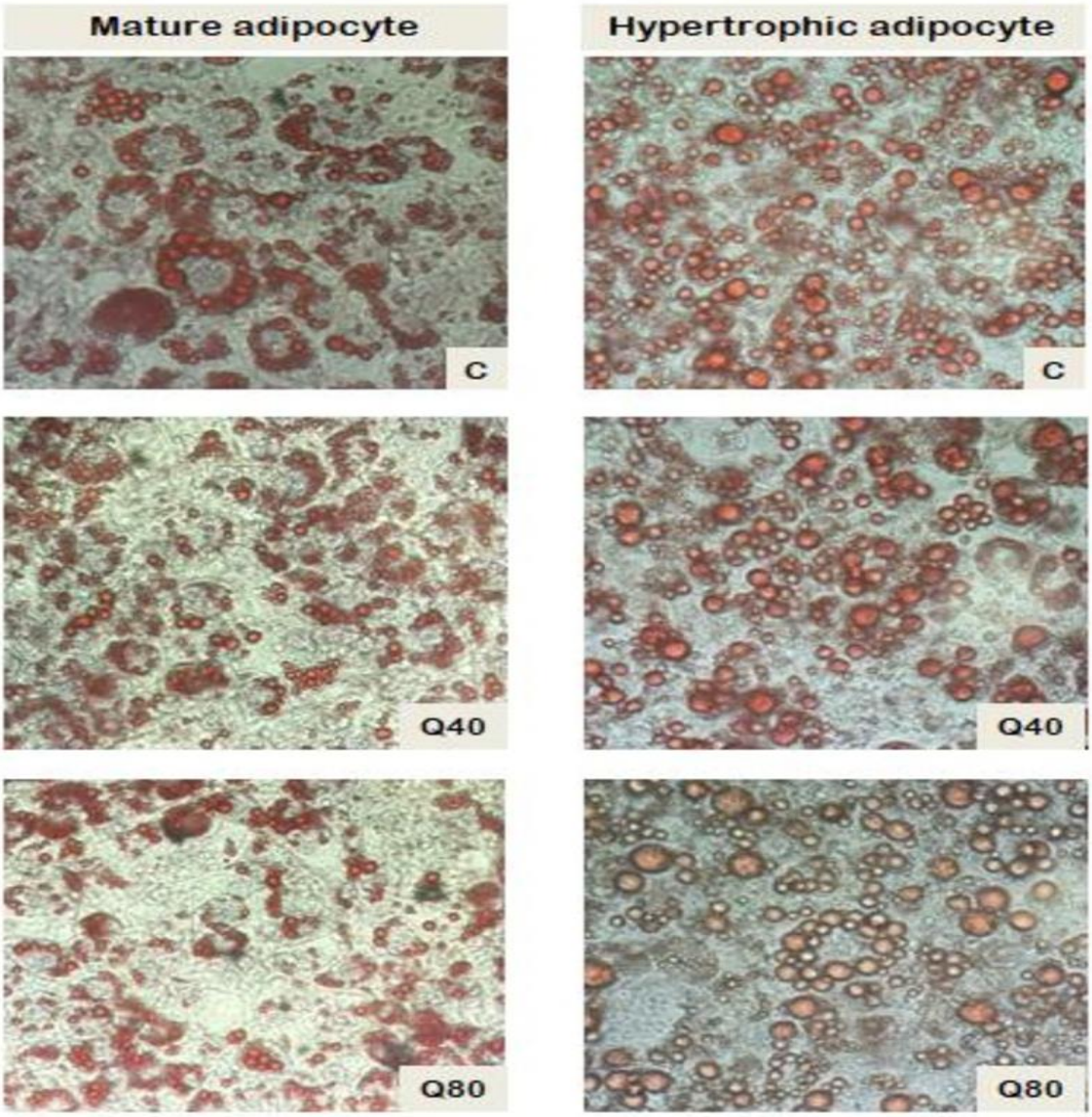

Figure 2. Oil-red-O stained inverted microscopy images of quercetin applied to mature and hypertrophic adipocytes (40× magnification)

C: Control, Q40: 40 μM Quercetin, Q80: 80 μM Quercetin

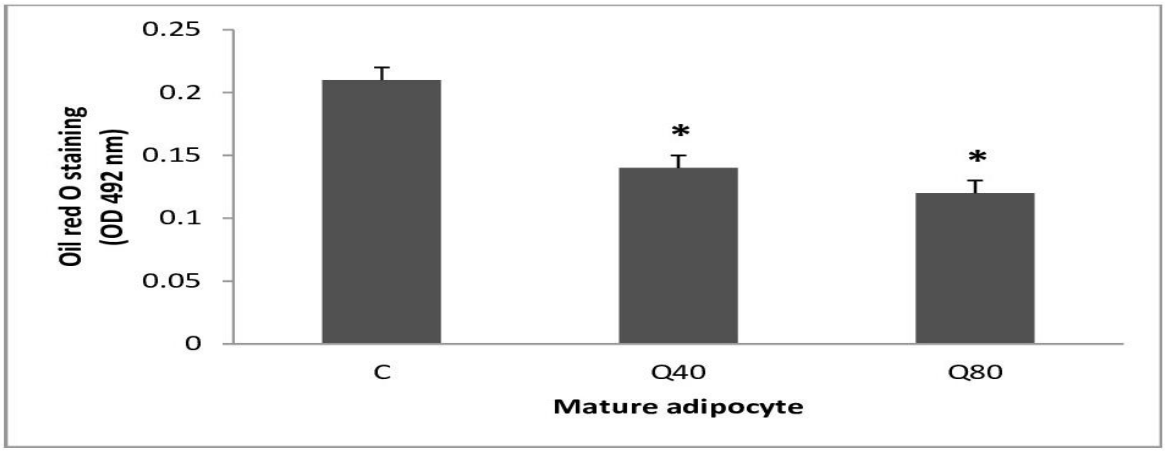

Figure 3. Lipid accumulation results of quercetin in mature adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results
Statistical significance was determined as $p \leq 0.05$ are represented by the *. *: Significantly from mature control (p=0.0001, F=125.842)
F: One Way Analysis of Variance, C: Control, Q40: 40 µM Quercetin, Q80: 80 µM Quercetin

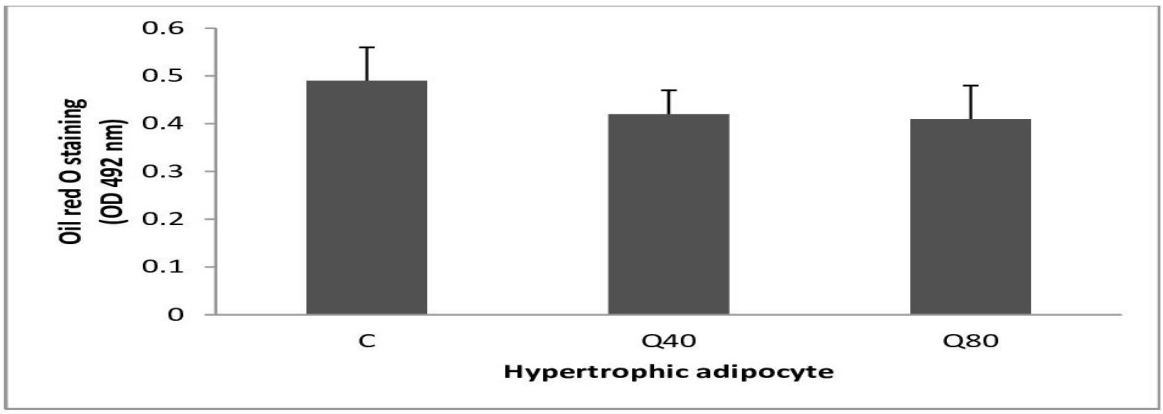

Figure 4. Lipid accumulation results of quercetin in hypertrophic adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results (p=0.369, F=1.181). F: One Way Analysis of Variance
C: Control, Q40: 40 µM Quercetin, Q80: 80 µM Quercetin

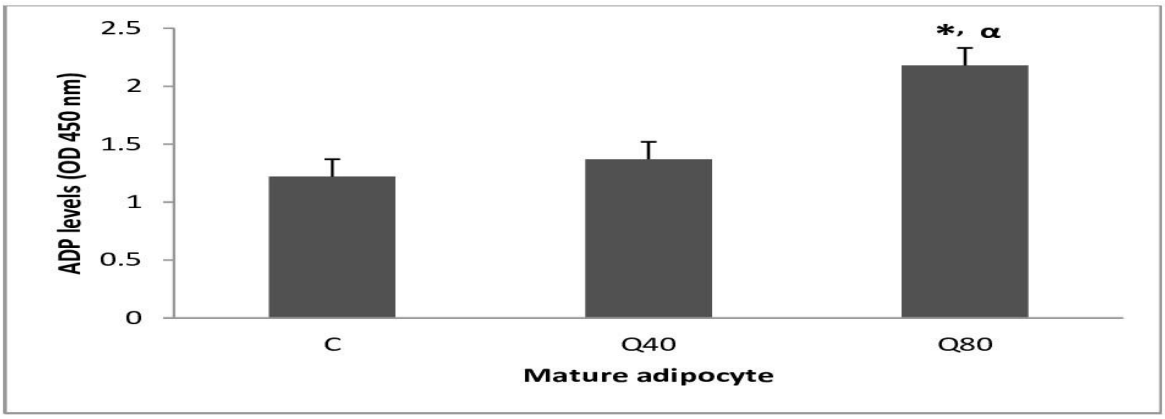

Figure 5. Effect of quercetin on ADP level in mature adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results
Statistical significance was determined as p≤0.05 are represented by the *,α. *: Significantly from mature control
α: Significantly from mature Q40 (p=0.0001, F=45.943). F: One Way Analysis of Variance, C: Control, Q40: 40 µM Quercetin
Q80: 80 µM Quercetin

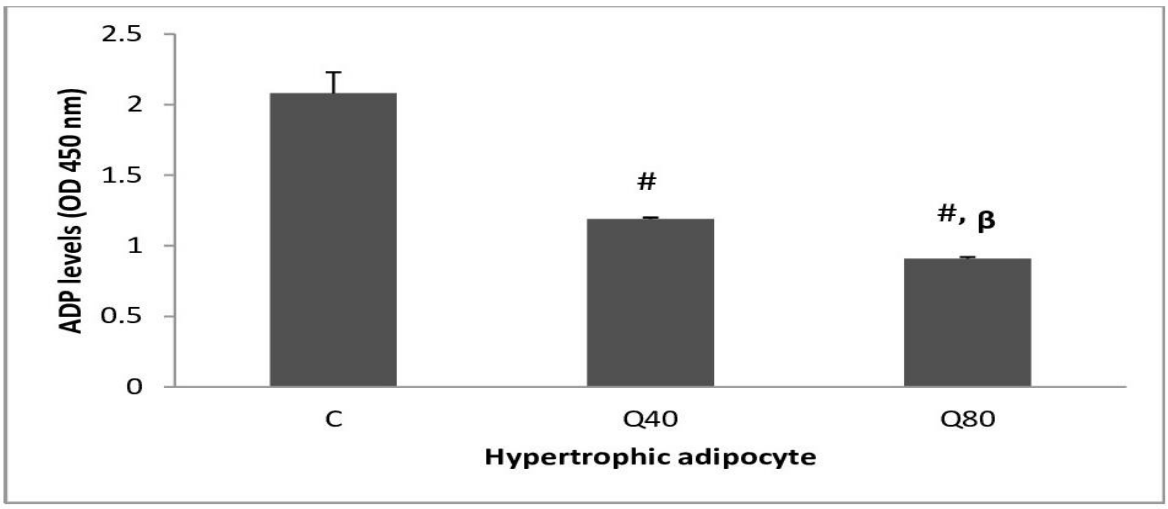

Figure 6. Effect of quercetin on ADP level in hypertrophic adipocytes

Arithmetic means and standard deviation (Mean±SD) were used to express the results
Statistical significance was determined as $p \leq 0.05$ are represented by the #, β. #: Significantly from hypertrophic control
β: Significantly from hypertrophic Q40 (p=0.0001, F=110.717), F: One Way Analysis of Variance, C: Control, Q40: 40 µM Quercetin
Q80: 80 µM Quercetin

Discussion

An energy imbalance between caloric intake and expenditure results in excessive fat formation, resulting in obesity. Obesity is an important and widespread public health problem globally due to its increasing incidence and adverse effects [14]. Obesity results in an increase in both the amount and size of adipocytes in the body. Adipocytes originate from preadipocytes and subsequently enlarge as they accumulate lipids. This process is often examined using models like the 3T3-L1 cell line [15].

Due to the challenges and long nature of managing obesity through diet, exercise, pharmacological interventions, and surgical procedures, alternative treatment methods have been explored, particularly the anti-obesity effects of polyphenols, which have long been studied for their health benefits in adipocytes. Tung et al. [16] demonstrated in their research that phytochemicals, such as polyphenols, reduce obesity in 3T3-L1 adipocytes by decreasing lipid accumulation via the activation of energy sensors (Adenosine monophosphate-activated protein kinase (AMPK), Mitogen-activated protein kinase (MAPK). Quercetin, a flavonoid belonging to the polyphenol group, is present in numerous vegetables, fruits, and plants, and has several health benefits, including anti-obesity actions [17]. Research on the 3T3-L1 cell line indicates that quercetin administration decreases lipid accumulation during adipocyte differentiation [18] and in completely distinct adipocytes [19]. Research on obese rats indicates that quercetin reduces adipocyte size, enhances adipose tissue hypertrophy [20], and lowers lipid accumulation [21]. Our study's observation of reduced lipid levels following quercetin administration to mature adipocytes aligns with previous studies.

Furthermore, only one study examined the impact of quercetin on lipid accumulation in hypertrophic adipocytes, revealing that the application of 100 µM quercetin decreased triglyceride levels in these cells [9]. In contrast to these data, quercetin administration to hypertrophic adipocytes did not affect lipid accumulation according to this study. The data indicate that this may result from the increase in cell density and size when cells undergo hypertrophy (progressive obesity), and that the administered doses may be inadequate to diminish lipid accumulation. Consequently, we suggest that future studies ought to focus on the administration of quercetin treatment during the initial or initial stages of obesity.

Adipose tissue is thought to have both endocrine and metabolic capabilities, significantly contributing to the development of obesity and related metabolic problems through the secretion of adipokines [22]. ADP is a polypeptide containing 244 amino acids, produced by adipose tissue and generated

and released in elevated quantities by mature adipocytes. In recent years, there has been an increase in studies investigating the effect of ADP on adipose cells, which is closely related to the size and structure of adipose tissue and is an important indicator of metabolic health [23-27].

Research investigating the impact of polyphenols on ADP levels in mature adipocytes revealed that the application of lipoic acid [23] inhibited ADP secretion, but the application of kaempferitrin [24] enhanced ADP secretion. Furthermore, the investigation of quercetin's impact on ADP levels in mature adipocytes revealed using ELISA that concentrations of 12.5 μM [25] and 5, 10, and 20 μM quercetin [26] elevated ADP levels in one research. A further investigation revealed that 100 μM quercetin elevated ADP mRNA levels as measured by PCR [27]. Our investigation revealed that the elevation of ADP levels after 24 hours of 80 μM quercetin administration to mature adipocytes consistent with the existing literature. According to Hwang et al. [28] (2009), AMPK is a crucial sensor for energy metabolism. In the literature, through dose-dependent phosphorylation, quercetin activated AMPK [9]. We speculate that by triggering AMPK signaling, quercetin may have raised ADP.

In the literature, adipocytes becoming mature is defined as the early stage of obesity, and becoming hypertrophic is defined as advanced obesity or insulin-resistant obesity model [10, 11]. In line with these findings, our study showed that the use of quercetin in the early stage of obesity may be more appropriate in terms of targeted effects. In advanced obesity, it can be said that quercetin is insufficient to produce the expected beneficial effects in terms of dose, duration and changing metabolic processes. This situation can be considered as one of the limitations of our study, in order to make clearer comments, it would be appropriate to test hypertrophic cells with quercetin at higher doses and different treatment durations and also to evaluate other pathways (such as AMPK, hormone sensitive lipase (HSL), Perilipin-1 (PLIN1), etc.). Our study's results from the mouse cell line could lead to variations in human adaption, which is another limitation.

As a result, it shows that similar metabolic functions may occur through different mechanisms in adipocytes undergoing hypertrophy and that further studies are needed to elucidate these mechanisms.

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Authors contributions: Conceptualization, E.K.T., M.T.A. and G.A. Formal analysis, M.T.A. and G.A. Investigation, G.A., M.T.A. and E.K.T. Methodology, E.K.T. Project administration, E.K.T. and G.A. Supervision, E.K.T. and M.T.A. Writing – original draft, E.K.T., M.T.A. and G.A. Writing – review & editing, E.K.T., M.T.A. and G.A.

Conflict of interest: The authors declare that there is no conflict of interest.

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Research Article

Pamukkale Medical Journal
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The effect of age on pain perception among patiens undergoing systematic ultrasonography guided transrectal prostate biopsy

Sistematik ultrasonografi eşliğinde transrektal prostat biyopsisi yapılan hastalarda yaşın ağrı algısı üzerine etkisi

Salih Bürlukkara, Coşkun Bostancı

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Abstract

Purpose: To investigate the effect of age on the pain levels caused by placing the transrectal probe and biopsy needle into the prostate tissue in transrectal ultrasound-guided prostate biopsy (TRUS-PB).

Materials and methods: The study included 308 patients. These patients were divided into groups based on age: Group 1 had 103 patients aged 65 or younger, Group 2 had 100 patients aged 66-70, and Group 3 had 105 patients over 70. 11 ml 2% lidocaine gel was administered intrarectally to the patients included in the study as anesthesia. Thirty minutes after the biopsy procedure, we measured each patient's pain using the VAS score, which ranged from 0 (no pain) to 10 (worst pain). Patients were asked for pain level during the insertion of the rectal probe and the maneuvers (VAS-p) and the pain level during the insertion of the needle through the prostate to take a biopsy (VAS-b).

Results: VAS-p score was lower in Group 2 than in the other age groups. When the groups were evaluated in terms of VAS-b, no significant difference was observed between the 3 groups. Across all patients, the pain felt during probe insertion was greater than the pain felt during biopsy and this was statistically significant (p=0.001). When prostate volume was compared with pain score, each unit increase in prostate volume increased the probability of pain in VAS-p by 1.014 times.

Conclusion: Pain sensation in patients undergoing biopsy is mainly felt during probe insertion, and that this pain sensation increases with increasing prostate size.

Keywords: Anesthesia, pain score, prostate biopsy, transrectal ultrasound.

Burlukkara S, Bostanci C. The effect of age on pain perception among patiens undergoing systematic ultrasonography guided transrectal prostate biopsy. Pam Med J 2025;18:189-194.

Öz

Amaç: Transrektal ultrason eşliğinde prostat biyopsisinde (TRUS-PB) transrektal prob ve biyopsi iğnesinin prostat dokusuna yerleştirilmesi sonucu oluşan ağrı düzeylerine yaşın etkisini araştırmak.

Gereç ve yöntem: Çalışmaya 308 hasta dahil edildi. Bu hastalar yaşlarına göre gruplara ayrıldı: Grup 1'de 65 yaş ve altı 103 hasta, Grup 2'de 66-70 yaş arası 100 hasta ve Grup 3'te 70 yaş üstü 105 hasta vardı. Çalışmaya dahil edilen hastalara anestezik olarak 11 ml %2'lik lidokain jel intrarektal olarak uygulandı. Biyopsi işleminden 30 dakika sonra her hastanın ağrısını, 0 (ağrı yok) ile 10 (en şiddetli ağrı) arasında değişen VAS skorunu kullanarak ölçtük. Hastalara rektal prob yerleştirilmesi ve manevralar sırasındaki ağrı düzeyi (VAS-p) ve biyopsi almak için iğnenin prostattan içeri sokulması sırasındaki ağrı düzeyi (VAS-b) soruldu.

Bulgular: VAS-p skoru Grup 2'de diğer yaş gruplarına göre daha düşüktü. Gruplar VAS-b açısından değerlendirildiğinde 3 grup arasında anlamlı fark görülmedi. Tüm hastalarda probun yerleştirilmesi sırasında hissedilen ağrı biyopsi sırasında hissedilen ağrıdan daha fazlaydı ve bu istatistiksel olarak anlamlıydı (p=0,001). Prostat hacmi ağrı skoru ile karşılaştırıldığında, prostat hacmindeki her birim artış VAS-p'de ağrı olasılığını 1.014 kat artırıyordu.

Sonuç: Biyopsi yapılan hastalarda ağrı duyusunun çoğunlukla prob yerleştirilmesi sırasında hissedildiği ve bu ağrı duyusunun prostat büyüklüğü arttıkça arttığı görülmektedir.

Anahtar kelimeler: Ağrı skoru, anestezi, prostat biyopsisi, transrektal ultrason.

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Introduction

Prostate cancer (PCa), which may even be classified as a geriatric illness, is second only to skin cancer in prevalence [1]. Prostate biopsy is the only method used for histopathological diagnosis, regardless of the biopsy method used. However, there is an ongoing debate regarding who should undergo a biopsy and what type is appropriate. According to European Association of Urology (EAU) guidelines, a multiparametric prostate magnetic resonance imaging (mpMRI) scan should be conducted on all biopsy candidates, and targeted fusion biopsy and additional systematic biopsy are recommended for patients with suspicious lesions on mpMRI [2]. Although prostate biopsy is typically recommended for patients with a life expectancy of 10-15 years, due to the increase in life expectancy and higher PSA values with age, there is a growing elderly population in need of prostate biopsies.

The EAU guidelines suggest transperineal biopsy as the first option due to its low risk of infection [2]. However, ultrasonography-guided transrectal prostate biopsy (TRUS-PB) is the most commonly used method, as it can be performed under local anesthesia in an outpatient setting [3]. This is advantageous for elderly individuals at risk of general or spinal anesthesia.

Pain management is crucial when performing biopsies since pain can affect the accuracy of the biopsy sample collection and may result in premature termination of the biopsy procedure. The two primary causes of pain during transrectal ultrasound-guided prostate biopsy (TRUS-PB) are the insertion of an ultrasound probe into the rectum and a puncture of the biopsy needle into the prostate tissue [4, 5]. The most conventional local anesthetic methods for TRUS-PB are intrarectal local anesthesia (IRLA), ultrasound-guided peri-prostatic nerve block (PNB), pelvic plexus nerve block (PPNB), and intraprostatic local anesthesia (IPLA). Lidocaine, available in gel, spray, or injectable form, is commonly used anesthetic agent. Studies have demonstrated that PNB, which involves the injection of lidocaine bilaterally along the apex to the base, is a superior option compared to IRLA [6-8]. However, IRLA, compared to PNB, is a noninvasive method and is also widely used to reduce pain during a prostate biopsy [9].

Numerous studies have indicated that younger patients undergoing a biopsy may experience more pain due to higher anal sphincter tone bion [5, 10]. Additionally, studies have found that acute pain decreases with age, while chronic pain increases with age [11]. These findings suggest that age can be a significant factor in the experience of pain during biopsy procedures.

This study aimed to investigate the effect of age on pain levels created by the transrectal probe and the biopsy needle insertion into the prostate tissue, in which 11 ml 2% lidocaine gel was used as an IRLA before TRUS-PB.

Materials and methods

The study was conducted by the Declaration of Helsinki and permission was obtained from Karabük University Non-Interventional Clinical Research Ethics Committee (approval number: 2023/1446, date:06/11/2023). Informed consent was obtained from all patients prior to the procedure.

Although this study is retrospective, we followed all patients prospectively. We recorded detailed data of all patients on electronic media from November 2020 to maintain a higher standard of care in our clinic.

Our study involved 308 patients who underwent TRUS-PB between November 2020 - March 2023. Our biopsy protocol included patients with a PSA level above 4 ng/ml, suspicious digital rectal examination (DRE), mpMRI PI-RADS score higher than 2, any mpMRI PI-RADS score with suspicious DRE or PSA >4 ng/ml, patients with previous suspicious pathology results, or who needed staging for prostate carcinoma. All patients were given prophylactic antibiotics, and an enema was applied on the morning of the biopsy. A pre-biopsy urine culture was obtained from all patients. Patients on anticoagulation therapy were stopped from taking anticoagulation drugs and switched to low molecular weight heparin five days before the biopsy.

Study inclusion criteria were the patients who were given 11 ml 2% lidocaine gel intrarectally as a local anesthesia. The study did not include patients who have undergone previous prostate biopsies or any prostate surgery, those with less than eight core biopsies, chronic prostatitis,

large hemorrhoids, and severe anal strictures impeding the insertion of a rectal probe, lidocaine or povidone-iodine allergies, active use of analgesic medications, urethral catheterization, and those who are incapable of indicating their pain level on the pain scale. Consequently, the study includes a remaining cohort of 308 patients. We divided these patients into groups based on age: Group 1 had 103 patients aged 65 or younger, Group 2 had 100 patients aged 66-70, and Group 3 had 105 patients over 70.

The same urology doctor (CB) performed 10-12 core systematic TRUS-PB procedures using a 7.5-MHz biplane probe (ProSound SSD-5500, Aloka, Tokyo, Japan) in the left lateral decubital position. A single-use automatic biopsy gun with an 18 gauge-24 cm needle and 11 ml of 2% lidocaine gel provided local anesthesia to the patient in the same outpatient room. The rectal probe's maximum diameter is 60 mm, increasing to 65 mm when the biopsy attachment is added.

The local anesthesia procedure begins with the perineal cleaning with a gauze soaked in povidone-iodine. Then, 11 ml of 2% lidocaine gel was applied intrarectally, followed by a digital rectal examination to distribute the gel on the prostate equally. After allowing 5 minutes for the anesthesia to take effect, pre-biopsy rectal cleaning was done by using sterile gauze soaked in 40 ml of povidone-iodine and 11 ml of 2% lidocaine gel. The gauze was manually inserted into the rectum and placed over the prostate for 2 minutes. The biopsy procedure started 10 minutes after the initial lidocaine gel application.

Thirty minutes after the biopsy procedure, we measured each patient's pain using the VAS score, which ranged from 0 (no pain) to 10 (worst pain). Patients were asked for pain level during the insertion of the rectal probe and the maneuvers (VAS-p) and the pain level during the insertion of the needle through the prostate to take a biopsy (VAS-b).

Statistical analysis

Statistical analyses were performed using IBM SPSS 22.0 (Armonk, NY: IBM Corp.). The normal distribution of data was analyzed using Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive data were expressed as mean ± standard deviation (SD) or median (25^{th}-75^{th} percentile) depending on the data distribution. Normally distributed variables were compared using the Independent Sample t-test. Quantitative variables that were not normally distributed and independent groups with ordinal data were compared using the Mann-Whitney U test. Categorical variables were compared using the Chi-square test. A value of $p<0.05$ was considered significant.

Results

The mean age was 67.5 (±6.95) years, the mean BMI was 26.6 (19.6-46.7) kg/m^2, the mean serum PSA level was 7.1 (0.2-575) ng/dL, and the mean prostate volume was 59.5 (10-220) mm^3. When the biopsy pathology results were compared, Group 3 had a higher incidence of PCa than the other groups (Table 1). However, the pathology results had no impact on VAS-p or VAS-b.

A statistically significant difference was observed between the groups in terms of VAS-p. Pain was observed in 83.5% of patients in Group 1, 70% in Group 2 and 81.99% in Group 3. VAS-p score was lower in Group 2 than in the other age groups ($p=0.038$). No significant difference was observed between Group 1 and Group 3. When the groups were evaluated in terms of VAS-b, no significant difference was observed between the 3 groups ($p=0.882$).

Pain during probe insertion developed in 74.5% of those who had no pain during biopsy, while pain during probe insertion developed in 90.9% of those who had pain during biopsy. The likelihood of pain during probe insertion was significantly higher in patients with pain during biopsy than in those without pain during biopsy ($p=0.002$)

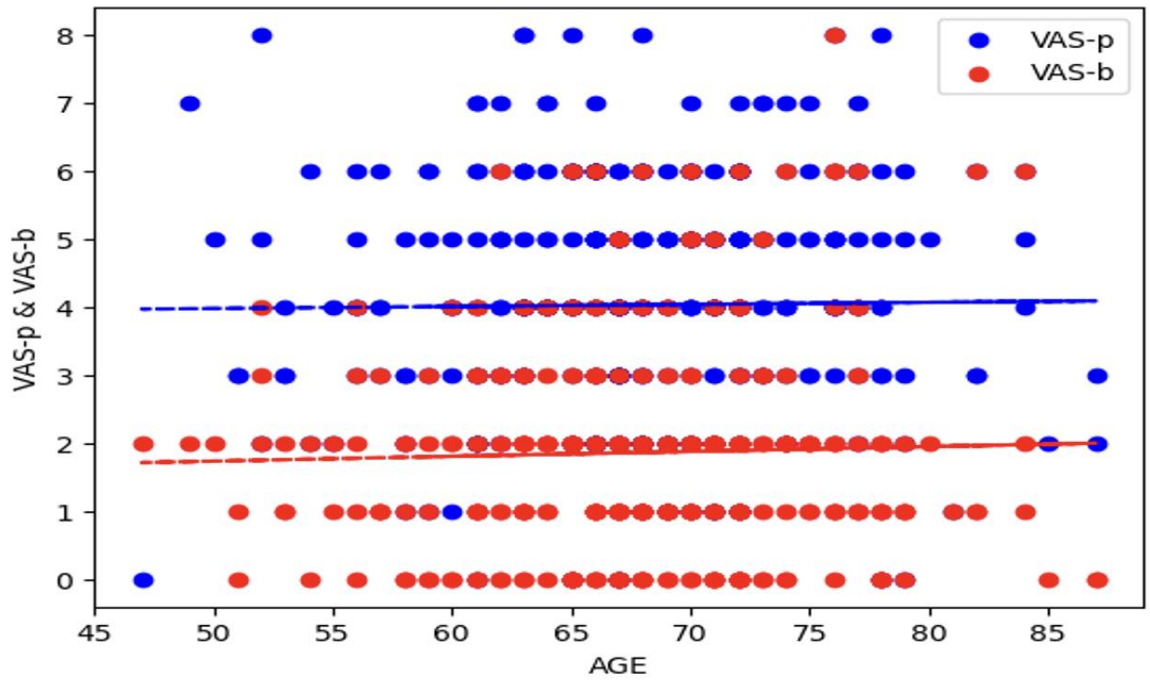
Across all patients, the pain felt during probe insertion was greater than the pain felt during biopsy, and this was statistically significant ($p=0.001$) (Figure 1).

When prostate volume was compared with pain score, each unit increase in prostate volume increased the probability of pain in VAS-p by 1.014 times ($p=0.015$).

Table 1. Main characteristics of groups. Group 1: patients aged 65 and under. Group 2: patients aged between 66-70 years old. Group 3: patients aged 71 and over

Parameters	Overall	Group 1	Group 2	Group 3	*p* value (Test value)
Number of patients (%)	308	103 (33.4%)	100 (32.5%)	105 (34.1%)	
Age mean (min-max)	67.5 (47-87)	62 (47-65)	67 (66-70)	74 (71-87)	0.479 (z:0.623)
PSA mean (min-max)	7.1 (0.2-575)	6.2 (0.2-151)	6.9 (1-575)	8.6 (2.4-456)	0.484 (z:-1.027)
PV mean (min-max)	59.5 (10-220)	55 (10-220)	66.5 (25-168)	60 (20-211)	0.008*(h:-0.536)
BMI mean (min-max)	26.6 (19.6-46.7)	27.7 (21.5-39.7)	26.7 (20.3-42.0)	25.5 (19.6-46.7)	0.075 (h:2.675)
No. of cores	12 (8-12)	12 (8-12)	12 (8-12)	12 (8-12)	0.054 (h:0.612)
Anormal DRE n, %	160 (51.9%)	43 (41.7%)	54 (54%)	63 (60%)	0.027* (cs:0.552)
Pathology results					
PIN n, %	13 (4.2%)	3 (2.9%)	6 (6%)	4 (3.8%)	
ASAP n, %	26 (8.4)	7 (6.8)	11 (11%)	8 (7.6%)	0.025* (cs:0.29)
BPH n, %	131 (42.5)	52 (50.5%)	47 (47%)	32 (30.5%)	
PCa n, %	138 (44.8)	41 (39.8%)	36 (36%)	61 (58.1%)	
DM n, %	111 (36%)	41 (39.8%)	35 (35%)	35 (33.3%)	0.602 (cs:0.282)
Biopsy time mean (min-max)	9 (7-11)	9 (7-11)	9 (7-11)	8 (7-11)	0.309 (h:2.349)
VAS- p mean (min-max)	4 (0-8)	4 (0-8)	4 (0-8)	4 (0-8)	0.372 (h:1.976)
VAS- b mean (min-max)	2 (0-8)	2 (0-6)	1 (0-6)	2 (0-8)	0.882 (h:0.250)

h: Kruskall Wallis test, cs: Chi-square test z: Mann-Whitney U test, PSA: prostate specific antigene, PV: prostate volume, BMI: body mass index DRI: digital rectal examination, PIN: prostatic intraepithelial neoplasia, ASAP: atypical small acinar proliferation, PCa: prostate carcinoma BPH: benign prostate hyperplasia DM: diabetes mellitus, VAS-p: visual analog scale for rectal probe VAS-b: visual analog scale during biopsy needle puncture



VAS-p: Visual Analogue Scale of Probe

VAS-b: Visual Analogue Scale of Biopsy

Figure 1. Scatter plot of VAS-p and VAS-b according to age

Discussion

Despite ongoing criticism, TRUS-PB remains the most widely used procedure for prostate cancer diagnosis. Since it is an invasive procedure, it is not without risks and can lead to complications, including sepsis. Additionally, the pain experienced during the biopsy procedure often discourages patients from undergoing it. For this reason, various forms of local anesthesia have been introduced to alleviate the pain and discomfort associated with prostate biopsy [12, 13]. Although advances have been made in the procedure over the years, pain and discomfort remain the most common side effects. Our study aimed to determine whether pain arises primarily during probe insertion or biopsy.

Desgrandchamps et al. [14] investigated the effect of 2% lidocaine gel on pain during the procedure and concluded that rectal administration of lidocaine had no effect on tolerance to prostate biopsy. Likewise, Peyromaure et al. [15] reported that only 51 of 275 patients (18.6%) did not feel any pain or discomfort during the biopsy procedure, while Aus et al. [16] reported that only 24 of 343 patients (7%) did not feel any discomfort. Pain developed during probe insertion in 25.5% of the patients included in our study and was consistent with the existing literature. Unlike the existing studies, we evaluated biopsy and probe pain separately and 90.9% of the patients who had pain during biopsy felt pain during probe insertion. Therefore, it was concluded that the main reason for the patients to feel pain was the probe insertion procedure.

In a study comparing anesthesia types by Kravchick et al. [17], in which the pain felt during probe insertion was evaluated, it was found that the lowest pain scores were observed in the perianal injection and DMSO/lidocaine groups, and that there was no correlation between the pain felt during probe insertion and biopsy. When we looked at the data we obtained, it was observed that the main reason why the patients felt pain during the biopsy was the pain felt at the probe entrance, and it was observed that as the pain score increased during the probe procedure, the pain score increased during the biopsy procedure.

Among the techniques to alleviate pain during TRUS-guided prostate biopsy, periprostatic nerve placement (PPNB) is the most effective method PPNB has been found to significantly reduce pain during biopsy [9, 18]. In addition, studies have reported that patients who underwent periprostatic anesthesia felt more pain during probe insertion than during the biopsy procedure [7]. 2% intrathecal lidocaine gel was administered as anesthesia to our patients before the biopsy procedure and 80% of the patients in all three patient groups felt pain during the procedure.

In the study in which the age of the patients and the pain felt during the biopsy procedure were evaluated, it was found that younger patients felt more pain due to high sphincter tone, while older patients felt less pain due to pain tolerance [5, 11]. When the data of our current study were examined, it was observed that the pain sensation was mostly in patients under 65 years of age, and they felt this pain especially during probe entry.

The strengths of our study are the separate evaluation of pain during probe insertion and biopsy procedure and its classification according to age groups. The study has several limitations, including its retrospective design and single-center setting, as well as the relatively small sample size. Another limitation is the use of VAS scoring, which is a subjective measurement tool. In addition to pain perception, which changes from patient to patient, sociocultural factors may also impact the results of this subjective assessment, potentially influencing the study's findings.

It has been concluded that pain sensation in patients undergoing biopsy is mainly felt during probe insertion and that this pain sensation increases with increasing prostate size. It can be concluded that younger and older patients are also more sensitive to pain. Larger prospective multicenter studies are needed to reach clearer conclusions.

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Research Article

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The relationship between systemic immune inflammation index and disease activity in ankylosing spondylitis patients

Ankilozan spondilit hastalarında sistemik immün inflamasyon indeksinin hastalık aktivitesi ile ilişkisi

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Abstract

Purpose: This study aimed to investigate the relationship between the systemic immune inflammation index (SII), platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), and disease activity and functional status in patients with ankylosing spondylitis (AS).

Materials and methods: This cross-sectional clinical study included a total of 90 patients diagnosed with AS according to the Modified New York Criteria, aged between 18 and 65, who presented to our outpatient clinics. Demographic data and laboratory parameters, including platelet, neutrophil, basophil, eosinophil, and lymphocyte counts, mean platelet volume (MPV), red blood cell distribution width (RDW), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), were recorded. NLR and PLR values were calculated. The SII was calculated by dividing the product of neutrophil and platelet counts by the lymphocyte count.

Results: The study included 90 AS patients (mean age: 42.9±11.3 years). Positive correlations were observed between SII and CRP (p=0.010, r=0.269) and ESR (p=0.007, r=0.282). No significant correlations were found between SII and BASDAI (p=0.323), BASFI (p=0.124) or BASMI (p=0.673). NLR and PLR values didn't differ significantly between active and inactive disease groups across all disease activity measures (BASDAI, ASDAS-CRP, and ASDAS-ESR; NLR: p=0.933, p=0.639, p=0.240; PLR: p=0.708, p=0.858, p=0.351; respectively). There was a significant correlation between SII and Ankylosing Spondylitis Disease Activity Score with erythrocyte sedimentation rate (ASDAS-ESR) (rho=0.282, p=0.007).

Conclusion: The study suggests that SII correlates positively with CRP and ESR, common inflammatory markers in AS. SII could be a potential marker for assessing inflammation, especially in patients with higher disease activity.

Keywords: Ankylosing spondylitis, complete blood count, inflammation.

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Öz

Amaç: Bu çalışma, ankilozan spondilit (AS) hastalarında sistemik immün inflamasyon indeksi (SII), trombosit-lenfosit oranı (PLR), nötrofil-lenfosit oranı (NLR) ile hastalık aktivitesi ve fonksiyonel durum arasındaki ilişkiyi araştırmayı amaçlamaktadır.

Gereç ve yöntem: Bu klinik kesitsel çalışmaya, Modifiye New York Kriterleri'ne göre AS tanısı almış 18-65 yaş aralığında 90 hasta dahil edildi. Demografik veriler, trombosit, nötrofil, bazofil, eozinofil ve lenfosit sayıları, ortalama trombosit hacmi (MPV), eritrosit dağılım genişliği (RDW), C-reaktif protein (CRP) ve eritrosit sedimantasyon hızı (ESR) gibi laboratuvar parametreleri kaydedildi. NLR, PLR ve SII değerleri hesaplandı.

Bulgular: Çalışmaya dahil edilen AS hastalarının yaş ortalamaları 42,9±11,3 yıl idi. SII ile CRP (p=0,010, r=0,269) ve ESR (p=0,007, r=0,282) arasında pozitif korelasyon saptandı. SII ile BASDAI (p=0,323), BASFI (p=0,124) veya BASMI (p=0,673) arasında anlamlı bir ilişki bulunmadı. NLR ve PLR değerleri aktif ve inaktif hastalık grupları arasında anlamlı bir fark göstermedi (BASDAI, ASDAS-CRP ve ASDAS-ESR için sırasıyla; NLR: p=0,933, p=0,639, p=0,240; PLR: p=0,708, p=0,858, p=0,351). SII ile ESR kullanılarak hesaplanan Ankilozan Spondilit Hastalık Aktivite Skoru (ASDAS-ESR) arasında anlamlı bir korelasyon vardı (rho=0,282, p=0,007).

Sonuç: Çalışma, SII'nin AS'de yaygın inflamatuar belirteçler olan CRP ve ESR ile pozitif korelasyon gösterdiğini ortaya koymaktadır. SII, özellikle daha yüksek hastalık aktivitesine sahip hastalarda inflamasyonu değerlendirmek için potansiyel bir belirteç olabilir.

Anahtar kelimeler: Ankilozan spondilit, tam kan sayımı, inflamasyon.

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease belonging to the spondyloarthritis group, primarily affecting the spine and sacroiliac joints [1]. Due to its progressive nature, disease activity needs to be regularly monitored, and the treatment plan should be adjusted according to changes in activity. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are commonly utilized markers for inflammation assessment during follow-ups. However, their elevation can occur due to various non-specific factors like infections, malignancies, and inflammation unrelated to AS. This highlights the necessity for more specific markers in evaluating disease activity in rheumatic diseases like AS and rheumatoid arthritis [2].

Complete blood count is an easily assessable, cost-effective, and straightforward test. There are changes in blood parameters during inflammatory processes, and these changes can be utilized to assess the level of inflammation [2]. The value obtained by dividing the number of neutrophils by the number of lymphocytes in a complete blood count, known as Neutrophil-to-Lymphocyte Ratio (NLR), has been found to be associated with the level of inflammation in diseases such as thyroid disorders [3], inflammatory bowel disease [4], and diabetes mellitus [5]. Another value obtained by dividing the platelet count by the lymphocyte count, known as Platelet-to-Lymphocyte Ratio (PLR), has found applications in conditions such as liver fibrosis [6] and diabetes [7]. The Systemic Immune Inflammation Index (SII), a novel marker, is calculated by multiplying the platelet count by the neutrophil count and then dividing this product by the lymphocyte count. SII has been found to be more successful in determining inflammation compared to NLR and PLR [8]. In studies, SII has been reported as a prognostic and activity determinant in conditions such as various types of malignancies [9, 10], Behçet's disease [11], vasculitis [12], lateral epicondylitis [13], rheumatoid arthritis [14] and post-stroke depression [15].

Recent studies have reported that data obtained through calculations of laboratory parameters such as the SII, NLR, and PLR can potentially be used to assess disease activity in AS, although this remains a topic of debate [16, 17]. Additionally, in the follow-up of AS patients, parameters that include assessments of symptom severity such as Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), the Ankylosing Spondylitis Disease Activity Score with C-reactive protein (ASDAS-CRP), and the Ankylosing Spondylitis Disease Activity Score with erythrocyte sedimentation rate (ASDAS-ESR) hold significant importance. The aim of this study is to evaluate the relationship between SII, PLR, NLR values and BASDAI, ASDAS-CRP, and ASDAS-ESR scores, which are used to determine disease activity and their applicability in patient monitoring among AS patients.

Materials and methods

Our study was designed as a cross-sectional clinical study. Between May 2, 2024, and May 20, 2024, a total of 90 patients between the ages of 18-65 who were diagnosed with AS according to the Modified New York Criteria and followed up at the Health Science University, Istanbul Physical Therapy and Rehabilitation Training and Research Hospital and Beylikdüzü State Hospital outpatient clinics were included in the study. Ethical clearance for this research, as per protocol number 2024/18, was granted by the Clinical Research Ethics Committee of Istanbul Physical Medicine and Rehabilitation Training and Research Hospital on April 30, 2024. Prior to the commencement of the study, participants provided informed consent. The study was conducted in accordance with the guidelines outlined in the Declaration of Helsinki.

Exclusion criteria for our study included the presence of acute or chronic infections, autoimmune diseases other than ankylosing spondylitis, pregnancy, diabetes, chronic kidney or liver disease, and the presence of cardiovascular or hematological diseases that could lead to changes in laboratory parameters. At the beginning of the study, sociodemographic data such as age, gender, marital status, body-mass index (BMI), smoking status, duration of disease diagnosis, and age at symptom onset were recorded for all patients.

Disease activity was assessed using the BASDAI, ASDAS-CRP, and ASDAS-ESR. The Turkish version of BASDAI, which has been validated and demonstrated reliability by Akkoç et al. [18], was utilized in the study . Scores of 4 or higher were considered indicative of active disease, while scores below 4 were classified as inactive disease. For ASDAS-CRP and ASDAS-ESR scores, patients with values of 2.1 and above were categorized as active, while those with values below 2.1 were placed in the inactive group [19]. Spinal mobility was evaluated using the Bath Ankylosing Spondylitis Metrology Index (BASMI), and functional status was assessed using the Bath Ankylosing Spondylitis Functional Index (BASFI) [20]. Furthermore, during the routine follow-up of patients, requested blood tests were examined to record platelet, neutrophil, basophil, eosinophil, lymphocyte counts, mean platelet volume (MPV), red blood distribution width (RDW), CRP, and ESR values. NLR, PLR and SII were calculated. SII is obtained by dividing the product of neutrophil and platelet counts by the lymphocyte count.

Statistical analysis

The study's sample size was determined based on Wu et al. [21] research, with correlations of 0.483 for SII and CRP, 0.374 for SII and ESR, and 0.667 for SII and BASDAI. With a significance level of 5% and 95% power, sample sizes for CRP, ESR, and BASDAI were calculated as 41, 73, and 19, respectively. A minimum of 73 participants was needed for all variables. Our study included 90 AS patients. We used G*Power 3.1.9.4 for sample size calculation. Data normality was assessed with Kolmogorov-Smirnov test. Descriptive statistics were used for quantitative data, presented as mean/standard deviation or median/min-max, and categorical data as frequency/percentage. Spearman test analyzed SII-AS disease activity correlation due to non-normality. Mann-Whitney U test compared active and inactive disease groups. SPSS 21.0 conducted statistical analyses.

Results

Our study included 90 patients diagnosed with AS. Detailed descriptive statistics are provided in Table 1. Of the participants, 61.1% were male, and 38.9% were female, with a median age of 45.5. The median BMI of the individuals was 27.7, with a minimum value of 11.5 and a maximum value of 41.6 (Table 1).

Table 2 illustrates the correlation between AS disease activity parameters and laboratory data. SII doesn't show significant relationships with BASDAI (p=0.323), BASFI (p=0.124), or BASMI scores (p=0.673), but it correlates positively with CRP and ESR values (p=0.010, r=0.269 and p=0.007, r=0.282, respectively). PLR and NLR ratios aren't correlated with any parameter. BASMI scores correlate significantly with CRP (p=0.026) and ESR (p=0.005), whereas BASFI and BASDAI scores don't exhibit such correlations. As expected, CRP and ESR values correlate with ASDAS-CRP (p=0.000 and p=0.000, respectively) and ASDAS-ESR scores (p=0.014 and p=0.000, respectively) (Table 2).

In Table 3, patients were examined in two groups based on BASDAI, ASDAS-CRP, and ASDAS-ESR scores, classified as active and inactive. The calculated SII value in patients with high ASDAS-ESR scores, indicating active disease, was significantly higher than in the inactive group (p=0.032). There was no significant difference in NLR (p=0.933, p=0.639, p=0.240) and PLR (p=0.708, p=0.858, p=0.351) between the groups across all disease activity measures (BASDAI, ASDAS-CRP, and ASDAS-ESR, respectively) (Table 3).

Table 1. Descriptive statistics

		Median (min-max) / n (%)	%
Age		45.5 (21-65)	
Gender	Male	55	61.1
	Female	35	38.9
Marital status	Married	68	75.6
	Single	22	24.4
BMI		27.7 (11.5-41.6)	
Smoking	Non-smoker	48	53.3
	<10 pack-years	13	14.4
	10-20 pack-years	15	16.7
	>20 pack-years	14	15.6
Duration of diagnosis		84 (1-396)	
Age at symptom onset		24.5 (8-57)	
HLA B27	Positive	51	56.7
	Negative	39	43.3
BASDAI		5 (0-10)	
BASFI		4 (0-10)	
BASMI		2 (0-8)	
CRP		3.9 (0.2-46.4)	
ESR		9 (2-60)	
MPV		9.2 (7.1-12.5)	
RDW		13.4 (11.9-21.2)	
NLR		1.7 (0.8-4.6)	
PLR		111 (45.6-258)	
SII		478 (198.6-1503.7)	
ASDAS-CRP		2.7 (1.1-5.2)	
ASDAS-ESR		2.7 (1-5)	

BMI: Body-mass index BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BASFI: Bath Ankylosing Spondylitis Functional Index BASMI: Bath Ankylosing Spondylitis Metrology Index, ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score with C-reactive protein ASDAS-ESR: Ankylosing Spondylitis Disease Activity Score with erythrocyte sedimentation rate, CRP: C-reactive protein ESR: Erythrocyte sedimentation rate, PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio SII: systemic immune inflammation index

Table 2. Correlation between laboratory parameters and parameters related to disease activity

	BASDAI		BASMI		BASFI		CRP		ESR		ASDAS-CRP		ASDAS-ESR	
	rho	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***
CRP	-0.029	0.785	0.234	0.026*	0.072	0.499	1	-	0.696	0.000*	0.473	0.000*	0.258	0.014*
ESR	0.016	0.879	0.296	0.005*	0.010	0.926	0.696	0.000*	1		0.374	0.000*	0.450	0.000*
RDW	-0.061	0.568	0.199	0.060	-0.100	0.348	0.124	0.245	0.315	0.003*	0.055	0.605	0.131	0.218
MPV	-0.049	0.649	0.041	0.701	-0.111	0.296	0.002	0.986	0.001	0.990	-0.009	0.932	-0.021	0.845
SII	0.105	0.323	-0.045	0.673	0.163	0.124	0.269	0.010*	0.282	0.007*	0.196	0.064	0.196	0.065
PLR	0.103	0.335	-0.019	0.860	0.112	0.295	-0.006	0.953	0.133	0.212	0.031	0.771	0.098	0.357
NLR	-0.007	0.949	-0.081	0.446	0.097	0.361	0.146	0.171	0.109	0.308	0.031	0.770	0.007	0.948

Spearman correlation test. Values with $p<0.05$ are marked with an asterisk (*). BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BASFI: Bath Ankylosing Spondylitis Functional Index
BASMI: Bath Ankylosing Spondylitis Metrology Index, CRP: C-reactive protein, ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score with C-reactive protein
ASDAS-ESR: Ankylosing Spondylitis Disease Activity Score with erythrocyte sedimentation rate, ESR: Erythrocyte sedimentation rate, PLR: platelet to lymphocyte ratio
NLR: neutrophil to lymphocyte ratio, SII: systemic immune inflammation index

Table 3. Changes in laboratory and clinical parameters according to disease activity

	BASDAI				ASDAS-CRP				ASDAS-ESR			
	<4 (n=27)	**≥4 (n=63)**	***p***	***z***	**<2.1 (n=22)**	**≥2.1 (n=68)**	***p***	***z***	**<2.1 (n=25)**	**≥2.1 (n=65)**	***p***	***z***
BASFI	1 (0-6)	5 (1-10)	0.000*	-5.427	1.5 (0-6)	5 (0-10)	0.000*	-3.524	1 (0-7)	5 (0-10)	0.000*	-3.912
BASMI	3 (0-6)	2 (0-8)	0.535	-0.621	2 (0-6)	2 (0-8)	0.404	-0.835	2 (0-6)	2 (0-8)	0.967	-0.041
RDW	13.4 (12.5-19.6)	13.4 (11.9-21.2)	0.558	-0.586	13.2 (12.5-19.6)	13.4 (11.9-21.2)	0.305	-1.025	13.4 (12.5-19.6)	13.4 (11.9-21.2)	0.708	-0.374
MPV	9.2 (7.2-10.7)	9.1 (7.1-12.5)	0.754	-0.313	9.2 (8-10.1)	9.1 (7.1-12.5)	0.764	-0.301	9.2 (7.8-10.1)	9.2 (7.1-12.5)	0.435	-0.780
CRP	4.2 (0.2-36.6)	3.6 (0.2-46.4)	0.853	-0.185	1.8 (0.2-12.2)	4.9 (0.2-46.4)	0.002*	-3.108	2.4 (0.3-23.1)	4.7 (0.2-46.4)	0.051	-1.951
ESR	8 (2-51)	10 (2-60)	0.363	-0.910	7 (2-35)	11 (2-60)	0.042*	-2.035	6 (2-35)	11 (2-60)	0.007*	-2.703
NLR	1.6 (0.8-4.4)	1.8 (0.8-4.6)	0.933	-0.084	1.6 (0.8-4.4)	1.76 (0.8-4.6)	0.639	-0.469	1.6 (0.9-4.4)	1.8 (0.8-4.6)	0.240	-1.176
PLR	105.7 (60.9-223.6)	111.6 (45.6-258)	0.708	-0.374	110.1 (60.9-195.3)	110.9 (45.6-258)	0.858	-0.178	105.7 (60.9-165.2)	111.8 (45.6-257.9)	0.351	-0.932
SII	438.7 (214.6-1017.4)	496 (198.6-1503.7)	0.410	-0.823	412.8 (205-847.3)	491.12 (198.6-1503.7)	0.233	-1.192	357.4 (205-847.3)	496 (198.6-1503.7)	0.032*	-2.139

Mann Whitney U test. Values with $p<0.05$ are marked with an asterisk (*). Bath Ankylosing Spondylitis Disease Activity Index, BASFI: Bath Ankylosing Spondylitis Functional Index, BASMI: Bath Ankylosing Spondylitis Metrology Index, ASDAS-CRP: Ankylosing Spondylitis Disease Activity Score with C-reactive protein , ASDAS-ESR: Ankylosing Spondylitis Disease Activity Score with erythrocyte sedimentation rate, CRP: C-reactive protein ESR: Erythrocyte sedimentation rate, PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio, SII: systemic immune inflammation index

Discussion

In this cross-sectional clinical study, a positive correlation was found between CRP and ESR values, which are frequently used in the follow-up and treatment decisions of AS patients and considered as markers of inflammation, and the SII value. Additionally, higher SII values were found in the patient group considered active according to the ASDAS-ESR score. However, there was no significant difference in SII values between active and inactive patient groups based on ASDAS-CRP and BASDAI scores. Furthermore, in our study, we did not find a significant difference in NLR and PLR levels between active and inactive groups for all three parameters.

There are many studies in the literature investigating the use of blood parameters to determine disease activity and periodic patient monitoring in rheumatological diseases [14, 15]. However, the number of such studies in AS patients is limited. In the follow-up and determination of activity in AS patients, not only laboratory parameters but also scoring systems such as BASDAI, ASDAS-CRP, and ASDAS-ESR are used. Limitation in spinal mobility can be evaluated with the BASMI index, and the patient's functional status can be assessed with the BASFI scoring. Scoring systems that rely on the patient's self-report, such as BASDAI and BASFI, are influenced by many additional factors beyond the inflammation caused by the disease, including the patient's psychological state, perception of the disease, and central sensitization. ASDAS-CRP and ASDAS-ESR values include both laboratory parameters and patient self-report when calculated [22]. The lack of correlation between SII and scoring systems obtained from subjective questioning, but its higher values in patients considered active according to ASDAS-ESR, may be attributed to the patient's mental and psychological factors. The correlation of SII with ESR and CRP values also suggests that it could be a biomarker with the potential to predict the current level of inflammation.

In a study, the SII value in AS patients was found to be correlated with ESR, CRP, and BASDAI scores [21]. In another study, SLE, RA, and AS patient groups were compared with healthy controls. SII values were higher in AS and RA patients compared to the control group. PLR was higher in all three groups, while NLR was significantly higher only in SLE patients compared to the control group. However, when the AS group was grouped according to the level of disease activity, there was no significant difference in SII, NLR, and PLR values. Additionally, in AS patients, SII and NLR values were correlated with CRP, ESR, and ASDAS parameters, while PLR was not correlated. On the other hand, MPV and RDW values showed changes consistent with disease activity in all three disease groups [23]. In contrast, in our study, RDW and MPV values in AS patients were not found to be associated with disease activity or laboratory parameters.

In the study by Liang et al. [24], PLR and NLR ratios were significantly higher in the AS patient group compared to the healthy group. Additionally, the AS patient group was categorized as active and inactive based on BASDAI scores, and the active group had significantly higher PLR values, but the same was not true for NLR. In our research, parallel to this study, NLR values were not found to be associated with disease activity, and similarly, PLR values were not found to be different between active and inactive patients, as was the case with NLR. In a study conducted by Osami et al. [25] in AS patients, NLR, PLR, and ESR values were found to be significantly higher in active AS patients compared to inactive ones. However, when compared to the healthy group, ESR was significantly higher, while NLR and PLR ratios were similar between the two groups. In contrast to this study, in our research, NLR and PLR values were not correlated with disease activity levels indicated by BASDAI, ASDAS-CRP, and ASDAS-ESR values.

Our study was conducted only with AS-diagnosed patients due to the absence of a control group. Additionally, the single-center nature of the study is one of its limitations. One of the strengths of our research is that we examined the correlation of markers by simultaneously using multiple methods for evaluating disease activity. The markers evaluated in this study are cost-effective parameters that can be easily obtained in all laboratories where complete blood counts are performed and are well-correlated with inflammation [23]. In our study, especially, SII has been shown to be a cost-effective marker that can be used for this

purpose. Further studies with a larger number of patients and multi-center studies are needed to establish its safe use in patient follow-up.

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Authors' contributions: The main idea and hypothesis of the study were formulated by B.S.A. and F.N.K. They also contributed to the development of the theory and organization of the material and methods section. Evaluation of the data in the Results section was conducted by B.S.A., F.N.K., N.P. and Y.C.A. The Discussion section of the article was authored by B.S.A., with input from Y.C.A., N.P. and F.N.K., who reviewed, corrected, and approved it. Furthermore, all authors collectively discussed the entirety of the study and endorsed the final version.

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

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Research Article

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Evaluation of Enterobacterales bloodstream infections in hematologic cancer patients

Hematolojik kanser hastalarında Enterobacterales kan dolaşımı enfeksiyonlarının değerlendirilmesi

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Abstract

Purpose: In this study, we aimed to evaluate the clinical and laboratory findings of hospitalized patients with Enterobacterales bacteremia/sepsis, the risk factors for mortality and the therapeutic options for treating bloodstream infections (BSIs) caused by Enterobacterales.

Materials and methods: Patients hospitalized in the Oncology Hospital between January 2021 and December 2022 whose Enterobacterales species were isolated in blood cultures were included in the study. Blood cultures were incubated in the Autobio BC120 device. Isolated microorganisms were named using a Vitek-2 (bioMerieux, France) automated system. Antibiotic susceptibility tests were performed in the Vitek-2 system (bioMerieux, France) and the disc diffusion method. In addition, the demographic and laboratory data of the patients were evaluated. A total of 103 patients were included in the study during the two years. Only the first isolates from each patient were included in the study.

Results: The distribution of Enterobacterales isolates grown in blood cultures, in order of frequency were *Escherichia coli* (n:74, 63.25%), *Klebsiella pneumoniae* ssp *pneumoniae* (n:27, 23.1%), *Klebsiella pneumoniae* ssp *ozaenae* (n:2, 1.71%), *Klebsiella oxytoca* (n:1, 0.85%), *Enterobacter cloaceae complex* (n:10, 6.84%), *Citrobacter freundii* (n:1), *Proteus mirabilis* (n:1), *Salmonella* spp (n:1). The median (min-max) white blood cell count was 1.51x103cells/uL (0.01-19.87), C-reactive protein (CRP) was 112.3 mg/L (0.06-546.0), procalcitonin was 7.35 µg/L (0.05-61.21), time between blood culture collection and growing signal was 11.33 (3-58) hours and the blood culture result report was three (1-8) days. Acute Myeloid Leukemia 40 (39.2%), B-cell Acute Lymphoblastic leukemia 18 (17.6%), Multiple Myeloma 11 (10.8%), Diffuse Large B-cell Lymphoma 11 (10.8%) were the most common diseases seen in Enterobacterales isolated patients from blood cultures.

Conclusion: Each hospital should conduct its evaluation and examine the patient profile to make the correct empirical antibiotic selection. It is crucial to develop a suitable algorithm for this purpose.

Keywords: Enterobacterales, bloodstream infections, hematologic cancer patients.

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Öz

Amaç: Bu çalışmada Enterobacterales bakteriyemisi/sepsisi nedeniyle hastaneye yatırılan hastaların klinik ve laboratuvar bulgularını, mortalite için risk faktörlerini ve Enterobacterales'in neden olduğu kan dolaşımı enfeksiyonlarının (KDE) tedavisine yönelik tedavi seçeneklerini değerlendirmeyi amaçladık.

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Gereç ve yöntem: Çalışmaya Ocak 2021 ile Aralık 2022 tarihleri arasında Onkoloji Hastanesi'nde yatan ve kan kültürlerinde Enterobacterales türlerine rastlanan hastalar dahil edildi. Kan kültürleri Autobio BC120 cihazında inkübe edildi. İzole edilen mikroorganizmalar Vitek-2 (bioMerieux, Fransa) otomatize sistem kullanılarak adlandırıldı. Antibiyotik duyarlılık testleri hem Vitek-2 sistemi (bioMerieux, Fransa) hem de disk difüzyon yöntemiyle yapıldı. Ayrıca, hastaların demografik ve laboratuvar verileri değerlendirildi. İki yıllık dönemde toplam 103 hasta çalışmaya dahil edildi. Her hastadan sadece ilk izolatlar çalışmaya dahil edildi.
Bulgular: Kan kültürlerinde üreyen Enterobacterales izolatlarının dağılımı sıklık sırasına göre; *Escherichia coli* (%63,25, n:74), *Klebsiella pneumoniae* ssp *pneumoniae* (%23,1, n:27), *Klebsiella pneumoniae* ssp *ozaenae* (%1,71, n:2), *Klebsiella oxytoca* (%0,85, n:1), *Enterobacter cloaceae* kompleks (%6,84, n:10), *Citrobacter freundii* (n:1), *Proteus mirabilis* (n:1), *Salmonella* spp (n:1) şeklindeydi. Ortalama (minimum-maksimum) lökosit sayısı 1,51x103 hücre/uL (0,01-19,87), C-reaktif protein (CRP) 112,3 mg/L (0,06-546,0), prokalsitonin 7,35 µg/L (0,05-61,21), kan kültürünün alınmasıyla üreme sinyali arasında geçen süre 11,33 (3-58) saat ve kan kültürü sonuç raporu üç (1-8) gün olarak belirlendi. Kan kültürlerinden Enterobacterales izole edilen hastalarda; Akut Myeloid Lösemi 40 (%39,2), B-hücreli Akut Lenfoblastik Lösemi 18 (%17,6), Multiple Myelom 11 (%10,8), Diffüz Büyük B-hücreli Lenfoma 11 (%10,8) en sık görülen hastalıklardı.
Sonuç: Doğru ampirik antibiyotik seçimini yapabilmek için her hastane kendi değerlendirmesini yapmalı ve hasta profilini incelemelidir. Bu amaçla uygun bir algoritma geliştirmek son derece önemlidir.

Anahtar kelimeler: Enterobacterales, kan dolaşımı enfeksiyonları, hematolojik kanser hastaları.

Tavukcu E, Arslan F, Süzük Yıldız S, Güreser AS, Mumcuoğlu İ, İnan N, Ulaş T, Dal T. Evaluation of Enterobacterales bloodstream infections in hematologic cancer patients. Pam Med J 2025;18:207-217.

Introduction

Hematological patients are prone to many infectious complications during their treatment, with bloodstream infections (BSIs) standing out as the most significant cause of mortality and morbidity in this patient group. In hematopoietic stem cell transplantation (HSCT) patients, more than 50% of deaths occur as a result of infections within the initial 100 days post-transplantation. Enterobacterales species are notably common culprits of BSIs in this population [1, 2].

The use of carbapenems in Enterobacterales infections has seen a considerable rise since the appearance of extended-spectrum beta-lactamases. There has been a noticeable increase in the prevalence of Carbapenem-Resistant Enterobacteriaceae (CRE) in the last few years. In 2017, the World Health Organization designated CRE as a pathogen of critical priority [3]. Due to plasmid-mediated horizontal gene transfer, CRE isolates have spread in hospitals, becoming a significant cause of death in immunosuppressive individuals. The most effective therapeutic approach for CRE bloodstream infections (BSIs) remains unknown. Therefore, this study aims to evaluate the clinical aspect and laboratory findings of hospitalized patients with Enterobacterales bacteremia/sepsis, identify risk factors for mortality, and propose possible treatment alternatives for the management of BSIs caused by Enterobacterales.

Material and method

Patients who were admitted to the hematology service and bone marrow transplant unit at Dr. Abdurrahman Yurtaslan Ankara Oncology Hospital between January 2021 and December 2022 and had Enterobacterales species isolated in blood cultures were included. The hospital primarily serves hematologic and oncologic patients in Ankara, Türkiye. Permission for the study was obtained from the Non-Interventional Clinical Research Ethics Committee of the University of Health Sciences Dr Abdurrahman Yurtaslan Oncology Training and Research Hospital (permission date: 11.01.2024, permission number: 2023-12/126).

Blood culture samples taken from the patients were sent to the Medical Microbiology Laboratory. Subsequently, blood cultures were incubated in the Autobio BC120 (Autobio, Chinese) device and the blood culture bottles indicating growth were inoculated onto 5% sheep blood agar, eosin methylene blue agar, and chocolate agar media. Isolated microorganisms underwent evaluation, and the Vitek-2 automated system (bioMerieux, France) was utilized for microbial typing. Antibiotic susceptibility was determined using both the Vitek-2 system and the disc diffusion method. Results of antibiotic susceptibility were interpreted following the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), with those classified as S (Susceptible) and I

(Intermediate) included in the sensitive group [4].

Furthermore, demographic data including age and gender, comorbid diseases, type of hematological malignancy, length of stay, empirical antibiotic use, antibiotics administered in post-culture treatment, concurrent infections, C-reactive protein (CRP) and procalcitonin levels, white blood cell counts and the duration times between blood culture collection and the growth signal of samples, were collected. The times and the laboratory's blood culture reporting data were also documented. The patients' immunosuppressive treatment options, bone marrow transplantation (BMT) status, type of transplantation, and neutropenia status were examined. Neutropenia was clinically categorized as mild when the absolute neutrophil count (ANC) ranged from 1000 to 1500/μL, moderate with an ANC between 500 and 1000/μL, or severe with an ANC below 500 /μL [5].

Statistical analysis

The data underwent analysis using SPSS (version 26) and were expressed as numbers, percentages, medians, minimum, and maximum values.

Results

During the two-year study period, a total of 103 patients were included and 117 blood culture growths were detected. The study included the first samples from patients with recurrent growth. The average age of the patients was 47.5 years, with 45 patients being male (43.7%) and 58 females (56.3%).

In the study, Gram-negative microorganisms were isolated from 44.6% of the blood culture isolates, and among the Gram-negative, the rate of Enterobacterales was 70.9%. The distribution of Enterobacterales isolates in blood cultures, in order of frequency, were as follows: *Escherichia coli* (n:74, 63.25%), *Klebsiella pneumoniae* ssp *pneumoniae* (n:27, 23.1%), *Klebsiella pneumoniae* ssp *ozaenae* (n:2, 1.71%), *Klebsiella oxytoca* (n:1, 0.85%), *Enterobacter cloaceae* complex (n:10, 6.84%), *Citrobacter freundii* (n:1), *Proteus mirabilis* (n:1), *Salmonella* spp (n:1) (Table 1). Additionally, non-Enterobacterales microorganisms were simultaneously isolated from blood cultures in nine patients [*Staphylococcus epidermidis* (n:4), *Staphylococcus hominis* (n:4), *Kocuria varians* (n:1)].

The antibiotic susceptibility rates of the Enterobacterales isolates were as follows ampicillin 8.6%, piperacillin/tazobactam 60.3%, gentamicin 65.5%, amikacin 92.1%, cefuroxime axetil 33%, ceftriaxone 42.1%, ceftazidime 42.5%, cefepime 52.7%, ciprofloxacin 23.4%, trimethoprim/sulfamethoxazole 13.8%, ertapenem 79.3%, imipenem 83.3%, meropenem 81.4%, ceftazidime/avibactam 91.8% (Table 2).

Table 1. Distribution of Enterobacterales isolates grown in blood cultures

Isolated microorganism	Number (n=117)	Percent (%)
Escherichia coli	74	63.25
Klebsiella pneumoniae* ssp *pneumoniae	27	23.1
***Enterobacter cloacae* complex**	8	6.84
***Enterobacter* spp.**	2	1.71
Klebsiella pneumoniae* ssp *ozaenae	2	1.71
Citrobacter freundii	1	0.85
Klebsiella oxytoca	1	0.85
Proteus mirabilis	1	0.85
***Salmonella* group**	1	0.85
Total	117	100.0

Table 2. Antibiotic susceptibility rates of the blood culture Enterobacterales isolates

Antibiotic	Resistance, n (%)	Susceptible, n (%)	Total
Amikacin	9 (7.9)	105 (92.1)	114
Ampicillin	96 (91.4)	9 (8.6)	105
Cefazolin	75 (97.4)	2 (2.6)	77
Cefepime	53 (47.3)	59 (52.7)	112
Cefoperazone/Sulbactam	27 (23.3)	89 (76.7)	116
Cefotaxime	12 (63.2)	7 (36.8)	19
Ceftazidime	65 (57.5)	48 (42.5)	113
Ceftazidime/Avibactam	6 (8.2)	67 (91.8)	73
Ceftriaxone	66 (57.9)	48 (42.1)	114
Cefuroxime axetil	75 (67.0)	37 (33.0)	112
Ciprofloxacin	85 (76.6)	26 (23.4)	111
Ertapenem	24 (20.7)	92 (79.3)	116
Gentamicin	38 (34.5)	72 (65.5)	110
Imipenem	18 (16.7)	90 (83.3)	108
Meropenem	22 (18.6)	96 (81.4)	118
Piperacillin/Tazobactam	46 (39.7)	70 (60.3)	116
Trimethoprim/Sulfomethoxazole	100 (86.2)	16 (13.8)	116

Antibiotic susceptibility percentages according to microorganism species are given in Table 3. Although colistin and tigecycline sensitivity has been studied with the device, it is not presented because it was not tested using the reference method recommended by EUCAST.

Demographic data, clinical characteristics, and laboratory findings of the patients are presented in Table 4. The median (min-max) white blood cell count was $1.51x10^3$ cells/uL (0.01-19.87), CRP 112.3 mg/L (0.06-546.0), procalcitonin 7.35 µg/L (0.05-61.21), with a time (hours) between blood culture collection and the growing signal recorded as 11.33 (3-58) hours. The blood culture result report took three (1-8) days. Comorbid disorders included diabetes in eight (8%), hypertension in 12 (12.1%), perianal abscess in 16 (16.8%) and other diseases (epilepsy, chronic kidney disease, rectum, thyroid diseases) in 17.5%. Regarding cancers, Acute Myeloid Leukemia accounted for 40 (39.2%), B-cell Acute Lymphoblastic Leukemia (B-ALL) for 18 (17.6%), Multiple Myeloma for 11 (10.8%) and Diffuse Large B-cell Lymphoma for 11 (10.8%) of the cases with Enterobacterales isolated from blood cultures. Hematopoietic stem cell transplantation (HSCT) was applied for 51 (50.5%) patients, with 36 (35.6%) receiving allogeneic and 15 (14.9%) receiving autologous HSCT. Immunosuppressive therapy with cyclosporine was administered to 28 patients (28.3%) and Graft Versus Host Disease occurred in one patient (0.97%).

One hundred (97%) patients were administered empirical antibiotic treatment, utilizing cefoperazone-sulbactam, piperacillin-tazobactam, meropenem, ertapenem, fosfomycin, colistin, vancomycin, teicoplanin, and linezolid either as monotherapy or in combination. For 72 (72%) patients, the antibiotic therapy was modified following the report of blood culture growth. Among them, 67 (67%) underwent escalation, while de-escalation was implemented for four (4%) patients. Antibiotic therapy choices remained unchanged for 29 (29%) patients. The overall 30-day mortality rate was 14.56% (Table 4).

Table 3. Antibiotic susceptibility of isolated strains in blood culture

Antibiotics	*Escherichia coli* (%)	*Klebsiella pneumoniae* ssp *pneumoniae* (%)	*Enterobacter cloacae* complex (%)	*Entero bacter* spp. (%)	*Klebsiella pneumoniae* ssp *ozaenae* (%)	*Citrobacter freundii* (%)	*Klebsiella oxytoca* (%)	*Proteus mirabilis* (%)	*Salmonella* group (%)
Amikacin	71/71 (100)	20/27 (74.1)	8/8 (100)	1/2 (50)	1/2 (50)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
Ampicillin	7/71 (9.9)	0/26 (0)	0/0 (0)	0/2 (0)	0/2 (0)	0/0(0)	0/1 (0)	0/1 (0)	1/1 (100)
Cefazolin	2/41 (4.9)	0/24 (0)	0/7 (0)	0/0 (0)	0/2 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/0 (0)
Cefepime	43/70 (61.4)	4/27 (14.8)	3/8 (37.5)	0/2 (0)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Cefoperazone/ Sulbactam	68/74 (90.7)	13/26 (50)	3/8 (37.5)	1/2 (50)	1/2 (50)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Ceftazidime	39/71 (54.9)	3/27 (11.1)	3/8 (37.5)	0/2 (0)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Ceftazidime/ Avibactam	45/46 (97.8)	13/15 (86.7)	4/7 (57.1)	1/1 (100)	2/2 (100)	1/1 (100)	-	-	1/1 (100)
Ceftriaxone	41/73 (56.2)	3/27 (11.1)	1/8 (12.5)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Cefuroxime axetil	30/72 (41.7)	2/26 (7.7)	1/8 (12.5)	-	1/2 (50)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Ciprofloxacin	19/69 (27.5)	3/27 (11.1)	2/7 (28.6)	1/2 (50)	1/2 (50)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
Ertapenem	70/74 (94.6)	14/27 (51.9)	3/8 (37.5)	1/2 (50)	1/1 (100)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Gentamicin	51/70 (72.9)	13/25 (52)	5/7 (71.4)	0/2 (0)	1/2 (50)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)
Imipenem	69/71 (97.2)	14/27 (51.9)	3/4 (75)	1/2 (50)	1/1 (100)	1/1 (100)	0/1 (0)	0/0 (0)	1/1 (100)
Meropenem	72/75 (96)	15/27 (55.6)	3/8 (37.5)	1/2 (50)	2/2 (100)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Piperacillin/ Tazobactam	56/74 (75.7)	8/27 (29.6)	3/8 (37.5)	0/2 (0)	0/1 (0)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)
Trimethoprim/ Sulfomethoxazole	7/74 (9.5)	6/27 (22.2)	1/8 (12.5)	1/2 (50)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	1/1 (100)

Table 4. Demographic, clinic and laboratory characteristics of the patients

Variables	Median (min-max)
Age, years	47.50 (20-77)
Laboratory findings during blood culture growth	
White blood cell (x10^{3}cells/uL)	1.51 (0.01-19.87)
CRP (mg/L)	112.3 (0.06-546.0)
Procalcitonin (µg/L)	7.35 (0.05-61.21)
Time between blood culture collection and growing signal (hours)	11.33 (3-58)
Blood culture result report (days)	3 (1-8)
	N (%)
Gender	
Female (n, %)	58 (56.3)
Male (n, %)	45 (47.7)
Comorbid Diseases (n, %)	
Diabetes	8 (8)
Hypertension	12 (12.1)
Perianal Abscess	16 (16.8)
Other diseases (epilepsy, chronic kidney disease, rectum, thyroid diseases)	18 (17.5)
Hematological diagnoses (n, %)	
Multiple Myeloma	11 (10.8)
Acute Myeloid Leukemia	40 (39.2)
B cell- Acute Lympholastic leukemia	18 (17.6)
T cell- Acute Lympholastic leukemia	4 (3.9)
Diffuse large B-cell lymphoma	11 (10.8)
Chronic Lymphocytic leukemia	2 (2.0)
Aplastic anemia	3 (2.9)
Marginal Zone Lymphoma	1 (1.0)
Burkitt Lymphoma	1 (1.0)
Hodgkin Lymphoma	5 (4.9)
NK/T cell Lymphoma	2 (2.0)
Mantle cell lymphoma	2 (2.0)
Thrombotic Thrombocytopenic Purpura	1 (1.0)
Graft Versus Host Disease (GVHD) (n, %)	1 (0.97)
Bone Marrow Transplantation (BMT) (n, %)	51 (50.5)
Allogeneic	36 (35.6)
Autologous	15 (14.9)
Patients receiving empirical antibiotic treatment (n, %)	100 (97)
Patients whose antibiotic therapy was changed after report of blood culture growth (n, %)	72 (72)
Immunosuppressive therapy use (n, %)	
Cyclosporine	28 (28.3)

Table 4. Demographic, clinic and laboratory characteristics of the patients (continued)

Variables	Median (min-max)
Antimicrobials used in empirical treatment	
	Cefoperazone/sulbactam
	Cefoperazone/sulbactam + Vancomycin
	Cefoperazone/sulbactam + Teicoplanin
	Piperacillin/tazobactam
	Piperacillin/tazobactam + Vancomycin
	Meropenem
	Meropenem + Teicoplanin
	Meropenem + Vancomycin
	Ertapenem
	Linezolid
	Fosfomycin + Colistin
Antimicrobials used in post-culture treatment	
	Cefoperazone/sulbactam
	Cefoperazone/sulbactam + Vancomycin
	Cefoperazone/sulbactam + Teicoplanin
	Cefoperazone/sulbactam + Linezolid
	Piperacillin/tazobactam
	Piperacillin/tazobactam + Vancomycin
	Imipenem
	Imipenem + Colistin
	Meropenem
	Meropenem + Teicoplanin
	Meropenem + Vancomycin
	Meropenem + Colistin + Vancomycin
	Meropenem + Colistin + Daptomycin
	Meropenem + Colistin + Fosfomycin
	Meropenem + Vancomycin + Metronidazole
	Meropenem+ Linezolid + Fosfomycin
	Meropenem + Tigecycline + Colistin
	Ertapenem
	Colistin + Fosfomycin
	Ceftazidime + Fosfomycin + Linezolid
Post culture antimicrobial therapy (n, %)	
Escalation	67 (67%)
Deescalation	4 (4%)
No change	29 (29%)
30 day-mortality (n, %)	15 (14.56%)

Discussion

In recent years, antimicrobial resistance has become a significant problem due to the common use of broad-spectrum antibiotics, particularly among gram-negative bacteria. These bacteria are the major causes of bloodstream infections in hospitalized patients, especially in hematology clinics. Therefore, it is crucial to enhance awareness of antimicrobial resistance by employing effective methods in education, training, and communication. It is necessary to ensure effective infection control and reduce the incidence of infection. It is important to increase economic opportunities to develop new antimicrobial drugs, vaccines, and diagnostic tools and ensure sustainability. A 2021 study conducted in Türkiye reported *K. pneumoniae* as the most prevalent cause of BSIs, followed by *S. aureus*. The reported rates of isolated microorganisms in the Turkish study were as follows: *K. pneumoniae* 18.42%, *S. aureus* 14.47%, *Acinetobacter* spp. 13.16%, *Escherichia coli* 13.16%, *Enterococcus faecalis* 11.39%, *Pseudomonas aeruginosa* 10.53%, *Candida* spp. 7.89% [6]. In a 2019 Italian study, *P. aeruginosa*, *E. coli*, *A. baumannii*, methicillin-resistant *S. aureus* and *K. pneumoniae* were identified as the most common causes of sepsis [7]. Various studies consistently report *E. coli*, *K. pneumoniae*, *Acinetobacter* spp., *Pseudomonas* spp. and *Enterobacter* spp. as the most frequently detected gram-negative agents in bacteremia and sepsis. According to a 2023 study conducted in our country, the frequency of causative gram-negative agents was 33.9% for *E. coli*, 19.1% for *K. pneumoniae*, 18.5% for *Acinetobacter* spp., 10.3% for *Pseudomonas* spp., and 4.6% for *Enterobacter* spp. [8]. In our study, 44.6% of blood culture isolates were identified as gram-negative microorganisms, with Enterobacterales constituting 70.9% among gram-negatives. The distribution of Enterobacterales isolates in blood cultures, in order of frequency, were *E. coli* (63.25%), *K. pneumoniae* ssp *pneumoniae* (23.1%), *K. pneumoniae* ssp *ozaenae* (1.71%), *Klebsiella oxytoca* (0.85%), *Enterobacter cloacae* complex (6.84%), *Citrobacter freundii* (0.85%), *Proteus mirabilis* (0.85%), *Salmonella* spp (0.85%). We emphasize the importance for each hospital to identify the most common causes of sepsis, as the distribution of microorganisms may vary among hospitals and countries.

Nowadays, multidrug resistant microorganisms are the major public health problem. Gram-negative bacteria are the major causes of bloodstream infections in patients hospitalized. A 2023 study in Türkiye revealed antibiotic resistance rates of Enterobacterales isolates ranging from 6.8% to 14.5% for carbapenems, 10.6% for amikacin, 70.3% for ampicillin, 63.6% for cefuroxime, 55.4% for ceftriaxone, 48.9% for cefepime, 44.1% for trimethoprim/sulfamethoxazole (SXT), 53.8% for ciprofloxacin and 27.5% for piperacillin-tazobactam [8]. In our study, the resistance rates (%) of Enterobacterales isolates were as follows: ampicillin 91.4%, cefuroxime 67.9%, ceftriaxone 57.9%, cefepime 51.7%, SXT 86.3%, ciprofloxacin 71.2%, piperacillin-tazobactam 40.2%. Additionally, resistance rates for ceftazidime-avibactam was found to be 8.2%. Meropenem resistance rates were 4% in *E. coli* and 45.4% in *K. pneumoniae* isolates. Ceftazidime/avibactam resistance rates were 2.2% in *E. coli*, 23.3% in *K. pneumoniae* and 42.9% in *Enterobacter cloacae* complex. Our study revealed that 97% of patients, with gram-negative bacteremia/sepsis developed under extended-spectrum empirical antibiotic treatment, with escalation applied for 67% of the patients. The study underscored the emergence of antibiotic resistance in Enterobacterales isolates, particularly in carbapenem-resistant *K. pneumoniae* (CRKP), as a pressing issue in our hospital. In response, hospitals should implement stringent infection control measures, including hand hygiene for hospital staff, training initiatives, patient isolation, and comprehensive disinfection sterilization practices.

Multidrug-resistant Enterobacterales BSIs have been related to a poor prognosis, with reported all-cause mortality rates ranging from 32.9% to 70% in severe CRE BSIs. A 2021 study by Zhou C, involving 208 CRE patients, found an overall 30-day mortality rate of 46.2%, with 85.6% of deaths attributed to CRKP isolated from blood cultures [3]. The study identified a short duration of antimicrobial therapy and empirical use of tigecycline as independent risk factors for mortality. Tigecycline treatment showed poor therapeutic effects on BSIs patients, whereas carbapenem treatment demonstrated better efficacy, especially in patients infected by meropenem minimum inhibitory concentration (MIC) ≤8 mg/L isolates.

Additionally, a shorter duration of antimicrobial therapy was associated with a poorer prognosis compared to longer-duration therapy [3]. In our study, the 30-day mortality rate was 14.56%, and this rate was higher for *K. pneumoniae* BSIs, emphasizing the clinical relevance of the findings in the context of Enterobacterales infections.

On the other hand, various factors, including individual risk factors, comorbid diseases, immunosuppression and the presence of cancer can significantly influence the prognosis of infection. Central venous catheters and urinary catheters, may contribute to mucosal damage, thereby increasing the incidence of BSIs [9]. A large-scale study by Sava et al. [10] showed that; BSI is a prevalent infectious complication after allogeneic HSCT, occurring in 20-60% of HSCT patients in the pre- and post-engraftment phases, as well as in patients with acute graft-versus-host disease. In the same study involving 1432 HSCT patients, acute leukemia was the most common underlying condition (53.2%), with 95.2% of patients undergoing a single allogeneic transplantation. The study reported that over a median follow-up time of 1.88 years, 33.1% of patients experienced at least one BSI. The highest incidence of BSI was observed in the peri-transplantation phase of the second transplant (30.6%). Many studies have indicated high BSI rates, particularly within the first 30 days after HSCT, even in cases where quinolone prophylaxis was used [11-13]. In our study involving patients with hematological diseases, Acute Myeloid Leukemia (39.2%), B-ALL leukemia (17.6%), Multiple Myeloma (10.8%) and Diffuse Large B-cell Lymphoma (10.8%) were the most common hematologic cancers in BSI patients. Of the patients, 28.3% received cyclosporine and bone marrow transplantation was performed in 50.5% of the patients, with 35.6% receiving allogeneic and 14.9% receiving autologous bone marrow transplantation. We recommend further BSI studies with a high number of HSCT patients, specifically evaluating the timing of BSI occurrence.

Blood culture is frequently the primary diagnostic method for identifying BSIs. Blood samples should be collected before administering medication, but the culture process is time-consuming, leading to delays in obtaining results [14]. In a 2023 study in Barcelona, the association between mortality and delays in reporting blood culture positivity in 6225 patients with bacteremia treated at a Barcelona hospital were evaluated, retrospectively. The study found that reporting delays for Enterobacterales increased the risk of death, and 77.8% of patients who died from an Enterobacterales BSI experienced delayed reporting [15]. In our study, the average time between blood culture collection and the growth signal was 11.33 hours, and the average blood culture result reporting time after signaling bacterial growth was three days. These results emphasize the need for an effective antimicrobial stewardship program and rapid molecular-based diagnostic methods to facilitate the early detection of causative agents in BSIs in our hospital.

In the early diagnosis of infectious diseases, various parameters are commonly utilized, with CRP being the most frequently employed among them. While some studies indicate that CRP's diagnostic value in sepsis is moderate and its predictive value for positive blood culture and disease prognosis is lower compared to procalcitonin, CRP levels generally show a decline within the first 48 hours following the initiation of infection treatment. Procalcitonin's advantage as a biomarker for predicting infection lies in its high in vitro stability and serum levels can elevate within a span of 2 to 3 hours following the onset of infection. Although procalcitonin's specificity for infection is not absolute, when the serum procalcitonin content exceeds 2.0 ng/ml, the risk of sepsis or septic shock increases significantly [16]. In our study, increased CRP and procalcitonin levels were observed in most patients [CRP in 101 patients (normal range: 0-5 mg/L), Procalcitonin in 97 patients (normal range: 0-0.1 µg/L)], with average CRP and procalcitonin levels of 112.3 mg/L and 7.35 µg/L, respectively. Our findings suggest that CRP and procalcitonin can serve as additional diagnostic tests for BSIs.

In conclusion, our study focused on patients with hematological cancer, revealing that *E. coli* and *K. pneumoniae* were the most commonly isolated microorganisms in BSIs among Enterobacterales. The resistance rates to meropenem were 4% in *E. coli* and 45.4% in *K. pneumoniae* isolates, while ceftazidime/

avibactam resistance rates were 2.2% in *E. coli* and 23.3% in *K. pneumoniae*. Notably, 97% of patients developed gram-negative bacteremia/sepsis under extended-spectrum empirical antibiotic treatment, with a 30-day mortality rate of 14.56%, which was higher for *K. pneumoniae*-associated BSIs. Hematologic cancers such as Acute Myeloid Leukemia and B-ALL were predominant among BSI patients. Cyclosporine was administered to 28.3% of the patients, and BSIs were common in BMT patients, with 35.6% receiving allogeneic and 14.9% receiving autologous BMT. Our study highlighted an average blood culture result reporting time of three days after signaling bacterial growth. Elevated levels of CRP and procalcitonin were observed in most patients, suggesting their potential as additional diagnostic tests for BSIs. The study emphasized the importance of an effective antimicrobial stewardship program and rapid molecular-based diagnostic methods for early detection of causative agents in BSIs within our hospital. Furthermore, the study underscored the emerging challenge of antibiotic resistance in Enterobacterales isolates, particularly in CRKP. As a response, strict infection control measures, including hand hygiene for hospital staff, training, patient isolation and comprehensive disinfection-sterilization practices, were recommended. Finally, the study proposed further research on BSIs, particularly focusing on a larger cohort of HSCT patients to evaluate the timeline of BSIs. Each hospital should conduct its own evaluation and examine the patient profile to make the correct empirical antibiotic selection. It is crucial to develop a suitable algorithm for this purpose.

There were some study limitations. EUCAST recommends the broth microdilution method for colistin antibiotic susceptibility testing. However, since the broth microdilution kit was not available in our laboratory, Vitek was used instead. Although colistin and tigecycline sensitivity has been studied with the device, it is not presented because it was not tested using the reference method recommended by EUCAST.

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Authors' contributions: T.D., E.T. constructed the main idea and hypothesis of the study. E.T., F.A. collected data. T.D., S.S.Y., T.U. developed the theory and arranged the material and method section. E.T., T.D. have done the evaluation of the data in the results section. Discussion section of the article was written by T.D. and E.T. Also I.M., A.S.G., N.I. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

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Selective embolization in renal angiomyolipoma with pseudoaneurysm

Psödoanevrizmalı renal anjiyomiyolipomda selektif embolizasyon

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Abstract
Angiomyolipomas are usually diagnosed incidentally and are usually asymptomatic. Dysmorphic blood vessels in angiomyolipomas usually do not contain an internal elastic lamina, so bleeding risks are high. Particularly, lesions larger than 4 cm have a tendency to become symptomatic and can present with life-threatening retroperitoneal or urinary bleeding. Although intrarenal or perinephric bleeding is the usual complication of angiomyolipomas, a pseudoaneurysm appears unusual. In this case, we aimed to present a patient with pseudoaneurysm, an unusual complication of angiomyolipoma. In this article, a patient who presented with complaints of left flank pain and hematuria and who underwent selective arterial embolization due to pseudoaneurysmatic angiomyolipoma in the left kidney is presented. The patient's angiography showed a large hypervascular mass filling the upper and middle segments of the left kidney. A pseudoaneurysmal filling originating from a subsegmental branch was observed in the mass. The patient underwent selective embolization. After 5 years of follow-up, the mass was observed to shrink. Angiomyolipomas are benign tumors, but especially symptomatic masses larger than 4 cm and especially masses with aneurysms larger than 5 mm can cause life-threatening retroperitoneal hemorrhages. Coexistence of angiomyolipomas with pseudoaneurysm is rare, especially. Safe, effective and minimally invasive selective arterial embolization can be safely performed to prevent massive bleeding and rupture.

Keywords: Angiomyolipoma, pseudoaneurysm, retroperitoneal hemorrhage, selective angiography.

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Öz
Anjiyomiyolipomalar genellikle tesadüfen teşhis edilir ve genellikle semptomsuzdur. Özellikle, 4 cm'den büyük lezyonlar semptomatik olma eğilimindedir ve yaşamı tehdit eden retroperitoneal veya üriner kanama ile ortaya çıkabilir. Anjiyomiyolipomaların tipik komplikasyonu intrarenal veya perinefratik kanamadır, ancak bir psödoanevrizma görünüşü olağandışıdır. Bu çalışmada anjiyomiyolipomanın olağan dışı komplikasyonu olan psödoanevrizmalı hastayı sunmayı amaçladık. Bu makalede, sol yan ağrısı ve hematüri şikayetleri ile başvuran ve böbrekteki psödoanevrizmatik anjiyomiyolipoma nedeniyle seçici arteriyel embolizasyon geçiren bir hasta sunulmaktadır. Hastanın anjiyografisi, sol böbreğin üst ve orta segmentlerini dolduran büyük bir hipervasküler kitleyi göstermiştir. Kütlede bir subsegmental dal kökenli psödoanevrizmal dolgu gözlemlenmiştir. Seçici anjioembolizasyon sonrasında, kitlede 5 yıllık takipte küçülme olduğu gözlemlenmiştir. Anjiyomiyolipomaların psödoanevrizma ile bir araya gelmesi nadirdir. Büyük kanama ve yırtılma önlemek için güvenli, etkili ve minimal invaziv seçici arteriyel embolizasyon güvenle uygulanabilir.

Anahtar kelimeler: Anjiyomiyolipoma, psödoanevrizma, retroperitoneal kanama, selektif anjiyografi.

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Introduction

Angiomyolipomas (AMLs) are benign mesenchymal tumors composed of adipose tissue, smooth muscle, and blood vessels [1]. They are found in approximately 0.3% of the general population and constitute about 3% of all kidney tumors [2, 3]. AMLs are often identified as solitary lesions, most commonly originating from the kidney, and less frequently occurring in the liver, lymph nodes, spleen, lungs, and retroperitoneal area [4]. While they are predominantly sporadic, around 20% of cases may be associated with tuberous sclerosis (TS) [5].

Angiomyolipomas are usually diagnosed incidentally and are usually asymptomatic [6]. Dysmorphic blood vessels in angiomyolipomas usually do not contain an internal elastic lamina, so bleeding risks are high. Particularly, lesions larger than 4 cm have a tendency to become symptomatic and can present with life-threatening retroperitoneal or urinary bleeding [7, 8]. Therefore, minimally invasive procedures such as selective renal artery embolization or nephron-sparing surgery can be performed for ineffective pain management, masses larger than 4 cm and with a risk of bleeding and whose pain cannot be relieved [6].

Although intrarenal or perinephric bleeding is the usual complication of angiomyolipomas, a pseudoaneurysm appears unusual. Intrarenal pseudoaneurysm is also a well-known complication of penetrating renal injuries, renal surgery and percutaneous renal procedures [9, 10].

In this case, we present the clinical follow-up and treatment process of a patient who was evaluated for flank pain and gross hematuria and underwent selective arterial embolization (SAE) due to a pseudoaneurysmal left renal AML, in line with the current literature.

Case presentation

A 46-year-old female patient presented with complaints of left flank pain and hematuria. Upon follow-up examinations, a mass suggestive of AML was detected in the left kidney, she was reffered to our department. The patient had no significant medical history except for a previous lumbar intervertebral disc herniation surgery. Physical examination revealed an immobile lobulated mass in the left upper quadrant of the abdomen. There were no signs of tenderness or defense in the abdomen. Arterial blood pressure was measured as 100/70 mmHg, and the patient had a body temperature of 37.1°C. Chest X-ray and electrocardiography did not reveal any abnormalities. Laboratory findings showed a white blood cell count of 14.25 K/uL, hemoglobin level of 10.3 g/dL, hematocrit of 30.7%, and creatinine level of 0.69 mg/dL. Other routine biochemical values and coagulation parameters were within normal limits.

Abdominal magnetic resonance imaging (MRI) was obtained after intravenous contrast agent (IVCA) injection. The MRI revealed a well-defined mass measuring 100x80x83 mm in size, located exophytically in the upper pole of the left kidney. The mass displaced the tail segment of the pancreas upward and anteriorly, and its borders could not be distinguished from the left suprarenal gland. The mass extended to the renal hilum and showed heterogeneous enhancement. The mass contained macroscopic fat (Figure 1), hemorrhagic signal intensity changes, and a pseudoaneurysm (Figure 2).

During the interventional radiology procedure, the patient's angiography revealed a large hypervascular mass filling the upper and middle segments of the left kidney. Within the mass, a pseudoaneurysmal filling originating from a subsegmental branch was observed (Figure 3). The microcatheter was advanced into the feeding vessel, and embolization was performed superselectively using four 3mm and two 4mm diameter coil embolization devices. Embolic particles with a size of 400 microns were used to perform embolization in the vascular bed supplying the mass through that branch, and achieving complete stasis (Figure 4).

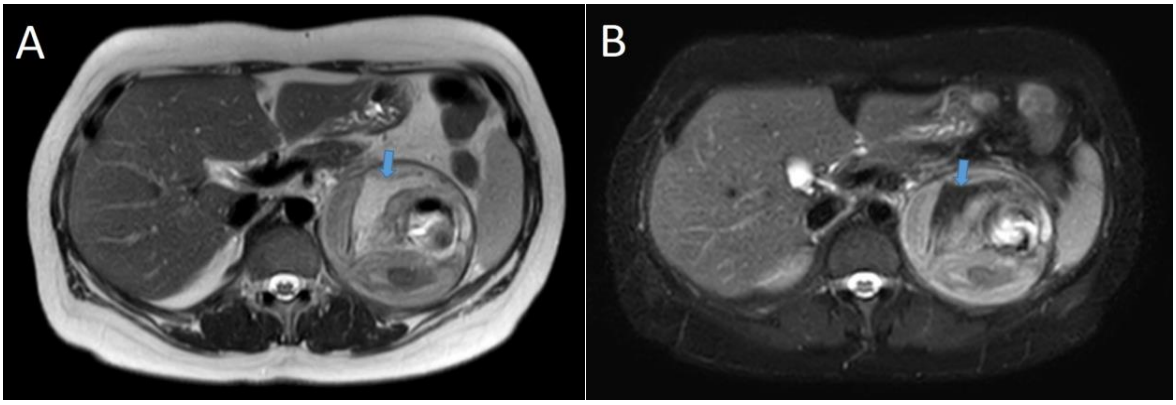

Figure 1. T2W image [A] and T2W fat saturated image, [B] showed macroscopic fat (blue arrow)

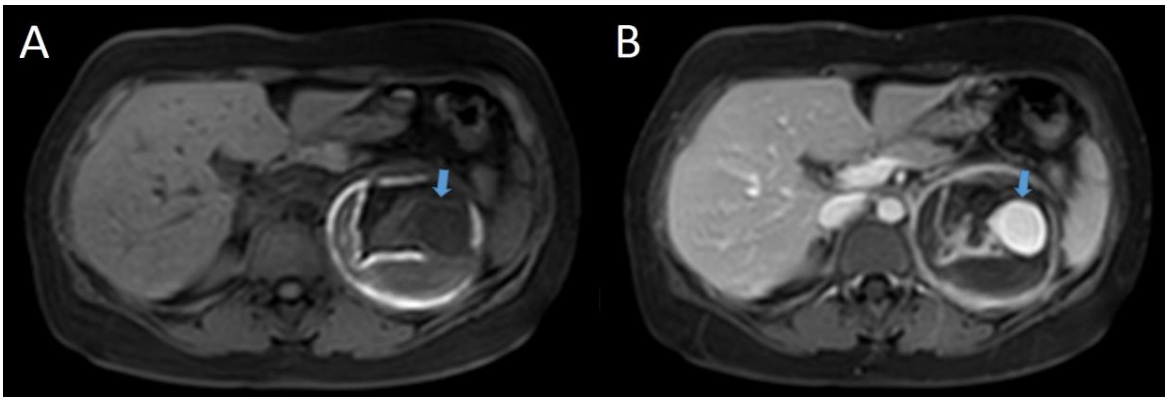

Figure 2. T1W pre-contrast fat saturated image [A] and T1W postcontrast fat saturated image [B] showed a pseudoaneursym (blue arrow) and peripheral hemorrhage

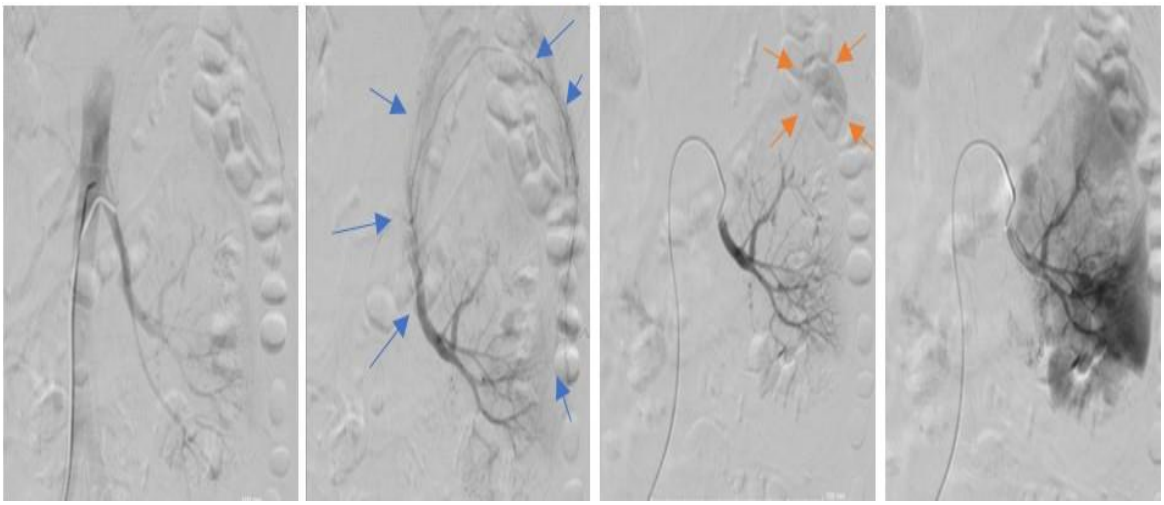

Figure 3. Angiography images after contrast agent injection through the catheter before the procedure

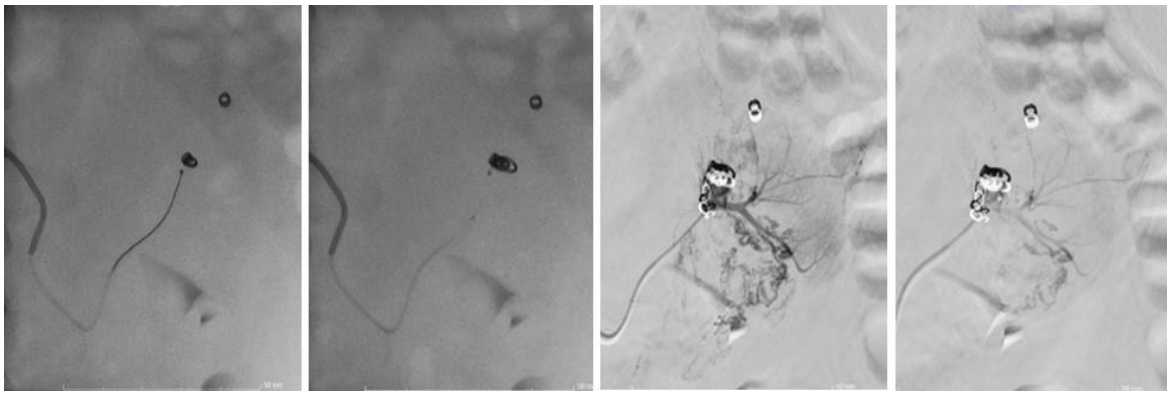

Figure 4. Angiography images after embolization

The patient, who did not experience any complications during the post-procedure follow-up, was discharged after one week. During the 5-year follow-up period, the patient remained asymptomatic, and no bleeding was observed in the mass. Imaging studies revealed a decrease in the size of the AML in the left kidney. 5 years after the procedure, the patient's creatinine level was measured as 0.79 mg/dL. Abdominal computed tomography (CT) was performed after the injection of IVCA showed densities that were likely artifacts related to the embolic agents in the posterior upper segment of the left kidney. A well-defined hypodense, similar density to fat, smoothly contoured lesion measuring approximately 4.8x3.3 mm, was observed in the upper segment of the left kidney (Figure 5).

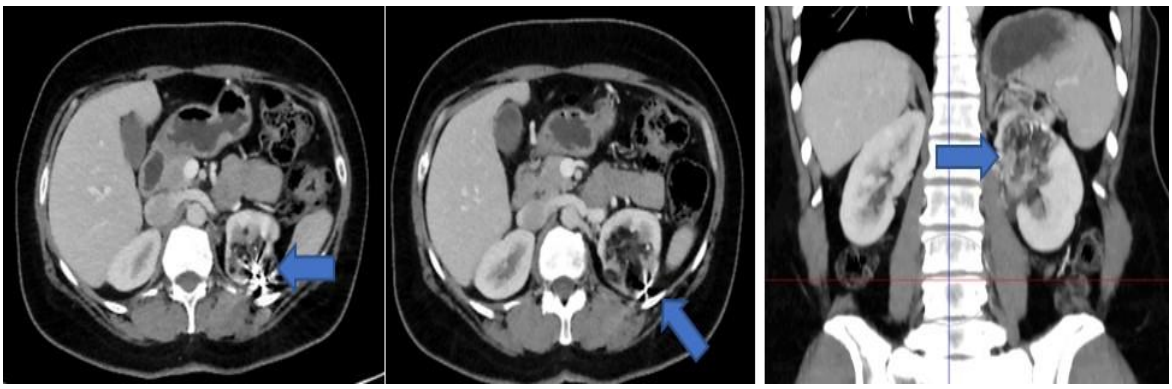

Figure 5. a. Axial section CT, b. Coronal section CT

Discussion

Although angiomyolipomas are mostly asymptomatic, they can pose a threat to life due to their fragile nature, leading to spontaneous bleeding or serious complications such as vena cava compression in large tumors [7, 8]. AML is the most common cause of spontaneous retroperitoneal bleeding, and factors such as larger size (>4 cm), multifocality, and association with tuberous sclerosis increase the risk of bleeding [11]. "Wunderlich Syndrome," also known as massive retroperitoneal hemorrhage, is the most serious complication of renal angiomyolipoma and has been reported in more than 10% of patients [11]. Wunderlich Syndrome manifests with the Lenk triad, which includes flank or abdominal pain, palpable mass, and gross hematuria. If it is untreated, Wunderlich Syndrome can lead to significant morbidity and potential mortality [11, 12].

Selective arterial embolization and percutaneous radiofrequency ablation are prominent minimally invasive options in the treatment of AML. Surgical options such as partial/radical nephrectomy are preferred in more severe cases and selected patients [13]. The presence of symptoms such as tumor size increase, bleeding, and pain, as well as suspicion of cancer on imaging, constitute the main indications for the treatment of

angiomyolipoma [14]. In cases of bleeding AML, embolization is implemented as the first-line treatment method and serves as a preventive treatment for masses at high risk of bleeding [15].

Although SAE is a minimally invasive procedure, it can still be associated with complications such as pain, post-embolization syndrome, hematuria, vascular injury, rupture during the procedure, infection, abscess formation, renal infarction, and kidney failure. Post-embolization syndrome (PES), which occurs as a result of renal tissue necrosis, manifests itself with symptoms like nausea, vomiting, fever, abdominal pain, and leukocytosis, and it is treated conservatively [16].

In a meta-analysis study conducted by Murray et al. [17] involving 524 patients, SAE performed for AML showed a success rate of 93.3% during an average follow-up period of 39 months. Different embolization agents were used in 46.8% of cases, with 2 or more agents used in some cases. Among the patients, 20.9% required retreatment due to revascularization, no change/increase in tumor size, persistent symptoms, or retroperitoneal bleeding. SAE was associated with low mortality and a 35.9% incidence of PES.

In a cohort study involving 41 patients with AML, SAE was performed on 48 kidneys, and no cases of retroperitoneal bleeding were observed during the follow-up period. There were no significant changes in creatinine levels (p=0.27). Five years after SAE, the rate of avoiding surgical treatment was reported to be 94%, and the disease-specific survival rate for the entire cohort was reported to be 100% [18].

In another single-center study, the results of 23 patients who underwent SAE for retroperitoneal bleeding (n=6) and prophylaxis purposes (n=17) were retrospectively evaluated. During an average follow-up period of 20.5 months, a 26.2% reduction in AML size was observed. Three patients experienced major complications, including renal abscess in 2 patients and femoral pseudoaneurysm in 1 patient. Additionally, 14 patients reported minor complications in the form of post-embolization syndrome (PES) [19].

Due to the hypervascularity of aneurysms, the risk of tumor rupture in AML is related to the presence and size of intratumoral aneurysms. The imaging findings, defined clinically as “aneurysm”, are divided into 2 pathological types: primary aneurysm and pseudoaneurysm [20].

Yamakado et al. [7] investigated a total of 29 kidneys with AML, with 8 being hemorrhagic and 21 being non-hemorrhagic. They reported that when a tumor size of 4 cm or larger and an aneurysm size of 5 mm or larger were used as rupture indicators, the probability of rupture could be predicted with higher specificity.

In a meta-analysis that examined 739 cases of AML, aneurysms were demonstrated in the interlobar and interlobular arteries in 71.4% of the cases based on selective angiography results [21]. In another study that evaluated 27 patients diagnosed with AML and 34 kidneys, aneurysms were detected in 29.4% of AML patients using CT angiography. However, in this study in which 6 kidneys were ruptured, it was stated that tumor size and aneurysm size cannot be used as indicators of spontaneous rupture of the tumor [22].

Renal artery pseudoaneurysms observed in AMLs have been defined as unusual vascular complications that can cause bleeding. Therefore, it is anticipated that appropriate treatment should be initiated based on the size of the AML [23]. Furthermore, in a study conducted by Albi et al. [12], it was stated that the presence of an intratumoral aneurysm on CT scan can predict a higher possibility of tumor rupture.

Sayin et al. [1], evaluated a 64-year-old patient who presented with severe abdominal pain, weakness, dizziness, and localized abdominal rigidity on the left side. The abdominal ultrasound (USG) revealed a well-defined AML that nearly filled the left half of the abdomen, measuring approximately 15 x 20 x 25 cm. Contrast-enhanced abdominal CT imaging showed a hyperdense area in the central region of the mass, consistent with a pseudoaneurysm. During the patient’s follow-up, there was a progressive decrease in hemoglobin levels and a deterioration in the overall condition, leading to the decision to perform a total nephrectomy. Sayin et al. [1], emphasized that for AMLs larger

than 4 cm and exhibiting symptoms, measures such as SAE or partial/total nephrectomy can be employed to prevent bleeding and rupture.

Esmat and Naseri [24], colleagues evaluated a 31-year-old patient who had been experiencing left-sided abdominal pain for a week and had recently developed hematuria. The patient had a medical history of bilateral AML and tuberous sclerosis. Four years ago, the patient had undergone SAE due to retroperitoneal hematoma. Contrast-enhanced abdominal CT images revealed findings consistent with AML in both kidneys. In the lower pole of the left kidney, a lesion was observed in the arterial phase, showing homogeneous contrast enhancement and being consistent with a pseudoaneurysm, with active extravasation observed in the adjacent area. No retroperitoneal bleeding was detected, and the patient underwent successful SAE. Esmat and Naseri [24], colleagues recommended SAE treatment for bleeding AMLs and those at risk of bleeding.

In this case in which SAE was performed due to pseudoaneurysm, no complications were observed during early and late follow-ups after the procedure. Additionally, there was no need for surgical intervention or any additional procedures during the follow-up period, and a significant reduction in lesion size was noted after 5 years. No renal function loss was observed during the patient's follow-up.

In conclusion, angiomyolipomas are benign tumors, but symptomatic masses larger than 4 cm and especially masses containing aneurysms larger than 5 mm can cause life-threatening retroperitoneal hemorrhages. Pseudoaneurysms seen in angiolipomas are an unusual component and should be followed carefully because of the risk of bleeding. Safe, effective and minimally invasive selective arterial embolization can be safely performed to prevent massive bleeding and rupture.

Informed consent: The patient gave informed consent for the publication.

Authors contributions: A.S. and M.K., constructed the main idea and hypothesis of the study. O.L.T, developed the theory and arranged/edited the material and method section. Discussion section of the article was written by M.B.D. and K.K. Y.O. and S.C. reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

List of abbreviations: AML: Angiomyolipoma, TS: Tuberous sclerosis, SAE: Selective arterial embolization, MRI: Magnetic resonance imaging, IVCA: Intravenous contrast agent, CT: Computed tomography, PES: Post-embolization syndrome.

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Case Report

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Unexpected color appearance of pleural fluid: a bilothorax case

Plevra sıvısının beklenmedik renkte görünmesi: bilotoraks olgusu

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Abstract

Bilothorax, or cholethorax, is a rare cause of exudative pleural effusion characterized by the presence of bile in the pleural space. Recognizing this condition is essential to prevent severe complications such as empyema and acute respiratory distress syndrome. This report presents a 70-year-old male patient who developed right-sided bilothorax following multiple biliary tract interventions. The patient presented with symptoms of jaundice, fever, and abdominal pain. Clinical and radiological evaluations revealed right-sided pleural effusion, and thoracentesis yielded dark yellow-green fluid. The diagnosis of bilothorax was confirmed by a pleural fluid/serum total bilirubin ratio >1.0. Early intervention was performed using a cystofix to drain the pleural fluid. However, despite all interventions, the sepsis condition could not be controlled, and the patient unfortunately passed away. Bilothorax is generally associated with hepatobiliary procedures and is mostly observed on the right side due to anatomical proximity. Diagnosis requires a high index of suspicion, especially in patients with relevant clinical histories and characteristic pleural fluid appearance. Rapid thoracentesis and pleural fluid analysis are crucial for diagnosis. Treatment typically involves pleural drainage and early administration of broad-spectrum antibiotics. In conclusion, bilothorax is a life-threatening condition requiring urgent diagnosis and intervention. This case highlights the importance of recognizing this rare condition and the necessity for early aggressive management in patients with a history of hepatobiliary procedures.

Keywords: Bilothorax, unilateral pleural effusion, biliopleural fistula.

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Öz

Bilotoraks, plevral boşlukta safra varlığı ile karakterize, eksüdatif plevral efüzyonun nadir bir nedenidir. Bu durumun tanınması ampiyem ve akut solunum sıkıntısı sendromu gibi ciddi komplikasyonları önlemek için önemlidir. Bu raporda, birçok safra yolu müdahalesi sonrasında sağ taraflı bilotoraks gelişen 70 yaşındaki bir erkek hasta sunulmaktadır. Hasta sarılık, ateş ve karın ağrısı şikayetleri ile başvurdu. Klinik ve radyolojik değerlendirme ile sağ taraflı plevral efüzyon görüldü ve torasentez ile koyu sarı-yeşil sıvı elde edildi plevral mayii/serum total bilirubin oranının >1,0 olması ile bilotoraks tanısı konuldu. Erken müdehale ie sistofix ile plevral mayi drenajı sağlandı. Ancak tüm müdehalelere rağmen sepsis tablosunun önüne geçilemeyerek hastamız kaybedildi. Bilotoraks, genellikle hepatobiliyer prosedürlerle ilişkilidir ve anatomik yakınlık nedeniyle çoğunlukla sağ tarafta görülür. Tanı, özellikle ilgili klinik öykülere ve karakteristik plevral sıvı görünümüne sahip hastalarda yüksek şüphe gerektirir. Tanı için hızlı torasentez ve plevral sıvı analizi kritik öneme sahiptir. Tedavi genellikle plevral drenaj ve geniş spektrumlu antibiyotiklerin erken uygulanmasını içerir. Sonuç olarak Bilotoraks, acil tanı ve müdahale gerektiren hayatı tehdit eden bir durumdur. Bu olgu, ilgili hepatobiliyer öyküye sahip hastalarda bu nadir durumu tanımanın ve erken agresif yönetimin önemini vurgulamaktadır.

Anahtar kelimeler: Bilotoraks, tek taraflı plevral efüzyon, biliyoplevral fistül.

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Introduction

The presence of bile in the pleural space is termed bilothorax or cholethorax, which is one of the rare causes of exudative pleural effusion. It is crucial to recognize it in order to make a proper differential diagnosis and to prevent complications such as empyema and acute respiratory distress syndrome [1, 2].

Bilothorax is primarily caused by congenital factors, iatrogenic injury to biliary tract, diaphragmatic ruptures, hepatic abscesses, and thoracentesis can be helpful to diagnose it [1, 3]. Therefore, bilothorax should be considered in cases of pleural effusion in patients with a history of liver and bile duct-associated procedures [4].

With educational purposes aimed at highlighting its diagnostic challenges, we present the case of a 70-year-old male patient who developed right-sided bilothorax following numerous biliary tract interventions, with a suspected diagnosis of primary sclerosing cholangitis.

Case report

The patient underwent evaluation following a consultation requested from the relevant clinic. This 70-year-old male, who has been monitored for jaundice at the Gastroenterology Department, exhibited right-sided pleural effusion on posteroanterior chest radiography and thoracic computed tomography (CT). The medical history revealed that he underwent coronary bypass surgery ten years ago, he has been consistently treated for coronary artery disease and hypertension. He ceased smoking 30 years ago after smoking a few cigarettes daily for 20 years. He has no prior history of lung disease.

Cholelithiasis in common bile duct and gallbladder was diagnosed through magnetic resonance cholangiopancreatography (MRCP) one month ago. Consequently, the stone was removed, and biliary stenting was performed during Endoscopic Retrograde Cholangiopancreatography (ERCP). Shortly after the procedures, the patient was discharged. Fifteen days later, he began experiencing jaundice, fever, and abdominal pain prompting a repeat MRCP and ERCP. Piperacillin tazobactam treatment was started with infectious diseases consultation. Cholangiocarcinoma was suspected due to the occurrence of primary sclerosing cholangitis. When a 44x30 mm irregular lesion was detected in the main ducts, a percutaneous transhepatic cholangiography and percutaneous biliary drainage was performed at the interventional radiology department. Three days later, t-tube cholangiography and biliary dilatation were performed as well. However, the bile drainage was significantly low. Two days after from these procedures, the patient began complaining of shortness of breath.

On inspection, the patient presented icteric findings. On auscultation of his chest, the left hemithorax appeared normal, but the lower right side of the lung exhibited diminished sound and dullness upon percussion. His oxygen saturation was 95% (with the support of 4 liters/minute of oxygen). Other vitals findings were within normal limits. Abnormal laboratory findings revealed as an elevation of CRP (248 mg/L), leukocytosis (WBC count: 20.170/mm^3), and a low hemoglobin level (9.2 gr/dl). Increased density consistent with right-sided pleural effusion was observed on chest radiography (Figure 1). On thoracic CT, a unilateral moderate pleural effusion approximately 6 cm in size was identified on the right side. Additionally, the catheter passage line was observed adjacent to the right hemi-diaphragm (Figure 2). Dark yellow-green colored 500 mL fluid drained during thoracentesis (Figure 3).

Considering the macroscopic appearance of the pleural fluid, the analysis of bilirubin levels was added to the routine laboratory testing for diagnostic approach to pleural effusions. The laboratory test results are summarized in Table 1.

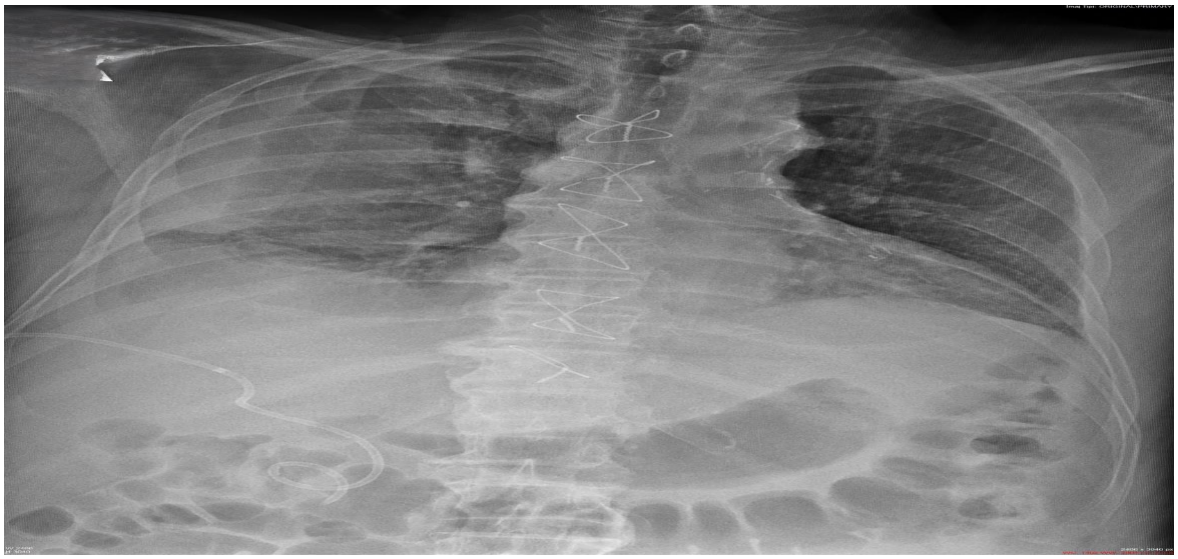

Figure 1. Posterior-anterior chest radiography

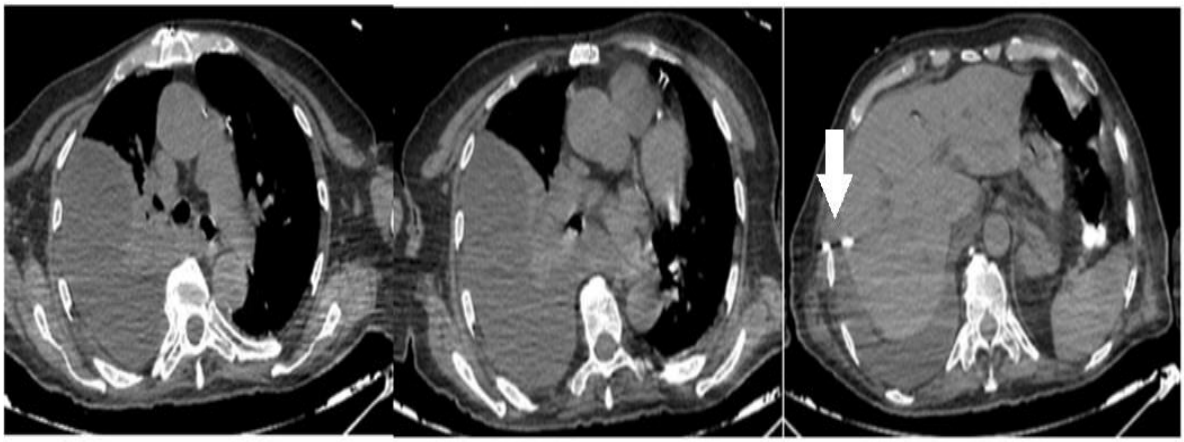

Figure 2. Computed tomography

Arrow is pointing to: hepatobiliary katater

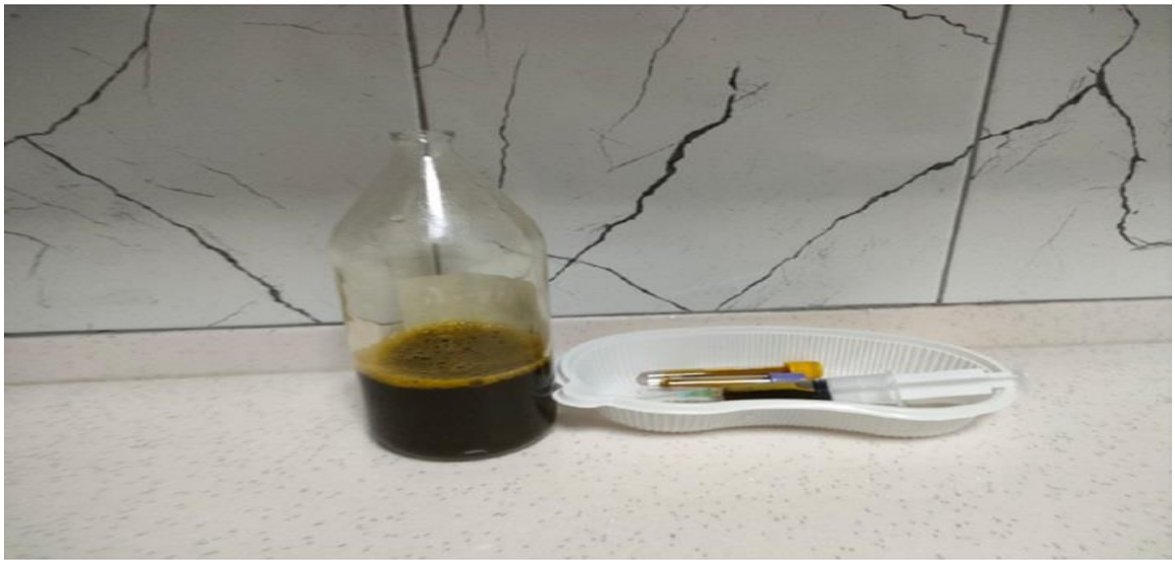

Figure 3. Thoracentesis fluid

Table 1. Laboratory results of blood serum and pleural fluid

Parameters	Pleural Fluid	Blood Serum	Pleura/Blood Serum Ratio
LDH (U/L)	688	189	3.64
Total Protein (g/L)	13.5	55.8	0.24
Albumin (g/L)	12.9	24.8	11.9
Glucose (mg/dL)	6	116	
Bilirubin (mg/dL)	53.45	5.94	8.99
pH	6.92		
Polymorphonuclear Leukocyte (%)	97.1		
Mononuclear Cells (%)	2.9		

The results revealed an exudative pleural fluid with an elevated percentage of polymorphonuclear cells, and the ratio of pleural fluid to serum bilirubin was higher than 1. These findings were favored the diagnosis of bilothorax. Tube thoracostomy was performed using 14F catheter by the thoracic surgeons. The patient had been given piperasilin-tazobactom during 7 days. But acute phase reactants had increased and the Infectious Disease Department changed antibiotics, and they started setazitim. Cystofix drain was monitored daily by thoracic surgeons. There was no drainage from thoracic sistofix. The patient's condition worsened and his symptoms had showed that sepsis. The patient was transferred to the intensive care unit for close monitoring. Meropenem treatment was started. Two days later Klepsiella pneumonia and Enterococcus Faecium growth was seen in the pleura culture. According to culture sensitivity, the meropenem treatment dose was increased and amikacin was added to the treatment. Bile flow through the cystofix stopped. However, there was no response to the patient's sepsis condition. Despite all the interventions, the patient suffered cardiopulmonary arrest days later and died.

Discussion

Bilothorax is a life-threatening condition as bile can increase the susceptibility to infection in the pleural space [5].

Diseases occur after penetrating or non-penetrating trauma. Biliopleural fistula is a rare condition that may occur. It can also be seen as bronchobiliary fistula after these diseases [4]. It should be always in differentials in patients who have pleural effusions. Even though patients usually present with pleuritic chest pain, respiratory distress, and a history of hepatobiliary procedures, few cases have been described with a spontaneous onset [5, 6]. Same as what occurred in our case, the localization is right-sided most of the time due to its anatomical nearity to the biliary tract, however, there are different cases that have been reported isolated left-sided and bilaterally [2, 5, 7]. As a rare incident, one case has reported a left-sided presentation of bilothorax due to cholangiocarcinoma which can occur because of a natural transition of the bile through the esophageal and aortic hiatuses [8]. Patients' medical history and chest radiographies should be key to suspect bilothorax for diagnosis and prevent further complications. Thoracentesis can be a part of the diagnosis seeing the green and viscous fluid. Additionally, after the tube insertion during the interventional procedures, realizing a low drainage of fluid than expected might be a cue as well. Observation of a pleural effusion should be followed by diagnostic thoracentesis with measurement of pleural total bilirubin. Visually, the pleural fluid will appear green-black, with an exudative profile on fluid studies. In various case reports, pleural-to-serum total bilirubin (P/S) ratio >1.0 has been reported as an indicator of the presence of bilothorax [6].

Prompt recognition of bilothorax is important as bile is a potent chemo irritant that can lead to a severe inflammatory response, such as acute respiratory distress syndrome [9]. In addition, given that bile is a medium conducive for bacterial growth, bilothorax places patients at higher risk for the development of bacterial empyema, especially those with chronic liver disease and

cirrhosis given their immunocompromised state [6]. Infections such as empyema can increase mortality 4-fold in patients with cirrhosis [10, 11].

Our case had a presentation of respiratory distress, a history of jaundice and fever, and multiple interventions to the hepatobiliary tract including ERCP, MRCP and, percutaneous transhepatic biliary drainage (PTBD). Bilothorax was suspected due to the development of pleural effusion in the case with known condition. The pleural fluid was drained immediately upon suspicion of bilothorax, the infection could not be controlled despite all treatment attempts. Unfortunately, our patient died due to sepsis.

There is no existing extensive experience regarding the best way of treatment but successful management requires a rapid and accurate diagnosis. Several reports describe conservative management by chest tube insertion and pleural drainage as a successful and appropriate way of solving the problem [12].

Additionally early administration of broad-spectrum antibiotics to prevent future infection is recommended as well [13].

Informed consent: Written informed consent was obtained from the patient.

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

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