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Research Article

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Significance of CD49f in diagnosis of minimal residual disease in pediatric acute leukemia

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Abstract

Minimal residual disease (MRD) is the most important prognostic indicator in acute lymphoblastic leukemia (ALL) in childhood. Multiparametric flow cytometry (FCM) is a technique that is often used to determine MRD, and many markers have been identified. Another marker examined in the MRD analysis is CD49f. We aimed to determine the importance of CD49f expression in MRD detection. Immunophenotyping MRD and CD49f expressions were performed in patients with Pre-B cell ALL at the diagnosis, and on the day 15. 27 patients were included (F/M: 10/17). The mean age was 6.6±4.8 years. 6 (22.2%) patients were in the standard risk group, 14 (51.9%) patients were in the intermediate risk group, and 7 (25.9%) patients were in the high-risk group. MRD was detected in 15 (55.6%) patients. Cytomorphological remission was observed in 21 (77.7%) patients on the 15th day. 10 of these patients (66.6%) were MRD positive. CD49f levels at diagnosis and at 15th day were mean 38.4 ± 22.1 and 5.4±12.6, respectively. A significant decrease in CD49f expression was observed at follow up (p=0.00). Mean CD49f levels in MRD positive and MRD negative patients were 7.8±17 and 2.8±2 at day 15, respectively (p=0.64). There was no correlation between MRD and CD49f at day 15 (p=0.54). We observed that leukemic blasts express CD49f at a high rate, and this expression continues to decrease on the 15th day. We concluded that studies including more patients are required to assess the performance and importance of CD49f as an indicator in MRD.

Keywords: acute lymphoblastic leukemia, minimal residual disease, CD49f

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most prevalent hematological malignancy in childhood (1). Understanding the clinical, immunological, and cytogenetic characteristics of the disease has highlighted the importance of disease risk categorization and risk-directed treatment (2). The prognostic risk factors are/include clinical presentation characteristics, genetic subtype, germline cancer predisposition, and minimal residual disease (MRD) (3). Early response to initial treatment has been demonstrated to be one of the important determinants of the outcome (4). Poor morphological response in the first month of treatment has been accepted as a poor prognosis indicator. However, morphological features were found to be insufficient, and more sensitive techniques were required to be developed in the assessment of remission (5). MRD can detect 10^{-3}-10^{-6} leukemic blasts. Therefore, MRD significantly reflects the response to treatment and serving as a good predictor (6).

In MRD studies, multiparametric flow cytometry (FCM), polymerase chain reaction, and next-generation sequencing methods are utilized (7). Immunophenotyping with FCM in MRD determination is fast, sensitive, and simple to use in most cases (7). Many studies have shown that combinations of CD10, CD20, CD22, CD19, CD34, CD38, CD45, and CD58 can be employed for MRD assessment (8). CD49f is an adhesion molecule expressed on T cells, monocytes, platelets, epithelial, endothelial cells, and perineural cells. Many studies have shown that CD49f is overexpressed on days 19 and 46 of induction therapy (9). However, the role of CD49f in MRD studies is still unexplained.

This study aimed to determine the importance of CD49f expression and its compatibility with FCM in the MRD detection with pre-B ALL patients.

2. Matherials and Methods

2.1. Patients

The study was conducted in the Pediatric Hematology and Oncology Clinic, Erciyes University Hospital between January 2012 and January 2013. All children newly diagnosed with B-ALL and treated under the Turkish Acute Lymphoblastic Leukemia Berlin Frankfurt Münster 2000 (TR-ALL BFM 2000) protocol were eligible for the study and included based on informed consent (10). Disease risk categories, demographic, and laboratory data were recorded from patient files. Risk groups were formed based on the clinical and laboratory findings of TR-ALL BFM criteria.

Immunophenotypic MRD was evaluated using monoclonal

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antibodies such as Cyto 16, CD45, CD19, CD20, CD10, CD22, and CD58 on 300000/mm^3 cells on day 15 using the Beckman Coulter FC500 device. Leukemic blasts containing more than 0.01% of mononuclear cells were regarded as positive for MRD (+).

CD49f expression was examined at the time of diagnosis and on the 15th day. Additionally, MRD and CD49f were assessed again and correlated on the 15th day.

The study was approved by the Erciyes University Ethics Committee and supported by Erciyes University Scientific Research Projects Coordination Unit (TSU-12-3805).

2.2. Studying CD49f with FCM

100 µL of filtered blood sample was taken and 5 µL of CD19 PC7, CD10 FITC, and CD49f PE moAbs were added to it and incubated for 10 minutes in the dark. After incubation, erythrocytes were lysed, leukocytes were stabilized, and cell membranes were fixed using immunoprep "Coulter" lysing reagents. 2 mL of isoflow was added and washed twice for 5 minutes at 1200 rpm. The pellet was poured out, and 1 mL isoflow was added to the tube and processed on the Beckman Coulter FC500 device. The analysis was performed using CXP software. Fig. 1-2 shows the CD49f examination of one patient.

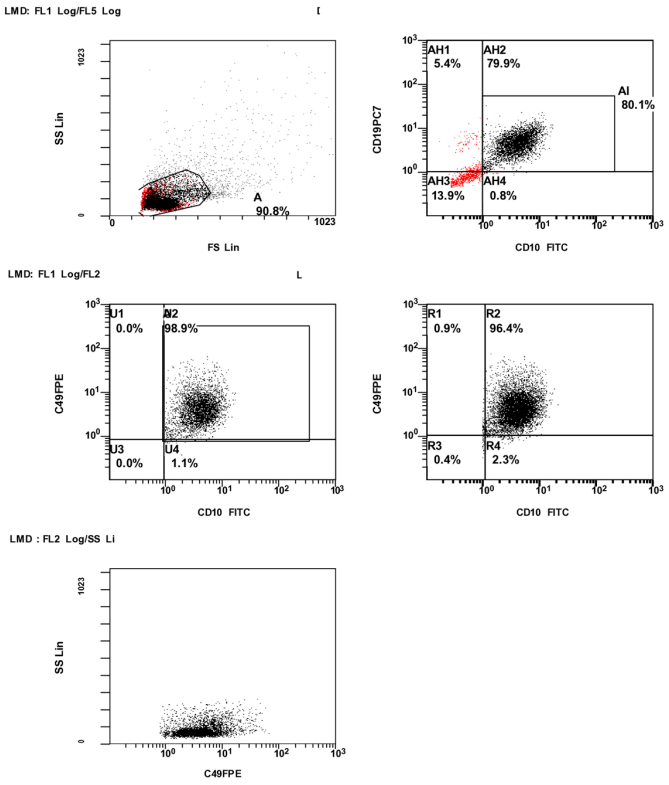

Fig. 1. CD49f analysis illustration in diagnosis

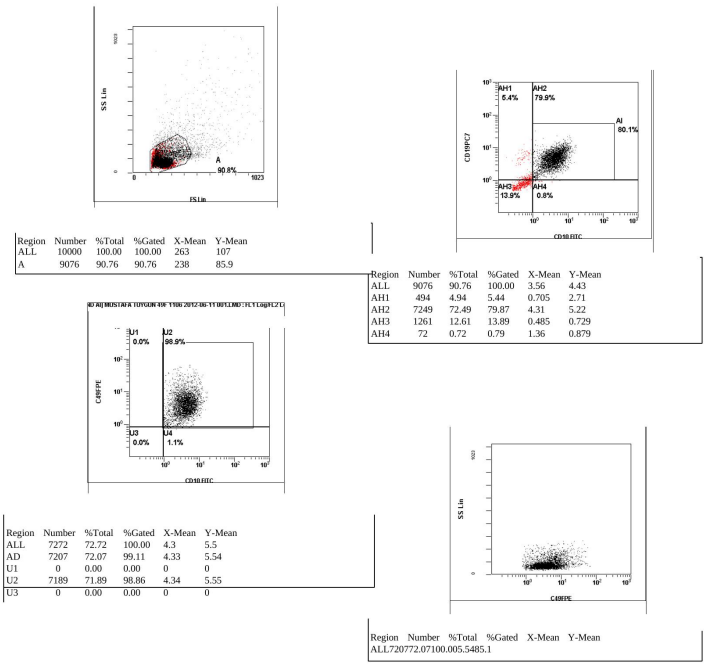

Fig.2. CD49f analysis illustration in diagnosis

2.3. Statistical Analysis

IBM SPSS Statistics for Windows, version 25 (SPSS Inc, Chicago, IL, USA) was used to perform the statistical analysis. The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov /Shapiro-Wilk's test) to determine whether they are normally distributed. Normally distributed data were expressed as mean±standard deviation, while non-normally distributed data were expressed as median [minimum-maximum]. The student's t-test was used for pairwise comparisons of normally distributed variables, and the Mann-Whitney U test was used for pairwise comparisons of non-normally distributed variables. Kruskal-Wallis test and Chi-square test were used to compare data from more than two groups. *p*-value <0.05 was considered significant.

3. Results

3.1. Patient characteristics

Twenty-seven patients were included in the study, of whom 10 (37%) were female. The mean age was 6.6±4.8 years. According to the risk classification used in the TR-ALL BFM treatment protocol, 6 (22.2%) patients were in the standard risk group (SR), 14 (51.9%) patients were in the intermediate risk group (IR), and 7 (25.9%) patients were in the high-risk group (HR). There was no central nervous system (CNS) involvement except for 1 (3.7%) patient in the high-risk group. BCR-ABL1 gene mutation was found in 1 (3.7%) patient in the genetic examination.

The median leukocyte, absolute neutrophil count, platelet count and, hemoglobin at the time of diagnosis were 6090/mm^3 (1720-215,000), 730/mm^3 (30-8500), 6.7 g/L (1,4-11,2), and 78,000/mm^3 (8000-818,000), respectively.

In the peripheral smear on the eighth day, a corticosteroid response was observed in 22 (81.5%) patients, while 5 (18.5%)

patients did not show a steroid response. At the end of the induction treatment, remission was achieved in 23 (85.2%) patients. Two patients who were not in remission underwent hematological stem cell transplants. 3 [11.1% (1 in the induction phase, 2 post-induction) patients died from sepsis.

The general characteristics of the patients are summarized in Table 1.

Table 1. The general characteristics of the patients

Patient No	Gender	Age, month	CNS involvement	BCR-ABL	15.th day MRD (%)	At diagnosis CD49f (%)	15.th day CD49f (%)	Risk group	Risk groups by MRD	8.th day MNC/mm^3	33.th day remission	Exitus	Relapse	HSCT
1	M	27	-		62.0	38.4	2.3	MR	HR	0	0	+ (at ind)	-	-
2	M	60	-		1.0	4.4	1.0	SR	HR	147	+	-	-	-
3	M	36	-		11.0	41	11	MR	HR	156	+	-	-	-
4	F	114	-		4.0	49	1.5	MR	HR	900	+	-	-	-
5	F	96	-		0.08	62.5	1.0	MR	SR	120	+	-	-	-
6	M	152	-		0.1	41	6.0	MR	SR	0	+	-	-	-
7	F	54	-		6.0	11.1	7.0	HR	HR	**4000**	+	-	-	-
8	M	48	-		13	26	1.8	SR	HR	0	+	-	-	-
9	M	14	-		4.0	52.5	1.6	HR	HR	278	+	+ (Post-ind)	-	-
10	M	21	-		0.1	8.0	2.0	SR	SR	95	+	-	-	-
11	F	168	-		0.02	57.7	3.8	MR	SR	0	+	-	-	-
12	F	132	-	]	40	44	1.0	HR	HR	**>1000**	+	-	-	+
13	F	94	+		2	22.5	2.92	HR	HR	**>1000**	0	+ (post-ind)	BM	+
14	M	17	-		78	73	66	SR	HR	0	+	-	-	-
15	M	32	-		18	88.5	-	HR	HR	**>1000**	+	-	-	-
16	M	132	-		0.06	30.9	1.2	MR	SR	0	+	-	-	-
17	M	84	-		22	49.8	4.5	HR	HR	550	+	-	-	-
18	F	60	-		0.16	33	0.8	SR	SR	0	+	-	-	-
19	F	32	-		0.01	79.2	3.0	MR	SR	200	+	-	-	-
20	F	48	-		0.02	56	6.0	MR	SR	0	+	-	-	-
21	M	18	-		-	22	1.0	HR	HR	**2133**	+	-	-	-
22	F	60	-		28	57	1.0	MR	HR	0	+	-	-	-
23	M	192	-		0.1	5	5.0	MR	SR	268	+	-	-	-
24	M	192	-		3.0	11.8	1.4	MR	HR	287	+	-	-	-
25	M	183	-		0.01	74.5	2.0	MR	SR	128	+	-	-	-
26	F	22	-		0.02	35	1.0	MR	SR	620	+	-	-	-
27	M	55	-		9.0	15	7.0	SR	HR	400	+	-	-	-

CNS: central nervous system, MRD: minimal residuel disease, MNC: mononuclear cell, HSCT: hematopoietic stem cell transplantation, M: male, F: female, Ph: Philadelphia chromosome, SR: strandard risk, MR: medium risk, HR: high risk, ind: induction, BM: bone marrow

3.2. MRD analysis

Fifteen (55.6%) patients were MRD$^+$. Among the MRD+ patients, five (33.3%) patients were female. No relationship was found between gender and the MRD$^+$ (p=0.68). The median age in MRD$^+$ patients was 5.6 (1.1-16) years.

Risk groups of MRD-positive patients were 4 (26.7%) SR, 5 (33.3%) IR and 6 (40%) HR respectively. Except for 1 patient, MRD could not be conducted due to lack of sample. All patients in the HR group were MRD positive. 5 (35.7%) IR, and 4 (66.6%) SR patients were classified as HR according to MRD assessment. Furthermore, 9 (64.2%) IR patients were assessed as SR according to MRD. A significant relationship was found between risk groups and the MRD positivity (p=0.02).

In the peripheral smear evaluation on the 8th day, 11 (73.3%) of MRD$^+$ patients had cytomorphological remission. All MRD$^-$ patients had cytomorphological remission on the 8th-day evaluation. No correlation was found between MRD positivity and 8th-day cytomorphological remission (p=0.11).

On the 15th day, 21 (77.7%) patients had cytomorphological remission. 10 (66.6%) of the MRD$^+$ patients and all the MRD$^-$ patients had cytomorphological remission. No correlation was found between MRD positivity and 15th-day cytomorphological remission (p=0.053).

Cytomorphological remission was achieved in 25 (92.5%) patients who achieved remission on the 33rd-day bone marrow evaluation. 14 (56%) of these patients had MRD$^+$.

3.3. CD49f analysis

CD49f levels at diagnosis and on the 15th-day were mean 38.4 ± 22.1 and 5.4±12.6, respectively. There was a statistically significant difference in leukemic blasts' expression of CD49f (p=0.00). In groups MRD$^+$ and MRD$^-$, CD49f levels at diagnosis were mean 38.9±24.0 and 43.8±24.5, respectively (p=0.57). In groups MRD$^+$ and MRD$^-$, 15th day CD49f levels were mean 7.8±17 and 2.8±2 respectively (p=0.64). This expression is not significant for minimal residual disease detection compared to day 15 MRD. No relationship was detected between MRD and CD49f on day 15 (p=0.54).

Therefore, no reclassification was performed according to CD49f. Fig. 3 shows the association between CD49f and MRD on day 15.

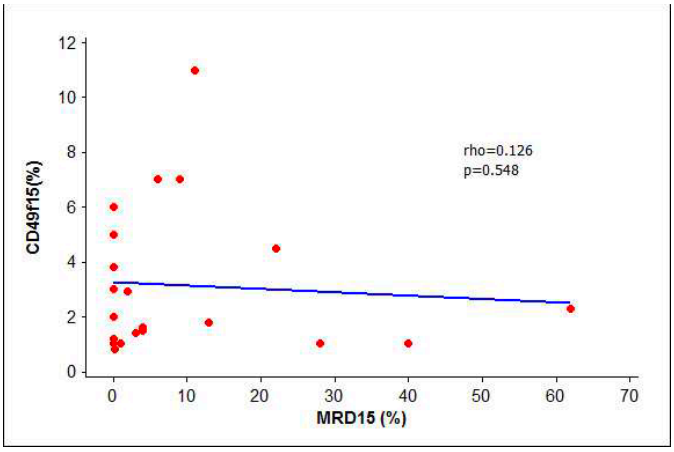

Fig. 3.15th day MRD and CD49f

4. Discussion

This study aimed to assess the performance and relevance of CD49f as a marker for the detection of MRD. Findings revealed that blasts initially exhibited high levels of CD49f, which then gradually decreased and persisted into the 15th day. However, no correlation was found between MRD and CD49f on day 15.

New approaches in risk classification for leukemia treatment have been developed. However, there was a need for more accurate and less leukemic blast identification techniques for identifying leukemic blasts. Detection of MRD has led to significant improvements in risk stratification and management of ALL (11, 12). Risk-targeted treatment strategies and improved overall survival have been aimed at using MRD detection, clinical, and cytomorphological features together (13, 14).

Numerous markers have been examined for MRD accuracy and usefulness during the last 2 decades. Macedo et al (1995) reported that CD34+ cells do not express CD3, CD20, CD22, CD14, CD65, and CD56, and their combination with CD34+ can be used in MRD examination (15). In another study, an MRD examination was performed with 30 different markers by the FCM method. This study determined that 22 different markers were expressed at different rates in leukemic cells and the relationship between some indicators and genetic abnormalities (8).

Disease recurrence is the primary factor influencing survival rates. Relapse was more likely in SR and IR patients when risk was classified based on cytomorphological characteristics (16). Therefore, MRD detection is very significant in patients in the low-risk group. Many studies have shown high relapse rates in MRD^+ (17). These recent data have provided a reclassification of SR patients with MRD^+. In our study, MRD^+ was in all the HR groups, but there was also a significant MRD^+ in the SR and IR groups. Furthermore, all MRD^- and the majority of MRD^+ patients had cytomorphologic remission. Due to our short follow-up time, we were unable to assess the relapse rate.

CD49f is also an investigated indicator for MRD (9, 18). DiGiuseppe et al. (2009) evaluated the expression of CD49f in normal B cell maturation and preB-ALL cells. In this study, low CD49f expression was detected in all stages of B cell maturation, as well as moderate CD49f expression in leukemic blasts at the time of diagnosis. CD49f expression had similar results with other antibodies used in MRD. In this study was observed that CD49f could be overexpressed during the induction period (9). In conclusion, this article highlighted that even if CD49f is not detected at diagnosis, it can still be a useful indicator for MRD in follow-up (9). In our study, CD49f expression was high at the diagnosis and significantly decreased by the time of follow-up. We did not observe similar results between CD49f and MRD. However, we found a correlation between a decrease in leukemic blasts and a reduction in CD49f expression.

A recent study reported that 22 markers, including CD49f, are expressed at different rates in normal B-cell and leukemic blasts (8). However, studies on CD49f are not sufficient. Our study is one of the few on CD49f and MRD. In our report, we detected high CD49f expression in leukemic blasts at the diagnosis. Also, we observed that CD49f was expressed higher in the MRD^+ patients on day 15 than in the MRD^- patients. We hypothesized that normal B cell expression could be correlated with low CD49f expression in MRD^-.

Recently, Collins et al (2021) reported an association between CD49f expression and genetic subgroups of ALL. In this study, significant differences in CD49f expression were detected in 5 genetic subgroups. Particularly in KMT2A-rearranged cases, decreased CD49f expression was revealed (19). Because there was only one patient in our study group with a genetic mutation, we were unable to assess the association between CD49f expression and the genetic subgroup.

ALL has a marked tendency to metastasize to the central nervous system. In a recent study, CD49f-laminin interactions were correlated to the CNS involvement (20). Yao et al (2018) emphasized that CD49f expression enables leukemic blasts to use neural migration pathways (20). In our research, one patient had CNS involvement. This patient had high CD49f expression, and this expression decreased on the 15^{th} day. This suggests that CD49f expressed in leukemic stem cells may be resistant to treatment, an increased likelihood of CNS metastases, and a potential? association with the ETV6-RUNX genetic group.

The most significant limitations of our study are the short follow-up time and the small number of patients. The impact of CD49f expression on relapse was not evaluated because of the short follow-up time.

In conclusion, this study demonstrated that CD49f was

significantly expressed in leukemic blasts but was also weakly expressed on the 15th day. We conclude that this expression alone is insufficient to define MRD. However, research with more participants, longer follow-ups, and sequential MRD and CD49f monitoring are required to assess the performance and relevance of CD49f as a marker in the detection of MRD.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: T.K., T.P Design: T.P., Data Collection or Processing: T.K, E.Y., Analysis or Interpretation: E.U., M.K., Literature Search: T.K., Writing: T.K.

Ethical Statement

Ethical permission required for the study was obtained by Ethic Committee from Erciyes University, with the decision numbers 2011/347.

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Demographic and clinical characteristics of cancer patients presenting to the emergency department: A single-center experience

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Abstract

Cancer is a major public health issue with a high mortality rate globally. Owing to advancements in diagnostic and treatment strategies, the survival rate of cancer patients has increased. This has also led to an increase in the frequency of emergency service use in cancer patients. Here, we retrospectively extracted and analyzed the data of patients who presented at the emergency department of Ondokuz Mayıs University's Faculty of Medicine as outpatients or via emergency services and then required oncology consultation between January 1, 2018, and January 1, 2019. Specifically, we analyzed their demographic characteristics and clinical data, including reasons for admission, cancer stage and performance status at admission, and emergency department stay length. In total, 542 visits by 376 patients (mean age, 60.8 ± 12.8 years; 162 women, 214 men) were recorded during the study period. The most common cancer types were breast cancer in women (30.9%) and lung cancer in men (33.2%). The most common reason for admission was signs of infection, whereas the most common diagnosis was neutropenic fever (17.3%). Furthermore, 223 (59.3%) visits resulted in hospitalization. Finally, 63.1% of the patients stayed in the emergency department for <24 h. The current results may aid in developing strategies for reducing workload and costs and improving service quality for cancer patients in emergency departments.

Keywords: cancer, medical oncology, emergency department, sociodemographic data

1. Introduction

Cancer is a leading health issue in both developed and developing countries. Advancements in diagnostics and therapeutics have led to increased life expectancy; as such, the number of patients diagnosed as having cancer has also increased (1). In Turkey, cancer is the second most common cause of death, preceded by cardiovascular diseases (2).

Cancer is a chronic disease, and patients with cancer may present to the emergency department for various reasons at the time of new cancer diagnosis, during cancer treatment, or during the palliative period. The aforementioned reasons may include mechanical effects of cancer (e.g., pain, bleeding, or compression), side effects of cancer treatment (e.g., hematological or metabolic issues or infections), or conditions or symptoms unrelated to their cancer (3, 4). At the time of new cancer diagnosis and during cancer treatment, the alleviation of oncologic emergencies is crucial; by contrast, during the palliative period, the focus is not only on symptom alleviation but also on the improvement of quality of life (5).

Cancer management requires a multidisciplinary approach, which may be designed for worldwide application and modified based on the requirements of each country. Under the Cancer Control Program, cancer registry data are used to determine the frequency of cancer occurrence, underlying causes, preventable etiologies, and appropriate screening programs for early diagnosis (6).

In this study, we investigated the demographic and clinical characteristics of cancer patients who presented to our emergency department. The current results may facilitate the development of appropriate cancer management strategies.

2. Material and Methods

This retrospective study was approved by Ondokuz Mayıs University's Non-Interventional Ethics Committee. We included the data of ≥18-year-old patients histopathologically diagnosed as having cancer, who presented to the emergency department of Ondokuz Mayıs University's Faculty of Medicine—a regional hospital in Samsun, Turkey—and required oncology consultation between January 1, 2018, and January 1, 2019. The demographic and clinical characteristics of the patients were extracted from their medical records and the hospital's electronic health records. The demographic characteristics included age, sex, and residence status, whereas the clinical characteristics included current pathological diagnoses, treatments received at the time of admission, reasons for admission, preliminary diagnosis after initial evaluation at the emergency department, interventions received, performance status at the time of admission, length of emergency department stay, metastasis status, laboratory results, and outcome of the emergency department visit.

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SPSS (version 21.0; SPSS, Chicago, IL, USA) was used for statistical analysis. Continuous variables are expressed as means ± standard deviations (SDs) or medians (minima–maxima), whereas categorical data are expressed as numbers and percentages. The normal distribution of variables was evaluated using the Kolmogorov–Smirnov test. Here, normally distributed variables are expressed as means ± SDs, whereas those that do not follow normal distribution are expressed as medians (minima–maxima). Independent group comparisons of non-normally distributed continuous variables were performed using the Mann–Whitney U test. Finally, all categorical data were evaluated using the Pearson chi-square test. A p-value of <0.05 was considered to indicate statistical significance.

3. Results

In total, 542 eligible emergency department visits were recorded during the study period; 376 of these visits were by unique patients who had presented to the emergency department as outpatients or through emergency services and then required oncology consultation.

Of these 376 patients, 43.1% were female and 56.9% were male, their demographic data, number of visits, and patient characteristics were examined (Table 1). Among women, the most common cancer was breast cancer (30.9%), followed by ovarian cancer (14.2%) and colorectal cancer (11.7%). By contrast, among men, it was lung cancer (33.2%), followed by stomach cancer (12.1%) and colorectal cancer (11.2%) (Table 2). At the time of emergency department admission, 70.5% of the patients had stage 4 cancer. As the stage advanced, the rate of repeated admissions remained similar (Table 3).

We next analyzed the Eastern Cooperative Oncology Group (ECOG) performance status scale scores at emergency department admission. The results indicated that most patients had an ECOG score of 2 or 3 ($p < 0.001$). Moreover, in those with an ECOG score of 4, the recurrent admission rate was only 1%.

The most common complaint during admission was fever or complaints suggesting infection (28.4%), whereas the most common diagnosis was neutropenic fever (17.3%). The length of emergency department stay was <24 h in most cases (63.1%). The most common intervention (50.2%) was multifaceted palliative support therapy, comprising fluid, electrolyte, and nutritional support (Table 4 and Table 5).

Table 1. Patient characteristics

	Patients with at least one application (376)	
	Number (n)	Percentage (%)
Gender		
Female	162	43.1
Male	214	56.9
Age		
20-30	9	2.4
30-40	18	4.8
40-50	34	9
50-60	103	27.4
60-70	112	29.8
>70	100	26.6
Cities of Residence		
Samsun	270	71.8
Ordu	39	10.4
Amasya	23	6.1
Sinop	21	5.6
Other	23	6.1
Living Area		
Rural District	193	51.6
City Center	183	48.7
Number of ED visit		
1	274	72.9
2	66	17.9
3 or more	36	9.2
Monitoring Center		
OMÜ	296	78.7
External Center	52	13.8
New Diagnosis	27	7.2
Unmonitored	1	0.3

Table 2. Distribution of cancer types by gender in patients admitted to the emergency department

Tumor location	376 Patient		Female		Male	
	n	%	n	%	n	%
Lung	86	22.5	15	9.3	71	33.2
Breast	52	13.8	50	30.9	2	0.9
Colon/Rectum	43	11.4	19	11.7	24	11.2
Stomach	39	10.4	13	8	26	12.1
Pancreas	24	6.4	9	5.6	15	7
Overian	23	6.1	23	14.2	0	0

Prostate	14	3.7	0	0	14	6.5
Kidney	13	3.5	4	2.5	9	4.2
Gallbladder/choledoch	11	2.9	4	2.5	7	3.3
Brain/spinal cord	11	2.9	5	3.1	6	2.8
Liver	8	2.1	0	0	8	3.7
Bladder	8	2.1	2	1.2	6	2.8
Other	44	11.7	18	11.1	26	9.8
Total	376	100	162	100	214	100

Table 3. Distribution of cancer patients' emergency visits according to tumor stages

		Total Patient (376)		One-Time Applicants (274)		Repeated Applicants (102)	
		n	%	n	%	n	%
State	1	4	1.1	2	50	2	50
	2	26	6.9	19	73.1	7	26.9
	3	44	11.7	31	70.5	13	29.5
	4	265	70.5	190	71.7	75	28.3
	Unknown	37	9.8	32	86.5	5	13.5
	Total	376	100	274	72.9	102	27.1

Table 4. Characteristics of emergency department admissions

Emergency Admission Complaints	Number (n)	Percentage (%)
Fever-Infection	154	28.4
General Condition Deterioration	69	12.7
Weakness Anorexia Oral Intake Reduction	68	12.5
Shortness of Breath	60	11.1
Nausea Vomiting Diarrhea	58	10.7
Pain	50	9.2
Neurological Causes	26	4.8
Bleeding	17	3.1
Other	40	7.4
Emergency Diagnosis	**Number (n)**	**Percentage (%)**
Neutropenic fever	94	17.3
Pneumonia	68	12.7
Drug side effect	57	10.5
Anemia/Thrombocytopenia	43	7.9
Mass effect	35	6.5
Pleural effusion	33	6.1
Acute abdomen/ Ileus	25	4.6
Electrolyte disturbance	25	4.6
Other	162	29.8

Table 5. Interventions performed. length of stay, and outcomes of emergency department visits.

Emergency Interventions		
Palliative supportive therapy	272	50.2
Antibiotic therapy	183	33.8
Blood transfusion	40	7.4
Interventional procedures*	27	5
Surgery	7	1.3
Paracentesis	6	1.1
Other	7	1.2
Length of Stay In the Emergency Department	**Number (n)**	**Percantage (%)**
Less than 24 hours	342	63.1
24-48 hours	111	20.5
48-72 hours	33	6.1
More than 72 hours	46	8.5
Not Determined	10	1.8
Outcome of emergency department visit (for each visit)		
Admission to medical oncology service	316	58.3
Discharge from emergency department	122	22.5
Death in emergency department	12	2.7
Other**	92	16.9

*Interventional Treatment Operations (pleural drainage, chest tube insertion, central catheterization) ** Non-oncological medical service hospitalization, transfer to intensive care unit, situations where the patient refuses hospitalization

Finally, anemia and thrombocytopenia were the most prominent findings in the laboratory tests among all 542 visits. In particular, grade-1, -2, and -3 anemia was noted in 33.9%, 29.3%, and 15.7% of the visits, respectively. Moreover, the platelet count was <150,000/mm3 at 43.7% of the visits, and thrombocytopenia of grade 3 or higher was noted at 12.7% of the visits.

4. Discussion

The increasing cancer survival rate is expected to result in an increase in the number of patient visits to cancer clinics, emergency departments, and palliative care centers. Samsun, a densely populated province in the Middle Black Sea region of Turkey, provides significant health services to its neighboring provinces. Therefore, understanding the relationship between patients and emergency services is important for healthcare management in this region.

Sex affects the pathophysiology, clinical symptoms, and treatment outcomes of cancer (7). An Ege University study reported that of the 34,134 patients with cancer, 56.6% were male and 43.4% were female (8); this result is corroborated by our findings: 56.9% male and 43.1% female. Increased life expectancy has resulted in an increase in the number of advanced-age cancer cases not only in Turkey but also worldwide: approximately half of all cancer cases occur in individuals aged ≥65 years (9). Bozdemir et al. reported that the average age of cancer patients presenting to their emergency department was 60 ±14.8 years (10). Similarly, 56.4% of our patients were aged ≥60 years when they presented to our emergency department.

Patients with cancer present to the emergency department for reasons directly or indirectly related to cancer (3). Mualloğlu et al. observed that 45.5% of 408 patients who visited the emergency department had at least two visits (average number of emergency department visits per patient = 2.08). This situation was attributable to the emergency services being more easily accessible than outpatient services, as well as to disease progression (11). In our study, of all 542 visits, 268 examined were repeat visits; the reasons for this included the palliative care needs of advanced-stage patients and easy access to the emergency department.

In one study, most cancer patients who presented to the emergency department had cancers of the respiratory system (including the lungs; 26%), followed by those of the gastrointestinal system (26%) and the genitourinary system (17%) (12). In the current study, most (22.9%) of the patients with cancer were diagnosed as having lung cancer, followed by breast cancer (13.8%) and colorectal cancer (11.4%). Moreover, the most common cancer was breast cancer among female patients (30.9%) and lung cancer among male patients (33.2%).

In patients with cancer, the need for emergency services may increase with the advancement of the cancer stage. In a Turkish study, 72.9% of cancer patients who visited the emergency department had advanced-stage cancer; however, none of them had in situ carcinoma (13). In their 2014 review, Lash et al. observed that patients with cancer at the T2a stage or lower below had more emergency department admissions than those with cancer at the T3 stage or higher (14). In the current study, of all 376 patients, 309 had stage 3 or 4 cancer. Moreover, of all 542 emergency department visits, 71.8% were for distant metastasis. As such, most of our patients with cancer had advanced-stage or metastatic disease—consistent with the previous results.

The ECOG Performance Status Scale score is widely used to assess the performance status of patients with cancer (15). In a Turkish study of 245 patients with cancer (with 324 emergency visits), most presented an ECOG score of 3 (10). In the current study, the ECOG score was available for 468 emergency department visits; at 33.3% and 29.5% of these visits, the ECOG scores were 2 and 3, respectively. Moreover, these scores were noted in patients with recurrent visits.

Patients with cancer may present to an emergency department for reasons that may or may not be related to cancer prognosis or treatment. A Canadian study demonstrated that among patients with cancer, the most common reasons for visiting the emergency department were nausea, fatigue, and shortness of breath (16). In contrast, a US study reported that pain, breathing difficulties, and gastrointestinal complaints were the most common reasons (17). In the current study, the most common reason was symptoms suggesting infection (e.g., fever, chills, and shaking), followed by weakness, loss of appetite, reduction in oral intake, and general health deterioration. In a few cases, these reasons included pain, bleeding, neurological symptoms, and non–cancer-related symptoms. Moreover, the most common diagnoses were neutropenic fever, pneumonia, cancer-treatment–related conditions, anemia, and thrombocytopenia. For these diagnoses, the most commonly used interventions included palliative or supportive therapy, oral or intravenous antibiotic use, and blood transfusion.

Swenson et al. reported that 55%, 10%, 6%, and 30% of 71 emergency department visits led to admission to the oncology unit, the oncology intensive care unit, the surgical unit, and other medical units, respectively (4). In the current study, the length of emergency department stay was <24 h in 63.1% of all visits. Moreover, of all visits, 59.3% led to the oncology department admission, 19.9% to discharge from the emergency department, 9% to non–cancer-related hospitalization, and 4.3% to intensive care unit admission. However, 1.9% of the visits resulted in death.

Anemia is commonly seen in patients with cancer over the course of their disease and as a side effect of their cancer treatment (18). In our study, 45% of the patients demonstrated anemia, and 15.7% of these patients had severe anemia. Thrombocytopenia is another hematologic disorder commonly seen among patients with cancer caused by systemic chemotherapy in most cases (19, 20). Among all our patients, 43.7% had thrombocytopenia; in particular, 12.7% had severe thrombocytopenia.

In conclusion, patients with cancer require effective disease management in terms of aspects such as diagnostics, therapeutics, and follow-up. In Turkey, additional comprehensive studies on the cancer burden on emergency departments and oncology services are required. Their results may aid healthcare professionals in designing and developing their future cancer treatment strategies and practices.

Conflict of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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Authors' contributions

Concept: M.U., T.A., Design: M.U., T.A., Data Collection or Processing: M.U., T.A., Analysis or Interpretation: M.U., T.A., Literature Search: M.U., T.A., Writing: M.U., T.A.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 27/02/2020 and the number of ethical committee decisions is 2020/93.

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Research Article

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A retrospective observational study of autologous peripheral blood stem-cell transplantation and long-term survival outcomes - An institutional experience

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Abstract

Autologous peripheral blood stem cell transplantation (PBSCT) has been employed in patients with various haematological and non-haematological malignancies. The present retrospective study aimed to examine the clinical efficacy and overall long-term survival outcomes of the patients who underwent autologous PBSCT. The clinical data of 49 patients with various haematological and non-haematological malignancies from the Department of Haematology of SMS Hospital from April 2015 to March 2021 were retrospectively analysed. The median age of our patients was 41.5 years. Among all indications, relapsed hodgkins lymphoma (10, 20.4%) and multiple myeloma (27, 55.1%) were reported to be high. The average engraftment was observed to be 11 days with no post-operative complications. The average follow-up period was 2.5 years with a mortality rate of 8.16% (4). Overall, a total of 43 (87.75%) patients showed a complete response with a relapse rate of 12.24% (6). In conclusion, autologous PBSCT can be an effective treatment option with good clinical efficacy, and long term survival outcomes. Our results are comparable to those of many national and international published reports. Overall, the results suggest that with improved management of conditioning-related toxicities and infections, it is possible to develop PBSCT programs in third-world countries and achieve outcomes comparable to those in the international data.

Keywords: autologous transplantation, peripheral blood stem-cell transplantation, conditioning regimen, retrospective observational study, survival outcomes

1. Introduction

Cancer is the leading cause of death worldwide with an estimated 19.3 million new cancer cases and 10 million cancer deaths in 2020 alone (1). Among all the cancers, hematological malignancies are a heterogeneous group of cancers that comprise diverse subgroups of neoplasms originated from uncontrolled growth of hematopoietic and lymphoid tissues (2,3). These biologically and clinically heterogeneous disorders account for 6.5% of all cancers around the world (2). They are commonly classified into four common subtypes: multiple myeloma (MM), non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), and leukemia.

In patients with hematological malignancies and disorders, bone marrow failure is a common phenomenon. In such patients, myeloablation or myeloablative therapy is initiated using very high doses of chemotherapy/radiation therapy to kill the cancer cells as a potentially curative treatment for a variety of hematological diseases. Such high doses also lead to death of normal bone marrow stem cells. In such cases, hematopoietic stem cell transplantation is done to restore those destroyed cells using either autologous or allogeneic stem cells through one of the following methods: bone marrow transplant (BMT), peripheral blood stem cell transplant (PBSCT), and cord blood transplant (CBT). The goal of the engraftment is for the transplanted cells in the bone marrow to grow/make healthy blood cells over time.

Among all hematopoietic stem cell transplantation methods, PBSCT has been preferred over others for more than 25-30 years, since its first reported use in 1989 (4,5). The use of PBSCT as a source is preferred and supplanted over BMT due to its higher potency to restore hematopoietic and immune functions, stem cell harvesting technique without the need of general anesthesia, and the discomfort associated with multiple BM aspirations (4). Other major benefits include graft-versus-tumor effect, fewer febrile days, a lower incidence of infections, a lower requirement of antibiotics, a lower number of platelet and red cell transfusions, and lower intensive care requirements leading to reduced costs (4). In contrast, the treatment with PBSCT is highly toxic and risky due to its severe immunological complications such as acute/chronic graft versus host disease (GVHD) (6,7). The most common causes of post-transplant mortality are organ toxicity, infections (11%), GVHD (12%), and relapse of neoplastic

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disease (41%) (6,8).

In the present study, we report our experience of autologous peripheral blood stem-cell transplantation, its clinical efficacy, and long term survival outcomes in our patients.

2. Matherials and Methods

2.1. Patient screening and selection

In this single-centre observational study, the medical records of all the patients who underwent autologous stem cell transplant from April 2015 to March 2021 were retrospectively analysed. The age range for the consideration was ≤75 years with a suitable Eastern Cooperative Oncology Group (ECOG) performance status, and the lack of significant comorbidities or multiple organ dysfunctions. Patients with only particular cancers (table 1) were included, while other active cancer patients were deemed ineligible.. The present study data was also keen on multiple patient specific characteristics as mentioned in table 1 such as type of transplantation, diagnosis, status of the transplant, chemotherapy regimen, etc. All patients gave their written informed consent. This retrospective evaluation has been approved by the institutional ethics committee. The objectives of our study were to investigate overall survival (OS), and treatment-related death (TRD) after transplantation.

2.2. Data collection and analysis

The patient's information such as socio-demographic profile, clinical history, diagnosis, type of transplant underwent, status of transplant, chemotherapy given, and follow-up details were collected, classified, and categorised. Procured data was analysed descriptively. OS was calculated starting from the day of the first PBSCT (day 0) until death from any reason with censoring of patients alive at their last follow-up. TRD was determined as death other than progression or relapse before day +100 from the last PBSCT. The results were compiled and statistically analysed using SPSS 22 (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1 shows the base-line characteristics of the 49 patients. Among the 49 patients, the youngest one was 5 years and the oldest was 67 years. Only patients undergoing their first transplant were considered for this present study and all of them underwent PBSCT using their own cryopreserved stem cells collected prior to conditioning. The most common diagnosis reported in our patients was multiple myeloma (27, 55%), relapsed (non-hodgkins and hodgkins lymphoma) (13, 26.53%), and multiple sclerosis (3, 6.1%). At the time of transplant, 67.34% of our patients were under remission. The average follow-up period post transplantation was 30 months. Among 49 patients, 4 patients were succumbed due to renal failure (2, 4%) and other GI infections (2, 4%). In the remaining, 43 patients have shown a complete response (87.75%) and 2 (4.4%) were under relapse. Overall, an average engraftment period for all the patients was reported to be 11 days.

Table 1. Baseline patient characteristics and transplant data of our autologous patients

Characteristics	Number of patients (n=49), %
Age (years ± SD)	41.5 (14.7)
Sex	
Men	36 (73.4)
Women	13 (26.6)
Chronological number of transplant - 1st transplant	49
Type of Transplant - Autologous	
Donor - Self - PBSCT	
Type - PB - Mobilized peripheral blood stem cells (all are cryopreserved stem cells)	
Diagnosis - conditioning regimen used	
Relapsed (non-hodgkins and hodgkins lymphoma) - BEAM	13 (26.53)
Relapsed non - hodgkins lymphoma - LACE	2 (4.08)
Neuroblastoma - CECy	1 (2.04)
Amyloidosis - Melphalan	1 (2.04)
Primary CNS Lymphoma - RCT	1 (2.04)
Relapsed seminoma testes - CECy	1 (2.04)
Multiple sclerosis - BEAM-ATG	3 (6.12)
Multiple myeloma - Melphalan	27 (55.1)
Day of engraftment (on average)	11
Status at transplant	
Remission	33 (67.34)
Non-Remission	16 (32.65)
Time (average days)	914
Status at LFU	
Alive	45 (91.83)
Dead	4 (8.16)
Cause of Death	
kidney failure	2 (4.08)
Infections	2 (4.08)
If Alive	
Complete response	43 (87.75)
Relapse	6 (12.24)

BEAM - Carmustine, etoposide, cytarabine, melphalan; BEAM ATG - Carmustine, etoposide, cytarabine, melphalan, anti-thymocyte globulin (ATG); CECy - Carboetocyclophasphamide; LACE - Lomustine, etoposide, cytarabine(Ara-C), cyclophosphamide; RCT – Rituximib, Carmustine, thiotepa; PBSCT - Peripheral blood stem cells transplantation; LFU – Last follow-up.

4. Discussion

The rationale of the present study was to compile and analyse the data to observe the survival outcomes our patients after autologous transplantation performed in the past decade at our centre. The four main indications were multiple sclerosis (MS), relapsed non-hodgkins lymphoma (NHL), relapsed hodgkins lymphoma (RHL), and multiple myeloma (MM). Over the past 20 years almost all the haematological malignancies are treated with PBSCT by largely replacing the bone marrow transplantation procedure for both autologous and allogeneic stem cell transplantation. Use of such mobilized PBSC were

proven to be highly efficient in terms of rapid restoration of the immune system, convenience of stem cell collection, fewer transfusions, shorter engraftment time, and a shorter hospital stay. In all of our patients, PBSCT were used.

Many studies have shown the preponderance of haematological and non-haematological malignancies in elders (60 years of age or older) compared to adolescents (0–19 years) and young adults (20–59 years) (9–11) However, some reports have made exceptions and have shown a higher frequency in adolescents and young adults (AYA, 0 – 39 years).(12,13) Our patients group median age was observed to be high in young adults as reported by previous studies (12,13). Many studies have confirmed the slight predominance of male over female patients.(2) In contrast, majority of our patients were male. Whereas with the current transplantations, almost all of autologous and a majority of allogeneic transplants are performed with mobilized peripheral blood stem cells due to their potential for tumor cell free collection and restoration of hematopoietic and immune functions more rapidly than BM (14,15,24,16–23). Before transplantation, conditioning regimens were given to all the patients with hematological indications using myeloablative conditioning chemotherapy using central lines by avoiding non-myeloablative or reduced intensity regimens and total body irradiation.

The preparative or conditioning regimen is a critical element that helps prepare patients for stem cell transplantation by killing any cancer cells that are in the body. As shown in table 1 and table 2, every indication has its own conditioning regimen for better outcomes. Most of our patients have four main indications and their conditioning protocols are presented in table 2.

Over the last three decades, BEAM and LACE have been the most widely used conditioning regimens before autologous stem cell transplantation for patients with NHL, relapsed NHL, and relapsed hodgkin lymphoma (HL).(2,3,11,13,25–30) In recent times, as a result of two major ground-breaking CORAL and PARMA trials, it has become a standard of care (SOC) in the treatment of chemotherapy-sensitive and relapsed NHL.(27,31,32) BEAM and LACE are generally very effective and well tolerated. In view of findings from previous studies, in our patients too we have adapted the BEAM and LACE protocols as SOC to treat HL, NHL, and relapsed NHL.(25,29,32–35)

Table 2. Disease indication conditioning regimens and their protocols

Conditioning regimens	Indications	Protocol
BEAM(25,29,33,34)	Relapsed hodgkins lymphoma and relapsed non-hodgkins lymphoma	Day-6: Carmustine (BCNU) (300mg/m^2) Days −5, −4, −3, −2: Etoposide (200mg/m^2) Cytarabine (Ara-c) (400mg/m^2) Day −1: Melphalan (140mg/m^2) Day 0: stem-cell transplant
BEAM-ATG(36–41)	Multiple sclerosis	Day -6: Carmustine (BCNU) (300mg/m2) Days −5, −4, −3, −2: Etoposide (200mg/m2) Cytarabine (Ara-c) (400mg/m2) Day −1: Melphalan (140mg/m2)] + peri-transplant ATG as an intermediate-intensity regimen. Day 0: stem-cell transplant
LACE(26,28,30,50–52)	Relapsed non - hodgkins lymphoma	Day -7: Lomustine (200mg/m^2) Day -7: Etoposide (1000mg/m^2) Day -6. -5: Cytarabine (Ara-C) (2000mg/m^2) Day -4, -3, -2: cyclophosphamide (1800mg/m^2) Day 0: stem-cell transplant
Melphalan (42,45,49,53–55)	Multiple myeloma	Day -1: Melphalan (200mg/m^2) or Melphalan (140mg/m^2) Day 0: stem-cell transplant

BEAM - Carmustine, etoposide, cytarabine, melphalan; BEAM ATG - Carmustine, etoposide, cytarabine, melphalan, anti-thymocyte globulin (ATG); CECy - Carboetocyclophasphamide; CM – Carbomelphalanetopocyte; LACE - Lomustine, etoposide, cytarabine(Ara-C), cyclophosphamide; RCT – Rituximib, Carmustine, thiotepa;

Recently, BEAM has been in the process of being replaced by a more economic and available fotemustine or bendamustine, etoposide, cytarabine, and melphalan (BeEAM) regimen. However, due to a lack of larger, prospective trials data on its risk-benefit ratio- instead of BeEAM, BEAM chemotherapy was used in our patients. In MS, we have used BEAM-ATG as conditional regimen due its high safety and efficacy profile. Its usage is also quite popular across Europe, North and South America. (36–41) Whereas in our MM patients, the current accepted standard conditioning regimen - high-dose melphalan (200 mg/m^2) was used (42). Previous trials attempting to replace this with oral and intravenous busulfan have failed, due to increased toxicity and a lack of superiority, respectively (42–44).

The average engraftment period after conditioning in our patients was observed to be 11 days and it was consistent and comparable with the reports published from multiple studies and other standard international data. On average, almost all

the studies have reported the median engraftment day of their patients as 13 days (± 4 days) (28,34,38,45–48). Overall survival of our autologous transplant patients was 91.83%. with a remission rate as high as 67.34% compared to the other studies where it was 30 - 65% (47,49). Complete remission means the disappearance of all signs of cancer, but it does not always mean that the cancer has been cured. A total of 87.75% of our patients have shown complete responses. On the other hand, transplant related mortality was reported in our patients, however, two patients were succumbed to kidney failure and infections due to non-transplant related complications. Such non-transplant mortalities due to various causes were reported to be up to 37% and they were 8% in our patient group (47).

In a developing and resource limiting settings like India, performing autologous PBSCT are complex due to various reasons such as a lack of expertise, knowledge, infrastructure, and high treatment related costs. However, reports from this study can assure the fellow clinicians and the patients about the positive outcomes and we encourage other centres to start performing such transplantations or refer eligible patients for this available important treatment option.

In conclusion, our results reinforced the evidence for encouraging autologous PBSCT for eligible patients. In our study with 49 patients treated with autologous PBSCT, the long-term overall survival and complete response were highly positive. Autologous PBSCT is now evolving as a highly efficacious and relatively safe therapeutic option for the treatment of patients with variable hematological and non-hematological malignancies. In autologous PBSCT, conditioning chemotherapy is of utmost importance and it plays a vital role in overall survival of the patient by avoiding various unnecessary post-operative and long-term complications. Even though the study is limited by its retrospective nature and some differences in cohort, the findings indicate that autologous PBSCT could serve as a best available treatment. Large prospective clinical trials and long-term registry data are required to ascertain its long-term safety, efficacy and to optimise the transplant techniques. It is of prime importance for scientists, healthcare organizations, haematological societies, and persons to organise such large prospective studies to demonstrate the effectiveness of Autologous PBSCT.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept - M.K., J.Y.; Design - M.K., J.Y.; Data Collection or Processing - M.K., S.J., A.M., K.G., R.K., L.M., S.K., J.Y.; Analysis or Interpretation - M.K., J.Y.; Literature Search - M.K., J.Y.; Writing: M.K.

Ethical Statement

This study was approved by the Institutional Review Board and Ethics Committee of SMS Hospital, Jaipur as part of project in accordance with the 1964 Helsinki declaration and later amendments. As the study was retrospective, there was no study-specific consent. All patients granted verbal or written consent prior to and investigation or treatment.

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The efficacy of propolis extracts as a food supplement in patients with COVID-19

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Abstract

Propolis is a natural immunomodulator with anticancer, antiviral, and anti-inflammatory effects. Propolis may be considered an agent in the supportive treatment of COVID-19. Propolis is known for its wide range of pharmacological properties, with many studies finding it effective in both the prevention and treatment of a variety of conditions. Forty-five patients who were hospitalized in our hospital and did not need intensive care were divided into groups of 15. The patients were receiving the standard COVID-19 treatment protocol. In the randomized, controlled study, one group of patients received 2 ml of water extract of propolis (WEP) (50 mg/ml) orally three times a day for one week, while another group received 1 ml of olive oily extract of propolis (64 mg/ml) plus 1 ml of olive oily Perga extract (120 mg/ml) (OEP). Hospital discharge times and changes in biochemical parameters were used as indicators of recovery. The WEP and OEP groups were found to have statistically significantly better D-dimer, CRP, and WBC results than the control group when the improvement in parameters between the groups were compared. Significantly different hospital discharge times in groups 1 and 2 were found compared to the control group. The addition of propolis to the treatment as a food supplement has a positive effect on the recovery of patients with COVID-19 and may shorten the treatment time. The use of propolis as a food supplement has been shown to have a range of health benefits, and the addition of it to the treatment regimen for COVID-19 may help to reduce the severity and duration of symptoms.

Keywords: caffeic acid, coronavirus, COVID-19, propolis

1. Introduction

Coronaviruses are a major human and animal pathogen that caused the pandemic that began in Wuhan, China's Hubei Province, at the end of 2019 and spread throughout the world (1). The virus, which is transmitted from person to person through the mucous membranes of the mouth, eyes, and nose, is highly contagious and infective (2). The coronavirus that causes COVID-19 is an envelope-positive-stranded RNA virus in the same subgenus as the severe acute respiratory syndrome virus (3). Over 200 million confirmed cases of COVID-19 have been reported worldwide since the first case reports at the end of 2019 (4). The coronavirus pandemic has caused immense global disruption, including to economies, societies, and individual lives.

Most patients develop serum antibodies against the receptor binding site of the viral spike protein and the associated neutralizing activity (5). The antibody response may be related to the severity of the disease, and the antibody may not be detected in those with mild disease (6).

It recommended various pharmacological agents for the prophylaxis of venous thromboembolism for hospitalized COVID-19 patients (7). Concerns about NSAIDs at the beginning of the pandemic do not seem significant when viewed with the new findings (8, 9). It advised using dexamethasone in patients with severe COVID-19 (10). Remdesivir is used as an approved treatment in some countries (11). Baricitinib, a Janus kinase (JAK) inhibitor used in the treatment of rheumatoid arthritis, is thought to prevent the virus from entering the cell with its immunomodulatory activity (12). Vitamin D, fluvoxamine, famotidine, zinc, and colchicine are some other agents tried in treatment (13–16).

Propolis is a product found in resins and plant exudates produced by plants to protect themselves, and these are collected by bees (17). Quercetin, myricetin, and caffeic acid, which are components of propolis, are thought to play a role in the treatment of COVID-19 (18). Bees show stronger immune properties with propolis (19). Berretta et al. evaluate propolis

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as an immunomodulator used in the treatment of microbial, inflammatory, oxidative stress, and cancer (20). The composition of propolis varies between regions (21).

The components in propolis have effects on the replication, virion integrity, endocytosis, and transcription of viruses (22–25). It forms a protective mechanism against COVID-19 by playing a role in the prevention of thrombosis (26,27), immunomodulation (28), inflammatory response (29), 3C-like protease inhibition (30), PAK-1 inhibition (31), TMPRSS2 down-regulation (32), ACE2 inhibition (33), 3a Channel Protein inhibition (34).

We aimed to investigate the contribution of propolis to the healing process of patients with COVID-19 with the current clinical study.

2. Materials and Methods

We conducted a pilot study with 3 participants to evaluate the required number of participants at a significance level of 0.05, using version 3.01 of the G* Power software (Franz Foul, Kiel, Germany), and found at least 12 participants for each group. In the current study, cases of COVID-19, who were treated at Trabzon Kanuni Training and Research Hospital and continued to take their medications according to the protocol of the Ministry of Health (favipiravir and paracetamol orally, corticosteroids intravenously), were divided into 3 groups of 15 subjects. All the patients were on oxygen therapy.

1st group: patients were given 2 ml of water extract of propolis (WEP) (50mg/ml) orally 3 times a day for 1 week. WEP was provided by Fanus Food Co. (Trabzon, Turkey) (manufacturer code: TR-OT-006-1364).

2nd group: Patients were given 1 ml olive oily extract of propolis (OEP) (64 mg/ml) + 1 ml olive oily extract of Perga (bee bread) (120 mg/ml) orally 3 times a day for 1 week. Olive oil extracts were provided by the same firm (Biopropolis, olive oil extract (25.36%), manufacturer code: TR-OT-006-1364, Bioperga, olive oil extract (12%), manufacturer code: TR-OT-006-1364). In the present study, two types of propolis extract were used: water extract of propolis and olive oily extract of propolis including perga which contributes to its antioxidant potential.

3rd group (control group): patients given only medications for COVID-19 and not given any investigational product.

Our inclusion criteria were patients over the age of 18 who did not need a ventilator while staying in the Covid ward. Exclusion criteria were patients with no oral intake, patients with high temperature (<37° C), patients whose informed consent form was not approved, patients in need of ventilators, and pregnant women. In addition, we did not include patients using warfarin in the study to prevent a possible liver interaction. If an allergic reaction developed in each patient, we planned to complete the trial for that patient or to complete the trial when the 7-day investigational product administration period was completed.

The age, gender, lung computed tomography findings, complete blood count (CBC), C-reactive protein (CRP), D-Dimer, Troponin, and oxygen saturation (sO_2) levels of the patient were all recorded. We analyzed the collected blood samples using the Abbott® chemiluminescence immunoassay method. Lung evaluation was performed by GE Revolution EVO 128-Slice Computed Tomography®. Lung tomography scoring normal=1, patchy atelectasis and/or hyperinflation and/or bronchial wall thickening=2, focal alveolar consolidation involving only one segment or one lobe=3, multifocal consolidation=4, and diffuse alveolar consolidation=5 (35). We recorded the length of the hospital stay in days. We made statistics in terms of compliance with the normal distribution between the groups.

Patient selection was randomized according to administration time, first patient was included in Group 1, second patient in Group 2, third patient in Group 3, and so on, until each group was completed with 15 patients.

After obtaining the approval number T140938 of the Ministry of Health of the Republic of Turkey for the current study, ethical approval was obtained from the Istanbul Medipol University Clinical Research Ethics Committee.

All data obtained were analyzed with IBM Version 23.0 SPSS Statistics for Windows. We examined the conformity to the normal distribution by calculating the mean and standard error of the initial and follow-up routine parameters. After finding out whether there was a difference among the groups for each parameter with ANOVA or the Kruskal-Wallis test (nonparametric), if there was a significant difference, the Mann-Whitney U test or posthoc Tukey test was applied between groups. The comparisons between the groups (before/after) were made with the Wilcoxon Signed Rank test. For the level of significance, $p<0.05$ was used.

3. Results

There were 45 participants in our study, 22 of whom were male and 23 of whom were female. Their ages ranged from 26 to 82. Each group showed a normal distribution according to age and gender. No patient was excluded from the study due to side effects or the need for intensive care. The demographic characteristics and medical histories of the patients are presented in Table 1. Table 2 shows the biochemical parameter results of the patients before and after treatment, as well as the one-week follow-up. During hospitalization, lung tomography scoring was performed on 1 in 10 patients, 2 in 16 patients, 3 in 14 patients, and 4 in 5 patients.

When CRP, D-dimer, Troponin, WBC, and sO_2 levels were compared before and after treatment, each group showed statistically significant improvement ($p<0.001$). When the improvement in parameters between the groups was compared, the WEP and OEP groups had statistically significantly better D-dimer, CRP, sO_2, and hospital discharge results than the control group.

Table 1. Demographic properties of the COVID-19 patients

	Group 1 (WEP) (n=15)	Group 2 (OOP) (n=15)	Group 3 (Control) (n=15)
Age(years) (mean±SD)	56.8±13.4	60.1±15.1	60.4±16.9
Female(n)	7	8	8
Male(n)	8	7	7
Coexisting Conditions (n)			
Diabetes	4	5	4
Hypertension	7	6	5
COPD/Asthma	1	2	1
Obesity	3	2	2
Oxygen therapy (n)			
Nasal canula	3	4	5
High flow nasal canula	12	11	13

COPD: Chronic obstructive pulmonary disease

Table 2. The arithmetic mean±standard error of the mean of study parameters of COVID-19 patients

	Group 1 (WEP) (n=15)	Group 2 (OOP) (n=15)	Group 3 (Control) (n=15)
CRP (before) mg/L	70.4±14.4	79.2±19.2	67.8±14 .0
CRP (after) mg/L*	15.1±8.5[a]	41.9±11.9	42.9±8.7
D-DIMER (before) ng/ml*	1475.3±1153.3[b]	2289.7±795.3	1806.3±822.2
D-DIMER (after) ng/ml*	798.6±643.1[a]	1442.6±501.0	1535.5±698.9
Troponin(before) ng/mL*	29.9±17.2[c]	11.3±1.1	7.7±1.4
Troponin(after) ng/mL	4.4±0.9	3.5±0.5	6.8±1.2
WBC (before) mcL	13.6±1.4	13.8±1.6	13.0±1.3
WBC (after) mcL	8.5±0.8	8.3±0.9	10.5±1.0
sO_2 (before) %	84.3±0.7	86.0±0.5	85.2±0.5
sO_2 (after) %*	92.1±0.8[d]	89.9±0.7	87.8±0.5
Hospital discharge time (day)**	5.5±0.2	5.7±0.2	9.5±0.4[e]

*Significant difference among groups by Kruskal-Wallis test

**Significant difference among groups by ANOVA test

[a]Significantly different from groups 2 and 3 ($p<0.003$), [b]significantly different from group 2 ($p<0.002$), [c]significantly different from group 3 ($p<0.01$), [d]significantly different from group 3 ($p<0.001$) by Mann-Whitney U test, [e]significantly different from groups 1and 2 ($p<0.001$) by post-hoc Tukey test

4. Discussion

In the current study, WEP and OEP were used/employed in addition to the standard COVID-19 treatment recommended by the Ministry of Health. There was no patient whose treatment was discontinued due to side effects during the treatment. When determining the doses, previous studies with propolis were considered (36, 37). We observed both rapid improvement in biochemical parameters and reduced hospital stays in the propolis groups. In another study with Brazilian propolis conducted in 2021, the ethanolic extract was used (400-800 mg/day) and a reduction in the length of hospital stay was reported (38). All of these studies support the use of propolis as an adjunctive treatment in hospitalized patients with COVID-19.

It was valuable for the study that there was no difference between the groups in age and gender. Because age and gender were parameters that could affect the course of the disease (39). Being elderly and having a coexisting disease increase the likelihood of requiring intensive care (40). However, there was no patient in need of intensive care among the patients included in the current study. The mean age of the patients in the present study was 59.1±15.2 years which could be attributed to the exclusion of patients who needed intensive care on admission.

The study's weakness is that it didn't include people who needed intensive care or who were living alone at home with the disease. However, it is not possible to apply oral treatment for those patients in need of intensive care and to follow-up patients at home who are already in relatively good condition. Therefore, this study may not be generalizable to a broader population that includes people who require intensive care or those living alone with the disease.

The propolis extracts used for the current study were prepared from samples obtained from plants native to Turkey and were obtained by completely dissolving in only water or olive oil. It should be considered that propolis components vary with climate and region (41). Both propolis extracts (WEP and OEP) used in the present study include phenolic acids, especially caffeic acid (approximately 200 µg/ml) (42) and flavonoids such as galangin, pinocembrin, and chrysin.

Using propolis not only in the treatment of COVID-19 but also in various cancers and inflammatory diseases has been studied (43, 44). It also has anti-ulcer activity (45). Combining current therapy with propolis in COVID-19 patients has been associated with earlier hospital discharge and lower mortality (46). Despite the promising results in laboratory tests and clinical trials, large-scale studies are needed to definitively establish the efficacy of propolis in the treatment of these diseases.

Limitation of the study: The patient numbers in each group were not sufficient because the study period of the present study was close to the end of the first wave of COVID-19 in Turkey.

As a result, the addition of propolis to the treatment as a food supplement has a positive effect on the recovery of patients with COVID-19. It should also be kept in mind that propolis, which is also used to treat many diseases, may be used as a preventive medicine application. While the evidence of propolis's effectiveness as a treatment for COVID-19 is still being studied, it appears to be an effective treatment in terms of increasing a patient's rate of recovery.

Ethical Statement

The current study obtained ethical approval from the Istanbul Medipol University Clinical Research Ethics Committee with the approval number T140938 issued by the Ministry of Health of the Republic of Türkiye.

Conflict of interest
The authors declared none.

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Authors' contributions
Concept: B.D.K., Design: B.D.K., Data Collection or Processing: B.D.K., E.S., D.K., Analysis or Interpretation: O.D., A.T.A., Literature Search: B.D.K., A.T.A., E.S., O.D., D.K., Writing: B.D.K., D.K.

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Retrospective analysis of inpatients' demographic and clinical characteristics at medical oncology service: A single-center experience

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Abstract

The cancer registry is a continuous, systematic process of collecting data on the occurrence and characteristics of reportable neoplasms to assess and control malignancies' impact on the population. It helps public health professionals understand the dynamics of cancer incidences so that they can formulate strategies. To contribute to the cancer incidence studies in our region, we aimed to define the demographic and clinical characteristics of patients who had been hospitalised at Ondokuz Mayıs University Medical Faculty Hospital Oncology Service between 2018 and 2019.This retrospective, descriptive study's participants comprised cancer patients over 18 years of age who had been admitted to the service and referred to our faculty's medical oncology department. In this study, 519 applicants who had been hospitalised by the service were evaluated. The data of 385 patients, 134 of whose hospitalisations had been repeated, were examined. Of these 385 patients, 226 (58.7%) were male and 159 (41.3%) were female. Their mean age was 59.74 ± 12.74 (21.0–86.0). The most common reason for their admission was palliative care (153 patients; 29.5%), infection (67; 12.9%) and treatment maintenance (65; 12.5%). Lung cancer was found to be the most common cancer type (21.0%), followed by gastric cancer (12.5%) and breast cancer (11.7%). The majority of this study's patients had been admitted to the medical oncology service with advanced metastatic disease requiring palliative support.Preparing algorithms through a multidisciplinary approach and determining the order of referrals between units will increase the quality of life for patients and their caregivers. Therefore, patient follow-up and care quality will increase when the frequency of hospitalisations and applications that exceed tertiary services' and outpatient clinics' capacities are reduced.

Keywords: cancer, clinical features, demographic features, oncology

1. Introduction

As they are in the rest of the world, chronic diseases are increasing in Turkey. Per the National Burden of Disease and Cost-Effectiveness Study's results, cancer is an important public health problem since it is the second-most-common cause of known death, after cardiovascular diseases, in Turkey (1). According to Global Cancer Observatory (GLOBOCAN) data, a total of 18.07 million new cancer cases developed while 9.5 million cancer-related deaths occurred globally in 2018 (2). The incidence of cancer has increased significantly due to prolonged life expectancies, advances in diagnosis and treatment and increased exposure to carcinogenic substances (3). Globally, cancers cause approximately 12% of all deaths. In developed countries, cancer is the second leading cause of death, accounting for 21% of deaths; in developing countries, it is the third, accounting for 9.5% of deaths (4).

Given studies' inadequacy in describing the characteristics of patients who have been hospitalised by oncology services, the current study aimed to define inpatients' demographic and clinical characteristics at Ondokuz Mayıs University Medical Faculty Hospital Oncology Service.

2. Material and Methods

Approval for this retrospective study was obtained from the Non-Interventional Ethics Committee of Samsun Ondokuz Mayıs University. The study's participants comprised cancer patients who were over the age of 18, had followed up with our faculty's medical oncology department and had been admitted to the oncology service between 1 January 2018 and 1 January 2019. Data regarding patients' demographic and clinical characteristics were obtained from the hospital's electronic database and patients' files. Patients' age, gender, cancer-affected organ and pathology type, date of diagnosis, presence of metastasis, reason for admission, hospitalisation laboratory results, time of admission, length of stay at the service (days), hospitalisation status and end-of-service hospitalisation were included in the data, which were collected through forms prepared in Excel.

2.1. Statistical analysis

The SPSS (Version 22.0, SPSS Inc.) program was used to statistically evaluate this study's data. Continuous variables were expressed as means ± standard deviations (SDs), medians and lowest-maximum values (minimum–maximum), while countable data were expressed using numbers and percentages (%). The variables' suitability for normal distribution in statistical analyses was evaluated with the Kolmogorov–Smirnov test. While descriptive analyses were expressed in the

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study, variables suitable for normal distribution were specified using arithmetic means ± SDs, and variables that did not conform to normal distribution were specified using median (minimum–maximum) values. When continuous variables were compared between independent groups, those that did not fit the normal distribution were evaluated with the Mann–Whitney U test. Pearson's chi-square test was used to evaluate categorical data. Statistical significance was accepted at $p < 0.05$ for all tests.

3. Results

In our study, 519 applicants who had been hospitalised with the oncology service between January 1 2018 and January 1 2019 were evaluated. The data of 385 patients, who had been hospitalised at least once and for whom 134 hospitalisations were repeated, were analysed. Within this study period, 694 patients had with consulted with the medical oncology department from our hospital's emergency department. During this time, 5,981 patients had presented at our hospital's oncology outpatient clinic, submitting a total of 24,688 presentations. Of the 385 patients included in the study group, 58.7% were male and 41.3% were female. Their mean age was 59.74 ± 12.74 (21.0–86.0). Meanwhile, the male patients' mean age was 61.62 ± 12.53, which was higher than the female patients' mean age (57.07 ± 12.59), representing a statistically significant difference ($p < 0.0001$).

When hospitalised participants at the oncology service were examined on a patient basis, 286 patients were found to have been hospitalised once, and 99 patients had experienced repeated hospitalisations. The mean stay length for the total 519 hospitalisations was 13.49 ± 14.00. Given patients' distribution by application place, 43.5% of the total hospitalisations were found to have occurred for outpatients (via an outpatient clinic or appointment system), versus 48.8% from the emergency department and 7.7% from other services (Table 1). The most common reason for admission was determined to be palliative care for 29.5% of hospitalisations, infection for 12.9% and treatment maintenance for 12.5%. Among the patients who had been hospitalised at least once, the most common reason for admission and hospitalisation was palliative care, at 20.4%, followed by maintenance or newly diagnosed treatment planning and initiation at 9.4% and febrile neutropenia at 9.2%. The reasons for readmission and repeated hospitalisation were most commonly palliative care at 9.1%. Meanwhile, the bleeding, malignant hypercalcaemia, tumour lysis, VCSS (vena cava superior syndrome), gastrointestinal obstruction, convulsion, spinal cord compression, interventional procedure and examination reasons were categorised as 'other (Table 2).

Table 1. Distribution of patients by place of application.

Variables		Total hospitalizations (519)	
		n	%
Distribution of hospitalizations	Emergency	253	48.8
	Outpatient Clinic	226	43.5
	Transfer	40	7.7
	Total	519	100.0

Table 2. Distribution of patients according to the reason for admission requiring at least one and repeated hospitalizations

Variables		Total hospitalizations (519)		At least one hospitalization (385)		Repeated hospitalizations (134)	
		n	%	n	%	n	%
Reason for application	Palliative	153	29.5	106	20.4	48	9.1
	Infection	67	12.9	47	9.0	20	3.9
	Maintenance of treatment	65	12.5	49	9.4	16	3.1
	Febrile neutropenia	59	11.3	48	9.2	11	2.1
	Acut renal failure	30	5.8	23	4.4	7	1.4
	Pleural/pericardial effusion	23	4.4	19	3.6	3	0.8
	Other	122	23.6	93	18.2	29	5.4
	Total	519	100.0	385	74.2	134	25.8

For 376 of the 519 hospitalisations, Eastern Cooperative Oncology Group (ECOG) data could be accessed. Among the ECOG scores, ECOG 4 was the most common at 34.5%, while ECOG 3 followed at 24.4% for patients who had been hospitalised at least once, and ECOG 4 was the most common for patients who had experienced repeated hospitalisations at 12.2%. The relationship and distribution between hospitalizations and stages are indicated in Table 3. When the distribution of 134 patients who had experienced repeated hospitalisations was examined by cancer stage, the patients who had undergone repeated hospitalisations were found to have the most advanced stages.

Table 3. The relationship and distribution between at least one and repeated hospitalizations of patients and their stages during hospitalization

Variables		Total hospitalizations (519)		At least one hospitalization (385)		Repeated hospitalizations (134)	
		n	%	n	%	n	%
Stage	Stage 1	11	2.2	8	1.6	3	0.6
	Stage 2	32	6.4	22	4.4	10	2
	Stage 3	74	15	53	10.7	21	3.1
	Stage 4	376	76.2	279	56.5	97	19.7
	Total	493	100	362	73.4	131	26.6

Of patients, 76.5% were determined to have been discharged from hospitalisation, and hospitalisation resulted in death for 17.5% of patients (Table 4). The cancer type distribution of the study's 385 patients revealed that 11.7% of female patients had been diagnosed with breast cancer, followed by gastric cancer (4.9%) and ovarian cancer (3.6%). Lung cancer was the most common cancer type for male patients (18.8%), followed by gastric cancer (7.6%) and prostate cancer (4.9%) (Table 5). Hospitalised patients' distribution concerning their additional disease status was also examined; 16.1% had hypertension, 13.5% had diabetes mellitus and 8.1% had coronary artery disease or congestive heart failure.

Table 4. The distribution of the patients' end-of-hospitalization status.

Variables		Total hospitalizations (519)		At least one hospitalization (385))		Repeated hospitalizations (134)	
		N	%	n	%	n	%
End of hospitalization status	Discharge	397	76.5	296	57	101	19.5
	Exitus	91	17.5	63	12.1	28	5.4
	Transfer	31	6.0	26	5.0	5	1.0

Table 5. Distribution of hospitalized patients by gender and region of diagnosis

Variables		385 people		Female		Male	
		n	%	n	%	%	n
Location	Pulmonary	81	21.0	9	2.3	18.8	72
	Gastric	48	12.5	19	4.9	7.6	29
	Breast	45	11.7	45	11.7	0.0	0
	Pancreas	31	8.1	12	3.1	4.9	19
	Colon / Rectum	25	6.5	11	2.9	3.6	14
	Prostate	19	4.9	0	0.0	4.9	19
	Bladder	17	4.4	4	1.0	3.4	13
	Ovary	14	3.6	14	3.6	0.0	0
	No diagnosis	10	2.6	2	0.5	2.1	8
	Other	95	24.7	42	11.1	13.6	53
	Total	385	100	158	41.1	58.9	226

4. Discussion

Cancer is an important, increasing health problem worldwide, and it remains among the leading causes of death despite improvements in cancer management. Prolonged life expectancies, thanks to early diagnosis and new treatments, have increased the number of cancer patients applying to hospital outpatient clinics and emergency services. In our study, we defined the sociodemographic characteristics of patients hospitalised at a medical oncology service in the Middle Black Sea Region, examining their reasons for hospitalisation, laboratory values, diagnosis and pathology dates, treatment type and duration, and their metastasis and post-hospitalisation status. We aimed to contribute to the epidemiological cancer studies in our region and guide physicians' future activities and plans by reviewing hospitalised patients' retrospective data. Thus, inappropriate practices can be corrected by revealing societal and professional habits concerning inpatient treatment. Additionally, our findings will help apply newly developed treatment methods and reveal which conditions require hospitalisation due to their side effects.

Our university hospital is an important oncology clinic not only for Samsun but also for its surrounding provinces. In our study, we examined 519 admissions to the oncology service between January 1 2018 and January 1 2019, finding that admitted patients were evaluated, and their follow-up and treatment conditions resulted in discharge, transfer or death. The most common reason for admission was palliative care (153 patients; 29.5%), which reveals the importance and need for palliative patient care centres. While the incidence of cancer in Turkey surpasses the global incidence for men, it is somewhat lower for women. According to SEER (Surveillance, Epidemiology, and End Results) data published in 2019 (5), new cancer cases totalled 481 for men and 417 for women per 100,000 cases per year, compared to the 2012–2016 cases. Overall cancer incidence rates are higher for men than women. Awad et al. (6) found that the male-to-female incidence ratio of 1.5:1. Meanwhile, Bozdemir et al. (7) found that 49.5% of cancer patients were male and 49.7% were female. In our study, 226 of 385 patients (58.7%) were male and 159 (41.3%) were female. Thus, the high male population in our study was consistent with previous studies.

Additionally, 26.1% of hospitalised patients in our study had been admitted repeatedly. In the literature, studies that retrospectively evaluated cancer patients who had presented at an emergency department found that 56% of applications were repeated admissions (5). Cancer patients who followed up at our hospital had experienced repeated hospitalisations, up to seven within a year, and a significant majority had advanced-stage, metastatic and palliative care needs. This finding suggests that appropriate, standardised palliative care will reduce the tertiary emergency and oncology services' workloads.

According to IARC data, the three most common cancer types in the world are lung, prostate and colorectal cancer for men, versus while breast, colorectal and lung cancer for women (9). The Ministry of Health Cancer Statistics 2017 report, published with data from the Turkey Unified Database in 2014, found that Turkey's cancer incidence for men of all age groups was 21.1% for trachea, bronchi and lung cancers, versus 12.7% for prostate cancer. For women, breast cancer is the most common, at a rate of 24.9%, while thyroid cancer is the second-most common at 12% (10). Kocak et al. (11) found that the three most common cancers were lung 30%, gastric 11% and breast cancer 11%, respectively. In our study, lung cancer (21.0%) was the most common, followed by gastric cancer (12.5%) and breast cancer (11.7%). We found that breast cancer was the most common among female patients, affecting 45 of 158 female patients (11.7%), followed by gastric cancer for 19 patients (4.9%) and ovarian cancer for 14 patients (3.6%). Of our 226 male patients, the most common cancer was lung cancer (72 patients; 18.8%), followed by gastric cancer for 29 patients (7.6%), prostate cancer for 19 patients (4.9%) and pancreatic cancer for 19 patients (4.9%). Thus, the prevalence of cancer types among women and men of all age groups in our study aligned with the literature.

Cancer patients' hospital presentations are increasing for many reasons, such as the development of early diagnosis opportunities, the increase in the elderly population, prolongated life expectancies due to new treatment methods and the side effects during treatment. In a previous study on cancer patients, the most common reasons for cancer patients to present at an emergency room or hospital in the previous six months were pain, confusion and decreased functional capacity (3). Bozdemir et al. (12) found that 245 patients sought readmission of a total of 24,903 patient applications in a six-month period; when the reasons for these recurrent admissions were examined, patients were found to have presented with complaints of pain and nausea or vomiting.

Erdem et al. (13) examined the most common causes of emergency admission, identifying pain at 28.7%, respiratory complaints at 19.7% and GIS (gastrointestinal system) complaints at 18.3%. In our study, the most common reason for 519 admissions was palliative care (29.5%), followed by infection (12.9%) and treatment maintenance (12.5%). Conditions such as nausea, vomiting, pain, poor oral intake and poor performance were categorised as needing 'palliative care' in our study. In the literature and similar studies, researchers have observed that the most common reasons for cancer patients to present to a hospital are nausea, vomiting, pain and shortness of breath. At our clinic, the most common causes for hospitalisation were treatment maintenance, new diagnoses and treatment planning due to our clinic's status as a central, oncological hospital in the Middle Black Sea Region; therefore, many patients are accepted from the surrounding provinces and districts. The hospitalisation of patients from surrounding areas is mandatory due to transportation problems

in chemotherapy protocols, which increases the clinic's number of inpatients seeking treatment.

Additional factors that affect oncology patients' hospitalisation are their stage of cancer diagnosis and the presence of metastasis (14). The main goal of cancer treatments is to prevent disease recurrence during the early stages, stop disease progression and – most importantly – relieve symptoms to increase patients' quality of life (15). In our study, the diagnosis stages of patients who had been hospitalised at least once and patients who had undergone repeated hospitalisations were examined; the majority of these patients had Stage 4 cancer during hospitalisation. Similar to our finding, another study found the majority of hospitalised cancer patients to have Stage 4 cancer (16). Evidently, the hospitalisation rates of patients with advanced cancer stages or metastases are significantly high due to both acute primary-disease complications and palliation problems, such as pain and nutrition. The hospital admissions and hospitalisations of patients with metastatic disease have been found to have increased significantly. Oncology patients' palliative admissions and hospitalisations are thought to be reducible by standardising follow-up and care for patients with metastases carefully at oncological outpatient clinics, algological outpatient clinics and palliative centres when necessary. Therefore, advanced cancer stages, metastasis status and performance status should be important prognostic markers in patient management for clinical departments involved in following up with, treating and caring for patients.

The presence of secondary disease among cancer patients affects their treatment and its effectiveness, as well as their survival (17). In our medical-oncology-service-centred study, patients' additional diseases were found to include hypertension (16.1%), diabetes mellitus (13.5%), cardiovascular diseases (8.1%) and COPD (chronic obstructive pulmonary disease) (5.2%). The number of patients with second malignancies that had been diagnosed in addition to their previous cancer diagnoses was 32 (8.3%). A report addressing diseases that accompany cancer (18) noted that the most common comorbidities were hypertension, diabetes and cardiovascular diseases, as we found in the current study. According to a report on the TEKHARF (2017) study in Turkey, cardiovascular diseases – such as obesity, diabetes and hypertension – and hyperlipidaemia are common in the total population (19). Thus, comorbid diseases play an important role in increasing the need for cancer patients' inpatient treatment.

In our study, we categorised patients' outcomes as 'discharge', 'death' and 'transfer to the intensive care unit'. We determined that 397 (76.5%) of hospitalisations resulted in discharge and 91 (17.5%) resulted in death. Swenson et al. (20) observed a 10% mortality rate in their study. By contrast, Swenson et al. (20) found that 77.8% of patients were discharged, while 18.6% died. Kocak et al. (11) observed that 35% of patients were discharged, while 19% died. Thus, oncology patients experience similar hospital mortality rates across similar studies.

As we determined in the current study, which defined the demographic and clinical characteristics of patients who had been hospitalised by our oncology service, the majority of hospitalisations were experienced by patients with advanced metastatic disease and low performance scores, and recurrent hospitalisations occurred. Therefore, cooperation between staff members at medical oncology, radiation oncology and related surgical clinics, algology clinics, psychiatry clinics, emergency and palliative centres and home-care services units, as well as dietitians, could encourage the dissemination of palliative care centres to provide easier access and more active, effective home-care services. The effective operation and implementation of necessary arrangements for patient care homes will increase the health system's efficient use of human and financial resources and the quality of diagnosis, follow-up and care for oncology patients.

Conflict of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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Authors' contributions

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

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 11/07/2019 and the number of ethical committee decisions is 2019/545.

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Investigating the relationship between anxiety caused by COVID-19 disease and academic burnout in Shiraz Nursing and Midwifery students

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Abstract

The emergence of COVID19 disease in 2019 in a short period of time caused many psychological consequences, including anxiety. Anxiety caused by this disease can endanger the mental health of people in a society, including students, and cause academic burnout by disrupting the process of education and learning. The present study aimed to investigate the relationship between anxiety caused by COVID-19 disease and academic burnout in Shiraz School of Nursing students. This descriptive cross-sectional study was conducted on 343 students. Data were collected using a demographic information form, Berso et al. academic burnout questionnaire and Corona disease anxiety scale questionnaire by census method and analyzed using SPSS statistical software (V22). Descriptive statistics, independent two-sample t-test, one-way analysis of variance and Pearson correlation were used to analyze the data. The general results of this study indicate a positive correlation between the total score of COVID-19 anxiety and academic burnout. The mean total score of anxiety in students was 12.24±10.19, and the mean score of academic burnout was in the range of 40.54±10.9. Based on the results, there is a statistically significant difference between the level of anxiety caused by this disease, the age of students and marital status ($p<0.001$). There was also no statistically significant difference between academic burnout and demographic characteristics. In general, the results of this study show that increasing the level of anxiety caused by COVID-19 causes an increase in students' academic burnout. Therefore, it is recommended that educational planners be considered to increase academic motivation and improve students' mental health.

Keywords: anxiety, burnout, COVID-19, mental health, education

1. Introduction

With the emergence of the COVID-19 epidemic in China in 2019 and the increase in the number of patients with this disease, people worldwide suffered adverse psychological consequences (1, 2). The uncertain nature of the disease and insufficient knowledge and implementation of quarantine measures affected many aspects of life and consequently caused physical problems and mental disorders such as anxiety and depression (3). With the prevalence of coronavirus, people's daily activities and social interactions with others were disrupted due to fears of being infected with the disease, resulting in increased health anxiety (4). When a disease occurs, anxiety as a common psychological response reduces the power of adaptation that can affect a person's mental health and cause irreversible complications in all aspects of health (5, 6). The results of studies conducted during previous epidemics indicate the extensive psychological effects at the individual, social and international levels (7).

Anxiety caused by COVID-19 is caused by being infected with a virus resulting from a lack of knowledge and the uncertain nature of the disease. Anxiety about disease and fear of death due to COVID-19 disease can cause fatigue, hopelessness, burnout, as well as mental health imbalances in a person's job and education (8). Based on Dick (1992), when a person is exposed to a stressful environmental situation with constant stress for a long time and cannot adapt to it, they will suffer burnout (9). Burnout manifests itself as a disorder that causes many problems, such as insomnia, depression, anxiety, and memory loss (10). Students with specific ages and social status typically experience a lot of stress that can cause physical and mental problems (11).

Psychological disorders such as stress, anxiety and depression during their studies disrupt their education, learning and academic performance (12). The main consequence of anxiety is academic burnout, which is caused by a decrease in adaptation to stressful conditions during the study period and causes feelings of inefficiency, mental fatigue and apathy in

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the individual (13). Academic burnout can lead to the wastage of the workforce and costs in addition to harming the process of education, learning, academic performance and motivation of students and their mental health (14, 15). Also, it causes turnover intention by reducing the person's mastery (16). In recent decades, one of the main challenges and issues in the educational system is academic burnout, which has had a significant impact on the academic performance and achievement of students (15, 17). The emergence of the COVID-19 pandemic caused dramatic changes in academic relations (18).

Since mental health includes all mental and physical dimensions, and anxiety is considered one of the most important determinants of mental health, the need to investigate the relationship between anxiety caused by COVID-19 disease and burnout seems essential. The results of this study can be used in the development of education and mental health of students as well as relevant officials to make changes in the educational and research environment and subsequently improve the quality of care services in the medical system.

2. Materials and Methods

This cross-sectional descriptive-analytical study was conducted on 343 students in Shiraz's Faculty of Nursing and Midwifery of Hazrat-e Fatemeh (PBUH). Sampling was done by census method. With the cooperation of the educational officials of the faculty, a list of names of third and above semester students along with their contact numbers in the fields of nursing, surgical technology, anesthesiology, midwifery and medical emergencies in bachelor, master and doctorate levels was first received. The reason for not selecting the first and second-semester students was due to their lack of exposure to COVID-19 disease and not being in the condition.

Questionnaires were provided to students online and through social networks, and informed consent was obtained from them in the form of one of the first questions of the questionnaire. Finally, 343 students participated in the project. This study was approved by the ethics committee of Shiraz University of Medical Sciences with the code of IR.SUMS.NUMIMG.REC.1400.057. Inclusion criteria included no underlying physical disease, no mental disease, studying at Hazrat-e Fatemeh School of Nursing and Midwifery in Shiraz and willingness to participate in the study. Failure to complete at least one of the two questionnaires and high missing data were considered exclusion criteria.

Data collection instruments were provided to students online, including a demographic information form, Corona Disease Anxiety Scale and Berso et al. (19) burnout questionnaire. Demographic information form includes information about age, gender, field of study, semester and level of education. Corona Disease Anxiety Scale was developed and validated by Alipour et al. in Iran to measure anxiety caused by the spread of coronavirus. The final version of this tool has 18 items and two components (agents). Items 1 to 9 measure psychological, while items 10 to 18 measure physical symptoms. The instrument is scored on a 4-point Likert scale (never=0, sometimes=1, most of the time=2 and always=3). High scores in this questionnaire indicate a higher level of anxiety in the subjects. The reliability of this tool was obtained at α=0.879 for the first agent and at α=0.861 for the second agent, and at α=0.919 for the whole questionnaire using Cronbach's alpha method. To check the confirmatory construct validity and to determine the fit of the data with the 2-agent model of this instrument, the confirmatory factor analysis method and LISREL-8.8 software were used (20).

The Burnout Questionnaire, developed by Berso et al. (2007), measures three areas of burnout: academic fatigue, apathy, and inefficiency. The questionnaire has 15 items scored by the subjects on a 5-point Likert scale ranging from strongly disagree to strongly agree. Academic fatigue has five items, academic apathy has four items, and academic inefficiency has six items, scored on a 5-point Likert scale from strongly disagree (1) to strongly agree (5). Questions about the academic inefficiency subscale are considered positive items, so they are scored reversely. Naami examined the psychometric properties of this questionnaire in Persian and calculated the reliability of this questionnaire as 0.79 for academic fatigue, 0.82 for academic apathy and 0.75 for academic inefficiency. He obtained the validity coefficients of this questionnaire by correlating it with the student stress questionnaire. It was calculated at 0.38, 0.42, and 0.45, respectively, which is significant at the level of p<001 (21).

To summarize qualitative and quantitative data, descriptive statistics indices of frequency (percent) and mean (standard deviation) were used, respectively. Independent t-test, one-way analysis of variance (ANOVA) and Pearson correlation were used to investigate the relationship between demographic variables and the main indicators of the study (COVID-19 anxiety and burnout). Also, the Pearson correlation coefficient test was used to investigate the relationship between these variables. The significance level for the tests used was considered at 0.05.

3. Results

Three hundred forty-three students participated in this study, including 231 (67.3%) females and 112 (32.7%) males. The female s group was twice as large as the male group (67.3%). Also, 287 participants (83.7%) were single and (16.3%) were married. Most participants were bachelor students (87.8%), doctoral students (9%) and master students. The highest frequency of students was related to surgical technology (31.5%) and the lowest frequency (7.3%) belonged to emergency medicine. Approximately 20% of the subjects were studying in the third, fourth and fifth semesters, and the eighth semester had the lowest frequency (3.8%) compared to other semesters among the samples (Table 1).

Table 1. Sociodemographic data of the examined samples

Variable	Categories	F	F(n)
Sex	Female	231	67.3%
	Male	112	32.7%
Marital Status	Single	287	83.7%
	Married	56	16.3%
Level of education	Continuous bachelor's degree	301	87.8%
	Non-continuous bachelor's degree	25	7.3%
	Masters	14	4.1%
	PhD	3	0.9%
Field of Study	Surgical Technology	108	31.5%
	Nursing	90	26.2%
	Anesthesia	65	19%
	Midwifery	55	16%
	Emergency Medicine	25	7.3%
Semester	3	92	26.8%
	4	69	20.1%
	5	81	23.6%
	6	37	10.8%
	7	51	14.9%
	8	13	3.8%

A comparison of the mean and standard deviation of academic burnout score and its sub-dimensions, including academic fatigue, academic apathy and academic inefficiency, showed that academic fatigue had the highest mean and effect on academic burnout (Table 2).

The mean and standard deviation of anxiety score along with its sub-dimensions, including psychological and physical anxiety, showed that the psychological dimension had the highest mean and effect on anxiety (Table 3).

According to the results and information of the correlation test, there is a significant and direct relationship between age and corona disease anxiety ($p<0.0001$, $r = 0.2$). In other words, anxiety increases with ageing. The mean anxiety of corona disease is not significantly associated with the variables of gender, level of education, field of study and semester. In other words, the mean of anxiety is the same between the levels of variables. Also, marital status is significantly associated with academic anxiety. Based on the results, married students had higher mean anxiety (Table 4). According to the results, the mean of burnout has no significant relationship with any of the variables of age, gender, marital status, level of education, field of study and semester. In other words, the mean of burnout is the same between the variables (Table 5).

The results and correlation test data indicate a significant and positive relationship between anxiety scores and academic burnout scores ($r=0.288$, $P<0.0001$). In other words, with increasing anxiety, academic burnout increases (Table 6).

Table 2. Description of the burnout and its sub-dimensions

Variable	Number of questions	Min	Max	Mean	SD	Mean based on the Likert scale
Academic fatigue	5	5	25	14.4956	4.40884	2.8991
Academic apathy	4	4	20	11.5277	3.99689	2.8819
Academic inefficiency	6	6	26	14.5190	3.99689	2.4198
Total burnout score	15	15	71	40.5423	10.90860	2.7028

Table 3. Description of quantitative anxiety and its dimensions

Variable	Number of questions	Min	Max	Mean	SD	Mean based on the Likert scale
Physiological	9	0	27	9.21	6.06	1.03
Physical	9	0	27	3.03	4.96	0.34
Total anxiety	18	0	54	12.24	10.19	0.68

Table 4. Relationship between demographic characteristics and corona disease anxiety

Variable	Ranking	n	Mean	SD	Statistical test
					p-value
Age	Total sample	343	23.76	4.91	§r=0.2, p<0.00001
Gender	Male	112	11.39	11.30	†t=-1.078, 0.282
	Female	231	12.66	6.61	
Marital status	Single	287	11.91	9.13	†t= -3.473, 0.001
	Married	56	17.64	13.3	
Level of education	Continuous bachelor	301	11.83	9.65	‡F=2.275, 0.32
	Non-continuous bachelor	25	17.92	17.75	
	Master	14	10.5	10.50	
	PhD	3	14.33	4.51	
Field of Study	Nursing	90	11.11	8.80	‡F=0.932, 0.473
	Midwifery	55	12.69	8.99	
	Surgical Technology	108	13.61	11.91	
	Anesthesia	65	11.60	9.33	
	Emergency Medicine	25	11.12	11.40	
Semester	3	92	11.40	10.26	‡F=1.72, 0.129
	4	69	13.88	12.86	
	5	81	13.75	9.99	
	6	37	8.86	6.71	
	7	51	11.94	8.59	
	8	13	10.92	6.64	

§Correlation Test

†Independent Sample T Test

‡One Way Analysis of Variance

Table 5. Relationship between demographic characteristics and academic burnout

Variable	Ranking	n	Mean	SD	Statistical test
					p-value
Age	Total sample	343	23.76	4.91	§r=-0.05, 0.359
Gender	Male	112	40.92	10.00	†t=0.446, 0.656
	Female	231	40.36	11.33	
Marital status	Single	287	40.76	10.71	†t=0.848, 0.397
	Married	56	39.4	11.92	
Level of education	Continuous bachelor	301	40.88	10.79	‡F=1.322, 0.267
	Non-continuous bachelor	25	39.92	11.98	
	Master	14	35.50	11.19	
	PhD	3	35.67	10.21	
Field of Study	Nursing	90	40.28	10.94	‡F=0503, 03.734
	Midwifery	55	40.71	11.16	
	Surgical Technology	108	41.07	11.54	
	Anesthesia	65	39.18	9.45	
	Emergency Medicine	25	42.36	11.36	
Semester	3	92	39.40	11.68	‡F=1.294, 0.266
	4	69	40.35	11.03	
	5	81	41.55	10.03	
	6	37	39.29	11.29	
	7	51	40.57	10.15	
	8	13	46.76	10.92	

§Correlation Test
†Independent Sample T Test
‡One Way Analysis of Variance

Table 6. Correlation matrix between anxiety score and academic burnout

	Anxiety	Academic Burnout
Anxiety	1	-
Academic Burnout	‡r=0.288, p<0.0001	1

‡Significant at the 0.01 level

4. Discussion

The present study aimed to investigate the relationship between COVID-19 anxiety and academic burnout in Hazrat-e Fatemeh School of Nursing and Midwifery students in Shiraz. The results showed that the mean score of students' academic burnout was in the average range (40.54 ± 10.9). These results were consistent with the results of similar studies (15, 22-24). The results of a study conducted by Sadoughi et al. (2019) at Kashan University of Medical Sciences reported low academic burnout (25). Since the instrument used in the mentioned study for measuring academic burnout is similar to the present study, the reasons for the inconsistency of the results might be attributed to differences in the psychological components studied in the study and the absence of Covid 19 disease at this time. Also, the results of a study by Da Silva et al. (2014) in Brazil showed that nursing students had a low burnout score, which was not consistent with the results of the present study (26). This could be due to differences in the instruments used or the relatively better conditions for students in Brazil.

Examination of the mean scores of the sub-dimensions of burnout showed that the highest mean based on the Likert scale was related to academic fatigue, and the lowest was related to academic inefficiency, consistent with similar studies' results (22, 24, 27). The mean total score of anxiety in students was lower than average (12.24±10.19). In the psychological dimension, the mean anxiety was 9.21±6.05; in the physical dimension, the mean anxiety was 3.02±4.96), which indicates a higher score of COVID-19 induced physiological anxiety than physical anxiety. Anxiety will negatively affect the mental health of people in the community. COVID-19 disease can also be considered a factor that leads to stress. In this regard, the results of a study by Alizadeh showed that anxiety negatively correlates with mental health (28). The results of a study conducted by Van et al. also showed that anxiety and mental disorder threaten mental health (29). Results of a similar study in Lebanon in 2020 indicated that students' mean anxiety score was below average (30). The results of a similar study by Rahmati et al. (2020) conducted by submitting online questionnaires showed that the level of anxiety in the study population was not high and was more favourable in students than in staff (31).

It might be since staff are in a different environment than students. In another similar study conducted in 2020 on 520 Lebanese students, the mean score of anxiety in students was reported at 24.74±7.4, which was associated with a sudden change in teaching methods to exclusive e-learning methods (30). According to the researcher, the low score of students' anxiety can be related to the use of e-learning methods and their reduced worries about getting coronavirus due to a lack of close and face-to-face communication with other friends and classmates. The results of a study conducted by Fitzgerald et al. (2020) revealed that students experienced high anxiety levels due to fear of corona disease (32). The results of the mentioned study are inconsistent with the results of the present study. Differences in the results of the present study with this study can be related to the research environment, the instrument used, and the studied universities.

The present study revealed that the level of anxiety experienced due to COVID-19 disease is directly associated

with the age of students ($p<0.0001$). It means that with ageing, the level of anxiety also increases. The results of a study conducted by Nasirzadeh et al. in 2020 indicated that there is a significant relationship between anxiety score and age. This study's results also showed a significant relationship between anxiety score and education level, which is inconsistent with the present results (16). Based on the results of the present study, marital status also affected anxiety ($p<0.001$), so married people experienced more anxiety. It might be because married people are in the process of living, and this factor has made them more worried about being infected with the coronavirus by other family members. Also, other demographic variables such as gender, level of study, field of study and semester did not affect the level of anxiety caused by COVID-19 disease in students.

The data of a similar study conducted on medical students of Hamadan University of Medical Sciences in 2020 by census method revealed no significant relationship between anxiety and level of education (32). A study conducted on 204 medical personnel working in hospitals and health centres of Jahrom city in 2020 showed that the mean score of psychological anxiety symptoms is higher than that of physical symptoms, similar to the present study. Also, the results of this study showed that the scores of anxiety, psychological symptoms and physical symptoms in women are significantly higher than in men ($P <0.05$), which is contrary to the present study (33). Since the present study was conducted in an academic setting, it seems that this discrepancy could be due to the greater vulnerability of women in coping with unfavourable working conditions.

Demographic characteristics of age, gender, marital status, level of education, field of study and semester had no effect on burnout. The results of similar studies showed that there is no significant relationship between age and academic burnout (34, 35). As mentioned, the present study showed that gender is not significantly different from any of the dimensions of burnout. These results are in line with the results of studies conducted by Sharifi Fard et al. in 2014 (34) and Sharif Shad et al. in 2017 (36). The reason for this discrepancy can be explained by the fact that gender differences are nowadays less effective in job and educational performance, and the ability of girls and boys in different fields has caused them to have almost the same functions. In studies inconsistent with the present study, a significant relationship was found between marital status, field of study, and semester and academic burnout (21, 34, 37-39). This discrepancy can be attributed to the use of different instruments and environmental conditions.

In this study, a positive association was reported between the total score of anxiety and academic burnout. It means that students with higher anxiety levels will experience more academic burnout. A study by Fitzgerald et al. (2020) showed that anxiety can divert students from their academic education. The strong association between anxiety symptoms and worry about academic issues showed that they are extremely anxious about academic issues (31). Based on the results of this study on the relationship between anxiety caused by Covid-19 and academic burnout, a positive step can be taken to improve student's mental health and increase their academic performance by designing educational programs in different academic courses, identifying the challenges of the diseases, and identifying the factors affecting academic burnout. Also, providing appropriate training to students to gain the necessary knowledge and skills when challenges arise will provide better clinical care for patients, followed by high-quality health services.

Ethical Statement

This study was conducted by Helsinki Principles, and ethical approval was taken from the local ethics committee (Decision Number: 2020/328, 27.08.2020).

Conflict of interest

None to declare.

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Authors' contributions

Concept: S.G., A.Y., Design: S.G., A.Y., Data Collection or Processing: J.E., K.R., Analysis or Interpretation: M.H., Literature Search: S.G., A.Y., Writing: S.G., A.Y.

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Cultural adaptation, validity and reliability of the Turkish version of the wheelchair skills test questionnaire (wst-q) 5.0 form in individuals with spinal cord injury

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Abstract

Paraplegic individuals with spinal cord injury (SCI) use a wheelchair to move around. The level of independence they have in the community is related to their wheelchair skills. Therefore, wheelchair skills are important for individuals with SCI. To increase the use of manual wheelchairs, it is important to evaluate the skills of wheelchair users. When the literature was examined, we could not find a scale for evaluating wheelchair skills on the Turkish Scale. Thus, this study aimed to determine the validity and reliability of the Turkish cultural adaptation of the Wheelchair Skills Test Questionnaire (WST-Q) as a questionnaire evaluating the skills of manual wheelchair users in Türkiye. This study was conducted with paraplegic manual wheelchair users. For the reliability of the questionnaire, internal consistency and test-retest reliability were examined. Internal consistency was evaluated with the Cronbach α coefficient. For the validity of the questionnaire, the data obtained from the WST-Q-Turkish were compared with the World Health Organization Quality of Life, Brief Version (WHOQOL-BREF). The study was completed with 20 cases with a mean age of 40±8.95 years. The general Cronbach α score of the questionnaire was calculated as 0.985. According to the results, the questionnaire was determined to be reliable. The currently widely used WHOQOL-BREF was used for the concurrent validity of the WST-Q-Turkish. A strong correlation was determined between the WHOQOL-BREF and the WST-Q-Turkish. In this study, the validity and reliability Turkish of the WST-Q form was performed/evaluated and the final form was named WST-Q-Turkish. The WST-Q-Turkish was determined to be a valid and reliable questionnaire for the evaluation of the skill capacity of paraplegic adults with SCI using a manual wheelchair.

Keywords: spinal cord injuries, paraplegia, wheelchairs, questionnaire

1. Introduction

Spinal cord injury (SCI) causes disruption in the transmission of neural signals, motor and sensory losses, and autonomic dysfunction, and therefore leads to severe functional failures (1, 2). In the rehabilitation process, the focus should be on gaining back the lost functions, especially motor functions such as walking, wheelchair mobilization, transfer, and functional use of the upper limb (3). The goal is to improve the activities and social integration of individuals to the most independent level possible. (4).

Individuals with SCI use a wheelchair to be able to move around. These users should have good wheelchair usage skills in order to reach the most independent social and cultural levels possible. One of the widely used tools to evaluate these skills is the Wheelchair Skills Test-Questionnaire (WST-Q), which was developed in Canada by the Wheelchair Research Team under the presidency of Prof. Ronald Lee Kirby. The WST-Q includes questions of capacity, safety, performance, and training goals related to the wheelchair use skills of disabled individuals. 33 skills are defined on the scale (5). There are different forms for manual wheelchair users and electrical wheelchair users. The WST-Q has been translated into French (6), Norwegian (7), and Portuguese (8).

WST-Q (manual chair) scoring is explained below. Scoring is done for each skill. Responses in the capacity section include: "Yes, very well "[3], "Yes, but not well" [2], "Yes, in part" [1], "No" [0], "Not possible with this wheelchair" [NP], "Testing error" [TE]. Responses in the confidence section include: "Very confident" [3], "Fairly confident" [2], "Somewhat confident" [1], "Not confident" [0], "Not possible with this wheelchair" [NP], "Testing error " [TE]. If the answer to the capacity question for a skill is “no [0]”, the confidence question for the same skill also has a score of 0. If the answer to the capacity question for a skill is “NP”, the score of the confidence question for the same skill is also NP. Responses in the performance section include: "Always" [3], "Usually" [2], "Occasionally" [1], "Never" [0], "Not possible with this wheelchair" [NP], and "Testing error" [TE]. If the answer to the capacity question for a skill is “0”, the performance question for the same skill also has a score of 0. If the answer to the capacity question for a skill is “NP”, the score of the

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performance question for the same skill is also NP. Training goals: This section is used if the WST-Q is being applied to identify the individual's potential educational goals. Responses include: "Yes" or "No". Scoring is not calculated for training goals. The total capacity score, confidence score, and performance score are calculated separately. Total percentage scores range from 0-100%.

• Total capacity or confidence or performance score = sum of individual skill scores / ([number of skills - number of NP scores - number of TE scores] x 3) x 100% (9).

In the literature, no questionnaire evaluating wheelchair skills has been found in Türkiye. Therefore, the aim of this study was to carry out the Turkish cultural adaptation, validity, and reliability of the WST-Q as a questionnaire evaluating the skills of manual wheelchair users in Türkiye.

2. Materials and Methods

This study was conducted with paraplegic manual wheelchair users. All the participants included were volunteers. The informed consent form was obtained online from all participants who participated in the study. Approval for the study was granted by the Non-Interventional Clinical Research Ethics Committee of Izmir Demokrasi University (Decision No:2020/14-06, Dated: June 29, 2020). To calculate the sample size of participants to be included in the study, the optimal design method for study reliability was used, as described by Walter et al. (10). The values used were α:0.05, β:0.20, H0: p=0.5 (acceptable level of repeatability), and H1: p=0.9 (expected level of repeatability). According to this calculation, it was necessary to have a sample size of at least 9 participants.

Inclusion criteria for this study were determined as being SCI, using a manual wheelchair, being paraplegic, being 18 years of age or older, having disabilities of at least one year, not having cognitive problems, having reading and writing ability, and participating in the study voluntarily. The exclusion criteria for this study were determined: unfamiliarity with or a desire not to use a wheelchair, having systemic diseases such as heart, lung, or kidney, having orthopedic or neurologic problems in the upper extremities, having communication or cognitive problems, and being an active wheelchair athlete.

All the participants were informed about the study and were then instructed on how to complete the questionnaires online. The digital formats of the questionnaires to be used in the study were prepared using Google Forms. The demographic data of the participants were recorded, including age, height, weight, and body mass index (BMI). The participants were also questioned in respect of how long they had been using a wheelchair and the mean number of transfers per day with a wheelchair.

The necessary permission was obtained to perform Turkish validity and reliability studies of the WST-Q 5.0 (manual chair), which was developed in Canada by Kirby et al. (11). Firstly, cultural adaptation of the questionnaire was made. The WST-Q was translated into Turkish and then adapted culturally according to the stages recommended by Beaton et al. (12). The translation into Turkish was made by two native Turkish speakers with a good level of English. One of these was a physiotherapist and the other was a university graduate of the English Language and Literature Faculty. The translations were completed independently. To prevent contextual errors and inconsistencies, the two translations were compared by a single person with good knowledge of both languages, and a single text was produced from the translation.

This final Turkish version was then back-translated into English separately by two native English speakers with good knowledge of Turkish. These two translators had no knowledge of the aim of the study or the original scoring. A committee formed of these four translators and the first author compared the translated version of the WST-Q with the original English version. The committee approved the Turkish version and named the questionnaire “Turkish Wheelchair Skills Test Questionnaire (WST-Q-Turkish). After approval, to pilot test the WST-Q-Turkish it was applied to 4 wheelchair users who met the study criteria. Items in the questionnaire that were difficult to understand were determined by these pilot users, and these were made more comprehensible.

2.1. Reliability

For the reliability of the questionnaire, internal consistency and test-retest reliability were examined. Internal consistency was evaluated with the Cronbach α coefficient. For the test-retest reliability, the WST-Q-Turkish was applied twice to the individuals at a 2-week interval. The data obtained were evaluated using the Spearman Correlation and Intraclass Correlation Coefficient (ICC).

2.2. Validity

The data obtained from the WST-Q-Turkish were compared with the World Health Organization Quality of Life, Brief Version (WHOQOL-BREF), for which Turkish reliability and validity studies were made by Eser et al. in 1999 (13). The questionnaire includes items evaluating the areas of physical health, psychological health, social relationships, and environmental fields. With the addition of a national question during the Turkish validity study, the questionnaire was formed/consisted of a total of 27 items. Each area on the scale was scored from a maximum of 20 or 100 points (13).

2.3. Statistical Analysis

All the statistical analyses were performed using SPSS version 26 software (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). Demographic data were reported as frequency (n) and percentage (%). The conformity of the data to a normal distribution was assessed with the Kolmogorov-Smirnov test. For reliability, test-retest reliability analyses were applied. The data obtained were evaluated using the Spearman Correlation and Intraclass Correlation Coefficient (ICC). The strength of the correlation was considered

negligible (<0.29), weak (0.3–0.49), moderate (0.5–0.69), strong (0.7–0.89), or very strong 0.9–1.0 (14). The Cronbach α coefficient was calculated for internal consistency. Validity was examined by correlation with a currently widely used questionnaire with proven validity and reliability.

3. Results

A total of 31 cases were identified who had SCI and used a manual wheelchair. After the exclusion of 11 cases, 8 with communication problems, and 3 who did not repeat the test, the evaluations were completed with 20 cases with a mean age of 40±8.95 years. The data on patient age, height, weight, BMI, years of wheelchair use, and the number of daily transfers are shown in Table 1.

Table 1 Demographic Data of the Cases

Variables (n=20)	Min-Max.	Mean±SD
Age (years)	25-58	40±8.95
Height (cm)	145-190	175±10.22
Body weight (kg)	53-110	75±13.02
BMI (kg/cm^2)	16-38	24.62±4.73
W/C usage (years)	1-27	14±8.55
The number of daily transfers	3-30	9±7.62

SD: Standard Deviation, BMI: Body Mass Index, W/C: Wheelchair

For the reliability of the questionnaire, internal consistency and test-retest reliability were examined. The internal consistency of the items in the questionnaire was evaluated with the Cronbach α coefficient. The general Cronbach α score of the questionnaire was calculated as 0.985. Also, the questionnaire was applied twice to the same participants at an interval of 2 weeks and was examined with ICC. ICC coefficients were 0.967 (95% confidence interval: 0.938-0.985). The correlations to test-retest reliability were calculated as a minimum correlation of 0.94 and a maximum correlation of 0.98, demonstrating a very strong correlation ($p<0.001$). According to these results, the questionnaire was determined to be reliable. The data obtained are shown in Table 2.

The currently widely used WHOQOL-BREF was used for the concurrent validity of the WST-Q-Turkish. The relationship between the two questionnaires was examined with the Spearman correlation coefficient (rs) to investigate the validity of the WST-Q-Turkish for use in Türkiye. All the sub-dimensions of the questionnaire were correlated at a high level, and all the sub-parameters were found to be significant. A strong correlation was determined between the WHOQOL-BREF and the WST-Q-Turkish. These data are shown in Table 3.

Table 2. Reliability of WST-Q Turkish*

Skill Description		Can you do it?	How confident are you?	How often do you do it?	Is this a training goal?
1	Moving the wheelchair straight forward for a short distance, for example along a short hallway.	0.98	0.98	0.97	0.98
2	Moving the wheelchair straight backward for a short distance, for example to back away from a table.	0.95	0.95	0.97	0.97
3	While moving the wheelchair, coming to a sudden stop to avoid people who do not notice you.	0.97	0.98	0.97	0.97
4	Turning the wheelchair around in a small space so that it is facing in the opposite direction.	0.98	0.97	0.96	0.96
5	Turning the wheelchair around obstacles while moving forward.	0.98	0.98	0.98	0.98
6	Turning the wheelchair around obstacles while moving backward.	0.96	0.96	0.96	0.96
7	Moving the wheelchair sideways in a small space, for example to get the side of your wheelchair next to a kitchen counter, and then back to where you started.	0.97	0.95	0.97	0.95
8	Moving the wheelchair to pick up a small dropped object, for example a cell phone, pen or coin, from the floor in front of you.	0.98	0.98	0.98	0.98
9	Removing the weight from your buttocks, either one at a time or both together.	0.98	0.97	0.97	0.98
10	Transferring from the wheelchair to a bench that is about the same height as the wheelchair and then getting back into the wheelchair.	0.98	0.98	0.98	0.96
11	Folding your wheelchair or taking it apart without tools, for example to store it out of the way, and then opening or reassembling it again.	0.98	0.97	0.97	0.97
12	Opening a hinged door, moving the wheelchair through it and closing it behind you, then coming back the other way.	0.98	0.98	0.97	0.97
13	Moving the wheelchair over a longer distance, for example on a smooth surface about half the length of a sport field.	0.98	0.98	0.98	0.98
14	Moving the wheelchair up a slight incline, for example a standard ramp (12 times longer than it is high).	0.97	0.96	0.96	0.97
15	Moving the wheelchair down a slight incline.	0.98	0.97	0.98	0.98

Table 2. Reliability of WST-Q Turkish* (Continue)

	Skill Description	Can you do it?	How confident are you?	How often do you do it?	Is this a training goal?
16	Moving the wheelchair up a steep incline (about twice as steep as a standard ramp).	0.97	0.95	0.95	0.97
17	Moving the wheelchair down a steep incline.	0.98	0.98	0.98	0.97
18	Moving the wheelchair across a slight side-slope, for example when crossing a driveway.	0.98	0.98	0.98	0.98
19	Moving the wheelchair, a short distance across a soft surface, for example gravel.	0.98	0.98	0.98	0.98
20	Getting the wheelchair over an obstacle that sticks up above the surface, for example a high door threshold.	0.98	0.98	0.98	0.98
21	Getting the wheelchair over a gap, for example a rut in the road that is too big to simply roll over.	0.98	0.98	0.96	0.98
22	Getting the wheelchair up a low curb, for example when entering a building.	0.98	0.97	0.97	0.97
23	Getting the wheelchair down from a low curb.	0.98	0.97	0.98	0.98
24	Getting the wheelchair up a high curb, for example at a street corner without a ramp.	0.96	0.96	0.97	0.96
25	Getting the wheelchair down from a high curb.	0.97	0.98	0.98	0.97
26	Getting down on the ground, then back into the wheelchair.	0.98	0.98	0.98	0.98
27	Doing a wheelie, balancing the wheelchair on its rear wheels, for 30 seconds.	0.95	0.94	0.95	0.97
28	Staying in a wheelie, turning the wheelchair around in a small space so that it is facing in the opposite direction.	0.98	0.98	0.98	0.98
29	Moving forward and backward in the wheelie position.	0.98	0.98	0.98	0.97
30	Staying in a wheelie, moving forwards down a high curb.	0.97	0.97	0.98	0.97
31	Staying in a wheelie, moving forwards down a steep ramp.	0.95	0.96	0.96	0.97
32	Getting yourself and the wheelchair up a short flight of stairs that has a rail.	0.94	0.95	0.94	0.97
33	Getting yourself and the wheelchair down a short flight of stairs that has a rail.	0.96	0.98	0.94	0.96

*Spearman Correlation Coefficient

Table 3. Comparison of WST-Q Turkish with the Results of the WHO Quality of Life Short Form

Total Scores of the Questionnaires	Physical Domain – Whoqol-Bref 0-100	Psychological Domain – Whoqol-Bref 0-100	Social Domain – Whoqol-Bref 0-100	Environment Domain – Tr Whoqol-Bref 0-100
	rs/p	rs/p	rs/p	rs/p
WST-Q Total Capacity Score	rs: 0.68 p: 0.036*	rs: 0.60 p: 0.012*	rs: 0.78 p: 0.071	rs: 0.67 p: 0.038*
WST-Q Total Confidence Score	rs: 0.64 p: 0.003*	rs: 0.61 p: 0.005*	rs: 0.72 p: 0.082	rs: 0.61 p: 0.092
WST-Q Total Performance Score	rs: 0.75 *p*:<0.001**	rs: 0.60 p: 0.010*	rs: 0.67 p: 0.034*	rs: 0.73 p: 0.026*

*p<0.05, **p<0.001, rs: *Spearman Correlation Coefficient*

4. Discussion

The results of this study demonstrated that the WST-Q-Turkish is a valid and reliable questionnaire for adults with SCI who use a manual wheelchair. The WST-Q-Turkish can be used to evaluate the skills, abilities, and capacity of individuals with SCI when using a manual wheelchair.

Following SCI, it is of great importance for the individual to be able to achieve mobility (15). Therefore, paraplegics with SCI are in the situation of using a manual wheelchair. However, the wheelchair skills of many individuals with SCI remain insufficient to be able to achieve social integration (16). These skills include functional activities such as maneuvers made with the wheelchair, transfers, ascending-descending ramps, and stairs, and folding and opening the wheelchair. No measurement tool was found by the researchers which could be used to evaluate the wheelchair skills of individuals with SCI in Türkiye. Therefore, there is a need for a valid and reliable scale to evaluate the wheelchair skills of these individuals in Türkiye.

From an examination of the literature, the Wheelchair Skills Test (WST) developed by Kirby et al. (2002) was seen to be a frequently used measurement tool for the evaluation of wheelchair skills (17). The validity and reliability of the WST have been proven in several studies in different languages (11, 17, 18). There are translations of the WST in Portuguese (19), Spanish (20, 21), and French (6).

The study by Ossada et al. of the Portuguese translation of the WST included 15 cases, 13 with SCI. It was concluded that the Portuguese WST was sufficient for the evaluation of wheelchair user skills and was useful for the planning of rehabilitation programs (19). Passuni et al. translated the WST into Spanish and evaluated its reliability. In the study of 11 cases with SCI, the Spanish version of the WST was determined to be a reliable evaluation tool for individuals using a manual wheelchair (20).

The WST provides objective evaluation through observation of the skills performance of the individuals. The WST-Q, which is the questionnaire version, is a subjective evaluation in which the responses of the individuals related to skill capacity are recorded. In a study by Kirby et al. (2016), the measurement properties of the WST and WST-Q were examined for the evaluation of wheelchair skills capacity and performance of wheelchair users with SCI. As a result of the study, the use of WST and WST-Q was proven to have good content and concurrent and simultaneous validity in individuals with SCI (22).

The WST-Q form was developed to be able to provide an evaluation when it is not possible to use the WST. The skills evaluated in the WST-Q questionnaire form are the same as the skills tested in the WST. In special conditions such as the COVID-19 pandemic, the WST-Q is a particularly useful scale. It is extremely practical for use in at-home evaluations, remote follow-ups, small rehabilitation centers which are not suitable for WST, and in situations when the WST is contra-indicated (e.g., during bed rest after a fall). The time needed to complete the WST-Q is shorter than for the objective test. There is no requirement for a specific area and equipment for scoring the skills. Failure originating from technical errors which can arise when applying the test is eliminated with the use of the WST-Q. Therefore, this study was planned to examine the validity and reliability of the Turkish version of the WST-Q, as it is more economical, and practical, and can be applied remotely during a pandemic. Thus, it can be considered a questionnaire that can be widely applied for evaluation in a short time with a low error rate and without the need for equipment in healthcare institutions and disability units.

Kirby and his team researched wheelchair mobility for many years. They examined the wheelchair skills required in daily living activities and social participation. The tests were created appropriate/according to the data obtained. In the scale, the capacity, confidence, frequency of application, and training target of the individuals are questioned related to the 33 skills determined (9, 23). The validity and reliability of the WST-Q have been researched in several studies in the literature (5, 24). The WST-Q has been translated into Portuguese (8), French (6), and Norwegian (7).

The cultural adaptation study by Campos et al. was planned by translating the WST-Q into Portuguese as a measurement tool that would be able to be used in Brazil. In the study, which included 46 manual wheelchair users, the Brazilian version of the WST-Q was proven to be reliable and have excellent internal consistency (8). In 2018, Moen translated the WST-Q into Norwegian. As the result of a study that included 50 participants, the Norwegian version of the WST-Q was reported to be a reliable test for use on adults with SCI (7). In the current study, the WST-Q-Turkish was determined to be a valid and reliable questionnaire. It can be used in research in Türkiye, in rehabilitation centers, and in situations requiring remote evaluation during the pandemic.

There are few paraplegics with SCI using a manual wheelchair in the community. This study was planned by evaluating previous studies and calculating the sample size. Therefore, to increase the number of cases, the level of injury was ignored. In addition, the study included cases who used both manual and electric wheelchairs. Although it was aimed to include cases involving people who only used a manual wheelchair both inside and outside the home, 75% of the participants reported that they used an electric wheelchair for long distances and a manual wheelchair for short distances and within the home. The reason for preferring an electric wheelchair outside was explained as the person not having the functional capacity for the wheelchair and that the outdoor environment is not standardized or suitable for disabled individuals. This study was conducted on individuals with SCI. However, future validity and reliability studies of the WST-Q-Turkish could be planned to include other wheelchair users, such as amputees and patients with cerebral palsy. In addition, future studies could be made of the validity and reliability of the cultural adaptation to Turkish of the WST-Q form for electric wheelchair users.

In this study, cultural adaptation to Turkish of the WST-Q form was performed and the resulting form was named WST-Q-Turkish. The WST-Q-Turkish was determined to be a valid and reliable questionnaire for the evaluation of the skills capacity of paraplegic adults with SCI using a manual wheelchair. The questionnaire was seen as practical and suitable for use in the evaluation of the skills of manual wheelchair users in hospitals, rehabilitation centers, disability units, and in situations requiring remote evaluation, such as during a pandemic. In addition, the effects of treatments applied on manual wheelchair skills can be evaluated, and changes in the functionality of patients can be determined.

Ethical Statement

Approval for the study was granted by the Non-Interventional Clinical Research Ethics Committee of Izmir Demokrasi University (Decision No:2020/14-06, Dated: June 29, 2020).

Conflict of interest

The authors report no conflict of interest.

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Authors' contributions

Concept: E.K., F.T., Design: F.T., B.T., Data Collection or Processing: E.K., H.G., Analysis or Interpretation: E.K., F.T., Literature Search: E.K., B.T., Writing: E.K., F.T.

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Pain catastrophizing, depression, and anxiety in fibromyalgia patients

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Abstract

Psychiatric findings may be seen in Fibromyalgia (FM) patients. We aimed to evaluate the relationship between anxiety, depression, and pain catastrophizing in FM patients and normal individuals. The study group consisted of FM patients and a healthy control group. Socio-demographic data form, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Visual Analogue Scale (VAS), Fibromyalgia Impact Questionnaire (FIQ), and Pain Catastrophizing Scale (PCS) were administered to the participants. It was evaluated whether there was a statistical difference between the groups in terms of BDI, BAI, PCS, VAS, and FIQ. Of the 178 patients in our study, 85 were FM patients and 93 were control groups. The BDI, BAI, PCS, PCSH (PCS Helplessness), PCSM (PCS Magnification), and PCSR (PCS Rumination) were found to be higher in FM patients compared to the control group. The FM patients were found to have moderate depression according to the BDI score (18.7±10.6) and moderate anxiety according to the BAI score (16.5±9.9). There was a positive correlation between BDI and BAI, FIQ and VAS, PCS and BDI, BAI and FIQ, and BAI and FIQ. It was found that the FM patients had "moderate" depression and anxiety, and their level of pain catastrophizing increased with the increase in depression and anxiety severity.

Keywords: fibromyalgia, depression, anxiety, pain catastrophizing

1. Introduction

Fibromyalgia (FM) is a chronic pain condition characterized by symptoms such as widespread musculoskeletal pain, the presence of tender points, fatigue, anxiety, sleep disturbances, and cognitive and mood disorders (1). According to the criteria of the American College of Rheumatology (ACR), FM patients should have tenderness in at least 11 of 18 specific tender point sites in addition to widespread pain (2). The etiology and pathogenesis of fibromyalgia have not been fully understood yet (3).

Since FM causes widespread chronic pain, it can seriously affect the quality of life (4). FM may be associated with some psychiatric disorders. FM patients' depression status can be evaluated by Beck Depression Inventory (BDI) and their anxiety status by Beck Anxiety Inventory (BAI) (5). Depression symptoms, which are quite common in the chronic course of pain, make it difficult for the patient to comply with the treatment (6).

Catastrophizing is the tendency to evaluate one's situation or physical complaint, fearing that it will worsen each time (7). As a result of the catastrophizing, individuals have difficulty suppressing thoughts about their pain (rumination), exaggerate the pain, and worry about the negative consequences of the pain (magnification), and believe that there is nothing they can do to relieve the pain (helplessness). This situation is evaluated by the Pain Catastrophizing Scale (PCS) (8). Catastrophizing pain has been associated with various diseases, chronic pain, deterioration of quality of life, increased disability, and more healthcare use (9). In individuals with FM, pain catastrophizing has been associated with poor response to treatment (10).

In this study, we aimed to compare the levels of anxiety, depression, and pain catastrophizing between FM patients and normal individuals.

2. Materials and methods

A total of 178 people were included in our study. Eighty-five people included in the study were FM patients who had previously been diagnosed with FM according to the ACR criteria (2). As the control group, 93 people without FM diagnosis and with similar socio-demographic characteristics were included. The data was collected using the socio-demographic data form, Fibromyalgia Impact Questionnaire (FIQ), Visual Analogue Scale (VAS), BDI, BAI, and PCS. Statistical differences between groups and the relationships

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between VAS, BDI, BAI, FIQ, and PCS were evaluated. A correlation analysis was carried out between BDI, BAI, PCS, FIQ, and VAS scores in the FM group.

The socio-demographic data form includes the following information: age, gender, marital status, history of chronic illness, and duration of illness.

Fibromyalgia Impact Questionnaire (FIQ) evaluates the following ten characteristics in FM patients: physical function, not going to work, feeling unwell, pain, difficulty at work, stiffness, fatigue, morning fatigue, anxiety, and depression. The maximum score for each subtitle is 10 points, so the total maximum score is 100. (11) In VAS, the pain level is scored between 0 (no pain) and 10 (unbearable pain) (11).

Beck Depression Inventory (BDI) is used to assess depression levels. In BDI, the scores within the range of 0-9 refer to "no depression", 10-16 to "mild depression", 17-23 to "moderate depression", and 24 or more to "severe depression" (12, 13).

Beck Anxiety Inventory (BAI) is used to assess anxiety levels. In BAI, 0-7 points refer to minimal anxiety symptoms, 8-15 points to mild, 16-25 points to moderate, and 26-63 points to severe (7).

Pain Catastrophizing Scale (PCS) is a self-administered questionnaire and includes the subscales of rumination, magnification, and helplessness. PCS is used to assess the patient's feelings and thoughts about pain. PCS score ranges between 0 and 52 points (8). The subscales "helplessness" (inability to cope with pain effectively), "magnification" (discontent created by focusing excessively on the negative consequences of pain), and "rumination" (inability to inhibit thoughts about pain) reflects the cognitive content of anxiety and depression accompanying headache (14).

Inclusion criteria: Participants (FM patients and control group) over 18 years of age, whose cognitive functions were sufficient to answer the questions, who did not have a history of psychiatric illness and who did not use psychiatric medication were included in our study. Exclusion criteria: Those with cognitive impairment that prevented them from answering the questions, those who did not fully answer the questionnaire, and those with missing socio-demographic data were excluded (Fig. 1).

Statistical data were analyzed using the IBM SPSS software package (v.22.0). To compare groups, a one-way ANOVA test was used for normally distributed values in non-categorical data based on the data distribution. Mann-Whitney U test was used for non-parametric data. Categorical data were compared with the Chi-square test. The Pearson Correlation test was used to analyze the relationship between scale scores. The values with $p<0.05$ were evaluated as statistically significant.

Ethical approval was taken from the local University Clinical Research Ethics Committee for this study (Approval letter number**:** 2020/327**,** Date of approval**:** 27/08/2020).

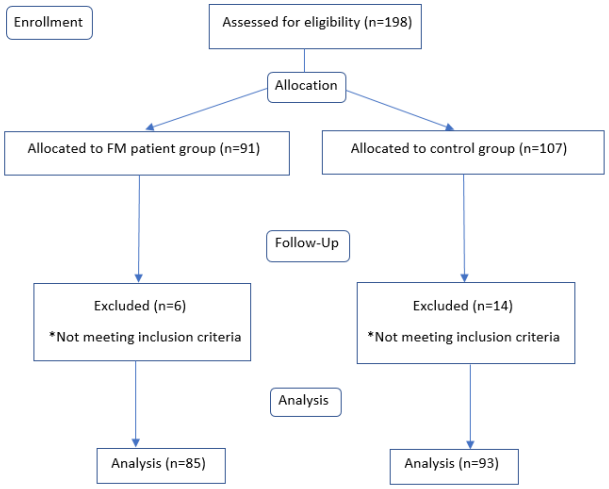

Fig. 1. Flow diagram of the study

3. Results

Of the 178 individuals participating in the study, 85 were FM patients and 93 were healthy controls. The mean age of the FM patients was 43.0±7.4 years. The mean duration of FM disease was 7.3±3.3 years (Table 1).

Table 1. Sociodemographic and clinical characteristics of FM patients and control groups

Variables	All participants (*n*=178)	FM patients (*n*=85)	Control groups (*n*=93)	*p*
Age (year), (mean ± sd)	42.0±7.3	43.0±7.4	41.1±8.5	0.121
Gender, *n* (%)				
Female	153 (85.9)	78 (91.8)	75 (80.6)	0.081
Male	25 (14.1)	7 (8.2)	18 (19.4)	
Marriage status, *n* (%)				
Single	24 (13.5)	15 (17.6)	9 (9.7)	0.120
Married	154 (86.5)	70 (82.4)	84 (90.3)	
Chronic disease history, *n* (%)				
Yes	27 (15.2)	7 (8.2)	20 (21.5)	0.051
No	151 (84.8)	78 (91.8)	73 (78.5)	
Duration of FM disease (year), (mean ± sd)	-	7.3±3.3	-	-

p, chi square test; *n*, number; sd, standard deviation

A statistically significant difference was observed between FM patients and the control group in terms of BDI, BAI, PCS Total (PCST), PCS Helplessness (PCSH), PCS Magnification (PCSM), and PCS Rumination (PCSR) (p<0.001, all). The BDI, BAI, PCST, PCSH, PCSM, and PCSR were higher in FM patients. FM patients were found to have "moderate" depression according to the BDI score (18.7±10.6) and "moderate" anxiety according to the BAI score (16.5±9.9) (Table 2).

Table 2. BDI, BAI, PCS, FIQ and VAS scores of FM patients and control groups

Scales	FM Patients (mean±sd)	Control Groups (mean±sd)	Mean Difference	95% CI	*p*
BAI	16.5±9.9	3.8±1.8	12.658	10.580, 14.737	<0.001
BDI	18.7±10.6	5.1±4.1	13.638	11.295, 15.981	<0.001
PCST	23.2±12.5	10.7±12.8	12.493	8.727, 16.260	<0.001
PCSH	9.6±6.4	3.8±5.4	5.754	3.989, 7.519	<0.001
PCSM	5.9±2.9	3.4±3.3	2.523	1.586, 3.459	<0.001
PCSR	7.6±3.8	3.7±4.4	3.963	2.730, 5.195	<0.001
FIQ	63.1±6.5	-	-	-	N/A
VAS	6.7±2.0	-	-	-	N/A

p, independent sample T-test; sd, standard deviation; CI, Confidence Interval; N/A, Not applicable; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PCST, Pain Catastrophizing Scale Total; PCSH, PCS Helplessness; PCSM, PCS Magnification; PCSR, PCS Rumination; FIQ, Fibromyalgia Impact Questionnaire; VAS, Visual Analogue Scale

The PCS subscale scores of the FM patients with and without depression were evaluated in terms of their total BDI scores, and those of the patients with and without anxiety in terms of their BAI total scores. The PCS total score (p<0.001) and the scores for the PCS subscales of "helplessness", "magnification", and "rumination" were found to be significantly higher in the FM patients with depression than in those without. Likewise, the PCS total score (p<0.001) and the scores for the PCS subscales of "helplessness", "magnification", and "rumination" were found to be higher in the FM patients with anxiety compared to those without (Table 3).

Table 3. PCS subscale scores according to the presence of depression or anxiety in FM patients

PCS Subscales	No Depression (BDI) (mean±sd)	Depression (BDI) (mean±sd)	Mean Difference	95% CI	*p*
Helplessness	7.5±6.4	13.4±4.6	-5.921	-8.565, -3.277	<0.001
Magnification	5.3±3.0	7.0±2.3	-1.721	-2.989, -0.454	0.008
Rumination	6.8±3.9	9.2±3.0	-2.430	-4.096, -0.765	0.005
Total	19.6±12.8	29.8±8.8	-10.145	-15.396, -4.895	<0.001
	No Anxiety (BAI) (mean±sd)	**Anxiety (BAI) (mean±sd)**	**Mean Difference**	**95% CI**	***p***
Helplessness	7.4±6.0	13.8±4.9	-6.416	-9.031, -3.801	<0.001
Magnification	5.2±2.8	7.3±2.4	-2.113	-3.363, -0.862	0.001
Rumination	6.6±3.6	9.7±3.4	-3.134	-4.757, -1.510	<0.001
Total	19.2±11.9	30.9±9.8	-11.733	-16.875, -6.873	<0.001

p, independent sample T-test; sd, standard deviation; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PCS, Pain Catastrophizing Scale

There was a statistically significant correlation between the PCS total scores and "severe depression" (p=0.006) from the BDI subscales and "severe anxiety" (p<0.001) from the BAI subscales. As the severity of BDI and BAI increased, the PCS total scores also increased statistically significantly (Table 4).

A correlation analysis was carried out between BDI, BAI, PCS, FIQ, and VAS scores and the duration of disease in FM patients. There was a positive correlation between the scores for BAI and BDI, PCS and BDI, FIQ and BDI, VAS and BDI, PCS and BAI, FIQ and BAI, and PCS and FIQ in FM patients. There was no correlation between the disease duration and the scales (Table 5).

Table 4. PCS Total Scores by BDI and BAI Subscale Scores in FM patients

Scales		PCS Total Score (mean±sd)	Mean Difference	95% CI	*p*
BDI	Normal	22.7±15.3	-	-	-
	Mild Depression	17.2±10.2	5.492	-1.437, 12.421	0.118
	Moderate Depression	22.2±7.0	0.550	-9.823, 10.932	0.915
	Severe Depression	33.6±7.0	-10.850	-18.362, -3.338	0.006
BAI	Normal	18.0±10.6	-	-	-
	Mild Anxiety	19.8±12.6	-1.785	-8.592, 5.022	0.601
	Moderate Anxiety	25.0±9.7	-6.947	-14.698, 0.803	0.077
	Severe Anxiety	35.1±7.8	-17.124	-23.498, -10.749	<0.001

p, independent sample T-test; sd, standard deviation; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PCS, Pain Catastrophizing Scale

Table 5. Correlation analysis between BDI, BAI, PCS, FIQ, VAS scores and duration of illness in FM patients

Variables		BDI	BAI	PCS	FIQ	VAS
BDI	***r***	-	0.803	0.424	0.385	-0.247
	95% CI	-	0.685, 0.894	0.224, 0.627	0.200, 0.544	-0.436, -0.070
	p	-	<0.001	<0.001	<0.001	0.022
BAI	***r***	0.803	-	0.507	0.528	-0.107
	95% CI	0.685, 0.894	-	0.323, 0.662	0.374, 0.656	-0.321, 0.081
	p	<0.001	-	<0.001	<0.001	0.329
PCS	***r***	0.424	0.507	-	0.351	0.025
	95% CI	0.224, 0.627	0.323, 0.662	-	0.163, 0.520	-0.192, 0.247
	p	<0.001	<0.001	-	0.001	0.823
FIQ	***r***	0.385	0.528	0.351	-	0.058
	95% CI	0.200, 0.544	0.374, 0.656	0.163, 0.520	-	-0.166, 0.269
	p	<0.001	<0.001	0.001	-	0.595
VAS	***r***	0.247	-0.107	0.025	0.058	-
	95% CI	-0.436, -0.070	-0.321, 0.081	-0.192, 0.247	-0.166, 0.269	-
	p	0.022	0.329	0.823	0.595	-
Duration of illness	***r***	0.071	-0.078	0.131	0.032	-0.026
	95% CI	-0.140, 0.260	-0.241, 0.086	-0.084, 0.334	-0.131, 0.181	-0.216, 0.153
	p	0.521	0.478	0.231	0.768	0.815

p value, Pearson Partial Correlation Test; *r,* Correlation Coefficient; CI, Confidence Interval; BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; PCS, Pain Catastrophizing Scale; FIQ, Fibromyalgia Impact Questionnaire; VAS, Visual Analogue Scale

4. Discussion

There is a strong relationship between chronic pain and depression, and both can aggravate each other (15). Chronic pain can occur as part of a pain syndrome such as fibromyalgia (16). A study published in 2014 showed a higher prevalence of anxiety and depression in FM patients (17). Our FM patients perceived higher intensity of pain due to increased levels of anxiety and depression. Similar to the studies in the literature, BDI and BAI levels were higher in FM patients compared to the control group in our study (18).

A study reported that, according to BDI, 10% of the FM patients had no depression, 50% had mild depression, 38% had moderate depression, 2% had severe depression, and the average BDI score was 15 (19). In our study, the FM patients were found to have "moderate" depression according to the BDI score and "moderate" anxiety according to the BAI score. It can be considered that FM patients are depressed due to having a chronic disease for a long time and have anxiety due to pain and other effects of the disease.

Our study observed a positive correlation between BDI and BAI, FIQ and VAS, and BAI and FIQ in FM patients. In a previous study, FM patients were reported to have fatigue, sleep disorders, and anxiety, and a significant correlation was found between their scores for FIQ and BDI (r=0.430, p=0.008) (20). One study reported that anxiety and depression correlated independently with pain and fatigue in FM patients (21). It has been reported that FM can be a symptom of psychiatric disorders or psychophysiological abnormalities since depression and anxiety often accompany chronic painful conditions (22). One recent study measured pain intensity. The results showed that the higher the pain score, the higher the mood disorder (23). Incompatible responses to pain can worsen the pain experience and further impair function. Excessive rumination about pain is associated with a magnification of distress and extreme helplessness, poorer response to pain treatments, and greater disability (24). One study reported that FM responds to anti-depressant drugs and is one of the medical, neurological, and psychological disorders that show high comorbidity with depression, known as "Affective Spectrum Disorder" and other affective spectrum disorders (25). Anti-depressants have the most evidence for treating chronic pain with accompanying anxiety or depression. Chronic pain has been shown to significantly impair dopamine activity in the limbic midbrain region (26). Depression can occur when monoamine neurotransmitters such as NE and 5-HT are decreased in the nervous system (27). The SNRIs have been shown to reduce FM and neuropathic pain with and without depression (28).

Pain catastrophizing is the tendency to feel increased pain, whether real or imaginary. This inability to distract the focus of attention from pain causes an increase in pain perception and sensitivity (29). In our study, according to the control list, a higher cognition was found in the PCS total score and the scores for the PCS subscales "helplessness", "magnification", and "rumination", which shows emotional and cognitive attitudes towards pain. In a study, catastrophizing was reported to be associated with pain activation, as it appeared to increase the perception of pain, according to brain MRI findings from FM patients (30). The same areas of the central nervous system are responsible for the sensation of pain and depression (15).

Our study found that anxiety and depression were associated with pain catastrophizing and increased helplessness, rumination, and magnification. The subscales "helplessness", "magnification", and "rumination" reflects the cognitive content of anxiety and depression accompanying headache (14). In our study, it was observed that the PCS total score of the FM patients increased in cases of "severe depression" from the BDI subscales and "severe anxiety" from the BAI subscales. This shows that the level of depression and anxiety increases the level of pain catastrophizing. Our study

observed A positive correlation between PCS and BDI, BAI and FIQ. This shows that pain catastrophizing increases with the effect of depression, anxiety, and illness.

As a result, given that catastrophe is associated with increased disease activity, depression, and anxiety in patients with fibromyalgia, the improvement and implementation of cognitive, psychosocial and medical interventions designed to reduce disaster in patients with fibromyalgia would potentially represent a significant improvement in disease management.

In most of the previous studies, anxiety, depression, pain level and quality of life were evaluated in patients with fibromyalgia, generally using one or both of the scales such as BDI, BAI, VAS, FIQ and PCS (10, 19-21). Similar to the study of Gürbüz at all (31) and Jesus at all (32), our study is one of the few studies in which this relationship can be explained by using scales such as BDI, BAI, VAS, and FIQ for pain status in patients with fibromyalgia.

Our study is limited in that it is single-centered. Future studies should be carried out in multi-centers with more participants. The strength of our study lies in that it is a prospective study and compares FM patients and normal individuals in terms of the symptom levels of anxiety, depression, and pain catastrophizing.

In conclusion, our study observed that the levels of depressive symptoms, anxiety, and pain catastrophic were significantly higher in the FM patients. It was found that the FM patients generally had “moderate” depression and anxiety. It was also found that as the severity of depression and anxiety increased in FM patients, their levels of pain catastrophizing, helplessness, magnification, and rumination also increased. In FM patients, in addition to medical treatments, psychiatric approaches should be incorporated into the treatment for patients who are indicated after psychosocial evaluations.

Ethical Statement

Ethical approval was taken from the local University Clinical Research Ethics Committee for this study (Approval letter number: 2020/327, Date of approval: 27/08/2020).

Conflict of interest

The authors declare no conflicts of interest.

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None to declare.

Authors’ contributions

Concept: H.İ., F.İ., Design: H.İ., F.İ., Data Collection or Processing: H.İ., F.İ., Analysis or Interpretation: H.İ., F.İ., Literature Search: H.İ., F.İ., Writing: H.İ., F.İ.

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Psychometric properties of the Comprehensive Diabetes Self-Management Scale in patient with diabetes

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Abstract

This study's objective was to investigate the psychometric characteristics of the "Comprehensive Diabetes Self-Management Scale," which was created by Mikhael et al. This scale provides a comprehensive screening tool for health promotion-oriented behaviors. With the involvement of 475 diabetic people, the study was undertaken between January and August 2022. The study's sample was formed by taking more than 33 times the amount of items on the original scale (14 items). "Diabetes Self-Care Scale" was used as a parallel form to calculate reliability coefficients. The scale's confirmatory factor analysis showed a very good fit [x^2 (53, N=475) p=0.014; x^2/ degree of freedom=1.477; chi-square:78.276; root mean square error of approximation =0.032; comparative fit index =0.970; goodness-of-fit index =0.975] with the results obtained in the first level factorial structure of 13 items and five sub-dimensions. There was a 57.16% overall variance, it was found. The Comprehensive Diabetes Self-Management Scale was shown to be a valid and reliable measuring instrument for people with diabetes. This scale will provide the opportunity to evaluate many important concepts and behaviors related to diabetes with a single tool.

Keywords: comprehensive, reliability, self-management, type 2 diabetes, validity

1. Introduction

One of the most dangerous and prevalent chronic diseases of our day, diabetes poses a risk to life, impairs function, results in expensive complications, reduces life expectancy (1), and affects daily activities related to self-care (2). Diabetes is an important public health problem that is increasing worldwide. There are 537 million diabetics worldwide, according to figures from "The International Diabetes Federation" for 2021. Additionally, it is anticipated that by 2045, there will be 783 million people worldwide with diabetes, with Turkey having one of the top 10 global rankings for the number of people with Diabetes Mellitus (DM) diagnoses (3).

It is very important for patients to maintain their diabetes management with determination in preventing or delaying the complications that may develop due to DM. A self-management approach should be developed in diabetic patients in order for them to continue with this determination (4). For those who have lived with chronic illnesses for a long time and who routinely make self-management decisions or take steps to solve problems, self-management is an essential component of daily life (5). Self-management, which is the cornerstone of diabetes care, provides routine glucose assessment and adherence to therapy, as well as careful planning of physical activity and diet and coping with low/high glucose levels. Effective self-management of diabetes will contribute to the maintenance of strict glycemic control and hence lower the risk of diabetic complications (6). More intense efforts are required to ensure the adoption of quality diabetic self-care tools, given the rapid expansion and serious effects of diabetes on world health. Effective diabetic self-care measurement will help to improve diabetes management by detecting self-care gaps (7). These tools for assessing health-promoting habits will assist in determining patients' behaviors and aid in developing interventions. Different studies have been conducted internationally to examine diabetes self-management. Some of these scales include the 40-item "Diabetes Self-Care Inventory" (8), 35-item "Diabetes Self-Management Scale" (9), 8-item "Perceived Diabetes Self-Management Scale" (10), 16-item "Diabetes Self-Management Questionnaire" (11), is a 28-item "Type 2 Diabetes and Health Promotion Scale" (12). There are several validity and reliability research (13–17) on diabetes in our nation; however, there is no comprehensive tool to evaluate diabetes self-management techniques. When all the above references are summarized, it is observed that many

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important notions and attitudes are spread across different assessment tools. Important factors in glycemic control include exercise, diet, medication adherence, blood glucose testing, risk avoidance, stress management, foot care, and patient adherence on sick days (such as the flu, diarrhea, or urinary tract infections). However, Turkey has a limited selection of assessment tools that combine these factors. The Turkish validity and reliability study of the "Comprehensive Diabetes Self-Management Scale (CDSMS)" addresses this need in this area. This scale offers a quick, simple, useful, and comprehensive screening tool for activities aimed at promoting health. Therefore, it is crucial to carry out a study on the tool's validity and reliability.

2. Materials and Methods

2.1. Study design and sample selection

This study is methodological research conducted to investigate the psychometric properties of CDSMS developed (18) by Mikhael et al. The study was conducted on patients with type 2 diabetes mellitus (T2DM) admitted to a State Hospital in northern Turkey between January and August 2022. The study sample consisted of 475 individuals who (i) were older than 18 years, (ii) had T2DM, (iii) had been taking antidiabetic medication for at least three months, (iv) could speak/understand Turkish, (v) could communicate effectively with health care professionals, and (vi) gave consent to participate in the study. Our study had a sample size that was more than 33 times the amount of items on the 14-item measure. It is advised that the sample size for scale studies be ten or fifteen times the number of each scale item. (19, 20). In the study, the sample was first taken 15 times, and since the "Kaiser-Meyer-Olkin (KMO)" values were below 0.50, data collection continued. It is stated that 0.50 should be the lower limit for the KMO test (21).

2.2. Data collection tools

Sociodemographic information form, the CDSMS, and for the calculation of reliability coefficients Diabetes Self-Care Scale (DSCS) (2, 22) has been used.

Sociodemographic characteristics questionnaire

A sociodemographic information form created by the researchers in accordance with the literature was employed, taking the study's goals into account. This questionnaire asked people with T2DM questions about their gender, age, educational background, occupation, and the year of their diabetes diagnosis.

"Comprehensive diabetes self-management scale"

To evaluate diabetes self-management techniques unique to persons with diabetes, Mikhael et al. created the CDSMS in 2019. It consists of a total of 14 items, including exercise (items 1 and 2), diet (items 3, 4, and 5), medication adherence (item 6), blood glucose testing (item 7), reducing diabetes risks (items 8-11), coping with stress (item 12) and solving problems (items 13 and 14). The scale's ten items were created using a multiple-choice format with five possible responses.; 4 items (8, 9, 11, 14) were designed using a style with dichotomous answer sub-questions. The items are scored between 0 and 4; zero is assigned to the response with the least accepted practice, while 4 is assigned to the answer with the most approved practice for multiple-choice items and 1 for dichotomous questions. By summing the scores of each sub-question, the score of the items containing sub-questions was obtained. Every item is computed inversely, with the exception of items 3, 4, 5, 10, 11D, and 14 B (18).

"Diabetes Self-Care Scale"

The 35-item DSCS, which Karakurt translated into Turkish, is a Likert-style scale. This scale deals with individuals' self-care and self-evaluation. The scale has four options: 'Never,' 'Sometimes,' 'Frequently,' and 'Always.' High scores on the scale, which has a maximum possible score of 140, show that patients are competent and autonomous in providing for their own needs (22).

2.3. Translation and cultural adaptation

For linguistic validity, the scale was translated to Turkish by three people fluent in Turkish and English, considering the use of appropriate sentence structures and the replacement of items that are foreign to the culture. Then, the researchers created the Turkish scale by analyzing these three translations. A native speaker of both languages who had never seen the English version of the scale before then compared the original and final versions of the scale after being translated back into English. Following the back translation, the scale items underwent grammar, comprehensibility, and cultural traits revisions. For content and language validity reviews, the final translation was presented to 15 health professionals (academics, physicians, and nurses with a focus on diabetes). Each scale item was graded ('no important omission,' 'partially important omission,' 'unimportant omission') by the experts for its content validity using the Lawshe approach (23). "The content validity index (CVI)" for 14 items was determined to be 0.88 as a consequence of the expert judgments, and the final form of the scale was developed in accordance with their suggestions. Then, the scale was pre-administered, and individuals with T2DM were asked about their thoughts on the items and the comprehensibility of the items (conceptual questioning). Since there was no negative feedback, the data collection phase started.

2.4. Ethical considerations

"The Bartın University Ethics Committee" received ethical approval (2021-SBB-0473) for this work. The study's goal was explained to the individuals who decided to take part, and their signed informed permission was acquired. Additionally, the authors who developed the scales granted permission for their use in the study by email.

2.5. Statistical analysis

With the help of "Amos version 24" and "SPSS version 26", the study's data were examined. Kurtosis and Skewness values were analyzed to determine whether the research variables

were normally distributed. In the related literature, the results of kurtosis and skewness values of the variables between +1.5 and -1.5 (24), +2.0 and -2.0 (25) are accepted as normal distributions. It was determined that the variables showed normal distribution. Frequencies and means ± standard deviations were used to define sociodemographic and clinical parameters. Firstly, the data were assessed to see if they were appropriate for factor analysis using the KMO value and "Barlett Test." "Exploratory Factor Analysis (EFA)" was used to determine the relationship between the variables. To determine if the conceptual model identified by EFA was supported or not, "Confirmatory Factor Analysis (CFA)" was utilized. To verify the reliability, Pearson correlation analysis was employed. The internal consistency of the scale's overall and sub-dimensions was examined using Cronbach's alpha.

3. Results

The mean age of the participants was 59.03±12.86 years (min=18, max=86), the mean years of illness were 10.29±7.70, and the mean duration of antidiabetic drug use was 8.91±7.11. The participants were 58.8% female, 41.2% male, 46.9% primary school graduates, 12.9% illiterate, and 12% high school graduates. When their employment status was analyzed, it was found that 42.5% were housewives, and 36.8% were retired. It was discovered that 70.6% of the individuals had a genetic susceptibility to diabetes and a family history of the disease (Table 1).

Table 1. The sociodemographic characteristics of the patients

Sociodemographic characteristics		n	%
Gender	Female	279	58.8
	Male	196	41.2
Age	18-34	22	4.6
	35-54	127	26.7
	55-64	135	28.4
	65+	191	40.2
Job	Retired	175	36.8
	Officer	15	3.2
	Employee	47	9.9
	Housewives	202	42.5
	Unemployed	12	2.5
	Other	24	5.1
Education status	Illiterate	60	12.9
	Only literate	46	9.7
	Primary school	223	46.9
	Primary education	55	11.6
	High school	57	12.0
	University and above	34	7.2
Body Mass İndex (BMI)	Underweight	3	0.6
	Health Weight	75	15.8
	Overweight	173	36.4
	Obesity	224	47.2

3.1. Reliability analysis

In the study, KMO and Bartlett's tests were used to evaluate the sampling adequacy of the data set for factor analysis. The KMO test value of CDSMS was found to be within the measurable range of 0.621. "The Bartlett Sphericity" test revealed that the variables were significantly correlated with one another and that the data were appropriate for factor analysis (x^2: 915.113, degree of freedom (df): 91, p<0.000). EFA factor analysis of the CDSMS was conducted. Tabacknick and Fidell (26) took this cut-off point as 0.32. The factor loadings of the items in a factor in the study are expected to be at least 0.40. Since the 13th item had a factor loading of 0.192, it was removed from the analysis, and the analysis was repeated. The total variation reported by the 13 items and 5 sub-dimensions (respectively "diet," "exercise," "reducing the risks of diabetes," "diabetes management," and "mental risk factors") of the scale was 61.22% as a result of this study.

An important criterion of factor analysis is that the variance explained should exceed 50% of the total variance. Because the generated factor structure has a limited ability to reflect the universe if it explains less than half of the total variable variation. In addition, the sum of the eigenvalues is expected to be above 1 (27). In the EFA, Factor 1 (F1: diet) had an eigenvalue of 2.27 and explained variance of 17.43%, Factor 2 (F2: exercise) had an eigenvalue of 1.94 and explained variance of 14.96%, Factor 3 (F3: reducing the risks of diabetes) had an eigenvalue of 1.45 and explained variance of 11.12%, Factor 4 (F4: diabetes management) had an eigenvalue of 1.26 and explained variance of 9.72%, Factor 5 (F5: mental risk factors) had an eigenvalue of 1.04 and explained variance of 8.00%.

The item correlation coefficient was found to be 0.755-0.795 in sub-dimension F1, 0.893-0.898 in sub-dimension F2, 0.788-0.851 in sub-dimension F3, 0.368-0.720 in sub-dimension F4, 0.808-0.812 in sub-dimension F5 and it was determined that the reliability of the scale was good. The resulting factors are named, and the model representing the properties of this structure is tested with the help of CFA (28). Therefore, the identified factors were tested again with CFA (Table 2).

3.2. Structure Validity

The CFA 13 items and five scale sub-dimensions were found to have a very good fit with the results obtained in the first level factorial structure [x2 (53, N=475) p=0.014; x^2/df=1.477; Chi-square (CMIN):78.276; Root Mean Square Error of Approximation (RMSEA)=0.032; Comparative Fit Index (CFI)=0.970; Goodness-of-Fit Index (GFI)=0.975]. These findings demonstrate that the study's data are consistent with the organizational structure (five-factor model) that the CDSMS scale is expected to have (Fig.1).

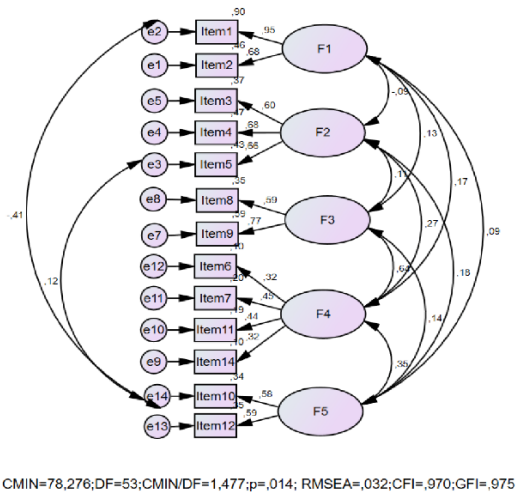

Fig. 1. First-level factorial structure of the Comprehensive Diabetes Self-Management Scale. CMIN: Chi-square; df: degree of freedom; RMSEA: Root Mean Square Error of Approximation; CFI: Comparative Fit Index; GFI: Goodness-of-Fit Index

When Table 3 is examined, x^2/df, RMSEA and Adjusted Goodness of Fit (AGFI), CFI, and GFI values show excellent fit, and Normed Fit Index (NFI) and Tucker-Lewis Index (TLI) show acceptable fit.

The findings suggest that the scale's original five-factor, 13-item format is also suitable for Turkish culture in terms of psychometric analysis.

The reliability coefficient is most frequently calculated in scale development and adaptation research using "Cronbach's alpha" (29). Cronbach's alpha was initially calculated for this reason. The total Cronbach Alpha of the scale was found to be 0.580. Cronbach's alpha for the F1, F2, F3, F4, and F5 sub-dimensions of the scale was 0.683, 0.785, 0.624, 0.407, and 0.511, respectively. The internal consistency coefficient Cronbach's alpha of the CDSMS scale was found to be lowly reliable for sub-dimensions F4 and F5 and highly reliable for sub-dimensions F1, F2, and F3.

Table 2. Exploratory factor analysis data of the Comprehensive Diabetes Self-Management Scale

Items	F1 (Diet)	F2 (Exercise)	F3 (Reducing the Risks of Diabetes)	F4 (Diabetes Management)	F5 (Mental Risk Factors)
Item 3*	.755				
Item 4*	.795				
Item 5*	.777				
Item 1*		.898			
Item 2*		.893			
Item 8*			.851		
Item 9*			.788		
Item 6*				.720	
Item7*				.510	
Item11*				.368	
Item14*				.681	
Item10*					.812
Item12*					.808
Eigenvalue*	2.27	1.94	1.45	1.26	1.04
Total Variance Explained (%61.22)*	17.43	14.96	11.12	9.72	8.00
Cronbach' alpha Total (.580) **	.683	.785	.624	.407	.511

*Factor Analysis, **Reliability Analysis

Table 3. Fit indices and scale values of The Comprehensive Diabetes Self-Management Scale

Fit indices	Perfect fit criteria	Scale values
$\chi 2$/df	≤ 2	**1.477**
p	≤ 0.05	**0.014**
RMSEA	≤ 0.05	**0.032**
AGFI	0.90 ≤	**0.958**
CFI	0.95 ≤	**0.970**
GFI	0.95 ≤	**0.975**
TLI	0.95≤	**0.955**
NFI	0.95≤	0.914
IFI	0.95≤	**0.971**

Confirmatory Factor Analysis
df: degree of freedom; RMSEA: Root Mean Square Error of Approximation; AGFI: Adjusted Goodness of Fit; CFI: Comperative Fit Index; GFI: Goodness-of-Fit Index; TLI: Tucker-Lewis Index; NFI: Normed Fit Index; IFI: Incremental Fit Index

In the study, the DSCS was used as a parallel form, which is another reliability criterion. Calculating the correlation coefficient between the data derived from these two parallel forms reveals if the parallel forms are equivalent (30). Depending on the data characteristics, Pearson correlation coefficients and equivalence coefficients are calculated (30). In the study, Pearson correlation analysis results between the two scales were found as (r:0.545; p=0.000). Although the equivalence coefficient varies between 0 and 1, being close to 1 indicates that the results obtained from parallel forms are reliable (31). These findings led to the conclusion that the CDSMS was reliable

4. Discussion

This study's objective was to evaluate the reliability and

validity of a brand-new tool for assessing diabetes patients' self-management. As a consequence, it has been shown that the CDSMS scale is reliable and acceptable in Turkish culture. It is expected to contribute to future research on this subject.

The CDSMS, originally in English and adapted to the Turkish population, was similar to the initial scale (14 items) in relation to sub-dimensions and a number of items. However, due to low factor loading within our analysis, the 13th item was eliminated. In addition, while the original scale had seven sub-dimensions and single-item sub-dimensions, the study consisted of 13 items and five sub-dimensions, with at least two items in each sub-dimension.

The homogeneous structure of the items is explained by "Cronbach's alpha coefficient," a metric of the items' internal consistency. The Cronbach's alpha coefficient is high when the items are consistent and contain items that measure the same trait. The literature has stated that this value should be more than 0.40 (32). In this study, the total Cronbach Alpha of the scale was found to be 0.580. The results of Cronbach's alpha for the scale's F4 and F5 sub-dimensions were found to be of low reliability, whilst those for the F1, F2, and F3 sub-dimensions were found to be fairly trustworthy. In our study, the sub-dimension encompassing behaviors related to physical activity (F2) had the greatest Cronbach's alpha coefficient (0.785), while the sub-dimension related to behaviors related to nutrition (F1) had a highly reliable Cronbach's alpha value (0.683). Similar findings were observed in the study by Chen et al., where it was discovered that Cronbach's alpha coefficient of the sub-dimension comprising dietary behaviors was 0.68 and that it was 0.86 for the sub-dimension including physical activity behaviors (12). In their analysis of the original scale's development, Mikhael et al. discovered that Cronbach's alpha coefficient was 0.704 (18). The fact that the scale in the study has a lower value than the original scale may be due to cultural factors. The dependability of the scale was further examined using a parallel scale. The parallel scale shows that the results obtained are reliable.

In more than one study in the literature, it is stated that 50% of the total variance explained is sufficient (27, 33, 34). Mankan et al. found that the total variance explained in the diabetes self-efficacy scale (8 items) was 52.38% (14). In the study of the type 2 diabetes self-management scale developed by Koç, it was detected that the 3-factor structure explained 50% of the total variance (17). In the study of Bakır et al. on the diabetic foot self-care behavior scale, it was found that the measurement tool consisting of 7 items explained 69.883% of the total variance (35). In the scale study developed by Chen et al., it was detected that the 28-item scale explained 56.7% of the total variance (12). Saffari et al. found that the scale was in the form of a structure explaining 54.6% of the total variance (36). Our study's total variance explained was determined to be 61.22%, and it is similar to other research on diabetes.

"The x^2/df ratio" [perfect fit (≤ 2.5-3)] should be as low as feasible for a good model fit. In the current research, x^2 /df =1.477, indicating a perfect fit. These findings were consistent with those of another Turkish study on validity and reliability that involved diabetic individuals (37).

The closer the RMSEA is to zero, the better the model-data fit (38). Since the RMSEA value was 0.032 in the study, there is a model-data fit. The CDSMS has strong internal consistency and steady dependability, as demonstrated by Mikhael et al. In their study, they reported that the scale is a reliable and valid tool and can be used to evaluate self-management practices among diabetic patients in their country (18). Similarly, our study concluded that the CDSMS is a reliable and valid tool that can be applied in the Turkish community.

The CDSMS scale raises intriguing questions about better diabetes management. Healthcare professionals and patients will have more detailed information through this scale. In addition, patients will be more aware of receiving these services (e.g., doctor's visits/regular tests) when certain tests are due. Moreover, this scale will provide an opportunity to examine many important concepts and behaviors in a single assessment tool.

When all of the data is taken into account, it is believed that the CDSMS scale is a valid and reliable measuring tool that can be applied and will close the gap in this area. To improve its evidential value, it is advised that it be used in many cultures and groups. In addition, in order to provide evidence for the validity and reliability of the Turkish version of the CDSMS Scale, the findings of this study should be supported by new studies that will include more samples.

Ethical Statement

"The Bartın University Ethics Committee" received ethical approval (2021-SBB-0473) for this work. The study's goal was explained to the individuals who decided to take part, and their signed informed permission was acquired. Additionally, the authors who developed the scales granted permission for their use in the study by email.

Conflict of interest

The authors declare that they have no conflict of interest.

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None to declare.

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Authors' contributions

Concept: Y.Ş.Y., B.A., H.Y.D., Design: Y.Ş.Y., B.A., H.Y.D., Data Collection or Processing: Y.Ş.Y., B.A., Analysis or Interpretation: Y.Ş.Y., B.A., H.Y.D., Literature Search: Y.Ş.Y., B.A., Writing: Y.Ş.Y, B.A.

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The effect of the basal frontal QRS-T angle on disease severity and mortality in Covid-19 patients

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Abstract

This study aimed to determine the relationship between the frontal QRS-T angle, the severity of the disease, and mortality, calculated with the ECG data taken during admission to the hospital. In this retrospective study, patients hospitalized in intensive care units and regular services with Covid-19 disease at Health Sciences University Mehmet Akif Inan Training and Research Hospital between April-September 2020 were included. Patients who were not given Covid-19 medication except for five days of Hydroxychloroquine (HC) and Azithromycin (AZ) with no cardiac disease history and daily taken ECGs were included in the study. A total of 135 patients were included in this study. While 45.9% of the patients received only HC treatment, 54.1% also received additional AZ treatment. It was observed that the frontal QRS-T angle was significantly longer in intensive care patients and intubated patients ($p =<0.001$). ROC curve analysis demonstrated that the best cut-off value for predicting mortality was 101.5º. The in-hospital mortality rate was significantly higher in patients with widened frontal QRS-T angle ($p = 0.008$). QRS widening, QTc prolongation, and QRS-T angle widening were substantially more frequent in intensive care patients ($p=0.001$,$p<0.001$, and $p<0.001$, respectively). Significantly QTc prolongation was observed more frequently in patients hospitalized in intensive care and followed up intubated ($p<0.001$ and $p=0.003$, respectively). The most common QTc prolongation time was on the 4th day of treatment in both groups (43.8% and 46.8%). Multivariate logistic regression analysis showed that frontal QRS-T angle ≥ 101.5º (OR: 7.08, 95%CI: 1.17-42.75, $P = 0.033$) was an independent predictor of mortality. The prolonged frontal QRS-T angle in Covid-19 patients increases the severity of the disease and mortality rates. The frontal QRS-T angle can be used to determine the prognosis of Covid-19 patients due to its advantages, such as easy evaluation and no extra costs.

Keywords: Covid-19 disease; frontal QRS-T angle; QTc prolongation; mortality

1. Introduction

Obviously, Coronavirus Disease 2019 (COVID-19) is the most significant health problem of the 21st century. Almost 35 million people worldwide have been infected with the virus, and one million people have died from this disease as of October 2020, since its origin in December 2019, according to the Johns Hopkins COVID-19 Resource Center (1). It may show a wide range between asymptomatic course and mild upper respiratory tract infection and severe ARDS (2). Myocardial damage is also one of the critical pathogenic features of COVID-19.

With the demonstration that Covid-19 caused cardiac damage, concerns were raised about Hydroxychloroquine (HC) and Azithromycin (AZ) used in the treatment because these drugs were known to prolong QT and correct QT (QTc) (3,4). Thereupon, studies reporting the use of these drugs may increase mortality in COVİD-19 patients have emerged (5). However, since many studies claim that it may increase mortality, some studies claim the opposite, so a definite opinion on this issue could not be concluded (6). The only precise information on the subject is that the cardiac impact of Covid-19 disease is severe.

The frontal QRS-T angle, which has recently become a popular field of study, shows the main direction of electrical cardiac activity (7). The QRS-T angle is defined as the difference between ventricular depolarization (QRS axis) and repolarization (T axis). An increased QRS-T angle is an indicator of abnormal ventricular repolarization. Electrocardiographic (ECG) risk indicators such as QT prolongation and other commonly used traditional cardiovascular risk factors have been recognized as strong and independent risk indicators for cardiac morbidity and mortality (8). Its predictive value and usability in many diseases, such as hypertension and coronary artery diseases, have been investigated and found useful (9-11). Since cardiac damage is common in Covid-19, there are many studies on cardiac biomarkers and QT-QTc prolongation, while there are only a

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few studies on the frontal QRS-T angle. Our study aimed to investigate whether the frontal QRS-T angle is beneficial in predicting in-hospital mortality in hospitalized patients due to Covid-19. We also planned to examine whether AZ and HC potentialize QTc prolongation and the effect of QTc prolongation on mortality.

2. Matherials and Methods

This study was carried out under the Declaration of Helsinki and approved by the Ethics Committee of Harran University (Approval Number E.4192). After obtaining the ethics committee's approval, patients who were hospitalized in intensive care and regular services in Şanlıurfa Health Sciences University Mehmet Akif İnan Training and Research Hospital due to Covid-19 disease between April 2020 and September 2020 were included in the study. Patients with Covid PCR (+) and who received HC with/without AZ in the treatment of Covid-19 were included in the study. Among these patients, those who were hospitalized for at least five days, those whose ECG was taken at the first admission to the hospital, and those whose ECG was taken every day during 5-day HC treatment were included in the study. The patients who are not receiving HC treatment, receiving other treatments other than HC and AZ in the treatment of Covid 19, using other drugs such as antidepressants, antibiotics, antiarrhythmic drugs known to prolong QTc, patients with previously known cardiac disease, patients who did not have a 5-day ECG examination, and the patient's under HC treatment for autoimmune diseases such as lupus were excluded from the study.

The data were obtained from patient files and the hospital data system. The condition of a 12-lead surface ECG was sought from all patients during admission to the hospital and every day during treatment. Demographic and biochemical data were collected, including basal troponin, creatinine, potassium, calcium, and C-reactive protein (CRP). As a result of the evaluations, serum creatinine, potassium, calcium, and CRP values of the day with the highest QTc prolongation were noted as maximum values.

2.1. Surface ECG and Measurements

A 12-lead surface ECG with a paper speed of 25 mm / s and a signal size of 10 mm / mV was provided to all patients during admission to the hospital and every day for five days of treatment.

Frontal QRS-T angle measurement: The ECG device automatically measured the frontal plane QRS angle and T angle. The absolute value of the difference between the QRS angle and the T angle was determined as the frontal QRS-T angle. (frontal QRS-T angle = | QRS axis – T axis |). If this difference was greater than 180 °, the frontal QRS-T angle was calculated again by subtracting 180 ° from this value (Fig. 1) (12). After the measurements, the best cut-off value of the frontal QRS-T angle for predicting mortality was determined with receiver operating characteristic (ROC) curve analysis (13). The best cut-off value of frontal QRS-T angle was found to be 101.5º. Our study population was divided into two groups according to this cut-off value as follows: patients with frontal QRS-T angle < 101.5º (absent QRS-T widening) and patients with frontal QRS-T angle ≥ 101.5º (present QRS-T widening).

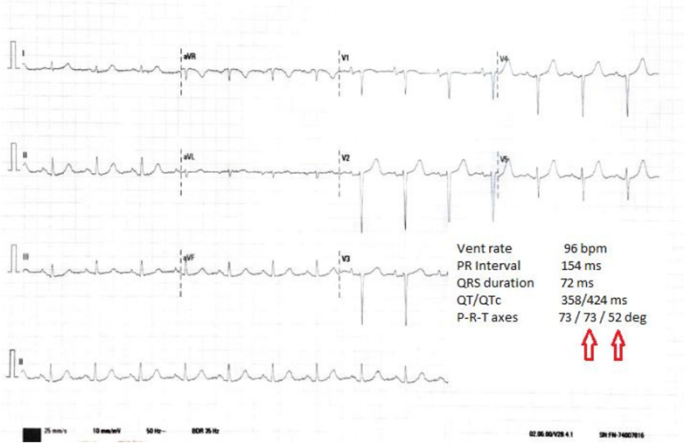

Fig. 1. Frontal QRS-T angle measurement

QTc prolongation: QTc ≥ 440 or 60 units higher than the previous value. It is based on the measurement automatically made by the ECG device.

QRS widening: QRS ≥120. It is based on the measurement automatically made by the ECG device.

2.2. Data Extraction

The patient's age, gender, and comorbid diseases were noted from the patient files. Heart rate, QT and QTc duration, QRS durations, and calculated frontal QRS-T angles were recorded from the ECG data of these patients. Troponin, creatinine, potassium, calcium, and CRP values were recorded from the hospital database. The ECGs taken during five days of treatment were interpreted, and the day with the highest QTc prolongation was determined. Creatinine, potassium, calcium, and CRP values were noted as maximum values on the day of the highest QTc prolongation.

2.3. Statistical Analyses

Statistical analysis was performed using SPSS version 23.0 software (SPSS Inc., Chicago, IL, USA). In descriptive statistics, the One-Sample Kolmogorov Smirnov test determines normal distribution, while categorical variables are expressed in numbers and percentages. Normally distributed variables were expressed as mean ± standard deviation and compared with independent sample t-test, whereas non-normally distributed variables were expressed as median (25-75th interquartile range) and compared with the Mann-Whitney-U test. The Chi-Square test calculates the difference between categorical variables. ROC curve analysis was performed to determine the best frontal QRS-T angle cut-off value for predicting mortality. Multivariate logistic regression analysis with backward elimination was used to determine the independent predictors of mortality. A p-value of <0.05 was considered statistically significant.

3. Results

After obtaining the ethics committee's approval, 302 patients

who were hospitalized for Covid-19, including intensive care units and regular services, were included in the study. One hundred and sixty-seven of 302 patients were excluded from the study according to exclusion criteria; a total of 135 patients were included. The mean age of these patients was 51.2 ± 19.5, and 63% were male. The most common comorbidity among the patients was hypertension (21.5%). While 45.9% of the patients received only HC treatment, 54.1% also received AZ treatment in addition to this treatment. Basal characteristics of the patients are shown in Table 1.

Table 1. Baseline characteristics of study population

	All patient n=135 mean±sd/ n(%)
Age	51,2±19,5
Gender	
Female	50 (%37)
Male	85 (%63)
Chronic diseases	
Diabetes Mellitus	16 (%11,9)
Hypertension	29 (%21,5)
COPD/Asthma	22 (%16,3)
Cancer	7 (%5,2)
Hospitalization	
ICU	48 (%35,6)
Regular service	87 (%64,4)
Intubated patients	19 (%14,1)
Medication	
HC+AZ	73 (%54,1)
HC	62 (%45,9)

AZ: Azytromycin, COPD: Chronic obstructive pulmonary disease, HC:Hydroxychloroquine, ICU: Intensive care unit

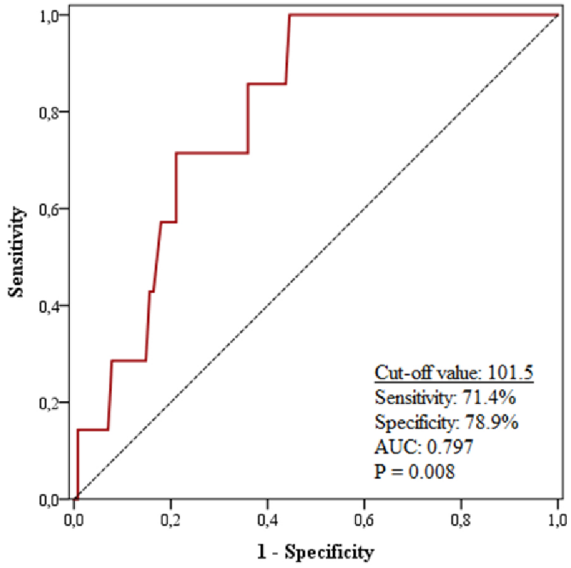

Fig. 2. ROC curve analysis of frontal QRS-T angle for predicting mortality

ROC curve analysis was performed to determine the best frontal QRS-T angle cut-off value for predicting mortality. The best frontal QRS-T angle cut-off value for predicting mortality was 101.5º (Fig. 2). Our study population was divided into two groups: patients with frontal QRS-T angle < 101.5º and patients with frontal QRS-T angle ≥ 101.5º. Basal characteristics of the patients according to the frontal QRS-T angle, laboratory, and ECG data evaluation are shown in Table 2. The mean age was significantly higher in those with increased frontal QRS-T angle (p <0.001). It was observed that the frequency of intubated patients was significantly higher in patients with widened frontal QRS-T angle (p = <0.001). It was observed from laboratory data that troponin (p <0.001) and CRP (p =0.002) values obtained during hospitalization were significantly higher in patients with widened QRS-T angle. In addition, QTc prolongation and QRS widening were significantly more frequent in patients with widened frontal QRS-T angle (p <0,001 and p = 0,001, respectively).

Table 2. Evaluation of baseline characteristics, laboratory and ECG data of the patients according to the frontal QRS-T angle

	QRS-t widening		
	Present (n=32)	Absent (n=103)	
	mean±sd / median (25-75th IQR) / n(%)		*p* value
Age	66,8 ± 14,6	46,4 ± 18,4	**<0,001**
Gender			
Female	13 (%40,6)	37 (%35,9)	0,630
Male	19 (%59,4)	66 (%64,1)	
Hospitalization			
ICU	28 (%87,5)	20 (%19,4)	**<0,001**
Regular service	4 (%12,5)	83 (%80,6)	
Intubated patients	14 (%43,8)	5 (%4,9)	**<0,001**
Chronic diseases			
Diabetes Mellitus	9 (%29)	7 (%6,8)	**0,002**
Hypertension	18 (%40,6)	16 (%10,7)	**0,003**
COPD/Asthma	11 (%34,4)	11 (%10,8)	**0,002**
Cancer	4 (%12,5)	3 (%2,9)	0,054
Labaratory initial			
Troponin-I	63,9 (26,3-117,8)	4 (3-8,5)	**<0,001**
Creatinin	1,13±0,49	0,91±0,27	0,025
K	4,49±0,89	4,25±0,50	0,153
CRP	62,1 (14,4-159,8)	23,0 (3,5-45)	**0,002**
QTc prolongation	26 (%81,3)	45 (%43,7)	**<0,001**
QRS widening	10 (%31,3)	7 (%6,8)	**0,001**
Mortality	5 (15,6)	2 (1,9)	**0,008**

AZ: Azytromycin, COPD: Chronic obstructive pulmonary disease, CRP: C-Reactive protein, HC: Hydroxychloroquine,ICU:Intensive care unit, K: Potasium, QTc: corrected QT

Baseline characteristics, and evaluation of laboratory and ECG data of patients with in-hospital mortality are shown in Table 3. All patients with in-hospital mortality were hospitalized in the intensive care unit (100%). In-hospital mortality was significantly higher in patients with diabetes mellitus and cancer (p = 0.036, and p = 0.003, respectively). The in-hospital mortality rate was significantly higher in patients with widened frontal QRS-T angle (p = 0.008).

Table 3. Evaluation of baseline characteristics, laboratory and ECG data of the patients with in-hospital mortality

	Exitus		
	Present (n=7)	Absent (n=128)	
	mean±sd / n(%)		*p* value
Age	63,9±13	50,5±19,6)	0,389
Gender			
Female	2 (%28,6)	48 (%37,5)	1
Male	5 (%71,4)	80 (%62,5)	
Hospitalization			
ICU	7 (%100)	41 (%32)	**0,001**
Regular service	-	87 (%68)	
Chronic diseases			
Diabetes Mellitus	3 (%42,9)	13 (%10,2)	**0,036**
Hypertension	1 (%14,3)	28 (%21,9)	1
COPD/Asthma	2 (%28,6)	20 (%15,6)	0,319
Cancer	3 (%42,9)	4 (%3,1)	**0,003**
Labaratory initial			
Troponin-I	41,5(23,4-87,5)	5 (3-23,6)	**0,008**
Creatinin	1,14±0,41	0,95±0,34	0,418
K	4,14±0,41	4,29±0,74	0,289
CRP	63 (29,8-172)	24,6(3,7-57,7)	**0,043**
Labaratory Max			
Creatinin	1,5±0,97	0,85±0,34	0,756
K	4,44±0,87	4,2±0,58	0,450
CRP	46 (22-238)	12 (2-51,3)	**0,018**
Medication			
HC+AZ	5 (%71,4)	68 (%53,1)	0,452
HC	2 (%28,6)	60 (%46,9)	
QRS max	102,7±10,3	97,3±33,8	0,565
QRS widening	1 (%14,3)	16 (%12,5)	1
QTc max	458,7±15	430,1±44,1	0,658
QTc prolongation	6 (%85,7)	65 (%50,8)	0,119
QRS-T	108,7±47,5	57,9±50	**0,010**
QRS-T widening	5 (%71,4)	27 (%21,1)	**0,008**

AZ: Azytromycin, COPD: Chronic obstructive pulmonary disease, CRP: C-Reactive protein, HC: Hydroxychloroquine, ICU:Intensive care unit, K: Potasium, QTc: corrected QT

Baseline characteristics, laboratory and ECG data evaluation of patients hospitalized in intensive care, and regular services are shown in Table 4. While the mean age of patients in the intensive care unit was higher (p <0.001), there was no significant difference in terms of gender. All chronic diseases were observed more frequently in intensive care patients. Considering the ECG data, QRS widening, QTc prolongation and widened frontal QRS-T angle were significantly more frequent in intensive care patients (p = 0,001, p <0,001, and <0,001, respectively).

Table 4. Evaluation of baseline characteristics, laboratory and ECG data of the patients staying in intensive care unit and regular service

	Intensive care unit stay		
	ICU	Regular	*p* value
	mean±sd / n(%)		
Age	66,3±14,7	42,9±16,7	**<0,001**
Gender			
Female	21 (42%)	29 (58%)	0,230
Male	27 (31,8%)	58 (68,2%)	
Chronic diseases			
Diabetes Mellitus	10 (62,5%)	6 (37,5%)	**0,016**
Hypertension	22 (75,9%)	7 (24,1%)	**<0,001**
COPD/Asthma	18 (81,8%)	4 (18,2%)	**<0,001**
Cancer	6 (85,7%)	1 (14,3%)	**0,008**
Medication			
HC+AZ	27 (37%)	46 (63%)	0,706
HC	21 (33,9%)	41 (66,1%)	
QRS widening	12 (70,6%)	5 (29,4%)	**0,001**
Max QRS	116±51	99±11	**0,011**
QTc prolongation	41 (57,7%)	30 (42,3%)	**<0,001**
Max QTc	459±33	433±22	**<0,001**
QRS-t widening	28 (58,3%)	4 (4,6%)	**<0,001**
QRS-t	104,4±45,3	36,4±35,7	**<0,001**

AZ: Azytromycin, Ca: Calsium, COPD: Chronic obstructive pulmonary disease, CRP: C- Reactive protein, HC: Hydroxychloroquine, ICU:Intensive care unit, K: Potasium, QTc: corrected QT

The evaluation of patients according to QTc prolongation is shown in Table 5. The mean age was significantly higher in those with QTc prolongation (p <0.001). Significantly, QTc prolongation was observed more frequently in patients hospitalized in intensive care, in patients who were followed up intubated, and in hypertensive patients (p <0.001, p = 0.003 and p = 0.016, respectively). It was observed that the troponin (p < 0.001) and maximum CRP levels (p =0.001) were significantly higher in the group with QTc prolongation.

Table 5. Evaluation of baseline characteristics, laboratory and ECG data of the patients with QTc prolongation

	QTc prolongation		
	Present (n=71)	Absent (n=64)	*p* value
	mean±sd / n(%)		
Age	57,3±19,3	44,5±17,6	**<0,001**
Gender			0,335
Female	50 (%37)	29 (%40,8)	
Male	85 (%63)	42 (%59,2)	
Hospitalization			**<0,001**
ICU	41 (%57,7)	7 (%10,9)	
Regular service	30 (%42,3)	57 (%89,1)	
Intubated patients	16 (%22,5)	3 (%4,7)	**0,003**
Chronic diseases			
Diabetes Mellitus	16 (%11,9)	11 (%15,5)	0,168
Hypertension	29 (%21,5)	21 (%29,6)	**0,016**

COPD/Asthma	22 (%16,3)	15 (%21,1)	0,109
Cancer	7 (%5,2)	6 (%8,5)	0,119
Labaratory initial			
Troponin-I	12 (3,9-50)	3,05 (3-7)	**<0,001**
Creatinin	1,02±0,39	0,91±0,28	0,072
K	4,31±0,89	4,25±0,47	0,509
CRP	29,8(5,4-92,4)	23,8(2,8-45)	0,075
Labaratory Max			
Creatinin	0,92±0,44	0,85±0,37	0,361
K	4,14±0,68	4,29±0,47	0,235
CRP	25,9(6,7-130)	9,4 (1,0-25,9)	**0,001**
Medication			
HC+AZ	73 (%54,1)	36 (%50,7)	0,408
HC	62 (%45,9)	35 (%49,3)	

AZ: Azytromycin, COPD: Chronic obstructive pulmonary disease, CRP: C-Reactive protein, HC: Hydroxychloroquine, ICU:Intensive care unit, K: Potasium, QTc: corrected QT

Elongation days of QTc in patients with QTc prolongation are shown in Figure 3. QTc prolongation was observed in 71 (52.6%) of the patients. There was no significant difference between those who only received HC and those who received HC+AZ in treatment (p = 0.408). In both groups, QTc prolongation was the most common on the 4th day of treatment (43.8% versus 46.8%).

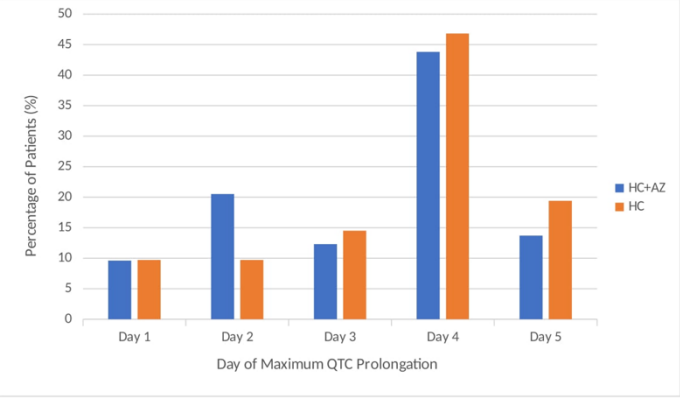

Fig. 3. Elongation days of QTc in patients with QTc prolongation

The independent predictors of mortality were determined by multivariate logistic regression analysis. It was found that frontal QRS-T angle ≥ 101.5 (OR: 7.08, 95%CI: 1.17-42.75, P = 0.033) and cancer (OR: 15.19, 95% CI: 2.17-106.40, P = 0.006) were the independent predictors of mortality (Table 6).

Table 6. Multivariate logistic regression analysis for demonstrating the independent predictors of mortality

	Odds Ratio	95% Confidence Interval	P
Frontal QRS-T angle ≥ 101.5º	7.08	1.17-42.75	**0.033**
Cancer	15.19	2.17-106.40	**0.006**

Entered variables: age, gender, QRS widening, QTc prolongation, frontal QRS-T angle ≥ 101.5º, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, cancer, initial troponin, creatinine and c-reactive protein, and maximum c-reactive protein

4. Discussion

In this study, we investigated the relationship between the severity of the disease and in-hospital mortality with the basal frontal QRS-T angle in the ECGs of Covid-19 patients during admission to the hospital. In addition, we examined whether the QTc prolongation was caused by AZ and HC, which are the drugs used in the treatment of Covid-19 disease, has a significant effect on mortality.

The rate of cardiac damage was around 20% in Covid-19 patients (14). Many studies investigating myocardial damage have shown that cardiac biomarkers, especially cardiac troponin I and T, increase in infected patients (15). However, there has not been a study based on ECG data to determine this cardiac damage at the time of admission to the hospital. This study was planned because it is thought that with the early detection of this cardiac damage, relevant measures can be taken earlier.

Although the spatial QRS-T angle is a better prognostic marker for cardiac risk estimation, in this study, we used the frontal planar QRS-T angle instead of the spatial QRS-T angle because special software is required for spatial QRS-T measurement, and the more complex special knowledge is required for this measurement (16). As non-cardiologist intensive care professionals, we found it appropriate to use the frontal QRS-T angle in our study because it has a more practical measurement and does not require special knowledge. Previous studies have shown that frontal QRS-T measurement is an appropriate clinical substitute for spatial QRS-T measurement in risk estimation (17). Previous studies have shown that the frontal QRS-T angle is useful in determining repolarization abnormalities before significant ECG changes occur (7). Damaged or inhomogeneous areas of the myocardium due to ischemia cause abnormal ventricular repolarization, and an increased QRS-T angle emerges (18). In recent years, many studies have shown that cardiac damage can be determined by widened frontal QRS-T angle. The clinical benefit of frontal QRS-T widening has been established in anterior myocardial infarction (19,20), hypertrophic cardiomyopathy (21), ischemic cardiomyopathy(22), and myocarditis (23). In our study, increased basal frontal QRS-T angle, QTc prolongation, and QRS widening were significantly common in intensive care patients. In addition, when we included all electrocardiographic parameters in the multivariate analysis, we found that only widened frontal QRS-T angle was the independent predictor of mortality. Therefore, it can be concluded that there is a significant difference between the prolongation of the basal frontal QRS-T angle at the time of admission to the hospital and the severity of the disease and in-hospital mortality.

Since the use of HC in autoimmune diseases is very old, it has been known for a long time that it can prolong QTc [3]. In addition, AZ is an antibiotic known to prolong QTc, and these

two drugs are often used in combination in Covid-19 patients. Studies have reported that the QTc prolongation effect of these drugs is more common in patients with known cardiac disease (4,24). It has also been shown that the use of these drugs together with other drugs known to prolong QTc (such as antiarrhythmics, antidepressants, and antibiotics) has been shown to potentialize QTc prolongation (25). We, therefore, did not include patients with known heart disease and those who normally use drugs known to cause QTc prolongation in this study, as we wanted to investigate whether adding AZ to the treatment in our study potentializes QTc prolongation, as the effect of HC administration. In addition, in order to understand which day of the treatment will QTc prolongation is common, we included patients whose ECG was recorded for five days of HC treatment in our study. In this way, we thought that in patients who are not monitored, such as intensive care, we could at least have an idea on which days ECGs should be taken. In correlation with previous studies (18), we found that QTc prolongation was more common in the ECG on our study's 4th day of treatment. In addition, we found that concomitant AZ use did not potentialize QTc prolongation in patients using HC. This finding was inconsistent with some previous studies (24,25). However, in none of these studies, patients with cardiac disease and those using drugs such as antiarrhythmic and antidepressants are known to prolong QTc were not excluded from the study. The different study results may be due to the different study designs.

As we wanted to examine the effects of drugs on QTc prolongation, it was important to have 5-day ECGs of the patients. Since there are reservations in terms of transmission of Covid-19 disease, having ECG from patients every day may increase the risk of disease transmission. By using methods such as telemetry, reservations such as the transmission of the disease can be removed, and ECGs of the patients can be accessed safely (26). However, in centres where such methods are not available, we believe that ECGs of the patients should be taken at least on the 4th day of the treatment to check whether there is QTc prolongation. It is important to determine if there is QTc prolongation in patients before home discharge and if necessary, it should be consulted with the cardiologist.

Our study has many limitations. The most important is the retrospective design of our research. In addition, patients who did not have a baseline ECG and did not have 5-day ECGs during treatment were not included in the study. This fact may present a bias. Our study was single-centred, and the limited number of patients was also an important limitation.

At the time this study was designed, hydroxychloroquine was routinely given to covid 19 patients in TÜRKİYE. Azithromycin could be added to the patient's treatment according to the clinician's preference. Other treatment methods (antiviral treatments, plasma therapy, etc.) were unavailable in our country at the time this study was designed. It is important to evaluate the study from this perspective.

As a result, the increased Frontal QRS-T angle in Covid-19 patients increases the severity of the disease and mortality rates. The frontal QRS-T angle seems to be a standard parameter that can be used in the follow-up of Covid-19 patients due to its advantages, such as not needing any extra knowledge about its evaluation, being easily calculated by anyone with standard ECG data, and not causing additional costs such as blood tests.

Conflict of interest

The authors have no conflicts of interest to disclose.

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The study was performed in Department of Intensive Care, University of Health Sciences, Mehmet Akif Inan Training and Research Hospital

Authors' contributions

Concept: G.P., B.C., Design: G.P., B.C., Data Collection or Processing: G.P., M.T., Analysis or Interpretation: G.P., M.T., T. B.T., F.T.B., Literature Search: G.P., B.C., T.B.T., F.T.B., Writing: G.P., B.C., M.T., T.B.T., F.T.B

Ethical Statement

Approval was obtained from Harran University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 15/06/2020 and the number of ethical committee decisions is HRU/20.11.18.

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Mineral composition, in-vivo hematinic and antioxidant potential of *Jatropha gossypiifolia* n-hexane root extract in hemolytic anemic rats

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Abstract

This study screened for the mineral composition and in-vivo hematinic and antioxidant properties of *J. gossypiifolia* root n-hexane extract in Wistar rats. Standard protocols were used for the mineral constituents of *J. gossypiifolia* root. Forty-five male Wistar rats were randomly selected for this study. They were administered 40 mg/kg phenylhydrazine hydrochloride to induce-hemolytic anemia for five days and thereafter treated with graded oral doses of 30, 50, and 100 mg/kg *J. gossypiifolia* extract for 14 days. Hematological, antioxidant, peripheral blood smear and histopathological evaluations of the blood cells and bone marrow were carried out. The results of the mineral composition of *J. gossypiifolia* physiological quantity include; calcium, potassium, sodium, chloride, manganese, iron, zinc, magnesium, and phosphorus. Calcium and magnesium were abundant at (33.14 and 8.34 mg/1kg) of the plant root. The results for the treatment groups at graded doses include; red blood cells at days 7 (4.68, 4.72, 4.82 x 10^6/ul) and 14 (6.51, 6.59, 6.82 x 10^6/ul), hematocrit at days 7 (40.30, 46.13, 48.63 %) and 14 (40.30, 41.47, 45.30%) and hemoglobin at days 7 (10.18, 10.92, 11.82 g/dl) and 14 (11.40, 11.87, 12.90 g/dl) when compared with untreated control ($p<0.05$). The peripheral blood smear showed normal blood morphology across the treatment groups compared to untreated control rats. The histopathological architectural framework of the bone marrow elicited an excitatory effect of myeloid/erythroid cell ratio in the treatment groups when compared with phenylhydrazine control. *J. gossypiifolia* extract displayed hematinic potential, which concurred with its ethnomedicinal report.

Keywords: mineral constituents, hematinic, antioxidants, *Jatropha gossypiifolia*

1. Introduction

In Nigeria, plants have been widely used as an alternative medicine since the event of orthodox medicine is prone to drug resistance, side-effect, non-economical, and non-readily available. Hence, herbal remedies with plant-based medicine have been scientifically proven with their potency, availability, less or no adverse effect, and very economical (1). This classification has helped World Health Organization set a standard in evaluating herbal products' possible safety, efficacy, and quality (2).

Jatropha gossypiifolia is a shrub plant that belongs to the family Euphorbiacea (2). It is generally known as Bellyache-bush, pignut, and wild cassava in English; botuje pupa (Yoruba), Cini da zugu, or Binidi zugu (Hausa), and ake mbogho (Igbo) (3). *J. gossypiifolia* has been used to treat several infectious diseases globally. Various parts of the plant have been used to manage several diseases, such as anemia, venereal disease, and so many diseases in ethnomedicines (4).

Anemia is a blood disorder that frequently affects people in all age brackets. People prone to higher risk include; adolescent women of child-bearing age, infants, and older adults (5). Anemia is of various types, such as hemolytic, sickle, and many more (6). Pregnant women are susceptible to anemia, resulting in several complications during fetal development (7). Anemia is one of the leading causes of death, either due to a lack of vital mineral constituents capable of triggering blood-related diseases like anemia. Thus, orthodox medicine has failed due to its verse adverse effect and drug resistance. This study aimed to screen for the mineral constituents of the plant and determine the hematinic and in-vivo antioxidant potential of *J. gossypiifolia* in rats. The investigation of the hematinic property of *Jatropha gossypiifolia* root extract was carried out.

2. Materials and Methods

2.1. Collection of Plant material

The young root of *Jatropha gossypiifolia* Linn. was collected in August from Oluku Estate, Ovia North East LGA, Benin City, Edo State. Dr. O. Timothy from the Department of Plant Biology and Biotechnology, Life Sciences, University of Benin, Nigeria, identified the plant. The plant was authenticated by Dr. H. A. Akinnibosun in the Herbarium Unit

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of Plant Biology and Biotechnology, Life Sciences, University of Benin, Nigeria, with voucher specimen number UBH-M511.

2.2. Preparation of Plant Material

The root of *Jatropha gossypiifolia* was rinsed, chopped into pieces, and air-dried at room temperature. The plant materials were further dried using an oven at a regulated temperature of 40°C for 10 minutes before being pulverized using a British mechanical grinder. Four thousand grams (4000 g) of the pulverized root was extracted with 5,000 ml of n-hexane via a Soxhlet extractor. The extract was concentrated semi-solid using (HH-S Water Bath; Search Tech Instruments) at a controlled temperature (45°C). Percentage yields were calculated via the formula (% Yield=extract weight/powder sample weight x 100/1).

2.3. Determination of mineral elements' composition

"The mineral element analysis was carried out using a modified procedure described by Silva et al. (8). The analysis of minerals, including calcium, potassium, sodium, chloride, manganese, iron, zinc, and phosphorus, was conducted using an Agilent FS240AA atomic absorption spectrophotometer. Magnesium was measured twice, once as a separate measurement and once as magnesium phosphorus. Approximately 2 g of the dried sample was placed into a digestion flask, and 20 ml of the acid mixture (650 ml conc. HNO_3; 80 ml perchloric acid; 20 ml conc. H_2SO_4) was added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 100 ml mark. A series of standard metal solutions in the optimum concentration range were prepared. The reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/liter. A calibration blank was prepared using all the reagents except the metal stock solutions. A calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations."

2.4. Experimental animals

Forty-five (45) healthy whisker (albino) rats (males) weighed 180-250 g. The animals were acquired from Animal and Environmental Biology at the University of Benin animal house. They were housed in well-ventilated wooden cages in a standard laboratory state (12 hours light/dark cycle: 23 ± 2°C) and fed using a standard diet. Food and water were administered at free choice (ad libitum) to the animals used for experiments. The animals were handled correctly using the ethics of Laboratory animals' approval from the ethical committee of the Faculty of Life Sciences with the ethical number LS21009.

2.5. Experimental Design

Phenylhydrazine hydrochloride was given to the entire group to induce anemia using a modified method by Sanni et al. (9). Five groups (n=9) received the following scheduled treatment. The reference group was pre-exposed to ferrous (iii) - hydroxide poly-maltose 5 mg/kg orally p.o., and the untreated group was administered with phenylhydrazine hydrochloride 40 mg/kg p.o. Other groups were pre-exposed to graded doses (30, 50, and 100 mg/kg derived from the pilot study) of *Jatropha gossypiifolia* n-hexane root extract. Animals were fasted overnight before administering phenylhydrazine hydrochloride for five days according to their body weight. Three (3) rats were sacrificed from each group on days 0, 7, and 14, and the blood sample, bone marrow, and other organs were analyzed for histopathological evaluation.

2.6. Hematological analysis

Blood samples in the EDTA bottles were injected into the chamber of the human-automated hematology system analyzer and diluted with an isotonic saline solution. Indices analyzed included hemoglobin, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration (it takes the volume or size of the red blood cell), mean corpuscular hemoglobin (mass or weight of red blood cell), platelet count, white blood cell count and differential white blood cell count (10).

2.7. Determination of antioxidant properties of the extract

Superoxide Dismutase (SOD) method was earlier reported by Bagul et al. (11) and Magili and Bwatanglang (12). Catalase assayed method was reported by Bagul et al. (11). Malondialdehyde activity was examined using a described method by Bagul et al. (11) and Magili and Bwatanglang (12).

2.8. Histopathological of Bone marrow

The isolated Bone marrows of Wistar rats were fixed in neutral buffered formalin. Affixed organs were utterly dehydrated, prepared, and interpreted via a modified method by Drury and Wallington (13).

2.9. Data Analysis

Results were analyzed with Graph pad prism version 6. Data were presented as Mean±S.E.M, and statistical significance was calculated using one-way ANOVA, followed by Dunnett's multiple comparison tests where $p<0.05$ were considered statistically significant.

3. Results

3.1. Mineral composition

Table 1 shows the mineral composition (calcium, potassium, sodium, chloride, manganese, iron, zinc, lead, magnesium, and phosphorus) of *J. gossypiifolia* n-hexane root extract at various concentrations.

3.2. Hematological Indices

The results in Fig. 1 showed a significant increase in red blood cell count and hemoglobin value on days 7 and 14 of 50 and 100 mg/kg root extract when compared with untreated control. This elicited that the plant at 50 and 100 mg/kg triggered the release or synthesis or rapidly matured the RBC and HGB, which could be responsible for the availability of the quantity of mineral content (iron) present.

Results in Fig. 2 exhibited a significant increase in

hematocrit and MCV values across the treatment groups on days 1, 7, and 14 of graded doses (30, 50, and 100 mg/kg) when compared with untreated control. It was observed that the plant extract displayed a quick onset of action of hematocrit and MCV, which will serve as a viable anti-anemic agent.

Table 1. Minerals composition of *Jatropha gossypiifolia* root extract

Minerals Composition	Concentration (mg/1kg)	Limit of Detection (LOD) ng/L	Limit of Quantitation (LOQ) ng/L
Calcium	33.14	11.16	33.82
Potassium	0.98	0.33	1.0
Sodium	0.1	0.03	0.10
Chloride	0.76	0.26	0.78
Manganese	0.021	0.01	0.02
Iron	0.65	0.22	0.66
Zinc	0.12	0.04	0.12
Magnesium	8.34	2.81	8.51
Phosphorus	0.73	0.25	0.75

The results in Fig. 3 indicated a significant increase in MCHC and MCH values across days 1, 7, and 14 of the treatment groups (30, 50, and 100 mg/kg) of *J. gossypiifolia* when compared with the untreated control.

Results in Table 2 showed a significant increase in white blood cell count in the treatment groups (30, 50, and 100 mg/kg) *J. gossypiifolia* on days 1, 7, and 14, compared with the untreated control, showed a significant decrease in WBC count.

3.3. In-vivo antioxidant

Glutathione and catalase are enzymatic antioxidants that elicited a significant increase in the scavenging capacity of graded doses of the extract when compared with the control in rats. Superoxide dismutase (SOD) is an enzymatic antioxidant that displayed a non-significant difference in the scavenging capacity of the extract when compared with the control in rats. Malondialdehyde (MDA) is a non-enzymatic antioxidant that exhibited a significant reduction, thereby enhancing the scavenging capacity of the extract when compared with the control in rats, as displayed in Table 3.

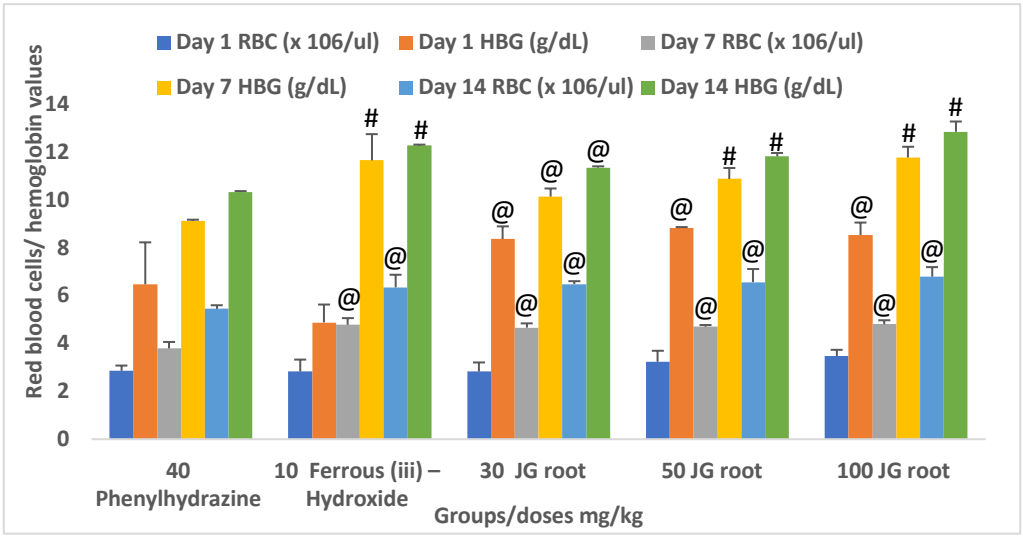

Fig. 1. Effects of *Jatropha gossypiifolia* root extract on phenylhydrazine-induced anemia in rats' red blood cells and hemoglobin. *p-value <0.05* showed the level of significant, superscript [@] and [#] indicated a significant increase (@ indicated significant increase while # indicated highly significant increase), JG; *Jatropha gossypiifolia*

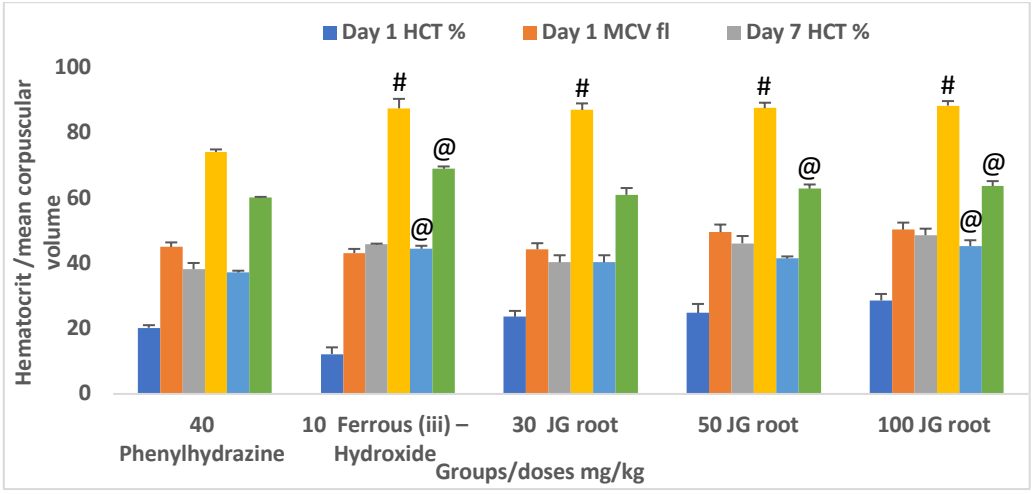

Fig. 2. Effects of *Jatropha gossypiifolia* root extract on phenylhydrazine-induced anemia in rats' hematocrit and mean corpuscular volume. *p-value <0.05* showed the level of significant, superscript [@] and [#] indicated a significant increase (@ indicated significant increase while # indicated highly significant increase) JG; *Jatropha gossypiifolia*

Table 2. Effects of *Jatropha gossypiifolia* root extract against phenylhydrazine-induced anemia in rats' white blood cells

Groups	Doses (mg/kg)	Mean±SEM DAY 1 WBC x 10^3/µl)	Mean±SEM DAY 7 WBC (x 10^3/µl)	Mean±SEM DAY 14 WBC (x 10^3/µl)
Phenylhydrazine	40	19.43±2.20[a]	6.70±5.24[a]	3.00±0.53[a]
Ferrous (iii) – Hydroxide	10	47.87±8.49[c]	7.80±0.40[a]	9.77±0.09[c]
JG root	30	44.13±3.64[b]	7.47±0.03[a]	7.40±0.12[b]
JG root	50	44.47±2.32[b]	7.80±1.27[a]	9.87±0.90[c]
JG root	100	48.27±0.72[c]	8.27±0.72[b]	10.30±3.52[c]

P-value <0.05 showed the level of significant, and superscript indicated non-significant JG; *Jatropha gossypiifolia*

Table 3. Effects of *Jatropha gossypiifolia* root extracts against phenylhydrazine-induced anemia on *in-vivo* antioxidant assay in rats

Groups	Doses (mg/kg)	Mean±SEM Glutathione (µg/ml)	Mean±SEM SOD (µg/ml)	Mean±SEM Catalase (µg/ml)	Mean±SEM MDA (10^{-4})
Phenylhydrazine	40	77.57±1.80[a]	0.043±0.00[a]	0.247±0.14[a]	5.80±0.10[a]
Ferrous (iii) – Hydroxide	10	85.37±1.93[b]	0.045±0.01[a]	0.480±0.01[b]	4.20±0.90[b]
JG root	30	83.40±0.51[b]	0.040±0.01[a]	0.466±0.02[b]	4.47±0.260[b]
JG root	50	81.01±1.01[a]	0.035±0.00[a]	0.480±0.01[b]	4.30±0.520[b]
JG root	100	82.25±0.95[a]	0.043±0.00[a]	0.483±0.01[b]	4.93±0.090[a]

P-value < 0.05 showed the level of significant, and superscript indicated non-significant JG; *Jatropha gossypiifolia*

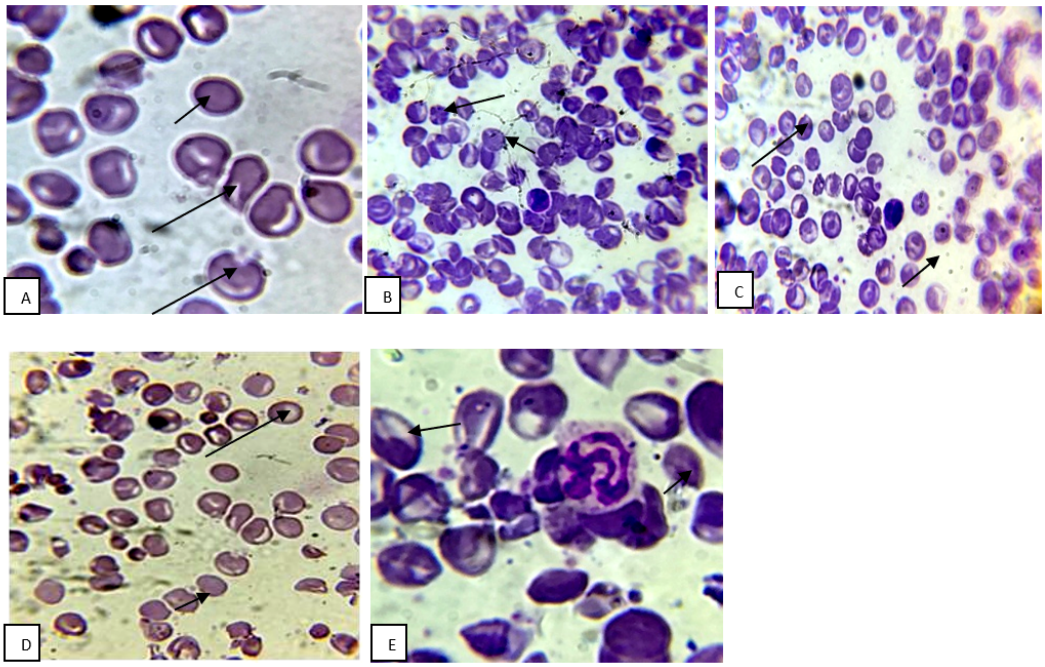

Fig. 4. Effects of the n-hexane root extract of *Jatropha gossypiifolia* on peripheral blood smear; **A,** Untreated group: Erythrocytes showed macrocytes, hypochromic, polychromatic cells, and Sickle shape. Normal WBC and platelet; **B**, Ferrous (iii) – Hydroxide: Erythrocytes showed normocytic and normochromic cells. Adequate and normal WBC and platelet; **C**, 30 mg/kg extract: Erythrocytes appeared normocytic, normochromic, and lysed cells with no polychromatic cells. Adequate and normal WBC and platelet; **D**, 50 mg/kg extract: Erythrocytes showed normocytic, lysed, and normochromic cells. Normal WBC and platelet; **E**, 100 mg/kg extract: Erythrocytes appeared in normocytic and normochromic cells with no polychromatic cells—adequate and normal WBC and platelet.

3.4. Peripheral blood smear

Fig. 4 displays normal blood normocytic, normochromic, and homochromatic cell morphology and structures across graded doses of the treatment groups (root extract of *J. gossypiifolia*) when compared with untreated control that displayed a macrocytic, hypochromic, polychromatic cells and Sickle blood shape.

3.5. Bone marrow analysis of rats fed with *Jatropha gossypiifolia*

The bone marrow in the treatment groups (root extract of *J. gossypiifolia*) of the extract displayed an increase in the level of Mylo-erythroid cells when compared with anemic control that had reduced Mylo-erythroid cells, as shown in Fig. 5.

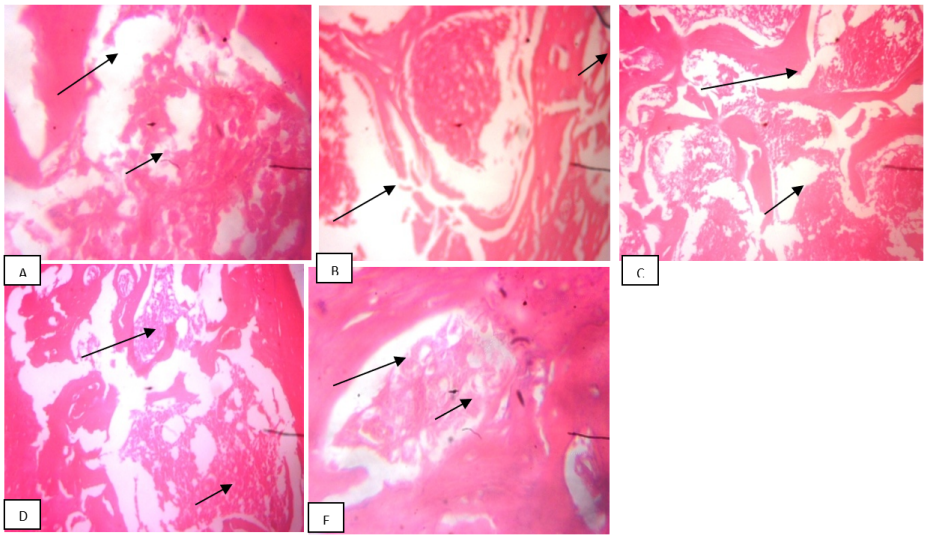

Fig. 5. Effects of n-hexane root extract of *Jatropha gossypiifolia* on the bone cell regeneration; **A**, Untreated group: The number of bone cells (Mylo-erythroid cells) decreased with cartilage surface areas appearing exfoliated with cracks. The bony trabeculae appear loose, irregular, and slender. All types of cells were scattered and sparsely distributed; **B**, Ferrous (iii) – Hydroxide: The bone marrow cavity was rich in bone marrow cells (Mylo-erythroid cells), with few fat cells—the visible histologic appearance of the bone trabeculae, bone marrow, and cartilage; **C**, 30 mg/kg extract: The bone marrow cavity has fewer bone marrow cells (Mylo-erythroid cells) and no fat cells. Normal morphology was noted, revealing a normal histologic appearance of bone trabeculae, bone marrow, and cartilage; **D**, 50 mg/kg extract: The bone marrow cavity has scanty bone marrow cells (Mylo-erythroid cells) with bone trabeculae, bone marrow, and cartilage; **E**, 100 mg/kg extract: The bone marrow cavity has fewer bone marrow cells (Mylo-erythroid cells) and no fat cells. Normal morphology revealed a normal histologic appearance of bone trabeculae, bone marrow, and cartilage.

4. Discussion

The present study showed that the calcium content level present in the root of *J. gossypiifolia* (Table 1) plays a functional role in strengthening bone marrow, which can stimulate erythrocyte regeneration, especially in anemic conditions. Additionally, as Faokunla et al. (14) reported, muscles, heart, bones, and teeth require calcium to function properly, with an increase in the quantity required for vital biological functions. Hence, the presence of this mineral content in *J. gossypiifolia* root, whose calcium level had a higher concentration than a similar report by Mustapha et al. (15) working on a related elemental constituent found in *Vitex doniana* sweet (black plum) stem bark, having calcium content (Table 1). The World Health Organization (WHO) places a safe calcium content limit at 3.6-80 mg/kg (16). The root of *J. gossypiifolia* showed the presence of magnesium content, which slightly exceeds the safety limits recommended by WHO. Therefore, caution is needed with the quantity consumed. The results of this present study are not significantly different from the recommended values, unlike the work of Faokunla et al. (14), who worked on Amaranthus hybridus leaves (23.18 mg/100g) and *Cassia siamea* (400 mg/100 g), whose results are greater than the recommended values. It is proposed that the root of *J. gossypiifolia* could be a viable source of magnesium (Mg) useful in managing extracellular fluid, bone, and plasma for osmotic balance. It is suggested that the safety limits for magnesium should not exceed 0.1-0.2 mg/kg, as recommended by WHO. Hence, the obtained doses present in the root of *J. gossypiifolia* slightly exceeded the recommended doses, and caution is needed with the quantity consumed (16).

The results obtained from this present study showed that the root of *J. gossypiifolia* possesses a slight increase in potassium content above the recommended safe limit (17). Therefore, the results obtained from the present study are better than those reported in the work of Uzama et al. (18) on *Securinega virosa* leaves at 3.67 mg/g. WHO (16) and Faokunla et al. (14) suggested that the harmless potassium limit is 0.01-0.1 mg/kg. The root of *J. gossypiifolia* is a restrained source of sodium with a slight reduction from the recommended safe limit. Moreover, the quantity of sodium in the root of *J. gossypiifolia* is almost within the safe limit, better than the reported values from the work of Swati et al. (17); Uzama et al. (18) and Okwu and Josiah (19), who compared the values of 20.31 mg/kg of *Securinega virosa* leaves with 0.02 mg/g in *Bryophylum pinnatum* and *Aspilia Africana*. WHO (16) suggested that the protective sodium limit is 0.4-0.5 mg/kg. Zinc reduces the amount of hemoglobin associated with red cell membranes, thereby inhibiting the effect of calcium in causing hemoglobin retention by membranes (20, 21). The zinc content in the root of *J. gossypiifolia* falls within the required safety limit. This present study concurred with the report of Faokunla et al. (14), which closely relates to the result of *Mucuna sloanei* content (0.25 mg/kg) and the leaves of *I. astragelina* (0.11 mg/kg), but is lower compared to the 6.85 mg/kg found in the leaves of *Cassia siamea*. Zinc is comparatively harmless despite being a microelement like manganese, which is vital to human health and cannot be overstated (22). "The root of *J. gossypiifolia* is rich in crucial micronutrients, as shown in Table 1, and falls slightly below

the recommended safe limit. This is consistent with previous studies on other plants, including *Securinega virosa* (1.50 mg/g), *Mucuna sloanei* (0.65 mg/100g), green vegetable leaves (0.98 mg/g), and the leaves of *I. astragalina* (0.43 mg/g). The leaves of *Momordica balsamina L*. were found to contain 11.6 mg/g, the root of *Boerhavia diffusa* had 9.09 mg/kg, *Catharanthus roseus* leaves had 37.2 mg/kg, and the leaves of *Phyllanthus amarus* had 64.5 mg/kg, according to a report by Djama et al. (24)." According to the World Health Organization (WHO) (16), the safety limit for manganese is 0.1-20 mg/kg (25). WHO also recommends harmless limit values for phosphorus at 0.1-0.2 mg/kg, while the National Research Council (NRC) (26) provides a Recommended Dietary Allowance (RDA) for phosphorus at 700 mg/day for adults. Phosphorus deficiency can lead to osteomalacia, tooth decay, and rickets, as previously indicated in the report by Michael (27). *J. gossypiifolia* root is an excellent source of phosphorus, as indicated in Table 1. The plant root's phosphorus values fall within the safe limit, stimulating the bone marrow to synthesize more red blood cells and promoting a healthy kidney to release erythropoietin. This study agrees with the NRC's report on the RDA values for phosphorus at 700 mg/day for adults (27).

This present study displays the results of the anti-anemic property of *J. gossypiifolia* root n-hexane extract, which significantly enhances the synthesis and regeneration of blood cells across the treatment groups when compared with the control. This could be due to certain phytochemical constituents or certain mineral components present in the extract that is implicated in the regeneration of blood cells from bone marrow. It could also stimulate the instigation of erythropoietin present in the kidney cells responsible for triggering the synthesis of blood cells from the mature bone marrow of the spleen. The results obtained from the hematological index in the untreated control at days 7 and 14 (RBC, HGB, and HCT) when compared with graded doses of the treatment groups (root extract) at days 7 and 14 (RBC, HGB, HCT), which elicited a significant increase as displayed in Figures 1 and 2. The presence of iron and other required mineral constituents in *J. gossypiifolia* root, though in smaller amounts, contributed to the synthesis and regeneration and facilitated the maturation of red blood and hemoglobin cells. The present study showed the efficacy of *J. gossypiifolia* root extract against hemolytic anemia, thereby agreeing with the work of Yamoto and Maude (28).

The anti-anemic properties of the standard drug (10 mg/kg ferrous (III) – hydroxide) and *J. gossypiifolia* root extract at a graded dose (30, 50, and 100 mg/kg) on days 1, 7, and 14 demonstrated a curative effect in blood regeneration and quick maturation of red blood cells, making it unique in its capacity to promote blood cells. The study had similar results to the report of Yakubu et al. (29). The recovery process of the blood cells could result from the mechanism of action from the mineral component or stimulation of the kidney to release erythropoiesis needed in the bone marrow to regenerate blood cells. Hemoglobin (which carries oxygen in the blood), RBC, HCT, MCV, MCH, MCHC, and WBC exhibited a significant increase in the treated groups, as shown in Fig. 1-3 and Table 2. These findings agree with the work of Cole (30) on the ethanol extract of *Bougainvillea spectabilis*.

Malondialdehyde (MDA) is a product of lipid peroxidation (LPO), which is shown to be reduced by *J. gossypiifolia* root extract. The extract reduces the over-synthesis of free radicals in erythrocytes, as evidenced by the significant reduction in MDA levels in the treatment groups compared to the control (Table 3). This suggests that *J. gossypiifolia* controls the level of oxidative stress, possibly due to the presence of natural antioxidants present in the plant extract. This finding is in agreement with the report of Bagul et al. (11) on the evaluation of the free radical scavenging properties of two classical polyherbal formulations. Glutathione (GSH) and Catalase (CAT) levels exhibited a significant increase in the treatment groups when compared with the control, as they have direct radical-scavenging properties and are crucial constituents of glutathione peroxidase (GPx), capable of eliminating diverse hydroperoxides. This is similar to the work of Dickinson and Forman (31) on cellular glutathione and thiol metabolism. Superoxide dismutase (SOD) levels showed no significant difference in the treated groups when compared with the untreated control (32).

The n-hexane extract of *J. gossypiifolia* root triggered the production and regeneration of blood cells, either due to the presence of mineral constituents or other unestablished mechanisms of action. This resulted in a normal blood cell morphology, with cells being normocytic and normochromic within adequate ranges compared to the untreated control and standard drugs in the peripheral blood smear (see Fig. 4). This study showed better results than the report by Yakubu and Afolayan (33), who worked on the effect of *Fadogia arggrestis* stem aqueous extract and *Bulbine natalensis* stem aqueous extract on anemic rats. The bone marrow is known to be a source of blood production, regeneration, and facilitation of immature cells. The treated groups' bone marrow (*J. gossypiifolia* root n-hexane extract) showed an increase in the level of myelo-erythroid (M.E.) cells when compared with the untreated control. Meanwhile, a decrease in M.E. cells level may lead to a severe anemic state. These findings agreed with the earlier study of Claro et al. (34), which reported significant changes and an increase in red blood cell percentage and morphology in myelo-erythroid cells ratio, as shown in Fig. 5. Hence, *J. gossypiifolia* root extract elicited hematinic properties specifically on days 7 and 14 of the treatment groups. MacDonald Idu et al. (35) also found similar results in their report on the phytochemical screening, antioxidant study, and hematinic properties of Mojeaga herbal remedy using an animal model, which showed an enhancement or stimulation of blood formations.

The root of *J. gossypiifolia* extract demonstrated the presence of essential mineral constituents responsible for various biochemical and hematological functions in anemic rats, particularly on days 7 and 14 of treatment. Hence, further investigation is needed to isolate, elucidate, and purify the compounds for potential clinical applications.

Ethical Statement

The animals were handled correctly using the ethics of Laboratory animals' approval from the ethical committee of the Faculty of Life Sciences with the ethical number LS21009.

Conflict of interest

No competing conflict of interest.

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Not applicable to this section.

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Authors' contributions

Concept: B.O.G., M.I., Design: B.O.G., M.I., Data Collection or Processing: B.O.G., M.I., Analysis or Interpretation: B.O.G., M.I., Literature Search: B.O.G., M.I., Writing: S B.O.G., M.I.

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Allogeneic peripheral blood stem-cell transplantation and long-term survival outcomes - A retrospective observational study

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Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HCT) has been extensively investigated as a potentially curative treatment option for hematological malignancies and other cancers. The current study aimed to investigate the long-term survival outcomes after allo-HCT. We retrospectively analyzed the long-term survival outcomes of patients who received allo-HCT from April 2015 to March 2021 at the Department of Haematology, SMS Hospital for different malignancies. Data from 51 patients with a mean age of 18.7 ±10.79 years who underwent peripheral blood allo-HCT as their first transplant were included for analysis. The average follow-up period was 30 months with a mortality rate of 23.52% (n=12). Overall, 39 patients were still alive and complete response was observed in 36 (92.3%) of patients. In summary, this retrospective study evidenced that peripheral blood (PB) derived allo-HCT has some survival advantages and can be successfully performed with the appropriate conditioning regimen and graft versus host disease (GVHD) prophylaxis.

Keywords: allogeneic transplantation, peripheral blood stem-cell transplantation, conditioning regimen, retrospective observational study, survival outcomes

1. Introduction

Hematologic malignancies (HMs) are heterogeneous disorders that accounts for 6.5% of all cancers around the globe and the incidence continues to rise (1). Although the treatment strategies for HMs have gained tremendous headway, the morbidity and mortality rate attributed to HMs still remain substantial (2). At this juncture, hematopoietic stem cell transplantation (HCT) using hematopoietic progenitor cells from a donor (allogeneic HCT) or the patient (autologous HCT) is a potential therapy, including HMs (3). However, transplantation type also significantly impacts the clinical outcomes in patients. In general, malignancy type, age of the recipient, status and stage of disease, and graft versus host disease (GVHD) effects are the factors to be considered for selection of transplantation type (4).

Although autologous stem cell transplantation (auto-HCT) offers lower treatment related mortality (<5%), rapid immune reconstitution, and lower risk of GVHD. On downside, auto-HCT has a higher relapse rate for disease dissemination due to contamination of autograft with clonogenic tumor cells(4). Allo-HCT includes irradiation-based conditioning regimens (non-myelo-ablative or ablative), and infusion of alloreactive HCT (for anti-tumoral effect and active immunological effect). In myelo-ablative regimen patients, 15–25% of early mortalities were reported to be due to GVHD, immunosuppression-induced infections, and drug-induced toxicities (5). Therefore, allo-HCT can be preferentially recommended only in patients with high-risk features after relapse, or after initial chemotherapy (6). Allo-HCT also offers a lower risk for disease recurrence and long-term survival benefits due to immune graft-versus-malignancy effect (4, 7, 8). However, the therapeutic potential of allo-HCT remains limited by both acute and chronic GVHD (9, 10). Besides, there have been many changes in transplantation practices over the past decade. Therefore, in the current study we investigate new insights into long-term survival after allo-HCT.

2. Matherials and Methods

2.1. Study design and population

In this single-center observational study, data on patient with specific cancer types (Table 1) who underwent allo-HCT at Department of Haematology of SMS Hospital from April 2015 to March 2021 were collected from the medical records and analyzed retrospectively. Before initiation, the study was cleared by IRB. Patient informed consents were taken from all the participating patients.

2.2. Data collection

Data collected for analysis included the patients' clinical characteristics such as age, gender, histological subtype of cancer, disease status, transplantation type and chronological

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order, conditioning regimens, acute GVHD (aGVHD) and chronic GVHD (cGVHD), cause of death, etc. The major patient dependent factors for allo-HCT include age, ECOG status, donor availability, and unfavorable prognostic factors (response to chemotherapy, bone marrow involvement, etc) Patients with missing required data were excluded.

Table 1. Baseline patient characteristics, transplant, and treatment data

Characteristics	Number of patients (n=51), %
Age (years ± SD)	18.7(±10.79)
Sex	
Men	34 (66.67)
Women	17 (33.33)
Acute graft versus host disease (GVHD)	
Yes	27 (52.94)
No	24 (47.05)
Chronic GVHD	
Yes	05 (9.80)
No	46 (90.19)
Time in days (range)	618(8-1920)
Status at last follow-up	
Dead	12 (23.52)
Alive	39 (76.47)
Cause of Death (n=12)	
Renal failure	3 (25)
Infection	2 (16.66)
GVHD	5 (41.66)
Progressive disease (PD)	2 (16.66)
If Alive (n=39)	
Complete hematologic remission	36 (92.30)
Relapse	3 (7.69)
Chronological number of transplant - 1st transplant	51
Type of Transplant - Allogenic	
Allogeneic (matched donor)	42 (82.35)
Haploidentical	9 (17.64)
Donor	
Identical sibling	30 (58.82)
Matched/Mismatched relative	21 (41.17)
Type	
Peripheral blood	51
Aplastic Anemia (AA)	24 (47.05)
Acute myeloid leukemia (AML)	8 (15.68)
Thalassemia	5 (9.80)
Myelodysplastic syndromes (MDS)	5 (9.80)
Acute lymphocytic leukemia (ALL)	3 (5.88)
CML with blast crisis	2 (3.92)
Chronic myeloid leukaemia (CML)	1 (1.96)
Dyskeratosis congenita	1 (1.96)
Congenital dyserythropoietic anemia (CDA) type II	1 (1.96)
Acute promyelocytic leukaemia (APML)	1 (1.96)
Status at transplant	
1st Complete hematologic remission	6 (11.76)
First relapse	14 (27.45)
Not applicable	31 (60.78)
Diagnosis - conditioning regimen used	
Flu-Cy-ATG-Fludarabine+Cyclophosphamide+anti-thymocyte globulin	23 (45.09)
Bu-Flu – Busulfan+Fludarabine	15 (29.41)
Flu-Mel – Fludarabine+Melphalan	7 (13.72)
Melphalan	2 (3.92)
Cy-TBI – Cyclophosphamide and total body irradiation	1 (1.96)
Bu-Flu-ATG – Busulfan+Fludarabine+ anti-thymocyte globulin	1 (1.96)
Bu-Cy-MTX–Busulfan+ Cyclophosphamide+Methotrexate	1 (1.96)
Flu-Cy-MTX–Fludarabine+Cyclophosphamide+Methotrexate	1 (1.96)

2.3. Statistical analysis

Descriptive statistics were used to summarize the data using SPSS 22 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Patients and transplant characteristics

Baseline patient characteristics, transplant, and treatment data are presented in Table 1. Of the 51 included patients for analysis, 34 (66.67%) were female and 17 (33.33%) were male and the mean age was 18.7 ±10.79 years. Further, all the included patient underwent peripheral blood allo-HCT (PBHCT, matched donor (82.3%) and haploidentical (17.64%) as first transplant. Majority of the included patients were diagnosed with aplastic anemia [24 (47.05%)] followed by acute myeloid leukemia [8 (15.68%)]. At the time of transplant, around 11.6% and 27.5% were in complete response (CR) and first relapse, respectively. Further, Flu-Cy-ATG – Fludarabine + Cyclophosphamide + anti-thymocyte globulin was used as conditioning regimen in majority of the patients (45.09%) followed by Bu-Flu – Busulfan + Fludarabine (29.41%).

3.2. Post-transplant outcomes

At a mean follow-up date 30 months' post-transplantation, 39 patients were still alive and CR was observed in 36 (92.3%) of patients. Further, 12 (23.52%) were succumbed and the causes of deaths were related majorly to GVHD [5 (41.66%)] followed by infection and progressive disease, 16.6 % each.

4. Discussion

Since its first application in 1957 between identical twins, the field of allo-HCT has made ground-breaking progress (11). Among various transplantation methods, peripheral blood stem cell transplantation has become preferred stem cell source largely replacing bone marrow due to its ease of collection and quicker engraftment kinetics. In the recent with the existing literature support, the use of Allo-HCT using hematopoietic progenitor cells has increased greatly as a potentially curative therapy for many nonmalignant disorders and life-threatening cancers. The Allo-HCT has the advantage over other techniques such as autologous transplantation in terms of its contaminating tumor cells free grafts and lower risk for disease recurrences, respectively. The allo-HCT outcomes have also improved over the years due to a variety of factors - including transplant techniques, matching of donor-recipient, and better patient selection (12). On the downside, allo-HCT is still associated with some potentially fatal complications such as GVHD, graft failure, and regimen-related organ toxicity (4). Overall, allo-HCT advantages outweighed its disadvantages and emerged as preferred transplantation technique. Over the

years, its usage was also projected to be increased proportionally with the advancement of technology, where patients were received a risk-adapted, individualized multidisciplinary follow-up care.

As per literature, large number of our patients also underwent matched unrelated or related donor HCT, to avoid allogeneic transplant related risks and for best possible outcomes (12). However, long-term follow-up reports from Hilgendorf et al. (13) have reported that patients who received a transplant before the age of 35 years were observed to have faced greater challenges in terms of long term complications. Such complications were quite evident in our patient population too, due to their lower age during the time of transplantation. Such chief risk factors reported from the studies and from our patient experiences were observed to be age, GVHD, infections, and progressive disease (7, 9, 10, 13).

A retrospective analysis from the EBMT registry have measured the non-relapse mortality over the years and it was observed to be decreased over time: 29.7% from 1980 through 1989 to 12.2% in 2010 through 2016 (14). Similar results were reported from our study but with a slight increase in mortality, possibly related to the lower age of patients at the time of transplantation. On contrary, in a retrospective study conducted by Greco et al. (15) a long-term disease remission with improved outcomes were reported in younger patients, especially in terms of toxicities and non-relapse mortality. Whereas, the prominence of GVHD and infections as a cause of death was no surprise and has also been noted earlier in multiple studies (7, 10). So clinically in allo-HCT, an emphasis on controlling and reducing GVHD should remain as high priority. Simultaneously, extra care should be taken in all the patients during the pre and post transplantation to avoid any such infections and their related deaths caused due to the insufficient immunologic recovery (7, 16, 17).

Overall, the literature suggests that the better supportive therapy, advanced intensive care medicine (to control infectious diseases, GVHD, etc), reduction in the intensity of conditioning regimens, adapted treatment protocols for induction/conditioning, improved risk stratification, and patient selection can play a likely vital role in contributing to better outcomes and survival pre and post allo-HCT. It was also evident from the literature that patients who underwent transplantation and survived the first 5 years without any recurrence of original disease were also projected to have high life expectancy (10).

Nevertheless, the limitations of the present study include its retrospective nature, small sample size, and non-heterogeneity.

In summary, this retrospective study evidenced the lower risk of disease recurrence and survival advantage in patients treated with PB-derived allo-HCT by successfully adapting the appropriate conditioning regimen and GVHD prophylaxis. PB-derived allo-HCT can also be preferentially recommended in patients with high-risk features after relapse, or after initial chemotherapy. Nonetheless, to optimise long-term outcomes and to avoid late life-threatening complications, regular follow-ups are highly recommended. In future prospective comparative studies, the role of each transplant modality in different subsets of patients receiving different conditioning regimens should be defined along with long-term follow-up outcomes.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: M.K., J.Y., Design: M.K., J.Y., Data Collection or Processing: M.K., S.J., A.M., K.G., R.K., L.M., S.K., J.Y.; Analysis or Interpretation: M.K., J.Y.; Literature Search: M.K., J.Y.; Writing: M.K.

Ethical Statement

This study was approved by the Institutional Review Board and Ethics Committee of SMS Hospital, Jaipur as part of project in accordance with the 1964 Helsinki declaration and later amendments. As the study was retrospective, there was no study-specific consent. All patients granted verbal or written consent prior to and investigation or treatment.

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Research Article

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Hydrogen peroxide-induced oxidative stress and apoptosis in SH-SY5Y cells: Protective effect of *Momordica charantia* fruit extract

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Abstract

Oxidative stress triggers apoptosis in neuronal cells, resulting in cellular loss, which is critical in the pathogenesis of neurodegenerative diseases. *Momordica charantia* (MC), a traditional medicinal plant, is famous for its variety of health benefits, including its anti-diabetic, anti-inflammatory, and antioxidant properties. The purpose of this study was to investigate on how MC might affect oxidative stress and apoptosis caused by H_2O_2. First, we investigated whether ethanol extract of MC in the presence of H_2O_2 attenuated cell death in human neuroblastoma SH-SY5Y cells. MC improved H_2O_2-stimulated intracellular reactive oxygen species (ROS) production. Additionally, MC reduced caspase activation, which greatly improved cell viability and avoided H_2O_2-induced apoptosis. Through its anti-oxidant and anti-apoptotic properties, MC ethanol extract has been shown to be effective in protecting against H_2O_2-induced cell death.

Keywords: Hydrogen peroxide, Oxidative stress, Apoptosis, *Momordica Charantia*

1. Introduction

Neurodegenerative diseases are typically associated with cytoplasmic protein aggregation in neurons caused by oxidative stress and are characterized by apoptosis-induced progressive cell loss in specific vulnerable neuronal cells (1). Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases with clinical findings such as progressive cognitive loss (dementia) and motor impairment (ataxia). The quality of life and longevity of elderly people are both impacted by these cognitive and motor impairments (2). It is critical to develop effective treatment strategies for neurodegenerative diseases that affect the elderly population. Therefore, antioxidants which protect neuronal cells from oxidative stress-induced apoptosis could potentially be used as therapeutic agents for neurodegenerative diseases.

Because of their rich antioxidant content, natural products have long been used as traditional medicines for the treatment of neurodegenerative diseases (3). Many studies conducted over the last several decades have demonstrated the protective effects of polyphenolic compounds extracted from natural products against neuronal cell damage caused by oxidative stress (4,5). Bioactive polyphenolic antioxidants have neuromodulatory properties, activating various intracellular signaling pathways that are important for neuroprotection. The regulation of the mitochondrial apoptosis cascade, which is finely tuned by the imbalance of apoptotic and anti-apoptotic proteins, is the molecular mechanism of neuroprotection. Neuroprotection mediated by antioxidant polyphenols can be achieved by increasing the expression of the anti-apoptotic Bcl-2, thus preventing apoptosis (6). Another neuroprotective mechanism is the suppression of caspases in controlling oxidative stress-mediated apoptosis, which is significant in the pathogenesis of neurodegenerative disorders (7).

Momordica charantia (MC), known as bitter melon or bitter gourd with its rich polyphenol content, is widely grown and generally consumed as an important medicinal plant in Asian countries (8). MC contains several bioactive compounds: triterpene, protein, steroids, alkaloids, inorganic, lipid, and phenolic compounds (9). Recent studies have reported the anti-bacterial (10), anti-oxidant (11), anti-inflammatory (12), and anti-diabetic (13) effects of various MC extracts.

The aim of this study was to determine the contribution of MC to the control of H_2O_2-induced oxidative stress and apoptosis. In our study, we report that MC has biological activities to reduce H_2O_2-induced cell death and cellular ROS production. We also demonstrated that MC decreased caspase-3 and caspase-9 expressions, inhibiting the process of H_2O_2-induced apoptosis in SH-SYY cells.

2. Materials and Methods

2.1. 2.1. Preparation *Momordica Charantia* Ethanol Extract

Fresh plant material (Ripe fruit without seeds) weighing 350 grams was dried under the shade. The plant parts were fully

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dried, then ground and the 31,7 g of the powdered plant was macerated in 70% ethanol for 72 hours. The extract was filtered using filter paper, the filtrate evaporated using a rotary evaporator, and then crude extract was obtained in a lyophilizer.

2.2. Cell Culture

SH-SY5Y (Human neuroblastoma) cells were cultured using DMEM supplemented with 10% heat-inactivated FBS and 0.1% penicillin/streptomycin in a 75 cm^2 culture flask at 37 °C with 5% CO2 humidified atmosphere. Every 2-3 days, the medium was changed, and cells were subcultured once they had reached 80–90% confluency. Cells were collected by centrifugation at 1000 rpm for five minutes after being digested with 0.25% trypsin and then resuspended in new media. In suitable assay plates, cells were seeded, and they grow overnight. For further research, adherent cells were used.

2.3. Cytotoxicity assay

For cytotoxicity analysis, the MTT reduction assay was performed (14). In 96-well plates with 2% FBS media, SH-SY5Y cells were seeded at a density of $1{,}5x10^3$ cells/well for the MTT assay. The cells were serum-starved before extract or H_2O_2 treatment for 24 hours. H_2O_2 was incubated for 24 hours in the presence of the extract in the study, where we demonstrated the protective action of the extract against the H_2O_2 cytotoxicity. Following that, the cells were treated with MTT (0.5 mg/mL) for an additional 2–3 hours. Each well's medium was removed and then DMSO was added to dissolve the purple formazan crystals and the absorbance of each well's solution was then determined using a microplate reader at 570 nm.

2.4. Measurement of Intracellular ROS

H_2DCF-DA Reagent was used to measure intracellular ROS production (15). Cells were initially exposed to H_2O_2 at various concentrations (0-800 µM) for 1 hour to determine the concentration at which H_2O_2 stimulated intracellular ROS generation. However, to evaluate the ROS scavenging ability of the extract, 800 µm H_2O_2 was applied to the cells in the presence of various concentrations of the extract for 1 hour. Subsequently, cells were incubated in DMEM (without phenol red) with H_2DCF-DA Reagent (25 µM) for 30 minutes in the dark. The change in fluorescence intensity was detected by fluorescence spectroscopy with excitation/emission at 485 nm / 535 nm.

2.5. Western Blot Analysis

The standard procedure for Western blotting was followed (16). Cells were treated with H_2O_2 (150 µM) alone or in combination with varying concentrations of extract for 3 hours. Following the treatment procedure, cells were lysed in RIPA buffer, and the protein content was determined using a BCA protein assay kit in accordance with the manufacturer's instructions. Proteins were separated on polyacrylamide gels with a 10% concentration before being transferred to the PVDF membrane. The membranes were blocked using a non-fat milk solution for 1 hour, and then they were treated with specific primary antibodies overnight. The next day, after the primary antibody was removed, membranes were incubated with the matched secondary antibody for 4 hours. ECL reagent was used to make the bands visible, and Image J software was used to measure the bands' intensity.

2.6. Acridine orange/Ethidium bromide (AO/EB) staining

Acridine orange/ethidium bromide (AO/EB) double staining was used to examine apoptotic and necrotic morphological changes in cells under fluorescence microscopy (17). 150 µM H_2O_2-exposed cells in combination with or without varying concentrations of extract for 3 hours were collected and washed three times in phosphate-buffered saline (PBS). Following centrifugation at 1000 rpm, the pellets were gently resuspended in 20 µL of medium containing 2.5 µL of dye solution (100µg/mL in equal concentration, AO/EB in DMEM w/o Phenol red). Each image was captured with excitation at 488 nm and emission at 520 nm on fluorescence microscope slides coated with a total of 10 µL of the cell-dye combination. Three or more random images were acquired for each well. At least 200 cells were counted on each slide during the triplicate testing.

2.7. Statistical data analysis

The data was analyzed using the "Student's t test" from the SigmaPlot 12.0 package program. $p<0.05$ was regarded as statistically significant.

3. Results

3.1. *Momordica charantia* extract protected SH-SY5Y against H_2O_2-Induced Cell Cytotoxicity

The cell viability test was utilized to assess the viability of SH-SY5Y cells after exposure to various concentrations of *Momordica charantia* extract and to establish whether nontoxic concentrations of *Momordica charantia* may alleviate the toxicity of H_2O_2 against SH-SY5Y cells. First, we investigated how varied H_2O_2 and extract concentrations affected the viability of cells. Fig. 1A shows the findings of the cell viability percentage following treatment with various concentration of H_2O_2 and extract. The viability of SH-SY5Y cells was reduced to 80.08% and 52.87%, respectively, when treated with 50 µM and 75 µM of H_2O_2, compared to 100% in the control group. However, up to a concentration of 15 µg/ml, *Momordica charantia* extract showed no evidence of cytotoxicity (Fig. 1B).

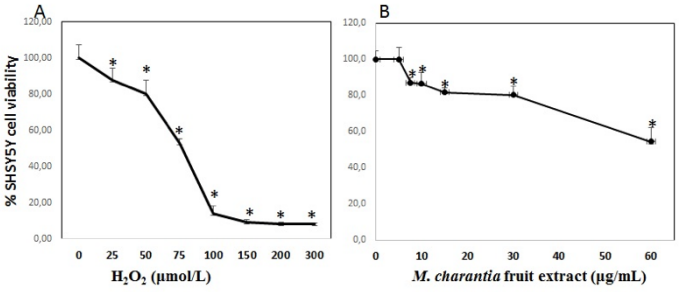

Fig. 1. A. The effects of H_2O_2 on the viability of SH-SY5Y cells. Cells were treated with indicated concentration of H_2O_2 for 24h. **B.** The effects of Momordica charantia fruit extract on the viability of

SHSY5Y cells. Cells were treated with a series of concentration of extract for 24 h. Data are expressed as mean ± SD of five independent experiments (n = 5). *p<0,05 vs. Control cell

As a result, the nontoxic concentrations of 5.0, 7.5, 10.0, and 15.0 µg/ml were selected for further tests to assess the protective effect of *Momordica Charantia* extract. However, *Momordica Charantia* extract treatment (at concentrations of 5.0, 7.5, 10.0, and 15.0 µg/ml) in the presence of H_2O_2 (50 µM and 75 µM) significantly inhibited in a concentration-dependent manner the cytotoxicity induced by H_2O_2 in SH-SY5Y cells vs. H_2O_2 treated cell. (Fig. 2).

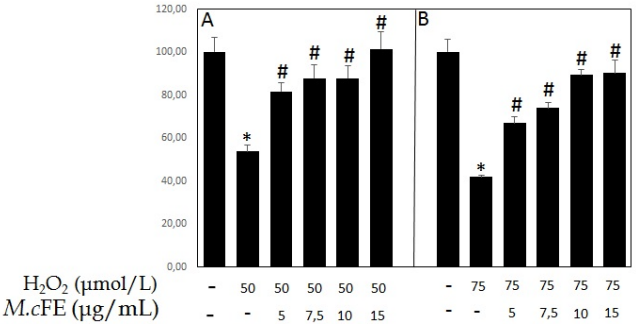

Fig. 2. Protective effects of Momordica charantia fruit extract on SHSY5Y cells against H_2O_2 -induced cell injury. Cells were treated with extract (5, 7,5, 10 and 15 µg/mL) in the presence of 50 and 75 µM H_2O_2 for 24 h. Data are expressed as mean ± SD of five independent experiments (n = 5). *p<0,05 *vs.* Control cell, #p<0,05

3.2. Effect of *Momordica charantia* extract on ROS production in H_2O_2-induced SH-SY5Y cells

The next step was to test the ROS production in cells using the H_2DCFDA reagent, a fluorescent dye that shows ROS, in order to see whether *Momordica charantia* extract might alleviate the oxidative stress caused by H_2O_2-induced ROS production. As can be shown in Fig. 3A, the H_2O_2-treated (0-800 µM) cells considerably raised the intensity of the DCF-liberated fluorescent signal in a dose-dependent manner, and the signal was markedly reduced in the presence of *Momordica charantia* extract. In the presence of Momordica charantia extract and H_2O_2 (800 µM) in the medium, DCF-liberated fluorescent signal decreased with increasing extract concentration (5.0, 7.5, 10.0, and 15.0 µg/ml), suggesting the H_2O_2 scavenging effect of the extract (Fig. 3B)

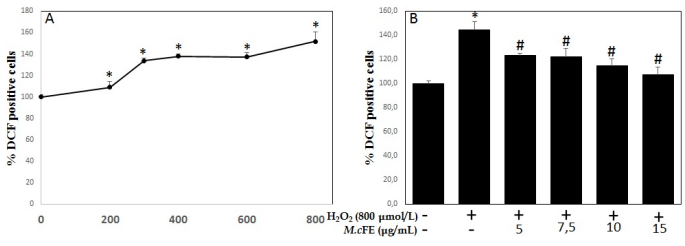

Fig. 3. A. The effects of H_2O_2 on ROS production in SHSY5Y cells. Cells were treated with indicated concentration of H_2O_2 for 1h. **B.** Effects of Momordica charantia fruit extract on intracellular ROS production. Cells were treated with extract (5, 7.5, 10 and 15 µg/mL) in the presence of 800 µM H_2O_2 for 1h. Data are expressed as mean ± SD of five independent experiments (n = 5). *p<0,05 *vs.* Control cell, #p<0,05 *vs.* H_2O_2 treated cell

3.3. H_2O_2 promotes caspase-3 and caspase-9 expression in SH-SY5Y cells: Effect of *Momordica charantia* extract

Apoptosis-associated protein (caspase-9 and caspase-3) levels were assessed to validate *Momordica charantia* extract protection against H_2O_2-induced apoptosis. Compared to H_2O_2 treatment alone, Western blot analysis showed that treatment with *Momordica charantia* extract reduced the expression of caspase-9 and caspase-3 (Figure 4).

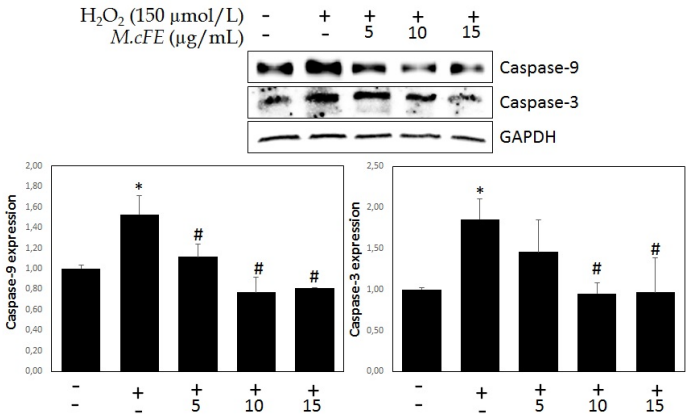

Fig. 4. Representative blots showing caspase 9 and caspase 3 expression. Normalized values of caspase 9 and caspase 3. Data are expressed as mean ± SD of five independent experiments (n = 3). *p<0,05 *vs.* Control cell, #p<0,05 *vs.* H_2O_2 treated cell.

3.4. H_2O_2 increased proportion of apoptotic and necrotic cells: Effect of *Momordica charantia* extract

Four cell phases upon exposure to H_2O_2-induced stress were detected using AO/EB double staining: While the nuclei of both live and dead cells are stained green by acridine orange (AO), only cells that have lost membrane integrity are stained red by ethidium bromide (EB). Consequently, whereas early apoptotic cells have fragmented nuclei that are brilliant green in color, living cells will appear to be uniformly green. Condensed and fragmented orange chromatin is a sign of late apoptosis in cells. The percentage of necrotic and apoptotic cells considerably increased after exposure to H_2O_2. Extract incubation in the presence of H_2O_2 partly but significantly enhanced the proportion of surviving cells and inhibited necrosis and apoptosis-related cell death (Fig. 5).

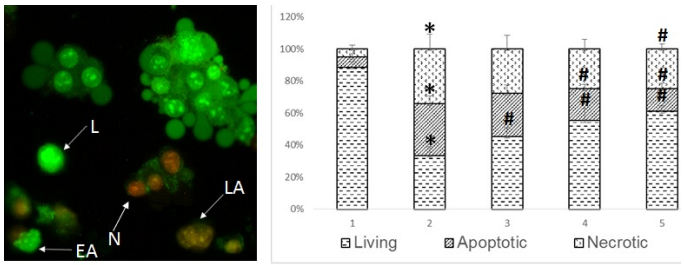

Fig. 5. Acridine Orange/Ethidium Bromide (AO/EB) dual staining of SHSY5Y cells. Bar 1: Control cell, Bar 2: H_2O_2 (150 µM) treated cell, Bar 3: Effect of McFE (5 µg/mL) at the presence of H_2O_2, Bar 4: Effect of McFE (10 µg/mL) at the presence of H_2O_2 Bar 5: Effect of McFE (15 µg/mL) at the presence of H_2O_2

4. Discussion

One of the main contributing reasons to neurodegeneration is the excessive creation of ROS, which results in oxidative damage to proteins, lipids, and DNA (18). A significant contributor to the production of intracellular ROS is the mitochondrial electron transport system, and the mitochondria are necessary for the process of ROS-mediated cell death (19). Furthermore, H_2O_2 immediately causes mitochondrial dysfunction, followed by an early release of intracellular ROS, which results in an immediate depolarization of the inner mitochondrial membrane (20). This procedure most likely accelerates the disruption of the mitochondrial membrane potential and the release of apoptosis-inducing compounds that activate caspase-dependent signaling cascades to trigger apoptosis (21). For the prevention and treatment of neurodegenerative diseases, it is therefore essential to find functional foods or bioactive substances that act against oxidative stress. It was determined in this study whether *Momordica charantia* extract inhibits H_2O_2-induced oxidative stress and apoptosis in SH-SY5Y cells. The results showed that H_2O_2 treatment caused intracellular ROS accumulation as well as apoptosis by preventing cell survival in SH-SY5Y cells. However, when SH-SY5Y cells were simultaneously exposed to H_2O_2 and *Momordica charantia*, H_2O_2-induced ROS generation, cell viability reduction, and increased apoptotic cell death were significantly attenuated, as previously reported in research on other SK-N-MC neuroblastoma cells (22). Therefore, we presume that *Momordica charantia* protects neuronal cells by inhibiting the oxidative effect of H_2O_2 and thereby reducing H_2O_2-induced apoptosis. *Momordica charantia* has a neuroprotective effect not just found in this study. It was emphasized in the previous study that charantin isolated from *Momordica charantia* demonstrated neuroprotective effects in SH-SY5Y cells by preventing nerurotoxin MPP+ and tunicamycin induced neuronal damage and endoplasmic reticulum stress (23). Despite the neurotoxicity caused by polycyclic aromatic hydrocarbons, *Momordica charantia* was found to prevent cell death in rat hippocampus neuronal cells by regulating the cell cycle and MAPK cascade with its ROS scavenging function (24). In a different study, lyophilized *Momordica charantia* juice was shown to have anti-oxidative and neuroprotective properties in diabetic cerebral ischemia reperfusion injury (25). *Momordica charantia* was used to create exosome-like nanoparticles, and their neuroprotective effects on brain ischemia reperfusion injury were also investigated. This study demonstrated how exosome-like nanoparticles could quickly cross the blood-brain barrier and inhibit neuronal apoptosis by modulating the AKT/GSK-3B pathway (26).

In conclusion, Overall, increased oxidative stress, as well as subsequent apoptotic neuronal cell loss, is the mechanism underlying the pathogenesis of neurodegenerative diseases. No specific drug has been developed yet for the treatment of neurodegenerative diseases. In the context, research on the neuroprotective properties of antioxidant-rich herbal extracts and plant-based compounds in neurodegenerative diseases has gained popularity. The results of our present study clearly demonstrated that *Momordica charantia* fruit extract exerted a neuroprotective effect against H_2O_2-induced cell death, ROS production, and apoptosis in SH-SY5Y cells. Our results also confirmed that fruit extract from *Momordica charantia*, a type of antioxidant, may have neuroprotective properties.

Conflict of interest

The authors declare that they have no competing interests.

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Authors' contributions

Concept: A.C., H.D., Design: A.C., H.D., Data Collection or Processing: A.C., H.D., Analysis or Interpretation: A.C., H.D., Literature Search: A.C., H.D., Writing: A.C

Ethical Statement

Ethics committee approval is not required for this study.

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Research Article

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Levels of serum copeptin in preeclampsia and association with maternal echocardiographic and doppler ultrasound parameters

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Abstract

Copeptin is a peptide that has been reported as a valuable tool in monitoring major cardiovascular diseases such as myocardial infarction and heart failure. Echographic examinations are useful in assessing alterations in cardiovascular structure and function during pregnancy. Having in mind the role of copeptin in cardiovascular abnormalities and the subtle changes in heart and spiral arteries that can be detected by echography, the present study aimed to evaluate copeptin concentrations in preeclampsia (PE) and to investigate the existence of an association between copeptin and maternal echocardiographic and Doppler ultrasound parameters. The current research was a case-control study. Fifty-five women with PE were examined. The mean age of patients was 24.9±6 years, and the mean age of the control group of 35 women with normal pregnancies was 24.7±5.4 years. The enzyme-linked immunosorbent assay (ELISA) was used to determine copeptin concentrations. An echocardiographic assessment of all subjects was performed. In addition, uterine (UtA) artery pulsatility indices (PI) were evaluated. Levels of serum copeptin in preeclamptic women were statistically insignificantly lower than these in women with normal pregnancy: 142.2 (131.4÷146.7) vs. 144.8 (138.5÷149.4) ng/l ($p>0.05$). Copeptin correlated with systolic blood pressure ($r=-0.41$; $p=0.0001$), diastolic blood pressure ($r=-0.30$; $p=0.004$), UtA PI ($r=-0.36$; $p=0.0005$), IVS ($r=-0.23$; $p=0.03$) and LVPWD ($r=-0.21$; $p=0.05$). We report the existence of a relationship between serum copeptin and maternal echocardiographic and Doppler ultrasound parameters in preeclampsia. The present study argues for a potential copeptin implication on maternal cardiac structures and spiral arteries. Our results also confirm that copeptin is associated with increased blood pressure in preeclampsia.

Keywords: copeptin, blood pressure, echography, preeclampsia, serum levels

1. Introduction

Preeclampsia (PE) is a pregnancy-associated hypertensive disorder after 20 weeks of gestation, characterized by the development of new-onset hypertension (140/90 mmHg) and either proteinuria (0.3g in a 24-hour urine sample) or end-organ dysfunction. Current evidence shows that PE complicates nearly 2–8% of all pregnancies worldwide (1). It's one of the leading causes of maternal and perinatal morbidity and mortality (2). It has been assumed that preeclampsia involves generalized vascular injury commonly associated with endothelial alteration (3). Significant pathways contribute to abnormal hemodynamic state, including increased circulating plasma volume and fine regulation of vascular tone (4).

Copeptin is a peptide also known as C-terminal of pre-pro-hormone of arginine vasopressin (CT-proAVP) (5). The molecule of copeptin involves 39 amino acid chains, derived by C-terminal of pre-pro-hormone of arginine vasopressin, neurophysin II, and copeptin. Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), plays a key role in many cardiovascular and renal conditions. Its abnormal levels have been associated with different myocardial and kidney abnormalities (6). Unfortunately, AVP measurement has not been incorporated into routine clinical practice because of its short half-life (7, 8). Contrary to that, immunoassays can easily detect copeptin and are also used as vasopressin secretion surrogate indicators (9, 10). Copeptin has been reported as a valuable tool in monitoring cardiovascular pathologies such as myocardial infarction, left ventricular hypertrophy, cardiogenic shock, and heart failure (11, 12). Its expression correlated with survival, severity, and disease prognosis (13, 14). For instance, copeptin also significantly correlates with 6MWD and New York Heart Association (NYHA) class (15-17) as well as with kidney function in pulmonary hypertension (18-21). Interestingly, data about maternal circulatory copeptin in preeclampsia are insufficient yet.

Echocardiography is a safe, noninvasive method for evaluating changes in cardiac structure and function in

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pregnancy (22-25). Uterine and umbilical Doppler ultrasound assessments are fast, harmless, and easily applicable diagnostic techniques to identify the compromised fetus and examine placental perfusion. It has been proposed that Doppler flow studies of the maternal uterine vessels could be used to detect women at higher risk for developing preeclampsia (26-31). Hence, these echographic methods allow the evaluation of the heart and vessels of pregnant women without exposition to X-rays and give valuable data referring to abnormal cardiovascular and hemodynamic changes during a healthy and complicated pregnancy. However, there are no data in the literature on a parallel examination of copeptin concentrations and maternal echocardiographic and Doppler ultrasound measurements in preeclampsia.

Having in mind the role of copeptin in cardiovascular abnormalities and the subtle changes in heart and spiral arteries that can be detected by echography in preeclampsia, this study aimed to: 1-) determine circulating copeptin levels in sera of women with preeclampsia and normal pregnancy; 2-) to investigate a possible relationship between copeptin and maternal echocardiographic and Doppler ultrasound parameters.

2. Materials and Methods

2.1. Study design

The current research was a case-control study**.**

2.2. Study setting

The current study was a part of the university scientific project N1/2020. The project was approved by the Ethics Committee of Medical University-Pleven with Protocol N51/2020. All participants signed informed consent. Study procedures followed all guidelines for ethical standards of the responsible committee on human experimentation as well as the Helsinki Declaration of 1975, as revised in 2000.

2.3. Study population

All patients were residing in the Clinic of Obstetrics and Gynecology, University Hospital "G. Stranski" Pleven. Sera of subjects were taken from October 2019 to March 2021. The study group consisted of 55 women with preeclampsia, the mean age of patients was 24.9±6 years, and the mean age of the control group of 35 women with normal pregnancies was 24.7±5.4 years.

2.4. Inclusion and exclusion criteria

The following criteria applied to inclusion in the study: Pregnant women with clinical symptoms who also met the laboratory criteria for preeclampsia [According to the European Society of Cardiology 2018 Guideline for the management of cardiovascular diseases during pregnancy was used for the diagnostic criteria of preeclampsia: gestational hypertension with significant proteinuria (>300mg/24h urine collection or the extrapolated amount from a timed collection)] (32); maintaining a regular diet and exercise routine throughout the research; signed informed consent form to take part in the investigation; dysfunction of mother's organ such as HELLP syndrome, kidney failure, neurological involvement, hepatic involvement, and fetal growth retardation. The following criteria applied to exclusion in the study: diabetes mellitus, renal and heart disease, signs of chorioamnionitis, and the presence of a fetus with a chromosomal abnormality.

2.5. Outcome measures and methods

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used for the determination of copeptin levels. Copeptin was measured in serum samples using an ELISA kit (RJ-HUFI02359 Human Copeptin/CPP ELISA Kit- Reagent Genie) according to the manufacturer's instructions.

Echocardiography

Echocardiography was performed with General Electric (Vivid S5) with a 4-MHz transducer. All measurements were obtained according to the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE) criteria for Cardiac Chamber Quantification by Echocardiography (33)

Doppler ultrasound of umbilical and uterine artery

Flow velocity waveforms of the uterine artery were performed by ultrasound apparatus using an AB 2–7 MHz convex abdominal probe. The mean pulsatility index (PI) was calculated. An abnormal Doppler of uterine artery result was diagnosed as a mean PI> the 95th percentile for each gestational age (34).

2.6. Statistical analysis

In order to analyze the research data following computer programs were used: Excel (Microsoft Corporation, Redmond, WA), SPSS, and Statgraphics Plus (Manugistics, Rockville, MD) for Windows. The level of significance was determined as ($p<0.05$). Stnd. Skewness and Stnd. Kurtosis tests were used to check the normality of distribution and equality of variances. To discover significant differences between groups, Student's t-test, and ANOVA with mean±SD were used in cases with normal distribution (LSD, Tukey HSD, Scheffe, Bonferroni, Newman-Keuls, Duncan). $\chi 2$ and Kruskal–Wallis H tests with median (M) value were used in cases with different from normal distribution, together with first and third quartile Q1 and Q3; (twenty-fifth and seventy-fifth percentile P25 and 75P). Pearson type of correlation was used. To confirm the existence of a significant relationship between the variables, linear regression analysis was carried out. All the linear regression assumptions were checked.

3. Results

Clinical data of women with preeclampsia and healthy pregnant women are presented in Table 1. Echocardiographic data of healthy pregnant women and patients with preeclampsia are described in Table 2. Levels of serum copeptin in preeclamptic women were statistically insignificantly lower than these in women with normal pregnancy: 142.2 (131.4÷146.7) vs. 144.8 (138.5÷ 149.4) ng/l

(p>0.05) (Fig. 1). Copeptin correlated with systolic blood pressure (SBP) (r=-0.41; p=0.0001) (Fig. 2), diastolic blood pressure (DBP) (r=-0.30; p=0.004) (Fig. 3), uterine artery pulsatility index (UtA PI) (r=-0.36; p=0.0005) (Fig. 4), interventricular septal thickness (IVS) (r=-0.23; p=0.03) and left ventricular posterior wall thickness (LVPWD) (r=-0.21; p=0.05).

Table 1. Clinical data of women with preeclampsia and healthy pregnant women

	Normal pregnant women	Preeclampsia	p
Maternal age	24.7±5.4	24.9±6	>0.05
BMI	26.7±4.2	34±7.3*	0.001*
Gravida	2(2) **	2 (2)**	
Parity	1(2) **	1(2) **	
SBP (mmHg)	116.1±9.55	157.8±22*	0.001*
DBP (mmHg)	75.3±7.76	100.5±10*	0.001*
Past history of PE	0/35	23/55	
Family history of AH	1/35	26/55	
AH before pregnancy	0/35	15/55	
Uterine artery PI	0.79±0.12	1.19±0.44*	0.001*
PP	40.8±7.32	57.3±16.1*	0.001*
MAP	88.8±7.69	119.7±13.1*	0.001*
Urea	2.96±0.78	3.75±1.63*	0.01*
Creatinine	75.78±14.45	73.33±15.33	>0.05
Uric acid	205.6±40.2	326.8±105.93*	0.001*
Total protein	68.89±3.16	58.71±8.78*	<0.01*
Albumin	37.31±2.78	31.67±4.98*	<0.01*
ASAT	8.43±2.33	20.67±7.82*	<0.01*
ALAT	9.83±2.50	27.76±8.25*	<0.01*
LDH	369±70.78	435.25±80.74*	0.04*
PLT	237.26±61.12	228.74±88.53	>0.05
Copeptin	144.8 (138.5÷149.4) **	142.2 (131.4÷146.7) **	>0.05
CPK	83.1±23.77	130.5±46.8*	<0.05*
CK-MB	15.3±3.3	24.3±7.9*	<0.05*
Number	(n=35)	(n=55)	

Abbreviations: BMI- body mass index; SBP- systolic blood pressure; DBP- diastolic blood pressure; PE-preeclampsia; AH-arterial hypertension; PI- pulsatility index; PP- pulse pressure; MAP- mean arterial pressure; ASAT- aspartate aminotransferase; ALAT-alanine aminotransferase; LDH-lactate dehydrogenase; PLT-platelets; CPK-creatine phosphokinase; CK-MB- creatine phosphokinase isoenzyme MB. Data are shown as the mean±SD; *p<0.05; **Data are expressed as median (interquartile range)

Table 2. Echocardiographic data of healthy pregnant women and patients with preeclampsia

	Healthy Pregnancy	Preeclampsia	p
LVEDD	46.06±1.51	47.67±2.83*	0.001*
LVESD	28.23±1.48	29.84±2.43*	<0.001*
IVS	9.47±0.86	10.74±0.93*	<0.01*
LVPWD	9.03±1.04	10.4±1.31*	<0.001*
EF%	68.28±1.98	64.69±5.14*	<0.001*
E/e'	9.64±1.02	11.76±0.77*	0.001*
Count	**35**	**55**	

Abbreviations: LVEDD- left ventricular end-diastolic diameter, LVESD-left ventricular end-systolic diameter, IVS- interventricular septal thickness, LVPWD- left ventricular posterior wall thickness, EF%- left ventricular ejection fraction, *p<0.05, Data are expressed as mean±SD

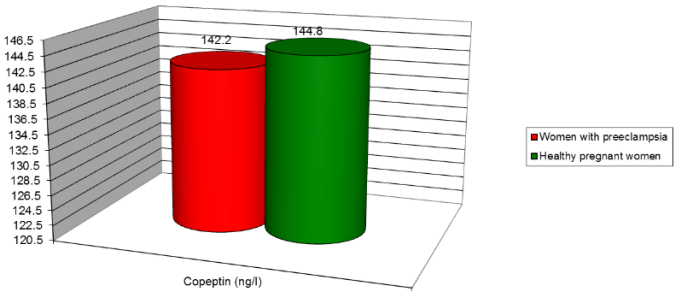

Fig. 1. Serum copeptin levels in preeclampsia and healthy pregnant women determined by ELISA

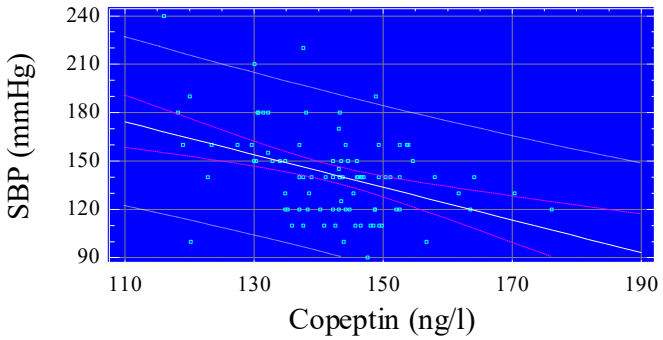

Fig. 2. Linear regression analysis, showing the results of fitting a linear model to describe the relationship between copeptin and SBP

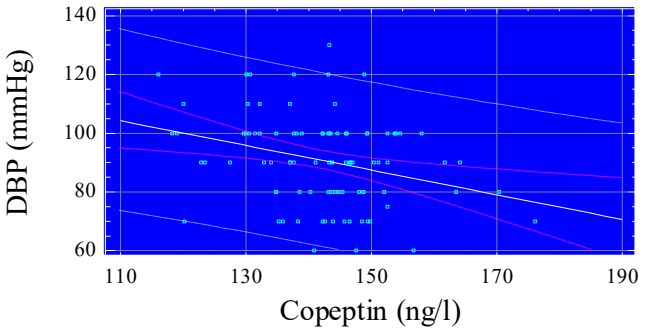

Fig. 3. Linear regression analysis, showing the results of fitting a linear model to describe the relationship between copeptin and DBP

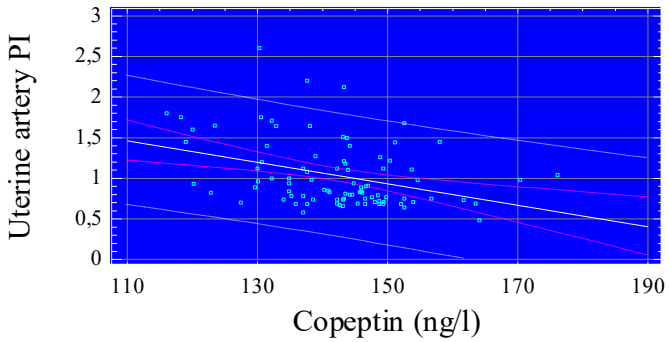

Fig. 4. Linear regression analysis, showing the results of fitting a linear model to describe the relationship between copeptin and Uterine artery pulsatility index

4. Discussion

Preeclampsia is one of the most common pregnancy disorders. It is a major cause of maternal and perinatal morbidity and mortality. According to the current understanding, preeclampsia is a systemic disease with generalized endothelial cell injury/dysfunction and multi-organ involvement. It has been reported by several studies that echocardiographic assessment of patients with preeclampsia indicates major findings such as increased ventricular mass, left ventricular hypertrophy, left atrial enlargement, and diastolic dysfunction (35-41).

Copeptin is a glycopeptide that forms the C-terminus of prepro-arginine vasopressin, which is the precursor protein of AVP, a vasoactive neuro pituitary hormone (42). Therefore, copeptin levels are used as a surrogate measurement for vasopressin secretion (43). Moreover, copeptin has also been previously investigated as a potential diagnostic and prognostic biomarker for various cardiovascular diseases (44-46). It has been theorized that copeptin is involved in the pathophysiology of preeclampsia. However, a possible relationship between copeptin and maternal echocardiographic and Doppler ultrasound parameters has not been explored.

A few researchers have assessed copeptin concentrations in healthy and complicated pregnancies so far. For example, Yeung et al. (2014), in the longitudinal study "Calcium for Preeclampsia Prevention trial," determined via BRAHMS Immunoluminometric Assay serum copeptin levels in 136 control subjects, 169 preeclampsia cases, 92 women with gestational diabetes, 101 with gestational hypertension and 86 with preterm birth. Authors found increased copeptin concentrations in pregnant women before the diagnosis of preeclampsia with "elevation specific to this pregnancy complication rather than hypertension alone"(47).

In 2015, Akinlade et al. considered the role of copeptin in PE and reported elevated maternal copeptin levels in preeclampsia. Moreover, copeptin concentrations increased with disease severity. Furthermore, the authors concluded that copeptin levels in the third trimester "could predict preeclampsia, and its elevation is associated with adverse perinatal outcome"(48).

In another research, Tuten et al. (2015) used ELISA and investigated serum copeptin levels in 80 pregnant women divided into the following subgroups: early-onset preeclampsia, late-onset preeclampsia, and two control groups of similar gestational ages for both preeclamptic groups. The mean copeptin levels in the early-onset and late-onset preeclampsia groups were higher compared with the control groups, but the difference was only statistically significant in the early-onset preeclampsia group. Copeptin levels were associated only with gestational age and systolic-diastolic blood pressure. The investigators suggested that "copeptin levels might be useful in evaluating the severity of preeclampsia." Based on their findings, authors concluded that copeptin could be involved in early- rather than late-onset preeclampsia (49).

Zulfikaroglu et al. (50) evaluated plasma levels of copeptin in preeclampsia patients and healthy pregnant women in 2011. Researchers used ELISA and measured higher plasma levels in mild and severe PE compared with normotensive pregnant women. Similarly, Santillan et al. (51) assessed copeptin levels throughout pregnancy in maternal plasma of women with preeclampsia and healthy controls in 2014. Authors reported that maternal plasma copeptin was significantly higher in preeclamptic pregnancies compared to control pregnancies. The researchers found that as early as the sixth gestational week, elevated maternal plasma copeptin concentration is an extremely important predictor of preeclampsia throughout pregnancy. Although these data suggest AVP as a novel predictive biomarker for preeclampsia very early in pregnancy, further larger clinical studies should be performed to confirm the prediction of preeclampsia by copeptin. Furthermore, new studies are needed to determine the reasons for the increased AVP production in these patients.

Recently, Hagras et al. (52) applied BRAHMS Immunoluminometric Assay in 2018 and reported that serum copeptin is higher as early as 13 weeks gestation in women who later developed preeclampsia than in cases who remained normotensive till full term and delivery and is higher in severe cases than mild cases of preeclampsia. In the same year, Mohamed et al. (53) found that serum copeptin level could be used as an important biomarker for the early diagnosis of preeclampsia.

To the best of our knowledge, the current research was one of the few in the literature to explore maternal serum copeptin concentrations in preeclampsia. In the present study, we reported statistically insignificantly lower serum copeptin levels in preeclamptic patients than in women with normal pregnancies. Our data demonstrated for the first time a relationship exists between serum copeptin and maternal echocardiographic and Doppler ultrasound parameters in preeclampsia. The current investigation argues for a potential copeptin implication on maternal cardiac structures and spiral arteries. These data were also validated by linear regression

analysis. The results obtained from our research also confirmed the findings of Tuten et al. (49) that copeptin is associated with increased blood pressure in preeclampsia. The relationship between copeptin levels and blood pressure implicates a possible copeptin role in the pathophysiology of hypertension in pregnancy and the development of preeclampsia.

Analyzing the results mentioned above, we can deliberate that the present findings agree with the findings of Tuten et al. (49), who represented that serum copeptin is associated with blood pressure in preeclampsia and might contribute to the diagnostic process of PE as for the measuring of copeptin levels in PE and healthy pregnancy, current data was not fully consistent with the reports of Yeung et al. (47), Zulfikaroglu et al. (50), and Santillan et al. (51), who demonstrated higher copeptin levels in PE vs. normal pregnancies. This difference can be explained by the usage of various laboratory methods (Yeung et al. [47] and Hagras et al. [52] used BRAHMS Immunoluminometric Assay, while our study, Akinlade et al. [48], Tuten et al.[49], Zulfikaroglu et al. [50] and Santillanet al. [51] used ELISA). Another explanation can be related to the usage of different sample types. For example, Zulfikarogluet al. (50) and Santillan et al. (51) used plasma while we investigated serum probes. The smaller sample size and measurement timing can also be important factors influencing the determination of circulating copeptin. Noteworthy, we explored blood samples after 20 gestational weeks, while Hagras et al. (52) analyzed probes before 20 weeks of gestation, and Mohamed et al. (53) collected samples in the third pregnancy trimester. It should also be highlighted that preeclampsia severity also impacts copeptin concentrations.

It has been reported by several studies that echocardiographic assessment of patients with preeclampsia indicates major findings such as increased ventricular mass, left ventricular hypertrophy, left atrial enlargement, and diastolic dysfunction (35-41). Our investigation found a relationship between copeptin and specific echocardiographic measurements such as interventricular septum thickness and left ventricular posterior wall diameter. This result assumes a possible interplay between copeptin and the above-mentioned heart structures. In addition, the relationship between copeptin and Doppler ultrasound parameters as uterine Doppler pulsatility index might reflect vascular changes responsible for abnormal remodeling and pathologically increased vascular resistance in preeclampsia. However, more specific methods like immunohistochemistry or immunocytochemistry with tissue samples analysis and evaluation of copeptin expression might be required to detect the exact structural alterations. This would help to assess exactly which tissues derive copeptin in serum during preeclampsia.

The current investigation demonstrated compelling evidence. To our knowledge, this is the first study reporting a significant relationship between serum copeptin and maternal echocardiographic and Doppler ultrasound parameters in preeclampsia. Another key finding is the association between serum copeptin concentrations and blood pressure values. The present results were validated by linear regression analysis. Our findings confirmed that copeptin might play an important role in blood pressure elevation in pregnancy. The presented data also demonstrate a potential copeptin effect on specific cardiac structures, such as the left posterior ventricular wall and interventricular septum. This might favor abnormal cardiovascular remodeling, the development of hypertension in pregnancy, and subsequent preeclampsia. Hereby, copeptin is proposed to be related to the process of altered spiral arteries' remodeling. All the factors mentioned above take part in the central pathways in the development and progression of preeclampsia.

Considering the correlation between copeptin and blood pressure found in the present investigation, we confirm that copeptin might be involved in the pathogenic mechanisms of the increase of blood pressure and the development of hypertension in pregnancy. The current study argues for a potential implication on maternal cardiac structures and spiral arteries based on the demonstrated relationship between copeptin and maternal echocardiographic and Doppler ultrasound parameters. However, larger-scale and longitudinal studies with more specific methods, such as immunohistochemistry or immunocytochemistry analysis of tissue samples, would allow a more precise assessment of the copeptin's role in the pathogenesis of PE and its interaction with the maternal heart and spiral arteries. This could provide a deeper understanding of the structural alterations and help identify the tissues involved in copeptin production during preeclampsia.

The present research had limitations. Firstly, it was a case-control study, and we could not perform serial measurements of copeptin. Secondly, the relatively small sample size also constituted a study design limitation. Thirdly, the variations in copeptin levels reported in different studies might be due to a lack of consistent laboratory methods and standardized timing of copeptin measurements. Using successive laboratory methods and validated determination timing would contribute to the comparability and reliability of copeptin level results across future studies.

Ethical Statement

The project was approved by the Ethics Committee of Medical University- Pleven with Protocol N51/2020.

Conflict of interest

The authors declare no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.N., N.P., Design: A.N., N.P., Data Collection or Processing: N.P., Analysis or Interpretation: A.N., N.P., Literature Search: N.P., Writing: A.N., N.P.

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Abnormal levels of serum N-terminal propeptide of collagen type IV (PIVNP) in women one year after preeclampsia

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Abstract

Proteins of the extracellular matrix (ECM) play an important role in normal pregnancy and preeclampsia (PE). Type IV collagen (COL4) is a major ECM structural element, uniquely presented in basement membranes. The N-terminal propeptide of collagen type IV (PIVNP) is a marker of COL4 synthesis. In the present study, we determined serum PIVNP levels in women one year after PE and tested whether PIVNP is related to hypertension development after preeclamptic pregnancy. The current research examined 32 women one year after PE (mean age 25.3±6.3 years) and a control group of 20 women one year after normal pregnancy (mean age 25.6±5.6 years). The enzyme-linked immunosorbent assay (ELISA) was used to determine concentrations of PIVNP. We found that at 1 year after delivery, 38.46% of women who suffered PE had developed arterial hypertension (AH), and 5.77% had developed diabetes mellitus. Women who had normal pregnancies developed neither AH nor diabetes mellitus 1 year after delivery. The distribution of women who had developed AH after preeclamptic pregnancy was as follows: Grade I AH-7; Grade II AH-4; and Grade III AH- 2. Serum PIVNP levels in women one year after PE were statistically significantly lower than in women one year after normal pregnancy: 0.26 (0.1÷0.65) vs. 0.45 ng/ml (0.3÷0.6) (KW= 5.342; p=0.02). PIVNP showed a correlation with creatinine (r= -0.26; p=0.05). Hypertensive women one year after PE showed the lowest levels of serum PIVNP.Our data showed decreased levels of PIVNP in the sera of women one year after PE. This finding demonstrated altered COL4 turnover after preeclamptic pregnancy. The diminished COL4 synthesis might play an important role in persistent vascular wall injury and dysfunction postpartum. We suggest that PIVNP might be involved in the pathogenic mechanisms determining the development of hypertension postpartum.

Keywords: arterial hypertension, collagen IV turnover, extracellular matrix, history of preeclampsia, N-terminal propeptide of collagen type IV

1. Introduction

Preeclampsia (PE) is a critical complication of pregnancy that develops after the 20th gestational week. It is described by the occurrence of new-onset hypertension (140/90 mmHg) and either proteinuria (0.3g in a 24-hour urine sample) or end-organ dysfunction (1). It is an important cause of maternal and perinatal morbidity and mortality (2,3). Patients with PE have an increased long-term risk of developing cardiovascular disease (CVD) (4). There is growing evidence that arterial hypertension (AH) is associated with decreased degradation of connective tissue proteins (5,6). However, little is known about the changes in the composition of the extracellular matrix (ECM), particularly Type IV collagen (COL4) after preeclampsia.

ECM is considered pivotal for maintaining tissue structure and modulating of cell differentiation (7). An important factor in the development of vascular wall alterations is the degradation of the extracellular matrix‘s major protein, collagen (8). COL4 is uniquely present in basement membranes of arteries and represents their predominant structural component (9,10). The uterine wall consists mainly of COL1 and COL3 (11). On the other hand, COL4 is a major component of arterial vasculature (12).

ECM plays a crucial role in normal pregnancy. It has been reported that ECM might modulate trophoblast invasion and contribute to the remodeling of the decidua at the maternal-fetal interface (13). Therefore, it can be concluded that the extracellular matrix of the uterus, placenta, and vasculature breaks down and remodels during physiological pregnancy (14,15). During preeclampsia, the uterine and spiral arteries' ECM metabolism has been found to be altered. Collagen metabolism is shifted, and the delicate balance between synthesis and degradation is disturbed (16). These processes are characterized by impaired collagen turnover, which might affect the remodeling of the uterine ECM and spiral arteries. Arterial vessels' collagen structure has been shown to be disturbed in women with PE. It is, therefore, possible that COL4 turnover is also affected. Hence, markers of COL4 turnover could be found in the sera of patients with PE.

Very few studies have investigated COL4 turnover in preeclampsia. Despite that, a consensus exists that marker of collagen degradation cannot be used as preeclampsia predictor biomarkers (17). Scientific efforts should focus on collagen

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synthesis markers- the collagen propeptides. In turn, the N-terminal propeptide of collagen type IV (PIVNP) has been reported as an important indicator of COL4 synthesis (18-19). Interestingly, changes in indicators of collagen synthesis and particularly PIVNP in preeclamptic women 1 year after delivery have not been explored yet. We studied PE patients one year later, because previous investigations focused on this pregnancy complication (20-21) have reported current time frame to be suitable for assessment of changes in serum markers and clinical parameters like blood pressure. This time point (1 year after delivery) is neither too early in the healing phase of the illness, nor too later in the postpartum period and allows alterations to be detected.

This study aimed to: (1) determine circulating PIVNP levels in the sera of women one year after preeclampsia and normal pregnancy; and (2) investigate the possible role of PIVNP in the development of hypertension in previously preeclamptic women.

2. Material and methods

2.1. Study design

The currentresearch was a longitudinal study.

2.2. Subjects

The current study included women who had preeclampsia symptoms 1 year ago and met the ACOG (2019) criteria: PE was defined as complication of pregnancy after 20 weeks of gestation,described by the occurrence of new-onset hypertension (140/90 mmHg) and either proteinuria (0.3g in a 24-hour urine sample) or end-organ dysfunction (1). All subjects signed and informed consent form to take part in the investigation and maintained a regular diet and exercise routine throughout the research. Women who had diabetes mellitus, renal and heart disease, signs of chorioamnionitis, or the presence of a fetus with a chromosomal abnormality were excluded. All patients were residing in the Clinic of Obstetrics and Gynecology, University Hospital "Georgi Stranski" Pleven one year ago.

The present study was part of a university scientific project under the national Program “Young Scientists and Postdoctoral Students-2“, approved by the Ethics Committee of the Medical University of Pleven (Protocol N70/2023). All participants signed informed consent. The study procedures were consistent with all guidelines for ethical standards of the responsible committee on human experimentation, along with the Helsinki Declaration of 1975, as revised in 2000. A sample of subjects was taken from February to March 2023 for the purposes of the present investigation. The study group consisted of 32 women one year after preeclampsia (mean age 25.3±6.3 years) and a control group of 20 women one year after normotensive pregnancy (mean age 25.6±5.6 years).

2.3. ELISA

For the purpose of the current investigation, an enzyme-linked immunosorbent assay (ELISA) was applied to determine PIVNP levels in serum samples. The following ELISA kit was used (RJ-HUFI02977 Human N-terminal Propeptide of Collagen Alpha-1 (IV) Chain / PIVNP ELISA Kit, AssayGenie, Dublin, Ireland), according to the manufacturer’s instructions.

2.4. Blood pressure

The arterial blood pressure was assessed by a standard aneroid sphygmomanometer, to the nearest 2 mmHg, in the dominant arm after at least 10 minutes of rest in the supine position. Blood pressure measuring was determined by the Riester blood pressure measuring tool, Type Precisa® N, 64 mm aluminum, single-tube, cotton hook cuff, adult, No. 1362–104.

2.5. Statistical analyses

In order to analyze the research data, following computer programs were used: Excel (Microsoft Corporation, Redmond, WA), SPSS, and Statgraphics Plus (Manugistics, Rockville, MD) for Windows. The level of significance was determined as ($p<0.05$). Stnd. Skewness and Stnd. Kurtosis tests were used to check the normality of distribution and equality of variances. To discover significant differences between groups, the Student's t-test and ANOVA with mean±SD was used in cases with normal distribution (LSD, Tukey HSD, Scheffe, Bonferroni, Newman-Keuls, Duncan). $\chi 2$ and Kruskal-Wallis H test with median (M) value were used in cases with a different normal distribution, together with the first and third quartiles Q1 and Q3 (twenty-fifth and seventy-fifth percentiles P25 and 75P). A Pearson type of correlation was used.

3. Results

Clinical data of women who had preeclampsia and women who had normotensive pregnancy at 1 year after delivery are presented in Table 1.

We found that at 1 year after delivery, 38.46% of women who suffered PE had developed arterial hypertension, and 5.77% had developed diabetes mellitus. Women who had normal pregnancies developed neither hypertension nor diabetes mellitus 1 year after delivery. The distribution of women who had developed AH after preeclamptic pregnancy was as follows: Grade I AH- 7; Grade II AH-4; and Grade III AH- 2 women (Fig. 1). Serum PIVNP levels in women one year after PE were statistically significantly lower than in women one year after normal pregnancy: 0.26 (0.1÷0.65) vs. 0.45 ng/ml (0.3÷0.6) (KW= 5.342; p=0.02) (Table 2) (Fig. 2). Hypertensive women one year after PE showed lower levels of PIVNP than normotensive women one year after PE, but not significantly: 0.22 (0.1÷0.4) vs. 0.25 ng/ml (0.1÷0.8) ($p>0.05$). PIVNP showed a correlation with creatinine ($r= -0.26$; $p=0.05$).

Table 1. Clinical data of women one year after preeclampsia and one year after normal pregnancy

	Women one year after normal pregnancy	Women one year after preeclampsia	P
Age	25.6±5.6	25.3±6.3	p>0.05
BMI	26.9±4.38	30.5±6.9*	P=0.03*
SBP (mmHg)	115±7.6	135.6±15.6*	p<0.001*
DBP (mmHg)	75±7.07	86.9±9.22*	p<0.001*
Past history ofPE	0/20	7/32	
Family history of AH	1/20	14/32	
AH before pregnancy	0/20	5/32	
PP	40.8±6.9	58.2±16.8*	P=0.001*
MAP	88.5±8.75	121.7±13.4*	P=0.001*
Urea	2.96±0.78	3.75±1.63*	p=0.01*
Creatinine	75.78±14.45	73.33±15.33	p>0.05
Uric acid	205.6±40.2	326.8±105.93*	P=0.001*
TCL	3.86±0.94	4.97±1.27*	P=0.001*
LDL	2.48±0.62	3.27±1.23*	P=0.01*
HDL	1.92±0.48	1.44±0.76*	P=0.02*
TG	1.35±0.37	2.12±1.01	P=0.002
AH GRADE I	0/20	7/32	
AH GRADE II	0/20	4/32	
AH GRADE III	0/20	2/32	
T2DM	0/20	3/32	
Number	(n=20)	(n=32)	

BMI- body mass index; SBP- systolic blood pressure; DBP- diastolic blood pressure; PE-preeclampsia; AH-arterial hypertension; T2DM- type 2 diabetes mellitus; PP- pulse pressure; MAP- mean arterial pressure; HDL- high density lipoprotein cholesterol; LDL- low density lipoprotein cholesterol; TCL- total cholesterol; TG- tryglicerides. Data are shown as the mean±SD; *p<0.05

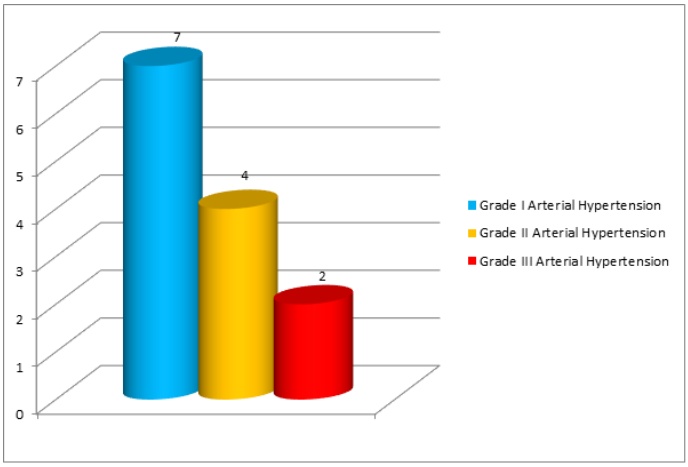

Fig. 1. Distribution of women who had developed hypertension one after preeclamptic pregnancy

7 women developed Grade I AH, 4 women developed Grade II AH and 2 women developed Grade III AH after preeclamptic pregnancy.

Table 2. Serum levels of PIVNP in women one year after preeclampsia and one year after normal pregnancy

	Women one year after normal pregnancy	Women one year after preeclampsia	P
PIVNP (ng/ml)	0.45 (0.3÷0.6)	0.26 (0.1÷0.65)	0.02

PIVNP- Human N-terminal propeptide of Collagen IV; Data are expressed as median (interquartile range)

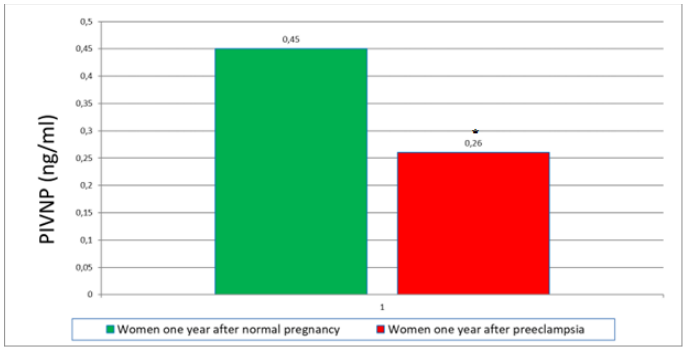

Fig. 2. Serum PIVNP levels in in women one year after preeclampsia and one year after normal pregnancy determined by ELISA

Serum PIVNP levels in women one year after PE were statistically significantly lower than in women one year after normal pregnancy 0.26 (0.1÷0.65) vs. 0.45 ng/ml (0.3÷0.6) (p=0.02). Values were presented as mean±SD; *P<0.05 compared with women one year after normal pregnancy.

4. Discussion

The primary component of basement membranes is COL4. It also supports the underlying endothelial cells. Therefore, COL4 is essential for the endothelial cells' function. Endothelial cells lie on and adhere to the basement membrane, a thin sheet of extracellular matrix (22–26). COL4 interactions with endothelial cells are important for maintaining endothelial cell function. "COL4 is also essential for cell adhesion and cell-matrix communication and plays an important role in maintaining endothelial cell function" (27). Abnormal changes in COL4 are likely to shift its interactions with endothelial cells and basement membrane components. These processes can lead to impaired basement membrane structure and endothelial cell dysfunction.

COL4 is only found in basement membranes, where it is their main structural constituent. In fact, the main component of the basement membrane is the network forming COL4, which comprises 50% of the basement membrane. Hence, COL4 is the most abundant collagen in basement membranes and is considered to be responsible for their mechanical stability (28). ECM remodeling plays an important role in the development and progression of AH. By means of vascular wall structural and functional alterations, ECM is involved in the pathological process of hypertension. AH is associated with

increased connective tissue proteins' degradation, loss of elasticity, elevated rigidity of the arterial wall, and an abnormal ratio of collagen to elastin. Interestingly, there is limited data in the literature about the ECM changes, and particularly COL4, after preeclampsia

One of the first examinations of COL4 in pregnancy was performed by Ogawa H. et al. (1994) when they localized COL3 and COL4 in the placenta by immunohistochemistry and measured by radioimmunoassay other collagen-related substances such as the amnio-terminal peptide of type III procollagen (P-III-P) and type IV collagen 7S domain (7S) in the serum of pregnant women. The authors used immunohistochemical techniques and studied the localization of COL3 and COL4 in normal and toxemic placentas. A special focus of interest were maternal serum levels of COL3 procollagen peptide and COL4 collagen 7S domain (7S) in non-pregnant women, normal term women, and cases of toxemia in pregnancy. "Immunohistochemical studies revealed that type III collagen exists in the connective tissues composing the villous core and type IV collagen in the basement membranes of trophoblast cells and fetal vascular elements. Even in normal-appearing toxemic placenta, the amount of type III and IV collagen appeared to be increased compared with that in normal-term placenta, but the amount of COL3 and COL4 appeared to be decreased in the necrotized chorionic villi of severe toxemia" (29). The results provided evidence that collagen-related substance levels in toxemic pregnancy were much higher than those in normal term pregnancy. The authors concluded that their data "support type III procollagen and 7S in maternal serum flow from the necrotized chorionic villi into the intervillous space and that these measurements are significant indicators of placental damage caused by toxemia in pregnancy" (29).

In the same year, Furuhashi et al. (1994) investigated serum COL4 and laminin levels in preeclampsia. The authors measured COL4 levels in the maternal serum of preeclamptic and normal pregnant women by radioimmunoassay. They found significantly higher serum COL4 levels in patients with PE than those in the normal pregnant group. Of note, in the preliminary investigations, when studying the changes in COL4 levels during gestation, they found a significant difference in the COL4 levels between the early gestational week and the late gestational week. These findings indicated that the serum COL4 levels of all pregnant groups were significantly higher than those of the non-pregnant controls. Moreover, the maternal serum COL4 levels of preeclamptic groups were significantly higher than those of the normal pregnant group. A correlation was found among the maternal serum COL4 levels in each period of gestation. The authors concluded that "COL4 may have an important role in the maintenance of pregnancy. These results suggest that there is early damage to endothelial cells in preeclampsia" (30).

In another study, Oefner et al. (2015) investigated COL4 at the fetal-maternal interface. Immunohistochemistry has been used by the researchers to examine the distribution of COL1, COL3, COL4, and COL6 in the endometrium and decidua during the menstrual cycle and the first trimester of pregnancy. Quantitative polymerase chain reaction and protein localization by immunohistochemistry were used to determine the expression of COL4 alpha chains during the reproductive cycle. In turn, of the COL4 structure of the placenta was examined using transmission electron microscopy. As for the expression of COL4 alpha chain NC1 domains and collagen receptors, it was localized by immunohistochemistry. The researchers found "a novel expression pattern of col-IV in the mesenchyme of placental villi as a three-dimensional network. NC1 domains of col-IV alpha chains are known to regulate tumor cell migration, and the selective expression of these domains in decidua basalis compared to decidua parietalis was determined" (31). The authors concluded that COL4 is expressed in novel forms in the placenta. These results show that COL4 is not merely a structural protein providing tissue integrity but also plays an integral role in invasive trophoblast cell behavior at the site of implantation.

As mentioned above, ECM proteins play an important role in normal pregnancy and preeclampsia. Because it is very important to find characteristics of COL4 metabolism after preeclamptic pregnancy and its role in hypertension development, we studied COL4 turnover postpartum via measuring PIVNP, a biomarker of COL4 synthesis. The present research found that at 1 year after delivery, 38.46% of women who suffered PE developed arterial hypertension (AH), and 5.77% developed diabetes mellitus. Women who had normal pregnancies developed neither hypertension nor diabetes mellitus 1 year after delivery. Our data showed decreased levels of PIVNP in women one year after PE. This can be partially explained by the current knowledge that arterial hypertension is connected with diminished degradation of connective tissue proteins. In light of these understandings, we propose decreased production of COL4 postpartum, which may contribute to COL4 alteration in the basement membranes and impaired structure of arterial walls. This could be one of the pathways favoring the development of AH after delivery. The lowest levels of serum PIVNP in hypertensive women one year after PE also provide arguments supporting that hypothesis. Our findings demonstrated altered COL4 turnover after preeclamptic pregnancy. The diminished COL4 synthesis might play an important role in persistent vascular wall damage postpartum.

The current evidence demonstrated, for the first time, decreased serum PIVNP levels and altered COL4 turnover in women who had PE. Such abnormal changes, expressed by diminished COL4 synthesis, can contribute to the processes favoring persistent vascular wall damage after preeclamptic pregnancy. We suggest that PIVNP might be involved in the mechanisms determining the development of hypertension

postpartum. Larger studies are warranted to clarify PIVNP's role in the ongoing vascular injury/dysfunction after preeclampsia and the pathogenic pathways of AH manifestation in previously PE women.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.N., N.P., Design: A.N., N.P., Data Collection or Processing: N.P., Analysis or Interpretation: A.N., N.P., Literature Search: N.P., Writing: A.N., N.P.

Ethical Statement

The Project was approved by the Ethics Committee of Medical University Pleven with protocal N70/2023.

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Assay of proteolytic activity of *Bacillus Subtilis* for collagen isolation from Snakehead Fish (*Channa striata*) scales

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Abstract

The collagen source could be derived from fish scales like snakehead fish (*Channa striata*). One of the possible methods can be used enzymatic methods. The objective of this research was to isolate and characterize the collagen from *C. striata* scale using *B. subtilis* protease. This study was an experimental method utilizing the protease enzyme produced by *B. subtilis*. The proteolytic activity of *B. subtilis* was seen from the ability of the bacteria to produce a clear zone on skim-casein agar and inoculated into tryptic soy broth (TSB) as a submerged medium to produce protease. The collagen isolation using *B. subtilis* protease (crude and freeze-dried enzyme) with ratios 1:1, 1:10, 1:100, and 1:1000 and incubation for 6, 12, 18, and 24 hours. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine the molecular weight (MW) of collagen proteins, protein determination by Bradford methods, and morphology was observed by scanning electron microscopy (SEM). All data were analyzed using using SPSS.16 software for windows. The highest collagen was at 12 hours (1:100) incubation using freeze-dried enzymes resulting in a mass of 0.10 grams and a protein content of 3.17 g/ml ($p<0.05$). SDS-PAGE and SEM visualization showed the collagen might be classified as type I collagen consisting of two chains (α1 and α2) with molecular weights approaching 118.03 kDa and 112.20 kDa. Collagen from Channa striata scales can be isolated enzymatically using a protease produced by B. subtilis and can be characterized as type 1 collagen.

Keywords: *Bacillus subtilis, Channa striata*, collagen, protease, scale

1. Introduction

Collagen is the fibrous protein that contributes to the unique physiological functions of connective tissues in skin, tendons, bones, cartilage, and others (1). It is also found in the interstitial tissue of all parenchymal organs, where they contribute to the stability of tissues and organs and maintain their structural integrity (2). Collagen is synthesized and secreted by mesenchymal and epithelial cells. Mesenchymal cells and their derivatives such as fibroblasts, osteoblast, odontoblast, chondroblasts, and cementoblasts are the source of collagen. A basic structural unit of collagen is tropocollagen - α-helix left-handed molecule with transverse bands repeating every 64–67 nm and composed of 3 bonds of collagen monomers (*triple-helix)*. *Triple helix* collagen is highly resistant to proteolytic attacks such as matrix metalloproteinases (MMP) (2).

Collagen is a long-chain protein composed of the amino acid alanine, arginine, lysine, glycine, proline, hydroxyproline, and hydroxylysine, but mostly consist of glycine (33%), proline, and hydroxyproline (22%) (3). The structure of Gly-Pro-Hyp amino acid sequence has been given in the Fig. 1 (4). The amino acid sequence is composed of collagen monomers with glycine in every three residues (5). The sequence of the peptide is (Pro-Lys-Gly) (Pro-Hyp-Gly) (Asp-Hyp- Gly). Furthermore, the characteristic feature of collagen is the presence of the amino acid hydroxyproline. The glycine-X-Y arrangement is continuously repeated, with X and Y being proline and hydroxyproline (5, 6).

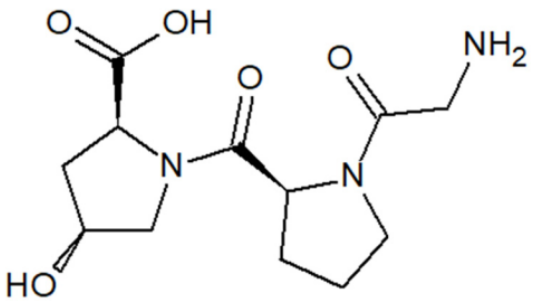

Fig. 1. The structure of Gly-Pro-Hyp amino acid sequence (4)

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Collagen is composed of connective tissue extracellular matrix (ECM) consisting of fibrillar collagen, forming collagen fibrils (3). The function of collagen fibrils is to produce structures with very high tensile strength without the ability to stretch. Collagen fibrils can support at least 10,000 times their weight. Collagen fibers are generally white and opaque and look like typical and repeating transverse lines when observed with scanning electron microscopy (SEM) (1). Collagen has been applied in food manufacturing, pharmaceutical, cosmetic, biomedical materials, and clinical fields (7). The most abundant primary collagens are types I, II, and III for health solution. It has low immunogenicity and is minimally rejected in the human body. It is widely used to help blood clotting, healing, and tissue remodeling. The major, most abundant collagens are types I, II, and III which form the structural fibrils of tissues, while the others only take part in the association of these fibrils with others (1).

Collagen is commonly isolated from plant-based and animal source such as skins and bones of mammals, especially bovine and porcine (1). Among these animal sources, bovine collagen is commonly used for extra-oral and burns wound healing. Porcine collagen is unacceptable for some religions, for example, Judaism and Islam. Moreover, bovine collagen has a risk of contamination from bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), foot-and-mouth disease (FMD), protein misfolding, and allergenicity (8). Consequently, much attention has been paid to the alternative sources of collagen such as bones, skin, fins, and scales of fresh or saltwater fishes (9,10). Fish collagens were considered good alternative sources because there is no risk of disease transmission, and they have a high yield (1). Fish scales are mainly composed of hydroxyapatite $Ca_{10}(OH)_2(PO_4)_6$ and type I collagen fiber with a lower denaturation temperature than the collagen from porcine (11). The source of fish scale collagen such as black drum, sheep's head sea bream, Red-seabream, Red Tilapia, sardine, Japanese sea-bass, skipjack tuna, yellow sea bream, and horse mackerel have been reported. Furthermore, there is limited information of collagen isolation from snakehead fish scales. Snakehead fish (*Channa striata*) is a freshwater fish from the *Channidae* family. This fish is a predator for other small fish and tadpole on fresh water ecosystem. In, Indonesia snakehead fish can be found throughout Indonesia, especially in Sulawesi and Papua (12).

The collagen isolation process needs to be noticed in order to get applicable collagen in society. Collagen can be obtained from the hydrolysis of fish scales with chemicals or enzymatic. Enzymatic hydrolysis using a biological process such as enzyme produced by organism is more promising due to generating less waste and may reduce the processing time (13). Proteases were the most important groups of extracellular enzymes which produced by animals, plants and microorganisms (14). Furthermore, microbial proteases have been exploited in the leather industries in many ways because their ability to produce protease in large proportions. A large proportion of the proteases are derived from Bacillus strains (15), such as *B. subtilis.* Therefore, the objective of this research was to isolate and characterize the collagen from *Channa striata* scale using *B. subtilis* protease

2. Materials and methods

2.1. Research design

This research was an experimental study with a completely randomized block design including incubation time (6, 12, 18, and 24 hours), the type of enzyme (crude and freeze-dried enzyme), and the ratio of the enzyme and substrate (1:1, 1:10, 1:100, and 1:1,000). In each group, 3 repetitions were carried out so the total number of samples was 96. This research was conducted from December 2014 to May 2015 at the molecular biology laboratory, animal anatomy and physiology laboratory and microbiology laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya.

2.2. *Bacillus subtilis* protease production

B. subtilis was grown in skim-casein agar containing (g/L) 5 caseins; 2.5 yeast extract; 28 skims; and 15 bacteria agar and incubated at 37°C for 24 hours. After 24 h incubation, plates were observed for each clear zone around the growth of the organism. If a clear zone is seen, it indicates that *B. subtilis* has the ability to degrade protein through the presence of proteases. Then, *B. subtilis* was inoculated into tryptic soy broth (TSB) as an optimal submerged medium containing (g/L) 170 triptone; 30 soy peptones; 25 dextrose; 50 NaCl; and 25 K_2HPO_4 and cultured in a shaker incubator (180 rpm) at 37°C for 48 hours (16). The whole fermented broth was centrifuged at 10,000 rpm at 4°C for 10 minutes and the supernatant was obtained. Crude enzymes from the supernatant were subjected to further research and were continuously lyophilized by freeze-drying machines as a source of proteases.

2.3. Protease assay

The protease activity from crude and freeze-dried enzyme from *B. subtilis* was measured using casein as a substrate. A mixture of 200 µl (500 ppm) of casein (in 300 ml phosphate buffer pH 7), and 100 µl protease (crude and freeze-dried enzyme) were incubated in an incubator at 37°C for 60 minutes. After that, the enzyme reaction was terminated by the addition of 400 µl of 4% (w/v) trichloroacetic acid (TCA) and was kept at room temperature for 30 minutes. Then, the mixture was centrifuged to separate the unreacted casein at 4,000 rpm for 10 minutes. The supernatant was diluted in 5 times phosphate buffer and measured the absorbance at 275 nm (16). The protease activity profile of the supernatant from *B. subtilis* on TSB media and measured by formula (Formula 1). One unit of protease is defined as the amount of enzyme that releases 1 µg of tyrosine per ml per minute under the standard conditions of supernatant solution (17).

$$\frac{[C]}{(Mr*t)} \quad \frac{x}{E} \quad H \quad x \quad Df$$

Formula 1. [C], concentration (U/ml); Mr, molecular weight of tyrosine (181.19); t, time (minute); H, total volume (ml); E, enzyme volume (ml); Df, dilution factor

2.4. Enzymatic isolation of collagen from snakehead fish scale

All preparation procedures were performed at 4°C. The powder of Kanjilo or snakehead fish scale was obtained from Makassar, South Sulawesi, and soaked in 100 ml of 0.5 M acetic acid for 24 hours and centrifuged at 4,500 rpm for 15 minutes. *B. subtilis* protease added into supernatant comparing of (v/v) 1:1, 1;10, 1:100 and 1:1,000 then incubated for 6, 12, 18, and 24 hours. The mixture was centrifuged at 4,500 rpm for 30 minutes to separate and precipitate the fibrous collagen. Pellets were mixed in 0.5 M acetic acid (1:9) and dialyzed using a cellophane membrane in 0.1 M acetic acid and deionized water to get pepsin solubilized collagen (PSC) (18).

2.5. Protein determination of collagen from snakehead fish scale

Total protein content was measured by Bradford methods using bovine serum albumin (BSA) as a standard protein. Collagen samples were taken100 µl and added 1 ml of diluted PRO-MEASURE solution. Then incubated for 2 minutes at room temperature and measured the absorbance at 595 nm (iNtRon Biotechnology, Inc) (19).

2.6. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by Laemli methods. 10 µl collagen samples were loaded onto polyacrylamide gel made of 12,5% separating gel and 3 % stacking gel. After electrophoresis, each gel was stained by Coomassie Brilliant Blue (CBB) R-250 for 15 minutes and destained for 30 minutes (20).

2.7. Morphology analysis

The morphology of collagen was observed by Hitachi High-Technologies scanning electron microscopy (HITACHI-TM3000 SEM).

2.8. Statistical analysis

The means of each data and standard deviation were calculated using Microsoft Excel. Furthermore, data from each group, including incubation time (6, 12, 18, and 24 hours), type of enzyme (crude and freeze-dried enzyme), and the ratio of enzyme and substrate 1:1, 1:10, 1:100, and 1:1,000, were analyzed using factorial test or completely randomized block design using SPSS.16 software for windows

3. Results

In this study, the extraction of collagen from snakehead fish scales supernatant was carried out using a protease enzyme from *B. subtilis.* The enzyme treatment was based on the ratio enzyme volume (µl) compared to snakehead fish scales supernatant volume (µl), which included 1:1, 1:10, 1:100, and 1:1,000.

Fig. 2 shows that at 1:1,000, the enzyme still works optimally and produces collagen mass mostly at 12 hours of incubation. The highest mass of collagen produced by crude enzyme was 0.34 gram, while for the freeze-dried enzyme, the collagen mass tended to increase following the incubation time. Furthermore, when compared at 1:1, 1:10, and 1:100 at 12 hours incubation, the highest collagen mass was found at 1:100 for crude enzymes and freeze-dried enzymes, which were 0.15 gram and 0.10 gram, respectively.

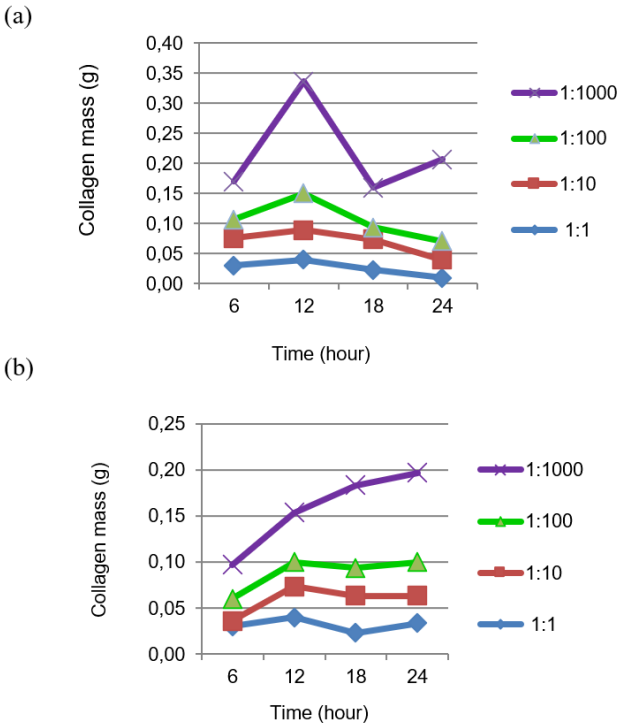


Fig. 2. Collagen mass produced by snakehead fish scales isolation with crude enzyme (a) and freeze-dried enzyme (b)

3.1. The protein content of snakehead fish scale collagen isolated using the *B. subtilis* protease

The protein content of snakehead fish scale collagen was determined by the Bradford method, which measured the total protein concentration in a solution by colorimetry.

Fig. 3 shows the protein content of snakehead fish scales collagen isolated using the *B. subtilis* protease, both crude enzyme (a) and freeze-dried enzyme (b). In this research using 1:1,000, the crude enzyme produced the highest levels of collagen protein at 12 hours of incubation (2.47 µg/ml), while the freeze-dried enzyme using 1:1,000 produced relatively lower that was 1.96 µg/ml at 24 hours of incubation. Moreover, the highest protein content of collagen was produced by 1:100 at 12 hours incubation using freeze-dried enzyme as much 3.17 (µg/ml) ($p<0.05$).

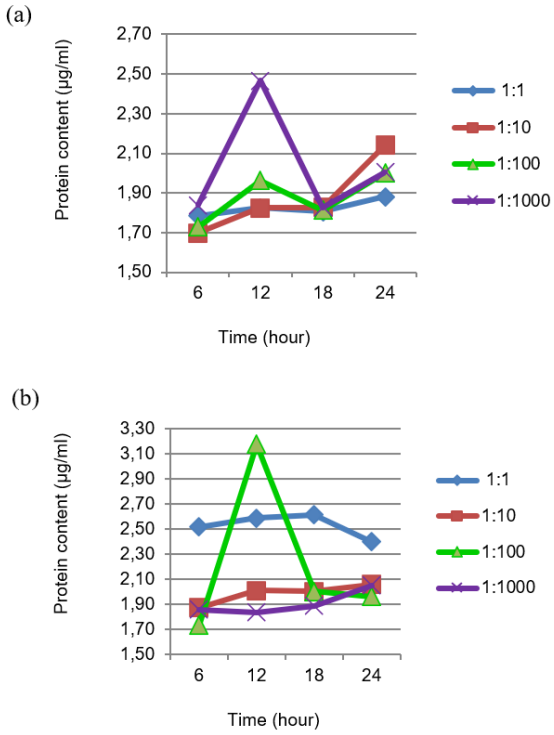


Fig. 3. Total protein content produced by snakehead fish scales isolation with crude enzyme (a) and freeze-dried enzyme (b)

3.2. Molecular weight of collagen from snakehead fish

The SDS-PAGE analysis of PSC from snakehead fish scale was shown in Fig. 4.

The protein banding pattern produced by SDS-PAGE in this study showed that the collagen residues from snakehead fish scales were composed of α- and β-chain that appeared to coincide. Collagen from the snakehead fish scale consists of two α-chains (α1 and α2) with the molecular weight of approximately 118.03 kDa and 112.20 kDa, respectively, and β chain with a molecular weight 137.40 kDa.

3.3. Snakehead fish collagen visualization

The fibrils formation profile shown in Fig. 5 from SEM visualization presented the collagen fibril morphology. The morphology showed random coil structure and obvious fibril networks with the rough membranous structure for the collagen membrane.

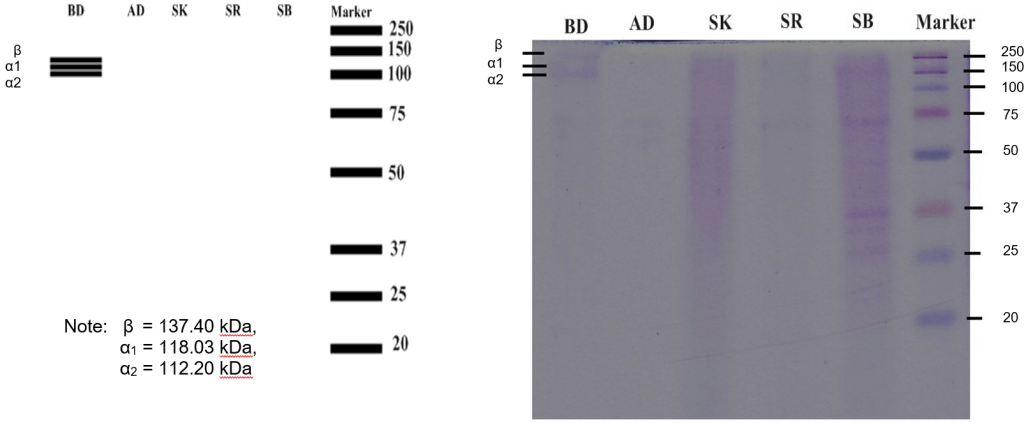
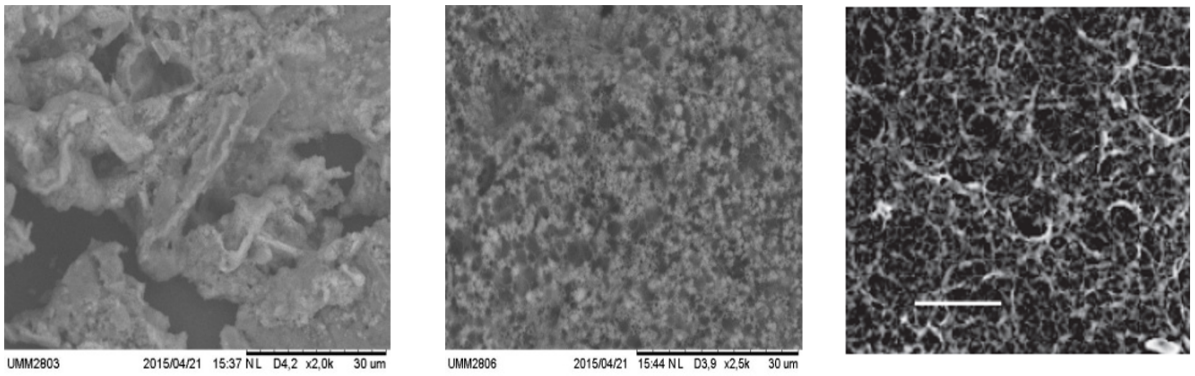


Fig. 4. The molecular weight of collagen type 1 from snakehead fish scales. *BD, before dialysis; AD, after dialysis; SK, sisik kering (dry scales); SB, sisik basah (wet scales)*



(a) (b) (c)

Fig. 5. SEM visualization of collagen from snakehead fish scales with mag. 2000x (a), 2500x (b), and collagen from bovine Bar 5 µm (30) (c)

4. Discussion

This research is a continuation of previous research carried out by the author (16). Relative protease activity was shown by clear zones around the growth of the *B. subtilis* colony on skim-casein agar plates. It was explained that the *B. subtilis* has the ability to degrade protein, which breaks the peptide bond CO-NH into free amino acids. Skim-casein agar was the media that contained skim milk and casein. The media had the function of inducing alkaline protease synthesis by bacteria. The protease activity of *B. subtilis* was influenced by temperature and incubation time. The increase of temperature which is more than optimum temperature will affect to the conformational changes of the substrate so that the substrate is inhibited and reduces enzyme activity. The protease activity of *B. subtilis* can be stable at temperatures between 30°C to 60°C and optimum at 37°C (21). Moreover, the optimum incubation time around 48 hours (22). Measurements of protease activity were interpolated against the standard tyrosine curve. Tyrosine standard curve was made with several variations of tyrosine concentration (10-100 ppm) and measured with absorbance of 275 nm. The absorbance value is the maximum wavelength for UV absorption by aromatic amino acids such as tyrosine, tryptophan, and phenylalanine (17).

Samples of crude enzyme and freeze-dried enzyme from *B. subtilis* were measured for their protease activity using casein as a substrate. The amount of protease activity was determined based on the amount of tyrosine produced from the hydrolysis of casein at a wavelength of 275 nm. One unit of enzyme activity is expressed as the volume (ml) of enzyme required to produce 1 mg of tyrosine per minute from casein substrate (Formula 1). The result showed that protease activity from crude enzyme and freeze-dried enzyme *B. subtilis* was significantly different ($p<0.05$). The crude enzyme produced 0,134 U/ml, while freeze-dried enzyme produced 0.106 U/ml. The protease activity was decreased in freeze-dried enzyme because the lyophilized or freeze-drying process induce the inactivation of the enzyme and was also reduced by the presence of a proteinaceous additive, such as sugar molecules that exists naturally (23). Furthermore, the stability of the enzyme during the freezing and freeze-drying process is affected by concentration (23), while in this research, the enzyme concentration was not measured.

Collagen extraction was carried out through a series of complex procedures, starting with the collection of raw materials, pretreatment, extraction, and purification. The two most common extraction methods are acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) (13). Based on the research of Schmidt (2016), it is known that PSC is the most ideal extraction method because the enzyme has a greater reaction selectivity. In this study, the extraction of collagen dissolved in the snakehead fish scales supernatant was carried out using a protease enzyme from *B. subtilis* which acts like pepsin. The enzyme treatment was based on the ratio (enzyme volume: snakehead fish scales supernatant volume), which included 1:1, 1:10, 1:100, and 1:1,000.

Collagen mass produced from snakehead fish scale influenced by time incubation, type of enzyme (crude or freeze-dried enzyme from *B. subtilis*), and ratio hydrolyzed sample volume from snakehead fish scales supernatant. Fig. 2 shows the different volumes of the substrate (1:1, 10, 1:100 dan 1:1,000) from snakehead fish scales supernatant can be hydrolyzed by the enzyme from *B. subtilis* as much as 1µl ($p<0.05$). Moreover, the figure shows that at 1:1000, the enzyme still works optimally and produces collagen mass mostly at 12 hours of incubation. The highest mass of collagen produced by crude enzyme was 0.34 grams, while for the freeze-dried enzyme, the collagen mass tended to increase following the incubation time. Furthermore, when compared at 1:1, 1:10, and 1:100 at 12 hours incubation, the highest collagen mass was found at 1:100 for crude enzymes and freeze-dried enzymes, which were 0.15 gram and 0.10 gram, respectively.

The extraction method in this study was in line with Matmaroh et al. (2011), which stated that collagen extraction from sharp nose stingray fish scales was more soluble in the presence of the pepsin (PSC) compared to the extraction method using only 0.5 M acetic acid (ASC). Pepsin is a type of protease enzyme. This enzyme plays a role in breaking down the collagen cross-links in the telopeptide region (18). Many studies have been carried out on collagen extraction using the PSC method, such as the cartilage of the brown-banded bamboo shark with the collagen yield of 9.59% (24), the jelly fish as much as 60% (25), the swim bladder of yellow-fin tuna as much as 12.10% (26), and so on. This shows that the use of pepsin in the hydrolysis of collagen can break the polypeptide chain in certain areas without destroying the integrity of the collagen triple helix structure, then resulting in higher PSC (27).

The protein content of snakehead fish scale collagen was determined by the Bradford method, which measured the total protein concentration in a solution by colorimetry. The composition of Bradford's solution was Coomassie brilliant blue (CBB) which was acidic and could bind to the proteins, then changed the solution color to blue. Fig. 3 shows the protein content of snakehead fish scales collagen isolated using the *B. subtilis* protease, both crude enzyme (a) and freeze-dried enzyme (b). The protein content measurement was carried out on the residual mass of collagen obtained from isolation method (fig. 2). However, the amount of residual collagen mass was not influence to the measurement of protein content ($p>0.05$). This could be seen in the comparison of 1:1,000, which produced the highest collagen residue mass (fig. 2) but produced fluctuating protein content (fig. 3). In this research using 1:1,000, the crude enzyme produced the highest levels of collagen protein at 12 hours of incubation (2.47 µg/ml), while the freeze-dried enzyme produced relatively lower that was 1.96 µg/ml at 24 hours of incubation. This happens because an

enzyme can become unstable in the freeze-thawing and freeze-drying process before being used (28). Proteins are also thought to be more susceptible to denaturation under various stress conditions (23). Moreover, fig. 2 and 3 shows the highest collagen mass and protein content produced by 1:100 at 12 hours incubation using freeze dried enzyme. The quantities of collagen mass and protein content were produced, approximating 0.10 gram and 3.17 (μg/ml) ($p<0.05$).

The protein banding pattern produced by SDS-PAGE in this study showed that the collagen residues from snakehead fish scales were composed of α- and β-chain that appeared to coincide. It is caused by the amino acid composition of α- and β-chain is not much different and has almost the same relative molecular weight. This figure also showed that the molecular weight of collagen could be classified as type 1. Collagen type I was the dominant type of collagen that used in cosmetics properties because the human skin was mainly built by type I, III, and V collagen. Moreover, collagen can accelerate wound healing and tissue regeneration. This collagen was composed of two different α-chain (α1 and α2) and β-chain. The molecular weight of β-chain was heavier than α-chain (4).

Collagen from snakehead fish scale consist of two α-chains (α1 and α2) with the molecular weight approximately 118.032 kDa and 112.201 kDa. Moreover, a small amount of β chain with molecular weight 137.404 kDa dimerized by components were obtained in this collagen due to hydrolysis by proteases. This pattern was similar to the collagen previously reported from the skin of *Priyacanthus tayenus* that had molecular weights 118 KDa and 111 KDa (29). The diversity of collagen types is also caused by differences in gene expression in protein biosynthesis. In addition, posttranslational modification of collagen also has a significant effect on collagen diversity and its groupings, such as collagen type I (found in skin, tendon, and bone tissue), type II (found in cartilage), and type III (found in skin and vasculature) (4).

The fibrils formation profile shown in Fig. 5 from SEM visualization presented the collagen fibril morphology. The morphology showed random coil structure and obvious fibril networks with rough membranous structure for the collagen membrane. The result suggested that this collagen might undergo partial cleavage in the telopeptide region by protease treatment (31). The formation of fibril on collagen is contains hydroxylysine and subsequent O-glycosidic bonds which is modulators of the fibrilllogenesis process (5). The collagen consists of the matrix at the later stage that take place as microfilaments, fibrils, and mature collagen filament (4). This formation was influenced by the isolation method. Collagen was extracted in an acidic solution containing pepsin in an isolation process which only attacks the non-triple helix domain of the original collagen. Therefore, it has a small molecular weight without a triple-helix structure. Collagen monomer bonds in the form of fibrils make collagen hydrophobic and have electrostatic interactions (30).

This research showed collagen from *Channa striata* scales can be isolated enzymatically using a protease produced by *B. subtilis* which can be characterized as type 1 collagen. Thus, collagen from snakehead fish scales can be an alternative source of collagen for further applications in the food and nutraceutical industries.

Ethical Statement

None to declare.

Conflict of interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

Concept: S.N., Design: S.N., N.W., Data Collection or Processing: S.N., Analysis or Interpretation: S.N., N.W., Literature Search: S.N., Writing: S.N., N.W.

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Clinical profiles of neuromuscular disorders: A tertiary hospital experience

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Abstract

This study aimed to identify the symptoms and disease-related features of the most common neuromuscular disorders (NMD) to establish an appropriate multidisciplinary approach in a specific field.A total of 46 patients, 26 (56.5%) women and 20 (43.5%) men with a median age of 46.0 (17.0-72.0) years were divided into three groups according to the affected part of the motor unit: Group I (neuronopathy group, n=11), motor neuron diseases; Group II (neuropathy group, n=13), peripheral neuropathies; and Group III (myopathy group, n=22), myopathies. Demographic and clinical features, functional level, muscular strength, balance, dexterity, activities of daily living, functional performance, global cognitive status test scores, respiratory symptoms, and pulmonary test results were recorded from patient files.There was a significant difference between the mean ages of Groups II and III (p =0.046). A significant difference was observed in muscle weakness between Groups II and III (p = 0.044). The prevalence of dysarthria and speech disorders was higher in Group III (33.3%) compared to Groups II (10%) and I (12.2%). The summarized mini-mental test scores were significantly lower in Group I than in Groups II and III (p =0.047 and p = 0.034, respectively). The nine-hole peg test scores were significantly longer in Group I than in the other groups (p = 0.034 and 0.038, respectively). In this study, NMD were divided into three main groups, and the most common symptoms and clinical findings were evaluated. Fatigue and muscle weakness were the most common symptoms shared by all groups. The mean age of the disease was lowest in the myopathy group. Dysarthria was most common in the neuropathy group. Cognitive impairment was most common in the neuronopathy group.

Keywords: neuromuscular disorders, neuronopathy, peripheral neuropathy, myopathy, clinical features, experience

1. Introduction

Neuromuscular disorders (NMD) are a complex group of heterogeneous, acquired, or hereditary disorders that affect different motor unit parts (1). Injury can occur in motor neurons, sensory neurons, axons, Schwann cells, neuromuscular junctions, muscles, or any combination of these regions (2). Therefore, the term NMD includes a wide variety of different syndromes (3). A systematic neurological evaluation is crucial in diagnosing these complex diseases (4).

The prevalence of NMD is not precisely known and differs across countries. In Turkey, there are approximately 100,000 (Social Security Institutions [SSI]) with NMD in different groups. It is known that there are 70,000 neuromuscular patients in Western Europe, 40,000 in the USA, and over 35,000 in the UK (1, 5). These disorders are rare, genetic, progressive, and untreatable. Some of these cases are autoimmune and can be treated. Symptoms can appear at any age, from childhood to adulthood, and range from mild to severe sensory and/or motor impairment and cardiac or respiratory involvement, which may require life support and/or result in death (6). Muscle weakness is the most common symptom shared by all these disorders (3). The onset and progression of muscle weakness provide some clues for the diagnosis of the disease. Proximal muscle weakness is a presenting symptom of myopathies such as Duchenne and Becker muscular dystrophies, fascioscapulohumeral, limb-girdle, juvenile spinal muscular atrophy, polymyositis, mitochondrial, glycolytic, or lipid storage myopathy, and also Lambert-Eaton myasthenic syndrome. Proximal muscle weakness may be described by patients as a complaint of difficulty combing hair, getting up from a chair, or climbing stairs. Distal muscle weakness can manifest as difficulty in performing finely coordinated movements, such as grasping, jumping off pavement, or descending stairs. Myotonic muscular dystrophy, distal myopathy, scapuloperoneal dystrophy, amyotrophic lateral sclerosis, and distal spinal muscular atrophy are predominantly associated with distal weakness. Distal weakness indicates neuropathy when there is an early loss of deep tendon reflexes and accompanying sensory loss. The trade of muscle weakness is also a guide for the diagnosis. Muscle weakness with remissions and flare-ups

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that fluctuate throughout the day, often worse in the evening than in the morning, is usually caused by myasthenia gravis (7). In addition to muscle weakness, patients should be questioned about common symptoms such as cramps, spasms, and stiffness (8). A comprehensive neuromuscular examination of each patient with NMD is required to determine an effective treatment plan and to initiate rehabilitation. A neuromuscular examination often includes cranial nerve function testing, manual muscle testing, an inspection of muscle atrophy, hypertrophy, and fasciculation, observation of gait, rising from a chair, evaluation of the activity of deep tendon reflexes and the presence of pathologic reflexes, as well as assessing sensory function (3, 7). A long-term multidisciplinary approach is often required (9). This study aimed to review the demographic and clinical features, functional status, current findings, and complications of patients with NMD, and to compare these findings of three main groups: neuronopathies, neuropathies, and myopathies.

2. Matherials and Method

2.1. Study design and patients

This was a retrospective study of all eligible patients admitted to the Physical Medicine and Rehabilitation Department of a tertiary hospital between September 2020 and October 2021. The data of the patients were analyzed from the patient files. The study was performed in accordance with the Declaration of Helsinki, and approval from the local ethics committee was obtained (No: 91/05, 06.07.2020). Medical files of all hospitalized neuromuscular patients with motor neuron involvement, peripheral neuropathies, or myopathies were reviewed. Patients with (i) involvement of the neuromuscular junction and (ii) missing information in their files were excluded. A total of 46 patients, 26 (56.5%) women and 20 (43.5%) men were included in the study. These patients were divided into three groups based on the affected component of the motor unit: Group I (neuronopathy group, n=11), motor neuron diseases; Group II (neuropathy group, n=13), peripheral neuropathies; and Group III (myopathy group, n=22), myopathies.

2.2. Demographic variables and clinical features

Demographic characteristics and clinical features of the patients, including the following variables: age (year), sex (male/female), job (white collar, blue collar, housewife, retired), weight (kilogram), height (centimeter), body mass index (BMI) (kg/m²), comorbidities (yes/no), age of disease onset (year), firt presenting symptom, disease duration (year), muscle weakness (yes/no), fatigue (yes/no), spasticity (yes/no), muscle twitching (yes/no), cramps (yes/no), pain (yes/no), pain level last week by a score from 0 to 10 on the Visual Analog Scale (VAS), dysphagia (yes/no), dysarthria (yes/no), speech disorders (yes/no), independent walking (yes/no), age at which independent walking ends (year), use of an assistive or adaptive device (yes/no), use of a wheelchair (yes/no), respiratory support (yes/no), enteral nutrition (yes/no), osteoporosis (yes/no), number of falls in the last six month, and history of bone fracture (yes/no), were recorded from the patient files.

2.3. Examination findings and clinical assessment tests

Muscle strength was evaluated using the Medical Research Council (MRC) test, bilateral upper and lower extremity range of motion (ROM) measurements with a goniometer, posture analysis according to the New York Postural Rating Scale, hand grip strength evaluation with Jamar hand dynamometer and pinch meter, and dexterity with nine-hole peg test (NHPT) (10). The 6-minute walking test (6 MWT) for functional capacity (11), 5-time sit and stand test (12), one-leg standing test (13), and stair and climb test for lower extremity strength and balance (14), Brooke and Vignos scale for functional status (15), Functional Independence Measure (FIM) for daily living activities (16), Functional Ambulation Scale (FAS) for ambulation levels (17), and standardized mini-mental test for global cognitive status (18) were obtained from patient files. Pulmonary function test (PFT) measurements were also performed.

2.4. Statistical analysis

SPSS version 22.0 software (IBM Corporation, Chicago, IL, USA) was used for the statistical analysis. Categorical variables and other discrete and continuous variables were represented as percentages, numbers, and medians (min-max), respectively. Variables with a normal distribution were represented as mean±standard deviation (SD). The Kolmogorov-Smirnov test was used for data distribution analysis. Continuous and non-parametric variables were compared using the Mann-Whitney U test. Fisher's exact and chi-square tests were used to compare the categorical variables. Comparisons among groups were performed using the Kruskal-Wallis test or ANOVA. Post-hoc analysis with Bonferroni correction was used for pairwise comparisons. A p-value of less than 0.05 was found to be statistically significant.

3. Results

Forty-six neuromuscular patients who met the inclusion criteria and were followed up in the neuromuscular disease unit of our hospital were enrolled in this study. The main pathologies were presented according to the group in which patients were included: Group I: Motor neuron involvement (11 patients), 5 Amyotrophic Lateral Sclerosis (ALS), 3 poliomyelitis, and 3 Spinal Muscular Atrophy (SMA). Group II: Peripheral neuropathies (13 patients): 6 diabetic polyneuropathies, 2 Charcot-Marie Tooth (CMT) diseases, 3 chronic inflammatory demyelinating neuropathies (CIDP), and 2 Guillain-Barré syndrome (GBS). Group III: Myopathies (22 patients): 8 myotonic dystrophies, 2 polymyositis, 5 muscular dystrophies, 3 core myopathies, and 3 mitochondrial myopathies.

The study population consisted of 26 (56.5) % women and 20 (43.5%) men. The median age of the population was 46.0 years (17.0-72.0). Age of patients in Group I: 54.0(17.0-72.0) years; Group II: 52.0 (21.0-67.0) years; Group III: 38.0 (17.0-

69.0) years. There was a significant difference between the mean ages of Groups II and III (p =0.046). The median age of onset of the first symptoms was 25.5 (1.50-68.0) years, while the median age of diagnosis was 27.5 (1.5-71.0) years. When comparing the median age of disease onset in the three study groups, a significant difference was observed between Group II and III (p = 0.028). The other demographic characteristics of the patients are presented in Table 1. When comparing the disease-related data of the three study groups, as shown in Table 2, a significant difference was found in muscle weakness between Group II and III (p = 0.044). The prevalence of dysarthria and speech disorders was higher in Group III (33.3%) when compared to Group II (10%) and I (12.2%) (Table 2).

Table 1. Demographic characteristics and clinical features of patients

Variables	All patients (n=46)	Group 1 (n=11)	Group 2 (n=13)	Group 3 (n=22)	P1	P2	P3
Age (year), median (min-max)	46.0 (17.0-72.0)	54.0 (17.0-72.0)	52.0 (21.0-67.0)	38.0 (17.0-69.0)	0.593	0.183	**0.046***
Gender, n (%) Female Male	 26 (56.5) 20 (43.5)	 6 (54.5) 5 (45.5)	 7 (53.8) 6 (46.2)	 13 (59.1) 9 (40.9)	 0.974	 0.811	 0.771
Occupation,n(%) White collar Blue collar Housewife Retired	 15 (32.6) 6 (13.0) 16 (34.8) 9 (19.6)	 3 (27.2) 4 (36.4) 2 (18.2) 2 (18.2)	 3 (23.0) 2 (15.4) 4 (30.8) 4 (30.8)	 9 (40.9) 0 10 (45.5) 3 (13.6)	 0.473	 0.384	 0.065
Age of disease onset (year), median (min-max)	27.5 (1.5-71.0)	42.5 (1.5-71.0)	44.5 (8.0-61.0)	23.0 (7.0-52.0)	0.857	0.057	**0.028***
Height (cm), mean±SD	164.28±9.02	161.44±9.47	167.60±8.60	163.78±8.95	0.145	0.523	0.145
Weight (kg), median (min-max)	66.0 (42.0-100.0)	63.5 (48.0-80.0)	67.0 (42.0-100.0)	67.0 (45.0-95.0)	0.556	0.502	0.992
BMI (kg/m²), mean±SD	24.41±5.38	23.66±3.59	23.41±6.68	25.11±5.43	0.995	0.534	0.507
Comorbidities, n (%)	24 (52.2)	11 (100)	3 (23.1)	10 (45.5)	0.111	0.116	0.924

Values are mean±SD (standard deviation), median (min-max) or percentage (n,%) *p values are statistically significant (p < 0.05) and are shown in bold. Group 1: Neuronopathy group, Group 2: Neuropathy group, Group 3: Myopathy group, P1: P value between group 1 and group 2, P2: P value between group 1 and group 3, P3: P value between group 2 and group 3.

Table 2. Disease-related symptoms and clinical features of patients

Variables	All patients (n=46)	Group 1 (n=11)	Group 2 (n=13)	Group 3 (n=22)	P1	P2	P3
Muscle weakness, n (%)	42 (91.3)	10 (90.9)	10 (76.9)	22 (100)	0.229	1.000	**0.044***
Muscle atrophy, n (%)	27 (58.7)	8 (72.7)	5 (38.5)	14 (63.6)	0.090	0.440	0.179
Fatigue, n (%)	37 (80.4)	9 (81.8)	10 (76.9)	18 (81.8)	0.604	0.998	0.653
Spasticity, n (%)	4 (8.7)	2 (18.2)	1 (7.7)	1 (4.5)	0.560	0.237	0.724
Muscle twitching, n (%)	21 (45.7)	7 (63.6)	4 (30.8)	10 (45.5)	0.100	0.280	0.427
Cramp, n (%)	20 (43.5)	4 (36.4)	6 (46.2)	10 (45.5)	0.691	0.690	0.895
Pain, n (%)	28 (60.9)	8 (72.7)	8 (61.5)	12 (54.5)	0.646	0.262	0.719
Disphagia, n (%)	9 (19.6)	2 (18.2)	4 (30.8)	3 (13.6)	0.646	0.637	0.211
Dysarthria, n (%)	10 (21.7)	2 (18.2)	6 (46.2)	2 (9.1)	0.204	0.572	**0.013***
Speed disorders, n (%)	13 (28.3)	3 (27.3)	7 (53.8)	3 (13.6)	0.231	0.346	**0.015***
VAS (0-100), median (min-max)	60.0 (0.0-100.0)	80.0 (0.0-80.0)	65.0 (0.0-80.0)	30.0 (0.0-100.0)	0.548	0.095	0.232
İndependent walking (year), median (min-max)	33 (71.7)	9 (81.8)	9 (69.2)	15 (68.2)	0.594	0.380	0.677
Age of ending independent walking (year), median (min-max)	41.0 (2.0-64.0)	42.0 (42.0-42.0)	52.0 (51.0-60.0)	25.0 (2.0-64.0)	0.163	0.517	0.060
Assistive technology, n (%)	8 (17.4)	2 (18.2)	1 (7.7)	5 (22.7)	0.931	0.863	0.637
Adaptive device, n	3 (6.5)	1 (9.1)	0	2 (9.1)	0.905	0.935	0.998

(%)							
Wheelchair, n (%)	7 (15.2)	10 (90.9)	1 (7.7)	6 (27.3)	0.998	0.142	0.387
Respiratory insufficiency, n (%)	1 (2.2)	10 (90.9)	0	1 (4.5)	---	0.493	0.473
Enteral nutrition, n (%)	2 (4.3)	2 (18.2)	0	0	0.214	0.091	1.000
Falls, n (%)	21 (45.7)	5 (45.5)	4 (30.8)	12 (54.5)	0.653	0.959	0.465
Bone fracture, n (%)	8 (17.4)	4 (36.4)	2 (15.4)	2 (9.1)	0.361	0.060	0.586

Values are median (min-max) or percentage (n,%) *p values are statistically significant ($p < 0.05$) and are shown in bold. Group 1: Neuronopathy group, Group 2: Neuropathy group, Group 3: Myopathy group, P1: P value between group 1 and group 2, P2: P value between group 1 and group 3, P3: P value between group 2 and group 3. VAS: Visual Analog Scale.

The SMMT scores were significantly lower in Group I than in Group II and III (p =0.047 and 0.034, respectively). NHPT scores for the left hand were significantly longer in Group I than in Group II and III (p = 0.034 and 0.038, respectively). No significant difference was observed in other clinical findings and physical examination tests, including the Jamar hand dynamometer and pinch meter, NHPT scores for the right hand, 6 MWT, five times sit and stand test, one-leg standing test, stair and climb test, Brooke and Vignos scale, FIM scores, FAS scores, and PFT measurements (p>0.05), as shown in detail Table 3 and 4.

Table 3. Clinical assessment tests and functional tests

Variables	All patients (n=46)	Group 1 (n=11)	Group 2 (n=13)	Group 3 (n=22)	P1	P2	P3
FAS level	5.0 (0.0-5.0)	5.0 (0.0-5.0)	4.5 (1.0-5.0)	4.0 (1.0-5.0)	0.731	0.511	0.771
Brooke score	1.0 (1.10-6.0)	1.0 (1.0-5.0)	1.0 (1.0-2.0)	1.0 (1.0-6.0)	0.567	0.570	0.217
Vignos score	3.0 (1.0-10.0)	2.0 (1.0-10.0)	2.0 8(1.0-9.0)	3.0 (1.0-10.0)	0.616	0.452	0.845
FIM-Motor	88.0 (21.0-91.0)	85.0 (33.0-91.0)	86.0 (42.0-91.0)	90.0 (21.0-91.0)	0.723	0.929	0.706
FIM-Cognitive	35.0 (18.0-35.0)	35.0 (35.0-35.0)	35.0 (30.0-35.0)	35.0 (18.0-35.0)	0.715	0.273	0.514
FIM-Total	121.0 (56.0-126)	120.0 (68.0-126.0)	121.0 (72.0-126.0)	122.0 (56.0-126.0)	0.681	0.853	0.773
SMMT score	28.0 (26.0-30.0)	27.0 (24.0-30.0)	29.5 (29.0-30.0)	30.0 (27.0-30.0)	0.047	**0.034***	0.737
Jamar-right hand (kg)	16.63 (2.83-36.3)	21.43 (3.2-36.3)	17.0 (11.6-32.3)	13.06(2.83-22.66)	0.882	0.054	0.117
Jamar-left hand (kg)	14.58(1.0-34.0)	24.15 (1.0-34.0)	18.0 (9.40-31.6)	10.36(2.40-24.33)	0.834	0.088	0.193
Pinchmeter-right hand (kg)	7.33 (2.6-23.0)	7.75 (3.0-23.0)	7.5 (2.60-14.83)	6.41 (2.66-17.33)	0.472	0.175	0.675
Pinchmeter-left hand (kg)	7.33 (2.5-22.6)	7.50 (2.5-22.6)	7.5 (3.0-12.0)	7.16 (2.66-14.33)	0.250	0.087	0.791
Nin-hole peg test-right (sec)	26.0 (19.28-43.70)	27.30 (20.0-43.7)	24.9 (21.0-39.0)	25.0 (19.28-43.0)	0.532	0.400	0.693
Nine-hole peg test-left (sec)	26.0 (19.0-65.0)	29.3 (20.0-65.0)	24.37 (21.0-27.0)	26.5 (19.0-35.0)	0.034	**0.038***	0.557
5-time sit-to-stand test (sec)	17.0 (0.0-60.0)	19.5 (10.0-54.0)	17.26 (11.0-60.0)	14.0 (0.0-43.0)	0.784	0.269	0.419
Stair-climb test-up (sec)	5.0 (2.52-24.0)	4.31 (3.0-10.0)	8.0 (3.0-20.0)	5.0 (2.52-24.0)	0.362	0.689	0.551
Stair-climb test-down (sec)	4.0 (2.0-57.0)	3.60 (2.80-9.60)	3.0 (2.0-14.0)	4.08 (2.0-57.0)	0.900	0.621	0.485
One-leg standing test-right (sec)	6.0 (0.0-77.0)	6.0 (0.0-69.0)	3.5 (0.0-77.0)	12.0 (0.0-52.0)	0.996	0.983	0.980
One-leg standing test-left (sec)	4.5 (0.0-180.0)	4.75 (0.0-14.0)	2.5 (0.0-180.0)	8.0 (0.0-57.0)	0.246	0.687	0.343
6MWT (m)	330.0 (9.0-630.0)	225.0 (9.0-560.0)	280.0 (30.0-510.0)	349.0 (90.0-630.0)	0.859	0.237	0.325

Values are median (min-max). *p values are statistically significant ($p < 0.05$) and are shown in bold. Group 1: Neuronopathy group, Group 2: Neuropathy group, Group 3: Myopathy group, P1: P value between group 1 and group 2, P2: P value between group 1 and group 3, P3: P value between group 2 and group 3. FAS: Functional ambulation scale, FIM: Functional Independent Measure, SMMT: Summarized Mini-Mental Test, 6MWT: 6 Minutes Walking Test. Kg: kilogram, sec: second, m: meter.

Table 4. Respiratory symptoms and pulmonary functional tests

Variables	All patients (n=46)	Group 1 (n=11)	Group 2 (n=13)	Group 3 (n=22)	P1	P2	P3
Cough, n (%)	27 (58.7)	5 (45.5)	7 (53.8)	15 (68.2)	0.462	0.209	0.117
Respiratory tract infection, n (%)	3 (6.5)	0	1 (7.7)	2 (9.1)	0.998	0.328	0.907
FEV1, median (min-max)	78.9 (49.5-120.0)	120 (120.0-120.0)	90.95 (78.9-103.0)	76.05 (49.5-98.0)	0.397	0.046	0.267

FVC, median (min-max)	78.0 (53.4-116.0)	116.0 (116.0-116.0)	88.7 (78.0-99.4)	75.45 (53.4-106.0)	0.380	0.130	0.550
FEV1/FVC, median (min-max)	86.0 (73.0-92.0)	86.5 (86.5-86.5)	86.35 (85.7-87.0)	82.5 (73.0-92.0)	0.133	0.704	0.599

Values are median (min-max) or percentage (n,%). P values are statistically significant ($p < 0.05$). Group 1: Neuronopathy group, Group 2: Neuropathy group, Group 3: Myopathy group, P1: P value between group 1 and group 2, P2: P value between group 1 and group 3, P3: P value between group 2 and group 3.

4. Discussion

We aimed to review the clinical features of patients diagnosed with NMD and compare the symptom findings of three main groups: neuropathies, neuronopathies, and myopathies. In this study, fatigue and muscle weakness were the most common symptoms shared by all groups. The mean age of the disease onset was lowest in the myopathy group. Dysarthria was most common in the neuropathy group. Cognitive impairment was most common in the neuronopathy group. Although there was no statistically significant difference between the three groups, the use of assistive technology was most common in the myopathy group, dysphagia was most frequent in the neuropathy group, pain was most common in the neuronopathy group. While the frequency of falls was highest in the myopathy group, the highest fracture rate was observed in the neuronopathy group.

In the present study of the three main groups of NMD, it was observed that the median age and diagnosis age of patients with myopathies (Group III) were significantly lower than in the neuropathy group ($p=0.044$ and 0.028, respectively). Disease onset and progression may differ in each group of NMD. Myopathies can be described according to their clinical manifestations, onset, histopathological features, or eponyms (19). In this study, except for two patients with polymyositis, all other patients had congenital types of myopathies in Group III. Therefore, the median age and age at diagnosis were lower in the myopathy group than in the other groups.

There are approximately 600 different NMDs that can be inherited or acquired despite differences in etiology and severity, and all NMDs share (progressive) muscle weakness as a clinical feature (20). In the present study, muscle weakness was the most common symptom in patients with myopathy, neuronopathy, and neuropathy (100%, 90.9%, and 76.9%, respectively). Muscle weakness was significantly higher in the myopathy group than in the neuropathy group ($p = 0.044$). The distribution of muscle weakness usually indicates the primary affected area within the neuromuscular system (21). Weakness of the proximal muscle groups, such as the hip or shoulder girdle, is generally due to myopathy or neuromuscular junction (NMJ) disorders, with few exceptions. Conversely, weakness of the distal muscle groups is usually, but not always, due to neurogenic causes, such as peripheral polyneuropathy or amyotrophic lateral sclerosis (4). Proximal weakness often first appears in the muscles of the lower extremities. Patients usually present with complaints of difficulty walking and climbing stairs. Proximal weakness in the upper extremities manifests as an inability to raise the arms above the shoulders. Limb weakness is the most common symptom, but there are several other symptoms, including weakness in the speaking, swallowing, and breathing muscles (7, 22). Muscle pain, cramps, extreme fatigue, atrophy or pseudohypertrophy, fasciculation, and stiffness are other significant symptoms that can be seen in NMD (8).

Fatigue was the most frequently reported symptom in patients with NMD (80.4%) in this study. Fatigue can be acute, chronic, central, or peripheral. According to some studies, as many as 80% of patients with NMD can report fatigue, similar to our study (23-25). Several studies have reported fatigue as an essential symptom, especially in NMD such as ALS, post-poliomyelitis syndrome, CIDP and GBS, CMT hereditary sensitive-motor neuropathies, and NMJ diseases (26-30). Fatigue may also be seen in NMD as the first symptom before any motor deficit develops. For example, fatigue is evaluated at onset because it is a crucial factor in diagnosing metabolic myopathies (31).

In this study, the incidence of dysarthria and dysphagia was highest in the neuropathy group (30.8% and 42.6%, respectively). A few studies have examined the prevalence of dysphagia and dysarthria in patients with NMD (32-34). In a study by Knuijt et al. (33) a prevalence of dysphagia of 36–58% and dysarthria of 46–62% was found in adult patients with NMDs. In the same study, there was a moderate but notable association between dysphagia and dysarthria ($rs = 0.40$; $p < 0.01$). While dysphagia is usually mild, dysarthria is moderate to severe in 15% of dysarthric patients. In a study by Audag et al. (32) dysphagia was observed in 45% of all NMD patients screened for dysphagia with the Sydney Swallow Questionnaire (SSQ). The median SSQ scores were higher than the cutoff value in patients with myotonic syndromes, ALS, and facioscapulohumeral dystrophy. In the literature, dysphagia in patients with polyneuropathy has been reported frequently in critical illness polyneuropathy and Guillain-Barre syndrome, especially in patients with prolonged requirements for orotracheal intubation and tracheostomy (35-37).

In this study, pain was most frequently observed in the neuronopathy group (72.1%). There are few studies investigating the prevalence of pain in motor neuron diseases, and they reported that the frequency of pain varies between 15% and 84% (38, 39). Pain can occur at all stages of the disease and is reported to be mild to moderate in severity (38). There are some differences between pain levels in the gradually progressing forms of NMD. It is seen that the intensity of pain is high in neuropathic diseases such as CMT, especially in demyelinating forms, and diabetic neuropathy

(40, 41). In addition, the most frequent forms of muscular dystrophy, myotonic type 1, and facioscapulohumeral are also present in painful NMD (42).

NMD significantly affects the affected person's independence in daily changes. Their gradually progressive nature reduces their functional capacity, and they need more support over time; this may be due to the caregiver and/or assistive devices. Therefore, this assistance is essential for maintaining a minimum level of personal autonomy (43, 44). In our study, the use of assistive technology was highest in the myopathy group (22.7%). With the progression of muscle weakness, more caregiver assistance, assistive devices, and environmental regulations are required for people with NMD to perform their professional activities and maintain their level of daily activities (43). Studies on the use of assistive technology in the literature have been most frequently performed in patients with Duchenne muscular dystrophy (45, 46). Falling is one of the most common clinical problems in patients with NMD. In our study, the highest rate of falls was observed in the myopathy group, whereas the highest rate of bone fracture was observed in the neuronopathy group. Data from case-control studies involving patients with axonal polyneuropathy and polio have shown an increased incidence of falls in specific patient populations (47, 48). In a study investigating fracture risk in NMD, 47% of the patients were shown to have a moderate to high fracture risk. In the same study, two-thirds of the patients were not included in the osteoporosis screening and treatment program. The authors stated that patients with NMD should be screened routinely for osteoporosis, and in this way, early treatment can be achieved to reduce the risk of fragility fracture (49).

The effects of NMD on the spectrum of cognitive function are not yet understood. While NMD is known to affect motor functions the most in patients, the cognitive effects of these conditions may be significant (50). In this study, the patients' SMMT scores were significantly lower in the neuronopathy group than in the neuropathy and myopathy groups ($p = 0.047$ and 0.034, respectively). In a study by Phukan et al. (51) comorbid dementia was observed in approximately 14% of newly diagnosed ALS patients. In the same study, cognitive impairments were observed in more than 40% of ALS patients without evidence of dementia. In a review by Consonnia et al (52), older age, rapidly progressing ALS, bulbar-onset, advanced disease stages were reported as related factors mainly associated with cognitive involvement. In the management of patients with motor neuron disease, it should be kept in mind that cognitive disorders are common, and patients should be evaluated in this respect.

NMD is a broad group of diseases separated into three main groups, and the most common symptoms and clinical findings were evaluated. In this study, fatigue and muscle weakness were the most common symptoms shared by all groups. The mean age of the disease onset was lowest in the myopathy group. Dysarthria was most common in the neuropathy group. Cognitive impairment was most common in the neuronopathy group. Although there was no statistically significant difference between the three groups, the use of assistive technology was most common in myopathy group, dysphagia was most frequent in the neuropathy group, pain was most common in the neuronopathy group. The frequency of falls was highest in the myopathy group, and the fracture rate was highest in the neuronopathy group. We believe that such demographic and clinical studies will be beneficial in promoting effective healthcare services for these patients and appropriate rehabilitation programs at each stage of the disease.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: Ö.Z.K., Design: Z.T.B., Data Collection or Processing: Y.T.Y., Analysis or Interpretation: D.C., Literature Search: E.U., Writing: Z.T.B.

Ethical Statement

The ethical committee of Dışkapı Yıldırım Beyazıt Training and Research Hospital approved the study protocol (Ethics Code; 91/05, 06.07.2020). All patients gave verbal consent as this was a retrospective study with an interview of the patient and a review of his medical file. No interventions were applied to the participants, and the institutional review board approved the study based on this verbal consent.

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Evaluation of oral cavity pathologies in pediatric dentistry patients: A 10-year retrospective study

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Abstract

This study examines oral cavity pathologies' prevalence and age distribution in pediatric patients. All biopsy records performed in Tokat Gaziosmanpaşa University Faculty of Dentistry, Oral and Maxillofacial Surgery clinic between 2013-2023 were obtained from patient files and electronic databases. The files of patients aged 0-18 were selected, and demographic data and biopsy results were examined. The study population was divided into three groups according to the condition of the dentition. Pathology results were analyzed in 4 groups: developmental odontogenic cysts, inflammatory odontogenic cysts, tumoral lesions, and others. Obtained data and classifications were subjected to statistical analysis. A total of 411 biopsy results in pediatric patients were included in the study. Compared to all biopsies taken, a rate of 17.5% was observed. Since only three patients in the 0-6 age group were observed, they were not included in the statistical analysis. 69.3% of all pathologies were observed in the 12-18 age group and 30% in the 6-12 age group. The most common pathologies were radicular cysts (37.9%), dentigerous cysts (17.9%), and giant cell granulomas (7%). An increase in the prevalence and diversity of pathologies was observed with increasing age. In addition, developmental odontogenic cysts had significantly larger lesion sizes compared to inflammatory odontogenic cysts and other groups in terms of pathology dimensions (p:0.001 and p:0.000). In patients under 18 years of age, the prevalence of pathologies and diagnostic diversity increase as age progresses. Radicular cysts, dentigerous cysts, and giant cell granulomas are among the most common lesions.

Keywords: child, odontogenic cysts, odontogenic tumors, pathology

1. Introduction

Oral and maxillofacial pathologies observed in pediatric dentistry patients differ from general populations. Oral and maxillofacial pathologies cover a broad spectrum and are diagnosed by clinical, radiological, and histopathological evaluations similar to adult patients. Incisional or excisional biopsies are often required. Studies have been carried out on evaluating oral and maxillofacial pathologies in pediatric patients in different geographical regions (1–7). Among the general criteria in the studies, age groups, gender, year intervals, diagnoses, and radiographic findings are observed. Pathology prevalences report different results in studies in other geographical regions (8). Although diagnosing various mucosal lesions observed in the oral cavity is essential in dental practice, the number of studies on the prevalence of such lesions in children and adolescents is insufficient (2,9). The frequency and probability of possible soft tissue lesions should be known to perform the appropriate diagnosis and treatment. Odontogenic cysts and tumors constitute an essential aspect of oral and maxillofacial pathologies. Although odontogenic tumors are relatively rare, odontogenic cysts are vital for dental practice. Only a few studies on oral and maxillofacial pathologies in pediatric populations in Turkey draw attention (4,10). Epidemiological data on oral and maxillofacial lesions in Turkey's pediatric populations are insufficient. The histopathological prevalence of the lesions is essential for oral and maxillofacial surgery, pedodontics and other dentistry branches, otolaryngologists, and pediatricians. The aims of this study are;

To evaluate pediatric oral cavity lesions treated at the Faculty of Dentistry in a province (Tokat) in Turkey over ten years,

To compare the prevalence of oral cavity lesions with similar studies in adult populations.

It compares the prevalence of pediatric lesions with similar studies performed on pediatric populations nationally and internationally.

2. Material and Method

The ethical suitability of this retrospective study was approved

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by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (Registration Number: 23- KAEK- 107, Date: 27.04.2023). The study was carried out per the Helsinki Declaration of Ethics for Medical Research Involving Human Subjects. Verbal consent was obtained from all participants or their relatives in the study, and all participants or their relatives were informed in detail about the study.

2.1. Patient Population

The pediatric population who underwent incisional or excisional biopsy in Tokat Gaziosmanpaşa University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery between January 2013 and June 2023 was included in this retrospective study. Considering similar studies, the age limit was determined as 18, and the patients were divided into three groups according to their dentition periods 0-6 years, 6-12 years, and 12-18 years (3,11). Age, gender, localization, and dimensions of pathologies were recorded from patient files. Diagnoses were classified under three headings: odontogenic cysts, tumors, and other oral cavity lesions. Odontogenic cysts were also grouped under two subheadings as developmental and inflammatory odontogenic cysts. The study did not include patients with missing or suspicious demographic data or biopsy results. Results with histopathology indicating normal tissues or without diagnostic validity were excluded from the study. Patients over 18, recurrent biopsies, and malignant lesions were excluded from the study.

2.2. Statistical analysis

Continuous data was presented as mean±standard deviation and categorical data in n(%) format. Descriptive, comparative, and correlation statistics were applied. Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, revealing that the data did not follow a normal distribution. Consequently, non-parametric tests were utilized. The Mann-Whitney U test was employed to evaluate the difference between means of continuous data obtained from two distinct groups. The Kruskal-Wallis H test was conducted for constant data obtained from more than two groups, and post hoc analyses were performed using the Games Howell test. The relationship between two or more categorical variables was assessed using the chi-square test. All statistical computations were utilized using IBM SPSS Statistics v26.00, and a p-value less than 0.05 was considered statistically significant.

3. Results

Included in the study were a total of 411 patients, 231 of whom were female (Mean age 14.43±3.303) and 180 were male (Mean age 14.09±3.580). The proportion of females and males did not differ significantly. Among the participants, 287 (69.3%) were in the age group of 12-18 years, and 124 (30%) were in the age group of 6-12 years. The distribution of pathological diagnoses based on age groups, gender, and localization is presented in Table 1a,1b,1c. Radicular cysts, constituting 37.9% of the pathological diagnoses, ranked first, followed by dentigerous cysts at 17.9% and giant cell granulomas at 7%.

Table 1a. Distribution of pathologies by age and gender

		Total	Age Range			Gender	
			0-6	6-12	12-18	Female	Male
DOC	Dentigerous Cyst	74	0	23	51	40	34
	Odontogenic Keratocyst	13	1	1	11	6	7
	Lateral Periodontal Cyst	1	0	0	1	1	0
	Calcified Odontogenic Cyst	2	0	0	2	1	1
IOC	Radicular Cyst	157	2	35	120	81	76
TUMORAL	Osteoid Osteoma	1	0	0	1	0	1
	Leiomyoma	1	0	1	0	1	0
	Compound Odontoma	24	0	15	9	11	13
	Complex Odontoma	10	0	5	5	6	4
OTHERS	Granulation	11	0	5	6	7	4
	Exogenous Pigmentation	1	0	1	0	1	0
	Squamous Papilloma	5	0	0	5	4	1
	Inflammatory Tissue	22	0	6	16	15	7
	Ulceration	1	0	1	0	1	0
	Traumatic Bone Cyst	2	0	1	1	2	0
	Pyogenic Granuloma	5	0	4	1	2	3
	Polyp	3	0	1	2	3	0
	Irritation Fibroma	4	0	0	4	4	0
	Mucocele	4	0	1	3	2	2
	Fibrotic Tissue	12	0	2	10	6	6
	Periapical Granuloma	3	0	2	1	3	0
	Tmj Ankylotic Joint Head	3	0	0	3	3	0
	Supernumerary Teeth	2	0	0	2	2	0
	Squamous Papilloma	6	0	3	3	4	2
	Simple Bone Cyst	4	0	0	4	1	3
	Retention Cyst	29	0	13	16	17	12
	Hemangioma	3	0	0	3	1	2
	Odontogenic Fibroma	4	0	2	2	4	0

	Ameloblastic Papilloma	1	0	0	1	1	0
	Odontogenic Myxofibroma	1	0	1	0	1	0
	Fibrous Dysplasia	2	0	0	2	1	1
	Cemento-Osseous Dysplasia	1	0	0	1	0	1

DOC*: Developmental Odontogenic Cyst,* IOC*: Inflammatory Odontogenic Cyst*

Table 1b. Localizations of pathology in the jaws

	Maxilla Anterior	Maxilla Premolar	Maxilla Molar	Mandible Anterior	Mandible Premolar	Mandible Molar
Dentigerous Cyst	14	2	3	4	17	33
Odontogenic Keratocyst	0	0	2	1	0	8
Lateral Periodontal Cyst	0	0	0	1	0	0
Calcified Odontogenic Cyst	0	0	1	0	0	1
Radicular Cyst	20	12	43	4	19	57
Osteoid Osteoma	1	0	0	0	0	0
Leiomyoma	1	0	0	0	0	0
Compound Odontoma	16	2	0	3	0	2
Complex Odontoma	4	0	2	0	0	4
Granulation	3	0	0	0	5	1
Exogenous Pigmentation	0	0	0	0	0	0
Squamous Papilloma	1	0	0	0	0	0
Inflammatory Tissue	1	0	3	0	4	12
Ulceration	0	0	0	0	0	0
Traumatic Bone Cyst	0	0	0	0	2	0
Pyogenic Granuloma	2	1	0	0	1	0
Polyp	1	0	1	0	0	0
Irritation Fibroma	0	0	0	0	0	0
Mucocele	0	0	0	0	0	0
Fibrotic Tissue	0	0	0	0	1	0
Periapical Granuloma	0	1	0	0	0	2
Tmj Ankylotic Joint Head	0	0	0	0	0	1
Supernumerary Teeth	2	0	0	0	0	0
Squamous Papilloma	0	0	0	0	0	1
Simple Bone Cyst	2	0	0	0	0	2
Retention Cyst	4	2	5	2	12	4
Hemangioma	0	0	0	0	0	0
Odontogenic Fibroma	1	0	1	1	0	1
Ameloblastic Papilloma	0	0	0	0	0	1
Odontogenic Myxofibroma	0	0	0	0	0	1
Fibrous Dysplasia	0	0	0	0	0	2
Cemento-Osseous Dysplasia	0	0	0	0	0	1

Table 1c. Localizations of pathology outside the jaws

	Maxillary Sinus	Buccal Mucosa	Palatal Mucosa	Gingiva	Floor of Mouth	Lip	Tongue	Condyle Head
Dentigerous Cyst	1	0	0	0	0		0	0
Odontogenic Keratocyst	1	0	1	0	0		0	0
Lateral Periodontal Cyst	0	0	0	0	0		0	0
Calcified Odontogenic Cyst	0	0	0	0	0		0	0
Radicular Cyst	2	0	0	0	0		0	0
Osteoid Osteoma	0	0	0	0	0		0	0
Leiomyoma	0	0	0	0	0		0	0
Compound Odontoma	1	0	0	0	0		0	0
Complex Odontoma	0	0	0	0	0		0	0

Granulation	1	1	0	0	0	0	0
Exogenous Pigmentation	0	1	0	0	0	0	0
Squamous Papilloma	0	0	0	1	1	2	0
Inflammatory Tissue	0	0	0	2	0	0	0
Ulceration	0	0	0	1	0	0	0
Traumatic Bone Cyst	0	0	0	0	0	0	0
Pyogenic Granuloma	0	0	0	0	0	0	0
Polyp	0	0	0	1	0	0	0
Irritation Fibroma	0	1	0	0	0	3	0
Mucocele	2	0	0	0	0	0	0
Fibrotic Tissue	0	1	2	7	0	1	0
Periapical Granuloma	0	0	0	0	0	0	0
Tmj Ankylotic Joint Head	0	0	0	0	0	0	2
Supernumerary Teeth	0	0	0	0	0	0	0
Squamous Papilloma	0	0	0	1	2	1	0
Simple Bone Cyst	0	0	0	0	0	0	0
Retention Cyst	0	0	0	0	0	0	0
Hemangioma	0	0	0	0	0	3	0
Odontogenic Fibroma	0	0	0	0	0	0	0
Ameloblastic Papilloma	0	0	0	0	0	0	0
Odontogenic Myxofibroma	0	0	0	0	0	0	0
Fibrous Dysplasia	0	0	0	0	0	0	0
Cemento-Osseous Dysplasia	0	0	0	0	0	0	0

When evaluating the prevalence of the most common pathological formation, radicular cysts, it was found to be 41.8% in the 12-18 age group and 28.2% in the 6-12 age group. The proportion of females with radicular cysts was 41.8%, while for males, it was 34.9%. Radicular cysts accounted for 70.5% of pathologies from maxillary molars, 60% from maxillary premolars, and 42.5% from mandibular molars.

As for the second most common pathological formation, dentigerous cysts, their prevalence was 17.8% in the 12-18 age group and 31.1% in the 6-12 age group. The proportion of females with dentigerous cysts was 17.2%, while for males, it was 18.7%. Dentigerous cysts represented 44.6% of pathologies from mandibular molars, 23% from mandibular premolars, and 18.9% from the maxilla anterior region.

Table 2. Pathology classification and distribution by age classification

		Diagnosis				P
		Developmental Odontogenic Cyst	Inflammatory Odontogenic Cyst	Tumoral	Others	
Age Range	6-12	24	35	7	58	.007
	13-18	65	120	6	96	

A chi-square analysis was employed to evaluate the effect of age groups on pathological diagnoses. Data from the 0-6 age group were excluded because only three patients (0.7%) fell into this category. The analysis results are summarized in Table 2, indicating a statistically significant difference among age groups concerning pathological diagnoses (P=0.007). Notably, 73.1% of developmental cysts were found in the 12-18 age group, while only 26.9% were in the 6-12 age group. Further detailed analyses were conducted using the Kruskal-Wallis H test (Table 3), revealing a statistically significant difference in pathological diagnoses based on age (P=0.003). The 'other diagnoses' group appeared in younger generations compared to developmental and inflammatory odontogenic cysts (P=0.023 and P=0.024, respectively). However, there was no statistically significant difference in age compared to the tumor and similar group (P=0.863).

Statistically significant differences among pathological diagnosis groups regarding lesion size (P=0.000) were also found. Developmental odontogenic cysts had significantly larger lesion sizes compared to inflammatory odontogenic cysts and other diagnoses groups (P=0.001 and P=0.000, respectively)

Table 3. Relationship between pediatric oral pathology groups and dependent variables

			Games-Howell					Kruskal-Wallis H
Dependent Variable	(I) Diagnosis	(J) Diagnosis	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		P
						Lower Bound	Upper Bound	
Age	DOC	IOC	.212	.438	.963	-.92	1.35	**.003**
		Tumoral	2.131	1.068	.233	-.94	5.21	
		Others	1.316*	.457	**.023**	.13	2.50	
	IOC	DOC	-.212	.438	.963	-1.35	.92	
		Tumoral	1.919	1.040	.295	-1.11	4.95	
		Others	1.103*	.386	**.024**	.11	2.10	
	Tumoral	DOC	-2.131	1.068	.233	-5.21	.94	
		IOC	-1.919	1.040	.295	-4.95	1.11	
		Others	-.815	1.048	.863	-3.86	2.23	
	Others	DOC	-1.316*	.457	**.023**	-2.50	-.13	
		IOC	-1.103*	.386	**.024**	-2.10	-.11	
		Tumoral	.815	1.048	.863	-2.23	3.86	
Diameter	DOC	IOC	.5094*	.1255	**.001**	.182	.837	**.000**
		Tumoral	-.2013	.3585	.942	-1.234	.832	
		Others	.6635*	.1245	**.000**	.339	.988	
	IOC	DOC	-.5094*	.1255	**.001**	-.837	-.182	
		Tumoral	-.7107	.3420	.213	-1.721	.299	
		Others	.1540	.0630	.071	-.009	.317	
	Tumoral	DOC	.2013	.3585	.942	-.832	1.234	
		IOC	.7107	.3420	.213	-.299	1.721	
		Others	.8647	.3416	.103	-.145	1.874	
	Others	DOC	-.6635*	.1245	**.000**	-.988	-.339	
		IOC	-.1540	.0630	.071	-.317	.009	
		Tumoral	-.8647	.3416	.103	-1.874	.145	

DOC: Developmental Odontogenic Cyst **IOC:** Inflammatory Odontogenic Cyst

4. Discussion

Similar studies examining the prevalence of oral and maxillofacial pathologies in pediatric populations report different prevalences of pathologies in the pediatric population compared to the general population, with rates varying between 2.48% and 20.6% (1,5,8,12). A similar study conducted in Chile observed this rate as 20.6%. In a similar study conducted in Thailand over a 15-year period, a rate of 15.05% was observed (11). In a similar study conducted in Turkey, which included odontogenic cysts and odontogenic tumors, this rate was 12.7%, but soft tissue lesions were not included (4). This study is generally similar to the literature, and a similar rate of 17.5% is observed in our study (2340/411).

A clear consensus on establishing study groups based on age needs to be established. The general idea is to classify age groups according to dentition periods. While some studies have evaluated pediatric patient populations as under 14 or 16, this limit has been accepted as 17 or 18 in some studies (1,3–7,11,12). In our current study, the age limit of 18 was determined. In our current study, this age limit was evaluated by considering the eruption times of the third molars. In the present study, the female-male ratio is generally similar to the literature (Female/Male: 1,2/1) (1,4,7,11). 69.3% of the total pathologies were observed in the 12-18 age group and 30% in the 6-12 age group. This result confirms that the frequency of pathology increases with age in pediatric populations in line with the literature (12–14). However, there are also studies in the literature stating that the prevalence of pathology is higher in different age ranges (11,15).

When the prevalences of pathologies were examined in our current study, the most common subtypes were radicular cysts (37.9%), dentigerous cysts (17.9%), and giant cell granulomas (7%). This result parallels a similar study conducted by Tekkesin et al. in Turkey (4). In the study of Tekkesin et al., the most frequently observed odontogenic lesions were radicular cysts at 48.4% and dentigerous cysts at 16.7%. Our present study and this study are similar in this respect. One of the main differences between these two studies is that soft tissue pathologies were not included in the study of Tekkesin et al. In a similar study conducted in Brazil with a much larger patient population (2,408 patients) covering 75 years, the most common pathologies were observed as salivary gland pathologies, reactive lesions, and odontogenic cysts (16). In similar studies performed in Chile and Brazil, the most frequently observed lesions were reactive soft tissue lesions (3,17); In a survey conducted in the USA, mucoceles (18); Reactive soft tissue lesions in a study conducted in Egypt (19); In a study conducted in South Africa(20), odontogenic cysts reported among the most common lesions. When different literature data are examined, different prevalences of pathology are observed in different geographies. This situation can be associated with various reasons, such as racial factors, socioeconomic status, awareness of dental treatment, routine dental check-ups, and environmental factors. Still, it isn't easy to make a definitive interpretation.

In our current study, the prevalence of radicular cysts was 28.2% in the 6-12 age group, while this rate was 41.8% in the 12-18 age group. This suggests that the prevalence increases with age, especially in inflammatory lesions. Adequate oral

hygiene education that can be given at an early age will be beneficial in reducing the incidence of dental caries and preventing the development of inflammatory lesions such as radicular cysts. Radicular cyst prevalence is 41.8% in women and 34.9% in men; the gender factor is insignificant. Radicular cysts in the maxillary molar (70.5%), maxillary premolar (60%), and mandibular molar (42.5%) regions constitute a serious pathology density.

Dentigerous cysts are odontogenic cystic lesions that start from the enamel-cementum junction of an unerupted tooth and involve the dental crown. It is usually observed around the lower third molars, upper canines, lower premolars, and upper third molars. Dentigerous cysts, one of the most frequently observed pathologies in our current study, constituted 17.8% (51 patients) of all pathologies in the 12-18 age group and 31.1% (23 patients) of the pathologies in the 6-12 age group. Although proportionally higher rates are observed in the 6-12 age group, this is the exact opposite numerically. As age progresses, the prevalence of dentigerous cysts decreases due to the increase in the majority and diversity of pathology.

Female/male ratios are similar in dentigerous cysts. Of the dentigerous cysts, 44.6% were observed in the mandible molar region, 23% in the mandible premolar region, and 18.9% in the maxilla anterior region. This condition can be associated with impacted mandibular third molars, mandibular premolars, and maxillary canines. Especially early loss of primary teeth may be related to an increase in the prevalence of impacted teeth in mandibular premolars and, accordingly, dentigerous cysts (Fig. 1). The fact that the data in the 0-6 age group is limited to only three patients prevents this group from being compared with other groups. When the developmental cystic lesions are examined, there is a similar result, and 73.1% of all developmental cysts are observed in the 12-18 age group and 26.9% in the 6-12 age group.

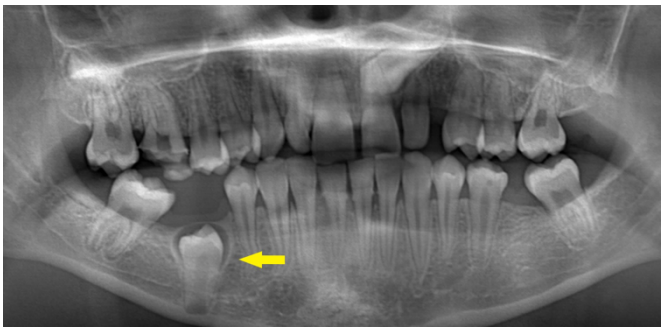

Fig. 1. Impacted premolar tooth and dentigerous cyst around the dental crown observed in the right mandible in a 15-year-old female patient (yellow arrow).

The high prevalence of dentigerous cysts among developmental cystic lesions is effective in this result. In addition, increasing the number and diversity of pathologies with increasing age is another factor. The "other" group, which includes soft tissue lesions, shows higher prevalences at younger ages than developmental and inflammatory odontogenic cysts, and these results are consistent with the literature (11,15). As another significant result, the size of cystic lesions can be evaluated. Developmental odontogenic cysts show larger dimensions than inflammatory odontogenic cysts and lesions in the other group. The nature and asymptomatic character of the developmental lesions usually cause the lesions to reach large sizes without symptoms. The fact that developmental odontogenic cysts, such as dentigerous cysts, are typically diagnosed during routine check-ups or when symptomatic reduces the chance of early intervention. At this point, the importance of regular dental check-ups emerges again.

In general, the limitations of the study include;

Short time interval (10 years)

Relatively low sample size compared to similar publications

There needs to be more observation of the number of patients in the 0-6 age group and the inability to include this group in the study entirely.

It can be considered as the exclusion of malignant lesions.

This study aimed to address a vast area that can attract the attention of dentistry professionals. The results describe the prevalence of pediatric oral cavity lesions over a period of time. The main criterion in the study is based on the histopathological examination results.

When the results of the study are evaluated, some basic conclusions emerge. In patients under 18, pathologies and diagnostic diversity prevalence increase as age progresses. Radicular cysts, dentigerous cysts, and giant cell granulomas are among the most common lesions. In the present study, the gender factor was not observed as an essential factor in the distribution of pathologies. Inadequate oral hygiene education and low socioeconomic status may be associated with inflammatory cystic or reactive soft tissue lesions. Premature loss of primary teeth, mainly observed in the mandible, may cause impacted mandibular teeth and increase the prevalence of dentigerous cysts. This case focuses on the personal experiences of the authors who have served in this region for a long time. In developmental odontogenic cysts, lesion sizes are observed at higher levels.

Conflict of interest
The authors declared no conflict of interest.

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None to declare.

Authors' contributions
Concept: S.Ç., Y.B., A.A., N.A., Design: S.Ç., Y.B., N.A., Data Collection or Processing: S.Ç., A.A., Analysis or Interpretation: Y.B., N.A., Literature Search: S.Ç., Y.B., Writing: S.Ç., A.A.

Ethical Statement

The ethical compliance of the study was approved by the Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (Registration Number: 23- KAEK- 107, Date: 27.04.2023). The study was conducted per the Helsinki Declaration of Ethics for Medical Research Involving Human Subjects.

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The effect of bone marrow reticulin fibrosis on survival in acute myeloid leukemia patients

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Abstract

Although acute myeloid leukemia is a rare cancer, it is a disease that deserves attention because of its increasing incidence and high mortality. Many prognostic factors, in particular age and cytogenetic classification, are important in the management of AML. Identifying new prognostic factors will enhance our understanding of AML and contribute to improved survival.For the purpose of investigating the impact of bone marrow reticulin fibrosis on overall survival, a total of 121 patients with acute myeloid leukemia were included in the study. Out of these 121 patients, 70 (57.9%) were male and 51 (42.1%) were female. The mean age of all patients was 57.72±16.3 years.There was no bone marrow reticulin fibrosis in 56 patients (47.9%), first degree fibrosis in 47 patients (40.2%), second degree fibrosis in 13 patients (11.1%) and third degree fibrosis in 1 patient (0.9%). No correlation was found between bone marrow reticulin fibrosis and age, gender, FAB classification, or bone marrow blast rate at diagnosis. Patients in the unfavorable cytogenetic risk group had more bone marrow reticulin fibrosis. The mean overall survival was 17.4±1.9 months. In the group without bone marrow reticulin fibrosis, it was 18.87±2.77 months, while in the group with bone marrow reticulin fibrosis, it was 11.09±1.51 months. This observed difference was determined to be statistically significant. Therefore, the presence of bone marrow reticulin fibrosis was considered an important prognostic factor for overall survival.Scientific publications, which have increased significantly in recent years, have contributed to a better understanding of acute myeloid leukemia and thus to the development of new approaches. Pharmacological inhibition of bone marrow reticulin fibrosis could potentially offer clinical utility and extend patient survival. Further studies are needed to incorporate bone marrow reticulin fibrosis into prognostic risk classifications, to develop appropriate chemotherapy regimens, and to improve the clinical efficacy of treatment in AML patients.

Keywords: Acute Myeloid Leukemia, bone marrow fibrosis, prognostic factors

1. Introduction

AML is a clonal disorder of hematopoietic stem cells that causes impaired differentiation and excessive proliferation of hematopoietic stem or progenitor cells from the myeloid lineage. The accumulation of immature and undifferentiated myeloid progenitor cells (blasts) in the bone marrow and sometimes in surrounding tissues leads to life-threatening complications such as neutropenia, thrombocytopenia, and anemia (1). AML may develop due to various environmental or genetic factors, although the etiology is unknown in most cases.

AML is slightly more prevalent in men than women, but the lifetime risk of diagnosis averages at approximately 0.5% for both sexes (2). The incidence rate of AML rose by 2% per year from 2007 to 2016; however, the mortality rate has remained stagnant. Significant breakthroughs in treatment have substantially increased survival rates for most forms of leukemia (3). AML, a condition that rarely occurs before the age of 45 years, primarily affects the elderly population, with 60% of diagnosed patients being aged 65 and above. Mortality rates are known to increase with age. The 5-year survival rates are 25% across all age groups (4).

When acute leukemia is clinically suspected, it is necessary to evaluate the morphology (bone marrow smear) and perform flow cytometry analyses. Additionally, cytogenetic and genetic testing are mandatory to aid diagnosis, assess prognosis, and determine the most effective therapeutic approach. The French-American-British (FAB) classification system was developed in 1976 to differentiate between AML subtypes. With advancements in immune phenotyping and cytogenetics, which increased during the latter part of the 20th century, the World Health Organization (WHO) introduced a new classification in 1999 that was later updated in 2022 (7). The WHO classification for acute leukemia is centered on clinical, morphological, immunophenotypic, cytogenetic, and molecular characteristics. However, these classifications do not provide prognostic and survival data.

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In AML treatment, achieving a cure is possible with standard treatment regimens for some patients, but some patients are unresponsive to intensive treatment regimens. It is crucial to anticipate these differences in response to treatment at the time of diagnosis to inform a treatment plan. Age is one of the most crucial prognostic indicators in AML and other diseases. AML has a poor outcome for patients over 60 years old (8). Patients with a low Karnofsky performance status score or an unfavorable Eastern Cooperative Oncology Group performance score are likely to experience a reduction in overall survival (9). The presence of pulmonary complications, hyperleukocytosis, or the expression of CD34, CD56, or CD25 at the time of diagnosis are associated with poor prognostic factors (10-12). It has been observed that patients who achieve complete remission with induction treatment generally have a more positive prognosis (11). Abnormalities in the number or structure of chromosomes are found in up to 60% of patients with AML and are considered the most important prognostic factor (13). The European LeukemiaNet (ELN) has categorized patients into three prognostic risk groups based on cytogenetic and molecular abnormalities in AML. These groups are further divided according to complete remission, disease-free survival, and overall survival rates. For acute promyelocytic leukemia (APL), the risk classification utilized is based on GIMEMA and PATHEMA studies (14). The presence of eosinophils (FAB M2 and M4Eo) is considered a positive prognostic factor, whereas the presence of dysplasia indicates a poorer prognosis. Consequently, FAB subtypes M0, M5, M6, and M7 demonstrate a less favorable prognosis compared to other FAB subtypes.

Increased fibrosis in the bone marrow has been linked to various benign and malignant conditions, but the underlying pathophysiology remains unclear (15). While bone marrow fibrosis is commonly observed in hematological diseases, its impact on disease prognosis is not fully understood. In AML, bone marrow fibrosis has been identified as a secondary reaction to clonal proliferation of hematopoietic cells (16). Bone marrow fibrosis has been found to be a negative prognostic factor in myelodysplastic syndrome (17). However, its effect on prognosis and survival in AML patients has been limited to a few studies in the literature (18, 19). The current AML diagnosis, classification, and treatment guidelines of ELN and WHO do not include an evaluation of bone marrow fibrosis. The purpose of this study is to investigate the impact of bone marrow reticulin fibrosis on overall survival among AML patients.

2. Materıals and Methods

A total of 487 patients with AML were followed up and treated at the Ondokuz Mayıs University Faculty of Medicine Hospital, Department of Hematology, between 2008 and 2019. After receiving approval from the Ondokuz Mayıs University Clinical Research Ethics Committee on January 16, 2020, with the reference number B.30.2.ODM.0.20.08/17, 121 patients from this group, for whom genetic results could be obtained, were included in the study. Patient data at the time of diagnosis and during follow-up were retrospectively reviewed using the patient management information system. For the cytogenetic risk classification of the patients, ELN guidelines were applied to non-APL patients, while GIMEMA and PATHEMA guidelines were used for APL patients. The bone marrow reticulin fibrosis grade of 117 patients who underwent bone marrow biopsies upon admission was categorized into grades 0, 1, 2, and 3, following the WHO 2008 classification criteria.

SPSS 22.0 (Statistical Package for the Social Sciences) package program was used for data analysis. Kolmogorov-Smirnov and Shapiro Wilk normality tests were applied to determine which test to use from the comparison tests. Independent two-sample t-test was used for two-category variables with normal distribution, and Mann-Whitney U-test was used for two-category variables that did not show normal distribution. For variables with more than two categories, ANOVA test was used for normally distributed variables, and Kruskal Wallis H test was used for non-normally distributed variables. However, Chi-square and Fisher tests were used to determine the relationship between categorical variables. Kaplan-Meier survival method was used for the analysis of patients' survival.

3. Results

Among the 121 patients diagnosed with AML who were over the age of 18, males accounted for 70 (57.9%) and females accounted for 51 (42.1%) of the population. The youngest patient was 21 years old, while the oldest patient was 83 years old. The patients included in the study had a mean age of 57.72±16.3 years. Among the patients, 55 (45.5%) were 60 years of age or older. The mean bone marrow blast rate was 53±25.8%, where the lowest was 20% and the highest blast rate was 95%. Eighteen patients were unable to attain a FAB classification following their bone marrow assessment. Among the patients who were successfully classified, the most prevalent subgroup was M0 with 37 patients (30.6%) and M1 with 22 patients (18.2%). The subgroups M5, M6, and M7 were the least commonly observed. The details regarding patient characteristics have been provided in Table 1.

At the point of diagnosis, the pathology department assessed reticulin fibrosis in 117 patients' bone marrow biopsy samples using silver stain, following WHO 2008 criteria. Of those, 56 patients (47.9%) didn't present any fibrosis, whereas 47 patients (40.2%) had first degree fibrosis, 13 patients (11.1%) had second degree fibrosis, and only 1 patient (0.9%) showed third degree fibrosis. When the patients were assessed based on age, gender, blast rate and FAB group in relation to the presence or absence of fibrosis in the bone marrow, no significant statistical difference was discovered. However, when categorized by risk groups, the presence or absence of fibrosis between the favorable and intermediate risk groups was similar. In the adverse cytogenetic risk group, bone marrow reticulin fibrosis was absent in one patient (9.1%),

whereas 10 patients (90.9%) exhibited fibrosis, which was statistically significant (p:0.021) (Table 2).

Table 1. General characteristics of patients

	Parameters		Number Of Patients	Percent
Patient Characteristics	Gender	Male	70	57,9
		Woman	51	42,1
	Age	<60	66	54,5
		≥60	55	45,5
	Cytogenetic Risk Group	Favorable	30	24,8
		Intermediate	80	66,1
		Adverse	11	9,1
	FAB Group	M0	37	30,6
		M1	22	18,2
		M2	12	9,9
		M3	12	9,9
		M4	14	11,6
		M5	4	3,3
		M6	0	0
		M7	1	0,8
		Unclassified	19	15,7
	Bone Marrow Reticulin Fibrosis Grade	Grade 0	56	47,9
		Grade 1	47	40,2
		Grade 2	13	11,1
		Grade 3	1	0,9
Survival	Overall Survival		**Average**	**Median**
			17,4 ± 1,9	11,3 ± 3,7 ay
Blood Count Parameters	**Parameters**		**Median**	**Lowest - Highest**
	WBC (thousand/µL)		10,9	0,47-237
	Monocyte (thousand/µL)		1,95	0,01-170
	Lymphocyte (thousand/µL)		2,43	0,09-96,62
	Neutrophil (thousand/µL)		1,73	0-74,7
	Eosinophil (thousand/µL)		0,03	0-6,2
	Basophil (thousand/µL)		0,03	0-22,89
	Hemoglobin (gr/dL)		8,7	3,5-14,7
	MCV (fL)		91,6	60-111
	Platelets (thousand/µL)		54	3-1106
Biochemical Parameters	Total protein (g/dL)		6,82	5,1-9,4
	Albumin (g/dL)		3,8	1,61-4,88
	B2 Microglobulin (ng/mL)		2.419	1.894-8.518
	Vitamin B12 (pg/mL)		520	7,45-2355
	Folic Acid (ng/mL)		5,2	0,6-20
	Iron (µg/dL)		103	105-362
	Ferritin (ng/mL)		780	21,3-21.000
	LDH (U/L)		407	132-4.700
	Uric acid (mg/dL)		4,8	0,7-14
	CRP (mg/L)		29	0,15-436
	Sedimentation (mm/h)		70	1-156

Table 2. Evaluation of patient characteristics with the presence of bone marrow reticulin fibrosis

Parameters		**No bone marrow reticulin fibrosis (Grade 0)**		**Bone marrow reticulin fibrosis present (Grade 1-2 and 3)**		**p**
		n	**%**	**n**	**%**	
Gender	<60 yaş	30	46,9	34	53,1	0,814
	>60 yaş	26	49,1	27	50,9	
Age	Erkek	30	43,5	39	56,5	0,342
	Kadın	26	54,2	22	45,8	
Cytogenetic Risk Group	Favorable	15	51,7	14	48,3	**0,021***
	Intermediate	40	51,9	37	48,1	
	Adverse	1	9,1	10	90,9	
FAB Group	M0	18	51,4	17	48,6	0,441
	M1	11	50	11	50	
	M2	5	41,7	7	58,3	
	M3	2	18,2	9	81,8	
	M4	5	35,7	9	64,3	
	M5	3	75	1	25	
	M6	0	0	0	100	

	M7	0	0	1	100	
	Unclassified	12	70,6	5	29,4	
Blast median percentage		50 (20-90)		50 (20-95)		0,837
Overall survival		18,87 ± 2,77 month		11,09 ± 1,51 month		**0,041***

The mean overall survival time equated to 17.4±1.9 months, whereas the median overall survival time was 11.3±3.7 months. When analyzing the impact of reticulin fibrosis in the bone marrow on overall survival, the group without bone marrow reticulin fibrosis had a mean overall survival of 18.87±2.77 months, while the group with bone marrow reticulin fibrosis had a mean overall survival of 11.09±1.51 months. This difference was statistically significant (p: 0.041).

4. Discussion

Bone marrow reticulin fibrosis is present to varying degrees in one-third to one-half of patients with AML at the time of diagnosis. Increased reticulin fibers in the bone marrow of AML patients are due to cytokine overproduction, which increases with CD34 and HLA-DR expression on leukemic cells (20, 21). The local bone marrow renin-angiotensin system plays a significant role in the onset of leukemia. Renin-angiotensin system mediates numerous biological processes involved in the formation and functioning of blood cells, including fibrosis. The local renin-angiotensin system is one of the causes of cytokine overproduction (22).

In a study of 34 patients by Islam et al, no reticulin fibrosis was found in 65% of patients, whereas grade 1 reticulin fibrosis was found in 26% and grade 3 reticulin fibrosis in 9% (23). In a study of 183 patients by Tang et al, 54% of patients had no reticulin fibrosis, 27.8% of patients had 1st and 2nd grade reticulin fibrosis, and 18% of patients had 3rd and 4th grade reticulin fibrosis (24). In our study, similar to the literature, 56 patients (47.9%) had no reticulin fibrosis, 47 patients (40.2%) had grade 1 reticulin fibrosis, 13 patients (11.1%) had grade 2 reticulin fibrosis, and 1 patient (0.9%) had grade 3 reticulin fibrosis.

It has been reported in the literature that fibrosis varies significantly according to the subgroup of leukemia; increased fibrosis is observed in the M7 subgroup, whereas increased fibrosis is rarely observed in the M3 subgroup (20). In our study, when the relationship between the degree of bone marrow reticulin fibrosis and the FAB groups was evaluated, no statistically significant difference was found between them. Among 11 patients diagnosed with APL in whom bone marrow reticulin fibrosis evaluation was performed, no fibrosis was observed in 2 (18%) and grade 1 reticulin fibrosis was observed in 9 patients (81.8%). Grade 2 fibrosis was observed in one patient diagnosed with M7. The presence of bone marrow reticulin fibrosis in APL can be a diagnostic challenge for clinicians. It may erroneously lower the index of suspicion for leukemiaand possibly delay the administration of appropriate treatment. It is important to consider the potential for bone marrow reticulin fibrosis in APL. Early initiation of ATRA following a correct APL diagnosis could improve the chances of a cure.

In our study, we classified patients into two groups based on the severity of their bone marrow reticulin fibrosis: those without fibrosis (grade 0) and those with fibrosis (grades 1-2-3). Our findings indicate that age, gender, and blast rate at the time of diagnosis did not differ significantly between these two groups. To our knowledge, no previous studies have examined this relationship.

In our study, an objective analysis of cytogenetic risk groups revealed that the presence or absence of bone marrow reticulin fibrosis was comparable in both favorable and intermediate risk groups. Technical term abbreviations will be explained upon initial use. However, within the unfavorable cytogenetic risk group, one patient (9.1%) displayed no signs of fibrosis, while fibrosis was present in the remaining 10 patients (90.9%), resulting in a statistically significant correlation (p:0.021). This study examines the correlation between cytogenetic risk classification, a crucial factor in determining AML prognosis, and bone marrow reticulin fibrosis.

The results reveal that patients without bone marrow reticulin fibrosis had an overall survival of 18.84±2.77 months, whereas those with the condition only had a survival rate of 11.09±1.51 months, a statistically significant difference (p:0.041). Our study showed that the effect of the presence of bone marrow fibrosis on overall survival was statistically significant. Therefore, the presence of bone marrow reticulin fibrosis was considered an important prognostic factor for overall survival.

A study by Zhang et al. involving 190 patients reported that patients with bone marrow reticulin fibrosis had a shorter overall survival. Three-year overall survival was 35.4% in the group without bone marrow reticulin fibrosis and 9.6% in the group with bone marrow reticulin fibrosis (18). The study by Wu et al, which included 152 patients, showed that overall survival decreased in the group with bone marrow reticulin fibrosis (19). Both studies showed increased bone marrow reticulin fibrosis in patients in the unfavorable cytogenetic risk group. The effect of the presence of bone marrow reticulin fibrosis on overall survival in AML patients was limited to these two studies. The results of our study support these two studies. The pathophysiology of bone marrow reticulin fibrosis in AML needs to be better understood, and new therapeutic strategies targeting bone marrow reticulin fibrosis may be promising to improve clinical outcomes.

Our study indicates that bone marrow reticulin fibrosis is an adverse prognostic indicator for AML patients. Further

research is required to incorporate bone marrow reticulin fibrosis into prognostic risk classifications, formulate appropriate chemotherapy regimes, and enhance the clinical effectiveness of treatment for patients with AML.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: Ö.Y.Ç., E.K., Design: Ö.Y.Ç., Data Collection or Processing: Ö.Y.Ç., E.K., Analysis or Interpretation: Ö.Y.Ç., E.K., Literature Search: Ö.Y.Ç., E.K., Writing: Ö.Y.Ç., E.K.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 16/01/2020 and the number of ethical committee decisions is 2020/17.

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The effect of technology-dependent behavioral disorders, including nomophobia, phubbing, fear of missing out, and netlessphobia, on quality of life and life satisfaction in desk-workers

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Abstract

This study aims to examine technology-dependent behavioral disorders and the effects of these disorders on the quality of life and life satisfaction of desk workers. The study sample consists of 850 (433 female, 417 male) desk workers. The Turkish Nomophobia Questionnaire, Fear of Missing Out Scale, Phubbing Scale, European Health Impact Scale and Life Satisfaction Scale were administered to the participants in the questionnaire. In addition, a question was asked to determine Netlesphobia. Correlation and path analysis were performed to determine the relationship between them. In the correlation analysis, the quality of life was negatively correlated with nomophobia, phubbing, FoMO, and netlessphobia and positively correlated with life satisfaction. Pairwise comparisons for all technology-dependent behavioral disorders showed a significant positive correlation. According to the path analysis, FoMO and netlessphobia reduce the quality of life, and netlessphobia reduces life satisfaction. Phubbing increases life satisfaction. Researching the effects of rapidly increasing internet and technological device use on individuals will be beneficial in terms of informing individuals about the correct use and preventing negative consequences that may arise in individuals' quality of life and life satisfaction.

Keywords: quality of life, life satisfaction, nomophobia, fear of missing out, phubbing, netlessphobia, desk workers

1. Introduction

In recent years, the use of technological devices has dramatically increased globally. According to the 2022 global digital report, there has been approximately a 1% (80 million) increase in internet users compared to 2021. Again, the same report states a 10.1% (424 million) increase in the number of active social media users (1). Internet usage is also increasing in Turkey, similar to the rest of the world. While the frequency of individuals using the internet in Turkey was 82.6% in 2021, this rate increased to 85% in 2022 (2).

The increase in the use of technological devices brings many problems and makes our lives easier. Some of these include weakening face-to-face communication, increased individualization, information pollution, addictive behaviors, and resulting psychological issues (3). Smartphones are considered among the most important non-drug addictions today (4). In the beginning, the negative situations caused by technological devices and the internet in people were measured in broad scopes, such as digital addiction (5) and internet addiction (6). As the use of technology increases, addiction-based behavioral problems experienced by individuals have begun to be measured in more specific and different ways.

Some behavioral disorders on the agenda that show negative influence from technology are nomophobia, phubbıng, FoMO, and netlesphobia. The first of these disorders, nomophobia, is an abbreviation of "No Mobile Phone Phobia" and is defined as the involuntary and irrational fear that individuals experience when staying away from mobile devices (7). In diagnosing nomophobia, which was first described in 2008, it is vital for individuals to spend a lot of time with their smartphones and to check their phones frequently. Still, the intense anxiety that occurs in individuals when the smartphone is lost and its place cannot be found is also important (8). The second disorder mentioned above, phubbıng, is derived from the words "phone" and "snubbing". The person doing phubbing is called a "phubber." The concept of phubbing can be evaluated as the individual's dealing with the phone in the social environment and avoiding interpersonal communication. Thus, phubbing reduces the quality of social interaction by reducing face-to-face communication between people. This situation can be defined as the isolation of the individual from the environment due to the smartphone (9-11). With the increasing use of technological devices and the internet, the follow-up of social networks has brought another behavioral disorder, Fear of Missing Out (FoMO), to the agenda. This situation causes people to ask questions such as "Did I miss something?", "Who shared what right now?" It causes them to spend a lot of time on social networks by experiencing fears such as (4,12). The concept of netlessphobia, a state-of-the-art addictive behavior disorder mentioned above, refers to the inability of the person to stay in an environment where there is no internet and to be worried

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about internet deprivation beyond excessive internet use (13).

It is known that individual with technology-dependent behavioral disorders is also at risk for others. Although the main causative factor is not fully clarified, studies show they play a correlation, mediator or moderator effect, or predictive role. Studies have found that nomophobia-FoMO (14,15), FoMO-phubbing (16,17), and nomophobia-phubbing (18) are associated. Although the relationship between netlessphobia and these technology-dependent behavioral disorders is not sufficiently clarified in the literature, one study found an association between nomophobia and FoMO and netlessphobia (19). Considering that anxiety without being in an environment without the internet is the main factor in the development of all of the above-mentioned behavioral disorders, and the necessity of using a smartphone and having the internet in the environment to follow the developments, it can be expected that the relationship between these disorders will be vital.

Satisfaction with life is one of the most prominent quality-of-life indicators and is accepted as a more subjective evaluation (20). In addition, life satisfaction is the result of comparing what an individual wants with what they currently have (21). Technology-dependent behavioral disorders may impact the quality of life and life satisfaction by creating an addiction or creating physical, mental, and social problems. In addition, the effects of these behavioral disorders on the quality of life and their effects on life satisfaction may occur in different directions. Some studies show that technology-dependent behavioral disorders lead to negative situations such as depression, which we can accept as indicators of low life satisfaction and quality, and studies show that negative psychopathological conditions cause technology-dependent behavior disorders (22,23). In the literature, rather than measuring the holistic effect of these disorders on quality of life, the relationship between diseases such as depression (23-26) and anxiety (24,25) has been investigated. Therefore, there is a need to examine the effects on quality of life and life satisfaction with a holistic approach.

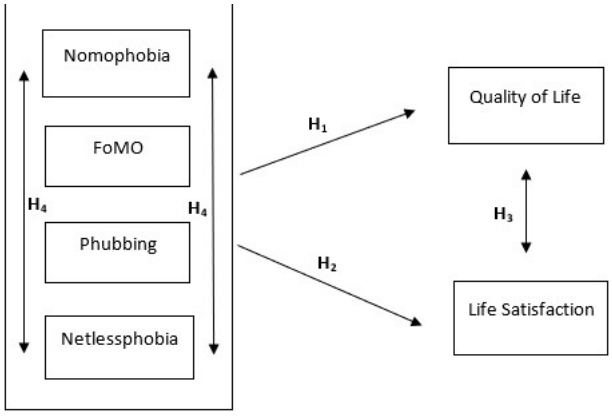

Fig. 1. Hypothetical model

Previous studies have focused on students who are considered to be at higher risk for technological device and internet use (25). However, we think that desk workers who spend most of their working lives in front of the computer are also at risk for technology-dependent behavioral disorders. For this reason, this study aimed to examine technology-dependent behavioral disorders and the effects of these disorders on the quality of life and life satisfaction of desk workers.

In this context, the following hypotheses were formed in the study: Technology-dependent behavioral disorders (nomophobia, phubbing, FoMO, and netlessphobia), quality of life (H1), and life satisfaction (H2) with the negative; the positive relationship between quality of life and life satisfaction (H3); Technology-dependent behavioral disorders also have a positive relationship with each other (Fig..1).

2. Matherials and Methods

2.1. The Procedure and Participants

The study's sample size was calculated as at least 768 by taking the minimum sample size of 50%, the margin of error of 5% with a 95% confidence interval and design effect 2. The data of this cross-sectional study were collected from 850 desk workers aged 19-63 between March and April 2021 via Google Forms in Turkey. The survey's participation criterion: Over 18 years old, still working, and spending more than half of the daily working time at a desk. Participants who did not meet these criteria were excluded from the study.

2.2. Measures

In the first part of the study, the socio-demographic characteristics of the participants (age, gender, marital status, income level), occupation, professional year, place of residence, daily-weekly working hours, time spent working at a desk/computer, and experience netlessphobia were questioned. In the continuation of the survey, the participants; Nomophobia, FoMO, Phubbing, European Health Impact Scale, and Life Satisfaction Scale were asked.

The World Health Organization Quality of Life Assessment (WHOQOL-8.Tr)

WHOQOL-8.Tr, the original version of the European Health Impact Scale (EUROHIS-QOL 8-item index), was used in the study. The original version is an index of the quality of life scale created by selecting some items from the WHOQOL-BREF scale (27). It was adapted into Turkish by Eser (2011) (Cronbach's alpha: 0.85) (28). The scale consists of 8 items and is a 5-point Likert type (1=not at all, 5=completely). Evaluation of the scale was made on a single dimension and total score. As the score obtained from the scale increases, the quality of life of individuals increases. In this study, Cronbach's alpha value was found to be 0.88.

Life Satisfaction Scale

The scale, which consists of 5 items and measures a single dimension, was developed by Diener et al. (1985) and adapted into Turkish by Dağlı and Baysal (2016) with a Cronbach's alpha of 0.88 (29, 30). The scoring system for the scale is a 5-point Likert scale ranging from 1 (Strongly disagree) to 5 (Strongly agree), with no reverse-scored items. The total score

obtained from the scale was used for evaluation, with higher scores indicating greater life satisfaction. For this study, the Cronbach's alpha value was 0.88.

Turkish Nomophobia Questionnaire

Yildirim and Correria (2015) developed a 20-item scale (Cronbach's alpha: 0.95), which was later adapted into Turkish by Yıldırım et al. (2016) (Cronbach's alpha: 0.92) (31,32). The scale uses a 7-point Likert scale (1=strongly disagree, 7=strongly agree), and the total score is used for evaluation. Higher scores on the scale indicate a greater level of nomophobia in individuals. For this study, the Cronbach's alpha value was 0.96.

Fear of Missing Out Scale (FoMOs)

Przybylski et al. (2013) developed a scale consisting of 10 items and a single sub-dimension, which was adapted into Turkish by Gökler et al. (2016) (Cronbach's alpha: 0.81) (12,33). The questions in the scale are in the 5-point Likert type (1=Not at all true, 5=Absolutely true), with no reverse-scored items. The total score obtained from the scale was used for evaluation, with higher scores indicating a greater level of FoMO in individuals. The Cronbach's alpha value for this study was 0.90.

Phubbing Scale

Karadağ et al. (2015) developed a 10-item scale with a 5-point Likert assessment (1=never, 5=always) (9) and a Cronbach's alpha of 0.86. The scale does not include any reverse-scored items, and higher scores indicate a higher level of phubbing in individuals. The total score obtained from the scale was used for evaluation. In this study, the Cronbach's alpha value was 0.89.

Netlessphobia

Since there was no scale to measure netlessphobia in the literature review conducted at the time the questionnaire was applied. The participants were asked to rate their fear of being without internet from 1 to 5 (1 = I definitely do not live, 5 = I definitely do).

2.3. Statistical analysis

The statistical analysis was conducted using IBM SPSS 25.0, Origin Pro correlation plot graph, and AMOS 23 package programs for path analysis. A significance level of $p<0.05$ was considered statistically significant. Skewness and Kurtosis values were examined to determine if the data followed a normal distribution, and it was found that the data were suitable for normal distribution. Descriptive analysis (number, percentage, mean, standard deviation), bivariate correlation, and path analysis were used to evaluate the data.

Path analysis was performed to test the study hypotheses. The goodness of fit of the analysis model was tested using chi-square ($\chi 2$)/degrees of freedom (d/f), comparative index of fit (CFI), Goodness of Fit Index (GFI), Adjusted Goodness of Fit Index (AGFI), Normed Fit Index (NFI), Non-Normed Fit Index (NNFI), and root mean square approximation error (RMSEA).

The study was approved by the Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee.

3. Results

The mean age of the 850 participants included in the study was 34.74 (SD=10.02), and the years of working in the profession were 9.85 (SD=9.35). Four hundred and thirty-three (50.9%) of them were women, and four hundred and twenty-nine (50.5%) were married. The majority of the participants (68.5%) reside in the city center. The socio-demographic characteristics and descriptive statistics of the participants are given in Table 1. The mean and standard deviations of the participants' total scores from the scales were as follows: For WHOQOL-8.Tr 21.96 (SD=5.71), life satisfaction scale 16.31 (SD=4.65), nomophobia scale 75.87 (SD=28.88), FoMO scale 24.72 (SD=9.20), phubbing scale was 26.32 (SD=9.17) and netlessphobia 2.83 (SD=1.24) was found.

Table 1. Socio-demographic characteristics of the participants

		n (%) / Mean±SD
Age, mean±SD		34.74 ± 10.02
Occupational year, mean±SD		9.85 ± 9.35
Daily working time (hours), mean±SD		8.12 ± 1.59
Gender, n (%)	Female	433 (50.9%)
	Male	417 (49.1%)
Marital status, n (%)	Single/divorced	421 (49.5%)
	Married	429 (50.5%)
Profession, n (%)	Physician	57 (6.7%)
	Other healthcare workers	58 (6.8%)
	Highly qualified white collar workers	206 (24.2%)
	Other white collars	529 (62.2%)
Living place, n (%)	City center	582 (68.5%)
	District center	268 (31.5%)
Percentage of time spent at the desk, n (%)	50-60%	266 (31.3%)
	61-80%	278 (32.7%)
	81-100%	306 (36.0%)
Percentage of time spent in front of the computer, n (%)	Less than 50%	97 (11.4%)
	50-60%	236 (27.8%)
	61-80%	222 (26.1%)
	81-100%	295 (34.7%)
Income, n (%)	Under 500 euros	200 (23.5%)
	500-1000 euros	444 (52.2%)
	Over 1000 euros	206 (24.2%)

n: Sample, %: Percent, SD: Standart Deviation

Quality of life, life satisfaction, technology-dependent behavioral disorders, numerical (Pearson), and ordinal (Kendall Tau) variables were analyzed in terms of correlation. Quality of life was positively correlated with life satisfaction and negatively correlated with nomophobia, phubbing, FoMO, and netlessphobia. In addition, pairwise comparisons for all technology-dependent behavioral disorders revealed a significant positive correlation (Fig. 2).

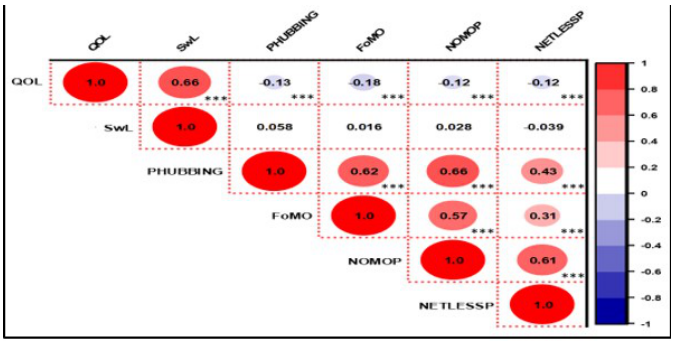

Fig. 2. Relationship between QOL (Quality of Life), SwL (Satisfaction with Life), Phubbing, FoMO, Nomophobia, Netlessphobia (correlation plot) (* <0.05, **<0.001, *** <0.0001

In this study, technology-dependent behavioral disorders (nomophobia, phubbing, FoMO, and netlessphobia) were negatively correlated with quality of life (H1) and life satisfaction (H2); the positive relationship between quality of life and life satisfaction (H3); A path analysis was performed considering that technology-dependent behavioral disorders would also be positively related between them. As a result of the path analysis: FoMO and netlessphobia decrease the quality of life (H1); netlessphobia reduces and phubbing increases life satisfaction (H2); a positive relationship between quality of life and life satisfaction (H3); all technology-dependent behavioral disorders were found to be positively associated (H4). The results are shown in Figure 3. The H1 and H2 hypotheses were partially supported, and the H3 and H4 hypotheses were fully supported.

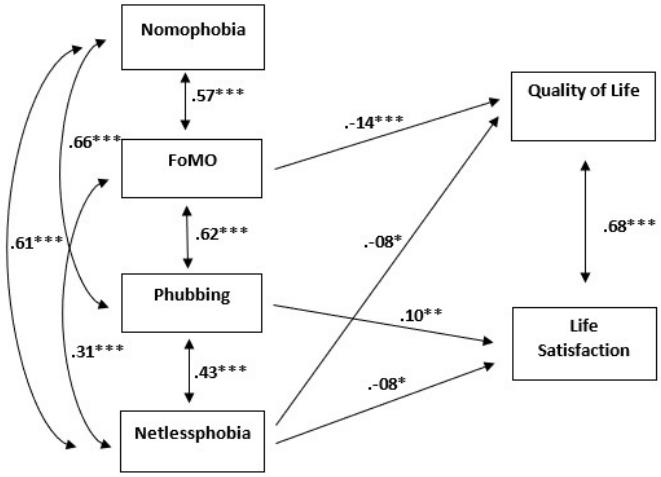

Fig. 3. Path analysis model (*p<0.05, **p<0.01,***p<0.001 The model created for Path Analysis was found to have a good fit (Table 2).

Table 2. Path Analysis Model Fitness Index

Model fit indexes	Good Fit	Acceptable Fit	Scale Values
NPAR			17
Chi-square (χ2)			1.277
P	0.05<p≤1	0.001<p≤0.05	0.865
Degrees of Freedom (DF)			4
Chi-square / Degrees of Freedom (χ2/DF)	0≤ χ2/sd ≤2	2<χ2/sd ≤3	0.319
Root Mean Square Error of Approximation (RMSEA)	0≤ RMSEA≤0.05	0.05<RMSEA ≤1	0.001
Comparative Fit Index (CFI)	0.95≤ CFI ≤1	0.90≤ CFI <0.95	1
Goodness of Fit Index (GFI)	0.95≤ GFI ≤1	0.90≤ GFI <0.95	0.999
Adjusted Goodness of Fit Index (AGFI)	0.90≤ AGFI ≤1	0.80≤ AGFI<0.90	0.997
Normed Fit Index (NFI)	0.95≤ NFI ≤1	0.90≤ NFI <0.95	0.999
Non-Normed Fit Index (NNFI) (TLI)	0.97≤ NNFI ≤1	0.95≤ NFI <0.97	0.999

The model created for Path Analysis was found to have a good fit (Table 2).

4. Discussion

This study determined a high correlation between technology-dependent behavioral disorders, FoMO, and netlessphobia decreasing the quality of life, phubbing increases, and netlessphobia reduces life satisfaction, and there is a positive relationship between quality of life and life satisfaction in desk workers. In the study, the participants' average scores from the scales were as follows: Nomophobia 75.87, FoMO 24.72, phubbing 26.32, and netlessphobia 2.83. In the literature, different scales are used to measure these disorders. When we look at the studies with the scales used in this study, it was between 26-27 (34-36) for phubbing and 21-27 (37-41) for FoMO. In a systematic review on this subject, nomophobia scores ranged from 51 to 82 (42). Since the result obtained from this study is close to the upper limit of the scores obtained in the literature, it can be thought that desk workers are especially at risk regarding nomophobia. The levels of FoMO and phubbing detected in the study are similar to the literature. There is only one study in the literature about netlessphobia. Compared to the scaled study, higher netlessphobia scores

were obtained in this study (43). This study will contribute to the determination of netlessphobia levels in the literature.

Technology-dependent behavioral disorders occur together due to frequent internet use and technological devices. As a result of this study, it was found that all technology-dependent behavioral disorders were positively correlated with each other. Considering the studies in these fields in the literature, nomophobia, and FoMO were positively related (15,44-46), and FoMO predicted nomophobia (14,47,48), nomophobia predicted phubbing (15), FoMO was positively related and predicted phubbing (16,17,49-53). In addition, it is thought that FoMO may be one of the psychological processes underlying problematic social media use (51). As FoMO increases the time spent both directly and with a smartphone, it can mediate the development of nomophobia and phubbing (14). In the literature, netlessphobia has been found to be positively associated with both FoMO and nomophobia (43). Examining these four technology-dependent behavioral disorders in future studies will clarify their relationship.

As technology-related behavioral disorders of individuals begin to occur, their quality of life and life satisfaction are affected. Quality of life is affected by physical, psychological, and social needs (54). Any situation that will negatively affect these requirements reduces the quality of life. In this study, it was determined that there was a negative correlation between the quality of life and phubbing, nomophobia, netlessphobia, and FoMO. In the path analysis, FoMO and netlessphobia decreased the quality of life. In two studies conducted with adolescents, nomophobia was found to be negatively related to the quality of life (55,56). Although there are not many studies in the literature that directly examine the relationship between these technology-dependent behavioral disorders and quality of life, there are also studies that examine the relationship with diseases such as anxiety, depression, stress, musculoskeletal problems, loneliness, sleep problems, which are indicators of decreased quality of life. Studies have shown that nomophobia is associated with depression, anxiety, and stress (57,58). A systematic review found that nomophobia is related to negative mental states such as stress, anxiety, and low self-esteem (25). According to the meta-analysis of Fioravanti et al. (2021), FoMO is positively associated with anxiety and depression (24), and according to another study, phubbing mediates the relationship between cell phone addiction and depression (26). Similarly, in another study, phubbing predicted loneliness, anxiety, and depression (23). This may suggest that technology-dependent behavioral disorders will affect the psychological state of individuals and cause a decrease in their quality of life. In addition, some opinions about reducing the quality of life may lead individuals to use technological devices and their addictions. In their study, Wegmann et al. (2017) stated that psychopathological symptoms cause FoMO (22). In future studies, it is essential to determine whether technology-dependent behavioral disorders negatively affect the psychological state or whether the opposite is true.

The relationship between technology-related behavioral disorders and life satisfaction is intricate, with a more nuanced effect than the overall quality of life. In individuals with a low quality of life, life satisfaction is also likely to be reduced. Quality of life encompasses various aspects of well-being, whereas life satisfaction is a more personal evaluation of one's life. Life satisfaction, on the other hand, is a more individualized assessment that depends on personal values and feelings. The study determined that netlessphobia decreased and phubbing increased life satisfaction, while nomophobia and FoMO did not affect it. Although the relationship between netlessphobia and life satisfaction has not been studied much in the literature (59), it was observed in a longitudinal study conducted on adolescents that internet addiction, which is a similar subject, reduces life satisfaction (60, 61). The use of desktop computers or laptop computers is as intense as the mobile phones of desk workers. It is crucial to have the internet active to perform online transactions on all technological devices. Therefore, the anxiety of being in an environment without the internet in the study group may have suppressed the negative effects of netlessphobia, FoMO, and nomophobia on life satisfaction. The fact that phubbing increased life satisfaction detected in this study is inconsistent with the literature. In the literature, there are studies in which phubbing is negatively associated with life satisfaction (62), and no relationship can be detected (23). It is stated that the negative relationship of phubbing with life satisfaction is due to the effect on the communication disturbance sub-dimension. It is noted that phubbing does not affect life satisfaction in cases where this sub-dimension is not involved (63). In another study examining the relationship between a different phubbing scale and life satisfaction, it was seen that the nomophobia sub-dimension was positively associated with life satisfaction, and the self-isolation and problem acknowledgment sub-dimensions were negatively related (23). All these results suggest that phubbing negatively affects life satisfaction when the individual's communication with the environment is negatively affected. Therefore, the life satisfaction of individuals who avoid face-to-face contact with their environment may not be affected. On the other hand, the life satisfaction of individuals who use online environments to socialize may increase. It is said that one of the underlying causes of phubbing behavior may be multitasking (64). In the case of desk workers, phubbing behavior can have a positive effect on life satisfaction as it enables them to cope with multiple tasks.

This is the first study to evaluate technology-dependent behavioral disorders in desk workers together and to examine the effects of these disorders on life satisfaction and quality of life. However, the study has some limitations. First, since the survey was designed to be cross-sectional, it is impossible to establish a cause-effect relationship between the variables. This part has been tried to be resolved by doing path analysis. However, studies to be conducted in a prospective design on

this subject will be more helpful in revealing causality. It should be kept in mind that the data are evaluated only based on their responses to the applied questionnaire, not by monitoring the individuals. Therefore, there may be partial subjectivity in the answers. In addition, the study was applied online. This may have led to the exclusion of individuals who do not prefer to use online environments. In order to prevent multiple replies from the same person, the e-mail addresses of the individuals were checked. Since the study is voluntary and does not benefit the participants, it is thought there will be no bias or misrepresentation. Within the framework of the hypothesis established at the beginning of the study, only the relationship between technology-related diseases and life satisfaction and quality of life was examined. While evaluating the results, it should be considered that other variables that may affect life satisfaction and quality of life are not included in the model.

As a result, the increasing use of technology in recent years has brought the negative situations of individuals on this issue to the public health agenda. Examining the effects of these disorders on life satisfaction and quality of life provides an opportunity for a holistic evaluation in terms of physical, mental, and social aspects. Reducing technology-dependent behavioral disorders should be included in health and anti-addiction policy intervention programs.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: Ö.Ö., Design: Ö.Ö., B.Ç., Ö.K., E.K., Data Collection or Processing: Ö.Ö., B.Ç., Ö.K., E.K., Analysis or Interpretation: Ö.Ö., E.K., A.N.K., Literature Search: B.Ç., Ö.K., E.K., Writing: Ö.Ö., B.Ç., Ö.K., E.K.

Ethical Statement

Approval was obtained from Süleyman Demirel University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 13/01/2021 and the number of ethical committee decisions is 7/143.

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The efficiency of the first trimester 50 grams glucose tolerance test for detection of the gestational diabetes and the outcome of the pregnancy

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Abstract

We aimed to evaluate the effectiveness of the first trimester's 50 g glucose loading as a screening test for gestational diabetes mellitus and to find a cut-off value for 50 g OGCT in the first trimester. This study was conducted on pregnant women at low risk for diabetes mellitus. A 50-g glucose load was done in the first trimester, and then pregnant women were followed up. A 100-gram diagnostic test for gestational diabetes mellitus was administered to all participants at 24–28 weeks of gestation. The sensitivity, specificity, and false-positive rate of the 50 g glucose challenge test were determined. A total of 454 pregnant women were assessed in this study. 34 women (7.5%) were diagnosed with gestational diabetes. 420 women have constituted the non-diabetic group. In patients with gestational diabetes, age, weight, polyhydramnios, and macrosomia rates were significantly higher than in the non-diabetic group. The discriminative power of the 50 g glucose test in the first trimester was found to be significant (p = 0.001) in gestational diabetic patients. The area under the ROC curve was 0.927. The best cut-off value was 143 mg/dl. In this value, the sensitivity and false-positive rate were 85% and 11%, respectively. A 50-g glucose challenge test done in the first trimester may contribute to the reduction of maternal and perinatal risks and prevent long-term consequences such as obesity, type 2 diabetes mellitus, and lipid profile disorders with early glycemic control and lifestyle changes.

Keywords: diabetic complications, first trimester pregnancy, gestational diabetes mellitus, 50 g glucose loading test

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia arising from inadequate insulin excretion or reduced biologic effectiveness of insulin due to various etiologic causes (1). The American Diabetes Association (ADA) describes GDM as diabetes mellitus diagnosed in the second or third trimester of pregnancy that is not clearly either type 1 or type 2 diabetes because GDM is usually diagnosed after 20 weeks of gestation and disappears either immediately or within 6 weeks after delivery. Pregnancies complicated with GDM require close monitoring to minimize maternal and neonatal morbidity, as maternal hyperglycemia increases the risk of preeclampsia, polyhydramnios, macrosomia, shoulder dystocia, and neonatal respiratory distress syndrome (RDS) (2).

There are two different approaches to identifying individuals with a high probability of having diabetes mellitus. Pregnant women with risk factors should be tested for overt diabetes mellitus at the first prenatal visit (3).

Universal screening for gestational diabetes is performed at 24 to 28 weeks of gestation. The American Diabetes Association (ADA) and American College of Obstetricians and Gynecologists (ACOG) recommend routine oral glucose tolerance tests (OGTT) with 50 grams of glucose between 24 and 28 weeks of gestation and with 100 grams of glucose for pregnant women whose plasma glucose is above 140 mg/dl in the first test, or they recommend directly applying oral glucose tolerance tests with 100 grams of glucose (4,5). Alternatively, a diagnostic test can be administered to all individuals, which is a one-step process.

The two-step approach is the most widely used approach for identifying pregnant women with gestational diabetes in the United Kingdom.

While there are no proven benefits to screening or testing for diabetes in early pregnancy, early diagnosis and treatment of maternal hyperglycemia may reduce fetal and maternal morbidity (6, 7). In the literature, there are quite a few reports investigating the association between 50-g glucose challenge test results and GDM in the first trimester (8–11).

In this study, we aimed to bring the old, safe, trendy, and most importantly, patient-friendly 50-gram OGCT and GDM screening to the first trimester. Especially the fact that 50 g of

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OGCT was easier to tolerate by the patient was one of the reasons for choosing this test. For these reasons, the 50-g OGCT test, which is usually applied between 24-28 weeks in GDM screening, could not be taken to an earlier period, for example, 11–14 weeks when aneuploidy screening was performed.

2. Materials and Method

This study was conducted prospectively on pregnant women who were admitted to our antenatal clinic for the first-trimester aneuploidy screen. It was planned to perform screening at 11–14 weeks of gestation to rule out early pregnancy losses, standardize the study group, and exclude patients with aneuploidy risk and the possibility of pregnancy termination. The time period for the study was two years. The Scientific Research Project Support Unit of Karadeniz Technical University supported this study (project number 2010.114.002). The local ethics committee reviewed and approved the study protocol. Informed consent was obtained from volunteers.

Calculation of sample size: $n = z^2pq / d^2$ (n = the number of individuals to be sampled, p = frequency of occurrence / probability of the event to be examined (0.5), q = frequency of absence of the event to be examined / probability of not happening (1-p = 0.5), z = theoretical value (1.96 for 95% confidence interval) found from the z table at a certain confidence level, d = standard error of the rate to be determined in the study (0.05 for 95% confidence interval)). $N = 1.96^2 \times 0.5 \times 0.5 / 0.05^2 = 384$ According to this calculation, 384 pregnant women will constitute the sample of the study. In order to prevent possible data loss, 10–20% more pregnant women will be sampled, and the study was planned with a total of 463 pregnant women.

Inclusion criteria: It was determined that pregnant women between the ages of 18 and 40 had a BMI of 30 kg/m2. Exclusion criteria: pregnant women with a history of pregestational or gestational diabetes, older maternal age, previously infant weight, members with a high prevalence of type 2 DM, medical conditions associated with the development of DM, hypothyroidism, hyperthyroidism, hyperprolactinemia, hereditary thrombophilia, polycystic ovary syndrome, multiple pregnancies, IVF (in vitro fertilization) pregnancy, recurrent fetal losses, steroid use, and diabetes mellitus in the first-degree relatives were not included in the study.

A 50-g oral glucose load was given after measuring the fasting plasma glucose level, and another blood sample was obtained to measure the 1st hour glucose level. The Gluc3 Cobas Integra Cobs C system was used for glucose measurement. Pregnant women with fasting glucose >125 mg/dl or 1st hour glucose >199 mg/dl were accepted as having pregestational diabetes mellitus and excluded from the study. Pregnant women were followed up. A 100-gram, three-hour oral glucose challenge test was performed for a diagnostic test at 24-28 weeks of gestation when two glucose values were elevated. We used thresholds for defining elevated values, which have been proposed by Carpenter and Coustan (12). After 50 g of GCT was performed in the first trimester, 100 g of OGTT was performed on the same pregnant woman at 24–28 gestational weeks. After this stage, the patients were divided into two groups: GDM and non-GDM. Maternal and fetal complications were investigated in all cases. Preeclampsia, premature rupture of membranes, preterm delivery, macrosomia (newborn weight > 4000 g), vacuum or forceps delivery, postpartum hemorrhage, intensive care unit needs for the newborn, fetal hypoglycemia, and hypocalcemia were monitored and recorded.

2.1. Statistical Analysis

SPSS 21.0 (IBM, USA) was used for statistical analysis. The Kolmogorov-Smirnov test was used to check the normality assumption. The student t-test was used in order to compare variables. Continuous data was given as mean ± standard deviation; ordinal and nominal data were given as medians or modes. The area under the receiver operating characteristic curve was used to determine the discriminative power of the first trimester's 50 g OGCT in the prediction of gestational diabetes. All p values were two-tailed, and statistical significance was set at $p<0.05$.

3. Results

Pregestational diabetes mellitus was detected in nine pregnant women at the time of the first trimester screen, and they were excluded from the study. Therefore, a total of 454 pregnant women were used in the final analysis. Clinical characteristics of the patients are shown in Table 1.

Table 1. The characteristics of the study population

	GDM Group (n=34)	Non-GDM Group (n=420)	p
Age (year)	33.38±5.29	28.84±5.34	
Gravida	3 (1-6)	2 (1-8)	0.558
Parity	1 (0-4)	2 (0-6)	0.442
Weight (kg)	72.81±8.08	62.87±6.5	**0.046**
Gestational age at delivery (day)	259	267	0.436
Neonatal birth weight (g)	3381.76±857.54	3220.5±555.21	0.582
APGAR 5	9	9	0.834
Mode of delivery			
Cesarean delivery (%)	58,8	47,4	**0.035**
Vaginal delivery%)	41,2	52,6	**0.033**

Data is presented as frequency and percentages or mean ± SD, n: number, g: gram, min-max. GDM: Gestational Diabetes Mellitus.

GDM was diagnosed in 34 (7.5%) cases. The mean serum glucose level at 1st hour following a 50 g glucose load was found to 169.5±28.95 mg/dl in 34 cases diagnosed with GDM. This value was obtained as 113.52±24.19 mg/dl for non-diabetic cases. The results demonstrated that the 1st hour serum glucose level following 50 g OGCT in the first-trimester was statistically significant in GDM cases (p<0.001). Maternal plasma glucose levels at the 1st hour following a 50 g glucose load at the first trimester and fasting, and 1st, 2nd, and 3rd hour glucose levels following 100 g OGCT at 24-28th weeks of gestation were presented in Table 2.

Table 2. The comparison of the mean glucose level measured at the first and second trimester

Serum Glucose (mg/dl)	GDM Group (n=34)	Non-GDM Group (n=420)	95% Confidence intervals	P
11-14 weeks 1.h-50g	169.5±28.95	113.52±24.19	-64.6 to-47.4	**0.001**
24-28 weeks fasting-100g	108.32±22.75	88.82±11.22	-23.9 to-15.1	**0.001**
24-28 weeks 1.h-100g	188.41±30.97	124.04±23.07	-72.7 to-56.1	**0.001**
24-28 weeks 2.h-100g	186.85±32.59	121.34±17.43	-72.2 to-58.9	**0.001**
24-28 weeks 3.h-100g	166.12±37.27	115.76±19.54	-57.8 to-42.9	**0.001**

Data are shown as mean ± Std

The mean age and the mean weight of the pregnant women were significantly higher in patients with GDM. Preeclampsia developed in 5 (1.1%) cases, and polyhydramnios was detected in 10 (2.2%) cases. Preterm birth occurred in a total of 48 (10.6%) cases. In a total of 28 (6.2%) cases, early rupture of the membranes complicated pregnancy. Macrosomia was detected in 23 (5.1%) cases. Of them, 9 (26.5%) were in the GDM group, and 14 (3.3%) were in the group without GDM. Shoulder dystocia developed in only one case without GDM. No woman required a vacuum or forceps delivery. In the GDM group, the rate of polyhydramnios, macrosomia, and neonatal metabolic complications was significantly higher despite treatment (Table 3).

Table 3. Maternal and neonatal morbidities of the study population

	GDM Group (n=34)	Non-GDM Group (n=420)	P
Preeclampsia, n (%)	0	5(%1)	0.435
Polyhidramnios, n (%)	6 (%17.6)	4(%1)	**0.001**
Preterm birth, n (%)	5 (%14.7)	43(%10.2)	0.546
Preterm rupture of membranes, n (%)	3 (%8.8)	25 (%6)	0.472
Postpartum hemorrhage, n (%)	1(%2.9)	6 (%1.4)	0.364
Macrosomia, n (%)	9 (%26.5)	14(%3.3)	**0.001**
Shoulder dystocia, n (%)	0	1(%0.2)	0.351
Respiratory distress syndrome, n (%)	2 (%5.9)	9(%2.1)	0.443
Neonatal metabolic complications, n (%)	3 (%8.8)	0	**0.001**
NICU admission, n (%)	6 (%17.6)	28 (%11.9)	0.523

Data are shown as percentage. n:number

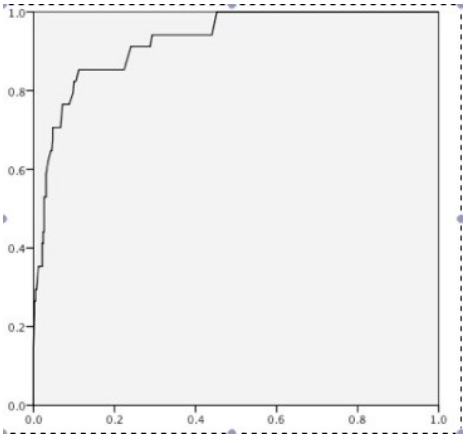

Fig. 1. Receiver operating characteristic curves for the first trimester 50 g glucose challenge test.

We calculated the area under the curve as 0.927 in the ROC analysis. 50 g of OGCT in the first trimester could discriminate GDM cases at a significance level of p<0.001 (Fig. 1). We calculated the best threshold value as 143 mg/dl for GDM screening with 50 g OGCT in the first trimester. The sensitivity of this value was 85%, and the false positivity was 11%. The 95% confidence interval value was found to be 0.886–0.968.

4. Discussion

GDM is an important health issue, as approximately 90% of DM seen in pregnancy is GDM, and it could significantly influence perinatal morbidity. Gestational diabetes mellitus (GDM) is the most common complication of pregnancy (13). The HAPO (Hyperglycemia and Pregnancy Outcome) trial, conducted in 15 centers in 9 countries between 2000 and 2006,

detected that elevated plasma glucose and increased pregnancy risks are directly interrelated. Many centers in the UK screen pregnant women who have risk factors (14, 15).

In contrast to the two-step or single-step diagnosis and screening performed at 24-28 weeks, in this study, we targeted both early diagnosis and planned to screen the patients with 50 g OGCT in the first trimester. Identifying and treating GDM earlier can have multiple benefits for patients (16). One possible explanation for the improvement in the results found may be the early treatment interventions in our study. Early screening increased the primary composite outcome (emergency caesarean section, neonatal hypoglycemia, and macrosomia; 41.2% vs. 30.3%) in the study on early detection of GDM reported by Ryan et al. (17).

Nahum et al. (8) performed a study involving 124 pregnant women using 1-hour oral glucose screening tests in the first and third trimesters (26–32 weeks). They reported that third-trimester glucose screening may be unnecessary for patients with first-trimester glucose screening test values of 110 mg/dL or less. In contrast, they reported that there was a high positive predictive value for high repeat glucose screening test results in the early third trimester for pregnant women whose first-trimester glucose test results were 135 mg/dL or above.

In a systematic review, at or after 24 weeks of gestation, oral glucose challenge tests with 140- and 135-mg/dL cutoffs had sensitivities of 82% and 93%, respectively, and specificities of 82% and 79%, respectively, against Carpenter and Coustan criteria. The 140-mg/dL cutoff had a sensitivity of 85% and a specificity of 81% against the National Diabetes Group Data criteria. (18). These studies were mainly conducted after the 24th week of pregnancy. Our study focused on GDM screening in the first trimester. In our study, we calculated the best threshold value as 143 mg/dl for gestational DM screening with 50 g OGCT in the first trimester. The sensitivity of this value was 85%, and the false positivity was 11%.

In a cohort study conducted in Finland, 75 g of OGTT was performed in the first trimester and at the 24th gestational week. While the rate of GDM diagnosed in the first trimester was 14.6%, the rate of GDM diagnosis in the 24th gestational week was 10.6%. According to the results of the study, they recommended the determination of new diagnostic cut-off values for the first trimester. The difference between this study and our study is that it should perform a single-step diagnostic test. Our study is screening by performing the 50 g GCT part of the two-step test in the first trimester (19).

Yeral et al. (9) carried out a randomized study involving 736 pregnant women. They performed first-trimester fasting blood glucose tests and two-stage 50-gram and 75-gram OGCT tests at 24-28 weeks. They reported the area under the ROC curves as 0.623, 0.708, and 0.792, respectively. They reported that 75 g of OGCT can be preferred for the GDM screening in the first trimester (9). In our study, we calculated the area under the curve as 0.927 in the ROC analysis.

In a randomized controlled study by Harper et al. (11) with obese women, it was found that performing early GDM screening did not decrease the incidence of primary results (56.9% in the early screen versus 50.8% in the routine screen). In obese women, primary outcomes may not be affected, but early glycemic control may improve primary outcomes in low-risk groups (11). We conducted our study not only on obese people but also on low-risk groups.

In the HAPO study, in the long-term results of GDM, the development of obesity in pregnant women with GDM was found to be much more significant than in pregnant women without GDM. (19.1% GDM (+) - 9.9% GDM (-)). If an early lifestyle change is made with an early diagnosis, the patient's compliance increases, and obesity can be reduced due to weight gain. Alyas et al. (17) proved that patients with GDM (+) had deterioration in blood lipid profiles, and this could lead to vascular damage. Early glycemic control would reduce these results.

Yalçın et al. (20) found the GDM incidence to be 6.6% in their study conducted at Ankara Zekai Tahir Burak Hospital in 1996. We obtained a higher GDM rate of 7.5% compared to the result of Yalçn et al. (20). We consider that the results of our study may reflect the GDM incidence in our region.

In our study, GDM (+) and GDM (-) pregnant women were compared in terms of fetal macrosomia, delivery type, preterm labor, preeclampsia, polyhydramnios, shoulder dystocia, RDS, neonatal hypoglycemia, and referral to neonatal intensive care. Fetal macrosomia, polyhydramnios, and neonatal hypoglycemia were seen more in the GDM (+) group, and this rate was statistically significant. These results were found to be similar to the current literature (20–26). There was no significant difference between the groups in terms of preterm labor, delivery type, shoulder dystocia, RDS, or referral to the NICU. These results may have been caused by the prevention of fetal macrosomia by strict glycemic control and good prenatal follow-up in our clinic.

While class B diabetics according to the White classification have similar risks as non-diabetics, hypertensive complications increase in patients in classes D, F, and R (14, 15). In this study, while preeclampsia was detected in 5 patients without GDM, it was not detected in patients with GDM. In addition, while the preeclampsia ratio is 1.1% among all patients, it was found to be 1.2% in patients without GDM, and the difference was not found to be significant. We considered that this resulted from the risk increasing by 60% due to the coexistence of GDM-related nephropathy and chronic hypertension, and we did not include pregnant women who had pre-gestational DM, previous GDM, or chronic hypertension in the study (22).

By performing 50 g of GCT in the first trimester, GDM complications that may occur up to the second trimester can be

prevented. In addition, with GDM screening in the first trimester, both aneuploidy and GDM screening will be performed in one visit, and glucose regulation will be started early since it has a high diagnostic reliability in the early period, and thus both maternal and fetal complication rates will decrease.

Limitations of our study: It was not randomized; patients with pregestational DM were excluded from the study; we did not treat those we thought to be GDM; and we waited 24–28 weeks for 100 g OGCT.

Strength: Contrary to the literature, we found that pulling the current cut-off value up rather than down reduced the sensitivity of the test and the rate of false positivity.

In conclusion, Making a 50-g glucose loading test together with the first-trimester combination test between 11 and 14 gestational weeks may contribute to making a diagnosis of GDM in the early weeks of gestation, planning the management of these cases, and reducing maternal and perinatal risks. During this period, if the 1st hour glucose threshold value is taken as 143 mg/dl, GDM cases can be detected with 11% false positivity and 85% sensitivity. These ratios are similar to the ratios of the 50-g loading test, which is routinely done between 24 and 28 gestational weeks. We believe that our study demonstrates the feasibility of early and one-step screening of GDM and can shed light on new research on this issue.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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

Ethical Statement

This study was approved by the clinical research ethics committee of the Karadeniz Technical University. Date: 01.05.2012, number: B301KTÜ0200000/489.

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Risk factors for voiding dysfunction following midurethral sling operations

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Abstract

We aimed to identify risk factors for postoperative voiding dysfunction following tension-free vaginal tape (TVT) or trans obturator tape (TOT). A retrospective case-control study was conducted on patients who underwent mid-urethral sling procedures over a four-year- period by the same surgical team. The patients were divided into two groups. Patients who needed to loosen the tape materials surgically before being discharged due to persistent postvoid residual bladder volume ≥150 ml and/or difficulty in emptying the bladder were described as postoperative voiding dysfunction (case group). The patients who did not need it were the control group. Demographic information, voiding symptoms, urodynamic evaluation, and intraoperative data were collected from the hospital's medical records. Of 167 patients, 29 (17.4%) were in the case group and 138 (82.6%) were in the control group. At univariate analysis, age, menopausal status, preoperative valsalva leak point pressure measurement, presence of preoperative ≥ grade 3 pelvic organ prolapse, TVT procedure, concomitant anterior colporrhaphy, and vaginal hysterectomy were associated with voiding dysfunction. Multivariate logistic regression revealed that menopausal status, TVT procedure, and concomitant anterior colporrhaphy were significant predictors of postoperative voiding dysfunction.The present study indicated that postoperative voiding dysfunction is more often after TVT than after TOT procedures. Menopausal status and concomitant anterior colporrhaphy increased the risk of postoperative voiding dysfunction. Recognition of these risk factors may enable surgeons to minimize this complication.

Keywords: postoperative complication, TOT, TVT, voiding dysfunction

1. Introduction

Urinary incontinence (UI) is identified as ‘‘objectively demonstrable unintended urinary leakage that can cause social and hygienic problems’’ (1). It is critical to accurately state the kind of incontinence so as to choose the cheapest and most effective treatment method for the treatment of UI (2). The most widespread kind of UI is the stress urinary incontinence (SUI). It is urinary incontinence during conditions that elevate intra-abdominal pressure and is defined as a form of UI that happens when the intravesical pressure greater than the urethral pressure without increased activity in the detrusor muscle (3). Urge urinary incontinence (UUI) is a urinary incontinence circumstance that accompanies a strong urge to urinate (4).

Among the types of incontinence, SUI patients are the patient group that can benefit most from surgery among the treatment options, and surgical treatment is often required. Many surgical techniques have been developed, vaginally and abdominally (5). Tension-free vaginal tape (TVT) surgery that was defined by Ulmsten in 1996, and Trans obturator tape (TOT) technique that was defined by Delorme in 2001 (6,7,).

Complications of TVT and TOT operations include bladder perforation, mesh erosion, bleeding, soft tissue infections, urinary tract infections, bowel injury, ureteral injuries, vaginal lacerations, and postoperative voiding disorders. The rate of voiding dysfunction after surgery ranges from 7.8% to 84%. This is associated with prolonged postoperative catheterization, increased urinary tract infections, the need for a second operation, higher healthcare costs, and reduced patient satisfaction. It will be helpful to state the risk factors correlated with postoperative voiding dysfunction in order to provide information to the surgeon in the preoperative period and appropriate counseling to patients who will undergo mid-urethral sling (MUS) operations (8). Thus, the purpose of the study is to determine the risk factors that contribute to postoperative voiding dysfunction in patients who have undergone TVT or TOT procedures.

2. Materials and Methods

This retrospective-case-control study was managed to patients who were diagnosed with SUI and underwent TVT or TOT operation from January 2013 to December 2016 in the gynecology department at a tertiary hospital. After obtaining ethical approval from Research Ethical Committee, the files of the patients who were operated on within the study period were

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reviewed from the hospital's archive (Approval number: 13.02.2017-21). This research complies with privacy legislation and follows the Helsinki Declaration.

The clinical, and sociodemographic data, laboratory findings, gynecological examination, and urodynamic examination results of each patient were recorded. Abstracted data contained age, menopausal status, obstetric history, body mass index (BMI), chronic systemic diseases, medications, and previous pelvic and urogynecological surgery history. Pelvic organ prolapse (POP) grading of the patients was done according to the Baden-Walker classification (9). In the preoperative evaluation, postvoiding residual urine volume was determined by applying a urinary catheter. A preoperative urodynamic evalutaion was applied according to ICS standards (10). The urinary incontinence kind of all patients was confirmed by urodynamic examination. Anesthesia type and duration of the patients' operations, MUS type, other accompanying surgical procedures (anterior colporrhaphy, posterior colporrhaphy, and vaginal hysterectomy), and the presence of complications were noted. TOT and TVT operations were applied to the patients as MUS operations. The TOT operation was performed by the same experienced team as described by Delorme and the TVT operation by Ulmsten, using the same type of mesh material (1.1 cm x 40 cm, polypropylene, monofilament braided) (6,7). The patients were followed up with urinary catheterization for 24 hours postoperatively, and then the catheter was removed. After catheter removal, the residual urine volume was measured. When the residual urine volume was above 150 ml or in the presence of voiding difficulty, urinary catheterization was applied again for 3 days. In case of residual urine excess or voiding difficulty despite this additional 3 days of catheterization, the mesh material placed during the MUS operation was surgically loosened for the patients. Patients who underwent mesh loosening were identified as patients who developed postoperative voiding dysfunction and constituted the case group in our study. The patients who were discharged without any problem after the operation constituted the control group. Patients with preoperative voiding dysfunction, neurological disease, or drug use that may cause urinary retention, intraoperative complications, postoperative urinary tract infection, and insufficient data were excluded.

2.1. Statistical Analysis

Statistical analysis was performed using IBM SPSS for Windows, Version 25.0 software. The distributions of the data were checked by the Kolmogorov-Smirnov test. In the case of normally distributed variables, parametric methods were applied, whereas in the case of non-normally distributed variables, nonparametric methods were applied. Normally distributed continuous variables were evaluated with the Independent Student's T-test. In order to determine the difference between categorical variables, we used the Chi-square test. Continuous variables were reported as mean±standard deviation, and categorical variables as numbers (n) and percentages (%). The multiple effects of possible risk factors for postoperative voiding dysfunction were evaluated by logistic regression analysis. A p-value less than 0.05 was deemed significant.

3. Results

Medical records of 338 patients were reviewed, of which 171 patients were excluded. The final analysis included 167 patients, 29 of whom developed voiding dysfunction after MUS surgery, and 138 were discharged without any problems. There was a statistically significant difference between the mean age of the patients in the case and control groups (54.44±11.54 vs. 49.36±8.55, p=0.007). It was also found that 21 (72.4%) of the patients in the case group and 45 (32.6%) of the patients in the control group were in menopause, and a statistically significant difference was found between the two groups (p<0.001). A statistically significant difference was not found between the two groups in terms of gravida-parity numbers, BMI, history of chronic diseases, a history of macrosomic birth, and previous urogynecological or pelvic surgery history (Table 1).

Table 1. Comparison of Study Groups by Demographic Characteristics (n=167)

	Case group (n=29)	Control group (n=138)	p
Age (years)	54.44±11.54	49.36±8.55	0.007
Gravida	4.34±1.67	4.47±1.34	0.798
Parity	3.24±0.83	3.08±1.01	0.575
BMI (kg/m^2)	30.49±3.88	29.67±3.76	0.291
Menopause status	21 (72.4)	45 (32.6)	<0.001
Diabetes mellitus status	4 (13.8)	13 (9.4)	0.479
Hypertension status	9 (31.0)	29 (21.0)	0.242
Asthma status	4 (13.8)	18 (13.0)	0.914
History of macrosomic birth	6 (20.7)	29 (21.0)	0.969
History of urogynecological surgery	2 (6.9)	4 (2.9)	0.293
History of pelvic laparotomy	5 (17.2)	26 (18.8)	0.840

Data are shown as Mean±Standard Deviation and number (n) and percentages (%). BMI: Body Mass Index p<0.05 was considered statistically significant.

Regarding accompanying POP, no significant difference was observed between groups, except POP grade 3 and above (p=0.004). In both groups, statistically significant differences were observed regarding valsalva leak point pressure (VLPP) (p=0.018), however, no differences were observed regarding detrusor overactivity, bladder capacity, or maximum urethral

closure pressure (MUCP) (Table 2).

Table 2. Comparison of Urogynecological Evaluation Results of Study Groups (n=167)

	Case group (n=29)	Control group (n=138)	p
Overactive bladder symptoms			
Urgency	12 (41.4)	46 (33.3)	0.408
Frequency	5 (17.2)	18 (13.0)	0.551
Nocturia	6 (20.7)	19 (13.8)	0.342
≥Grade 2, presence of POP	24 (82.8)	108 (78.3)	0.589
≥Grade 3, presence of POP	13 (44.8)	27 (19.6)	0.004
Bladder capacity (ml)	453.52±54.34	457.02±49.78	0.735
Presence of detrusor overactivity	10 (34.5)	33 (23.9)	0.237
VLPP (cm H_2O)	70.55±34.86	54.92±31.49	0.018
MUCP (cm H_2O)	63.34±23.75	67.96±24.72	0.329
Type of incontinence			
SUI	19 (65.5)	105 (76.1)	0.237
SUI+UUI	10 (34.5)	33 (23.9)	

Data are shown as mean±standard deviation and number (n) and percentages (%). POP: Pelvic Organ Prolapse VLPP: Valsalva leak point pressure MUCP: Maximum Urethral Closure Pressure SUI: Stress Urinary Incontinence UUI: Urge Urinary Incontinence p<0.05 was considered statistically significant.

The frequency of TVT and anterior colporrhaphy accompanying MUS operation was significantly higher in the case group than the control group (p<0.001; p=0.024) (Table 3). Factors that may be effective in the postoperative voiding dysfunction were investigated by multiple regression analysis. Age (p=0.487), VLPP value (p=0.15), presence of ≥G3 POP (p=0.253), and vaginal hysterectomy (p=0.452) were not detected to be significant factors for postoperative voiding dysfunction. Moreover, the presence of menopause (Wald=6.20, OR=4.59, 95% CI=1.38-14.73 P=0.013), TVT (Wald=15.77, OR=16.26, 95% CI=4.12-64.37 P<0.001) and anterior colporrhaphy (Wald=4.10, OR=4.51, 95% CI=1.05-20.05 P=0.043) were detected to be significant independent variables (Table 4).

Table 3. Comparison of Study Groups by Intraoperative Characteristics (n=167)

	Case group (n=29)	Control group (n=138)	p
Type of anesthesia			0.949
General	9 (31.0)	42 (30.4)	
Regional	20 (69.0)	96 (69.6)	
Anesthesia time (min)	102.93±35.34	100.04±37.06	0.497
MUS type			<0.001
TVT	26 (89.7)	50 (36.2)	
TOT	3 (10.3)	88 (63.8)	
Concomitant operations			
Anterior colporrhaphy	20 (69.0)	68 (49.3)	0.024
Posterior colporrhaphy	14 (48.3)	60 (43.5)	0.816
Vaginal hysterectomy	6 (20.7)	12 (8.7)	0.058

Data are shown as mean±standard deviation and number (%). MUS: Mid-urethral Sling TVT: Tension-free Vaginal Tape TOT: Trans Obturator Tape p<0.05 was considered statistically significant.

Table 4. Multiple Regression Analysis Results of Factors That May Be Effective in the Development of Postoperative Voiding Dysfunction

	Wald	P	OR	95% CI
Age	0.48	0.487	0.97	0.90-1.05
VLPP	2.07	0.150	1.01	1.00-1.03
Menopause status	6.20	0.013	4.59	1.38-14,73
≥Grade3, presence of POP	1.31	0.253	2.11	0.59-7.64
TVT	15.77	<0.001	16.26	4.12-64.37
Anterior colporrhaphy	4.10	0.043	4.51	1.05-20.05
Vaginal hysterectomy	0.57	0.452	0.53	0.10-2.74

OR: Odds Ratio, CI: Confidence Interval POP: Pelvic Organ Prolapse VLPP: Valsalva leak point pressure TVT: Tension-free Vaginal Tape p<0.05 was considered statistically significant.

4. Discussion

Although UI is a common health concern that reduces the quality of life and negatively affects social life (10). Therefore, UI should be considered as a health problem, regardless of the age of the patient. An accurate diagnosis is critical and patients should be directed to appropriate treatment. Patients with SUI can benefit most from surgery among the treatment options (5). MUS operations can be applied and voiding dysfunction may occur after these operations.

The incidence of postoperative voiding dysfunction ranges

from 7.8% to 84% (8). In our study, this rate was 17.4%. The difference between studies can be attributed to patient characteristics, the use of different surgical techniques, and the use of different standard definitions of voiding dysfunction among different teams. According to Groutz et al. (11) and Ambroise et al., (12) a postoperative residual urine volume of 100 ml and above was considered the lower limit for the development of voiding dysfunction; this limit was 200 ml according to Stanton et al. (13) and Chang et al. (14) By applying ICS standards in our study, we defined the presence of residual urine excess or voiding difficulty despite 3-day urinary catheterization as postoperative voiding dysfunction when the postvoid residual urine volume is above 150 ml or voiding difficulty (10). We considered that this describtion is helpful for standardizing the describtion of postoperative voiding dysfunction.

Salin et al. (12) and Mutone et al. (15) demonstrated that aging is a critical risk factor for postoperative voiding dysfunction. Similarly, Vervest et al. (16) showed that concomitant menopause with increasing age is a significant risk factor for developing postoperative voiding dysfunction. In our study, the case group was older and menopausal compared to the control group. We assumed that advanced age and the presence of menopause may be risk factors for postoperative voiding dysfunction. As age advances, bladder capacity, compliance, and urinary flow rate decrease, and post-void residual urine volume increases. In addition, MUCP and functional urethral length decrease with age (17). Aging patients may experience an increase in symptoms associated with the lower urinary system due to anatomical and physiological changes (18). Vaginal atrophy that occurs with menopause causes frequent urination, dysuria, urinary incontinence, and difficulty in urination. Studies have shown that vaginal estrogen therapy improves lower urinary tract symptoms in postmenopausal women (19). In patients with voiding dysfunction following MUS surgery who are in menopause, we may consider vaginal estrogen therapy as a treatment option.

Previous studies found that low BMI in addition to increasing age was a risk factor for postoperative voiding dysfunction (20,21). However, we found no difference in BMI in our study. Similar to our study, Dawson et al. (22) and Park et al. (23) did not detect a relationship between BMI and voiding dysfunction. Therefore, in our opinion, studies with larger patient populations are needed to determine the definitive effect of BMI on voiding dysfunction. In line with our study results, Dawson et al. (22) and Karin et al. (24) could not find a relationship between parity and the development of voiding dysfunction.

In previous studies, it was stated that there was a statistically significant difference between mean bladder capacity (25). Even though the authors have suggested that having a high bladder capacity in the preoperative period is a protective factor with reference to the risk of postoperative voiding dysfunction, they emphasized that it was important to work with a much more patient population in the conclusion of their study, since the total number of patients was 100. Despite a larger number of patients included in this research, mean bladder capacity of the case group was greater than the control group, but there was no statistically significant difference between the two groups. Chang et al. (14) showed that the presence of detrusor overactivity among the preoperative urodynamic findings was significantly higher in the case group than the control group. In our study, however, there was no difference in terms of the presence of detrusor overactivity. Because this study was limited to patients who developed voiding dysfunction after TOT operation. Also, Chang et al. (14) defined preoperative voiding difficulty and a history of urinary retention as independent risk factors for postoperative voiding dysfunction. In our study, however, preoperative voiding dysfunction or urinary retention were among the exclusion criteria. Previous studies indicated that the risk of developing voiding dysfunction increases in patients with postoperative urinary tract infections in the early postoperative period.[26] In our study, urinary tract infection is among the exclusion criteria as it may cause the development of voiding dysfunction independent of the operation.

A study by Park et al. (23) examined the urodynamics of patients in the preoperative period and reported that the mean VLPP and MUCP values did not differ between the groups. In this study, a difference in mean VLPP and MUCP measurements between the case and control groups was not demonstrated. These results reflect those of Ripperda et al. (8) who also did not find any significant differences in preoperative urodynamic examination findings. It can thus be suggested that preoperative urodynamic parameters do not play a defining role in postoperative voiding dysfunction.

A recent study reported a higher rate of voiding dysfunction after MUS operation in patients previously operated for POP (26). The main weakness in their study is that they overlooked the effect of the presence of POP accompanying existing urinary incontinence on the development of voiding dysfunction after MUS operation. An even greater source of the issue was that no classification was used for the grading of POP. Our results differed slightly from those of Hayser et al.'s study findings. Our analysis showed significant differences between the case and control groups in the presence of POPs accompanied by grade 3 and higher. However, not with an accompanying POP grade 2 or higher.

In the current study, evaluating anesthesia types and average anesthesia duration did not demonstrate any difference between the two groups. Although there has been no study of the literature concerning anesthesia types and durations of the cases in detail, Risperda et al. (8) stated that spinal anesthesia and Chang et al. (14) general anesthesia might increase the risk for postoperative voiding dysfunction. Regression analysis in

these studies, however, was also statistically insignificant.

A study emphasized that the rate of development of voiding dysfunction after the TVT operation was higher than after the TOT operation (TVT 18.3%, 11.0% after TOT, $p < 0.05$) (27). This finding was also reported by Tahseen et al. (28) and Jeffry et al. (29). In our study, the prevalence of TVT was higher in the case group than in the control group. It can thus be suggested that it may cause more post-operative voiding dysfunction since the sling in TVT is more upright and stronger than in TOT. In their multicenter case-control study, Molden et al. noted that the presence of simultaneous surgery accompanying the MUS operation increased the risk of developing postoperative voiding dysfunction. However, they did not differentiate between the types of accompanying operations (30).

In the present study, the incidence of anterior colporrhaphy accompanying MUS operation was higher in the case group. On the other hand, no significant difference was found in postoperative voiding dysfunction in posterior colporrhaphy or vaginal hysterectomy accompanying MUS operation. This can be explained by the fact that anterior colporrhaphy causes a wider incision, more local edema and local inflammation, and more fibrosis development after the surgery.

Retrospective design was the main weakness of our study. As anticipated, it was not possible to assess conditions such as race, ethnicity, and genetic factors that may cause voiding dysfunction due to the study design. On the other hand, it was an advantage in terms of the correct interpretation of the study results, that there are more study groups compared to similar studies in the literature and that the applied procedures were performed by the same team with the same standards.

Postoperative voiding dysfunction was associated with prolonged postoperative catheterization, increased urinary tract infections, the need for a second operation, and decreased patient satisfaction. In our study, performing a TVT operation, the presence of a simultaneous anterior colporrhaphy procedure, and being in the postmenopausal period were significant risk factors for the development of postoperative voiding dysfunction. Therefore, patients who are scheduled for a MUS operation due to incontinence are in the postmenopausal period or who will undergo a TVT operation or simultaneous anterior colporrhaphy operation, may have prior knowledge of voiding dysfunction that may develop after the operation. In addition, it provides an advantage for the surgeon to apply the necessary preventive approaches against this complication that may occur. As a result, determining which patient will develop postoperative voiding dysfunction will help the surgeon to provide appropriate counseling to the patients in the preoperative period.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: U.K.O., E.K., C.M.A., M.K.K., O.S.A., Design: U.K.O., E.K., C.M.A., M.K.K., O.S.A.., Data Collection or Processing: U.K.O., E.K., C.M.A., M.K.K., O.S.A. Analysis or Interpretation: U.K.O., M.K.K., O.S.A., Literature Search: U.K.O., E.K., C.M.A., M.K.K., O.S.A, Writing: U.K.O., E.K., C.M.A., M.K.K., O.S.A

Ethical Statement

Approval was obtained from Health Sciences University Zekai Tahir Burak Women and Children Diseases Training and Research Hospital Ethics Committee, the study started. The ethics committee decision date is 13/02/2017 and the number of ethical committee decisions is 21.

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Evaluation of type differences of arteria vertebralis with C2 vertebral artery groove variation origin

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Abstract

Vertebral artery Groove (VAG) variations at the C2 vertebral level are one of the most important factors determining the risks of C2 pedicle screw surgery. However, there are no accepted guidelines for radiographic parameters that can predict C2 screw placement and probabilty of fracture risk. In our study, we evaluated the type differences in the C2 pedicle caused by the C2 VAG variation of the arteria vertebralis, which is specific to our population, with three-dimensional computed tomography angiography (3D-CTA). Therefore, we aimed to easily estimate the risks of C2 pedicle screw placement by type in patients scheduled for surgery. Measurements were made on a total of 200 patients in 100 female and 100 male patients who underwent cervical 3D-CTA. C2 level VAG variations were categorized into four groups as Type I, Type II, Type III and Type IV. Female, male, right pedicle and left pedicle type comparisons were statistically analysed. It was predicted that screw placement was possible with low risk in Type I, with high risk in Type III and Type IV, and impossible in Type II. Type I pedicle was the most common variation with a rate of 66.5% in men and 48 iin women. There was no significant difference between men and women in the distribution of Type I C2 pedicles in our population (p=0.067, p=0.138). There were statistically significant differences between men and women in the distribution of Type II, Type III and Type IV variations (p=0.008, p=0.037, p=0.0069). Type II pedicle variation was 5% in men and 15% in women. We think that the evaluation of the type differences of the C2 VAG variation origin of the arteria vertebralis in our population will be a guide for surgical interventions in this region.

Keywords: C2 pedicle screw, C2vertebral artery Groove, HRVA, narrow pedicle

1. Introduction

Variations in the anatomical C2 vertebral artery groove (C2 VAG), observed in preoperative imaging, have been reported to influence the surgical plan for C2 pedicular. No guidelines have been established as to which radiographic parameters can be used as predictors of the risk of pedicle fracture with C2 pedicle insertion. The diameters of the C2 pedicles vary according to various researchers, often due to the use of alternative methods and images from different slices of a computed tomography (CT) scan. Hassan et al. reported in 2010 that a thin-section CT scan before surgery can predict the risk of C2 pedicle screw placement and that the risk of cortical fracture is two times higher in pedicles with a C2 pedicle diameter of less than 6 mm[1). According to Wang et al. describes as the greater the vertical distance from the VAG apex to the upper facet joint surface and the horizontal distance from the VAG entrance to the vertebral canal, the safer pedicle screw implantation will be. If any of these distances is less than 4.5mm, there is a high risk of inserting a screw with a maximum diameter of 3.5mm[2).

Preoperative thin-section three-dimensional computed tomography angiography (3D- CTA) to measure pedicle diameter can be a sensitive parameter for assessing screw placement risks. As a result, we used 3D-CTA to assess the type differences of Arteria vertebralis originating from C2 VAG variation unique to the Turkish society in the C2 pedicle. Thus, we aimed to easily predict the risks of C2 pedicle screw application due to type differences in patients who are planned to undergo for a C2 instrumentation.

2. Material and Method

For this study, permission was obtained from the ethics committee University of Health Sciences İzmir Tepecik Training and Research Hospital, with Decision No: 2023/05-18. Cervical 3D-CTA images of 200 patients obtained in our institution between January 2017 and January 2023 were evaluated retrospectively.

3D-CTA application technique: All patients' neck regions and craniocervical regions were scanned with 64-channel dual-source computed tomography (GE company, USA). A 20- 22 G intravenous cannula was used to administer contrast agent injection from the right upper extremity. An iodized contrast agent (Iohexol) was administered at a rate of 5 ml/sec using a 60 ml 350 mg/ml auto-injector. The scan lasted for

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approximately 6-10 seconds. Following extraction, a 20 ml saline infusion was administered intravenously to the patients. The area between the arcus aorta and the vertex was examined. Curved reformat reconstruction, color coding, maximum intensity projection (MIP), and 3D volume rendering algorithms were applied to 0.5 mm thick images in all three planes. Digitally generated 3D-CTA images were used for measurements.

We used 0.5 mm 3D-CTA slices obtained at the C2 pedicle direction in cervical region to perform a type of CT scan spectrum generation method of C2 VAG used by Wang et al. in 2013(2). Wang et al (2013)'s study is the result of a series of 45 patients they operated on, and the current study is a retrospective study of 200 patients in whom patients under the age of 18, those who had upper cervical surgery, those who had trauma or tumors in the upper cervical vertebrae, and those who had congenital anomalies were all excluded from the study. Whereas vertical height from the apex of the C2 VAG to the upper facet joint surface was referred to as the 'e' parameter, a horizontal distance from the entrance of the C2 VAG to the vertebral canal was referred to as the 'a' parameter. Three-dimensional CT reconstruction was performed based on the defined 'e' and 'a' parameters. A C2 VAG model figure was used to create a safe zone area for pedicle screw placement.

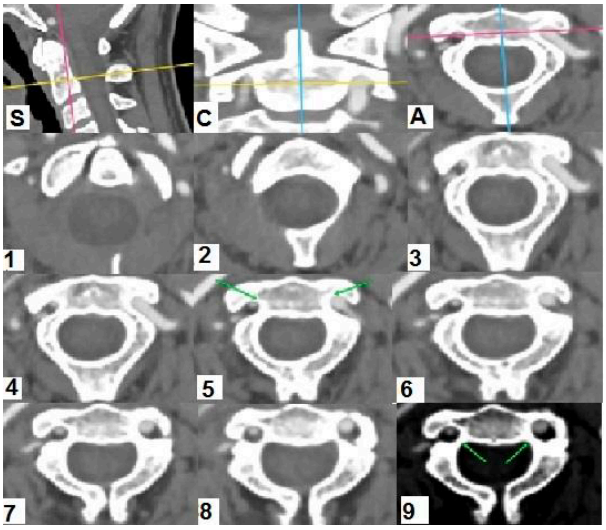

Fig. 1. 0.5mm 3D-CTA sections taken along the axis pedicle direction. S: Sagittal image, C: Coronal image, A: Axial image. Numbered photographs are 0.5mm axial sections parallel to the pedicle. The arrows in image 5 indicate the peak of VAG and then the parameter 'e' is calculated according to the number of slices. The parameter 'a' can be measured in slice 9 ofm Fig.1, where the markings indicate the distance from the entrance of the VAG to the vertebral canal.

Curved reformat reconstruction images were obtained from 0.5 mm sequential cervical 3D-CTA images along the C2 pedicle direction. The arrows in Fig. 1, Slice 5 indicate the peak of the VAG, and the parameter 'e' is calculated based on the number of slices. The 'a' parameter can be measured in the slice where the marks in the 9th slice of Fig. 1 demonstrate the distance from the VAG entry to the spinal canal. In Fig. 2, the 'e' and 'a' parameters are shown in the photograph coded 'C'. Four anatomical typings in terms of C2 pedicle screw insertion specific to the Turkish population were made by measuring "e and 'a' parameters in the coronal and axial sections of the pedicle. These typings were described as follows: TYPE I: wide and low (a > 4.5mm, e ≥ 4.5mm), TYPE II: narrow and high (a ≤ 4.5mm, e<4.5mm), TYPE III: narrow and low (a < 4.5mm, e ≥ 4.5mm), TYPE IV: wide and high (a ≥ 4.5mm, e < 4.5mm). Measurements were grouped according to sex (female or male) and based on the measurement direction (right or left). Type I was estimated to have the lowest risk of C2 screwing complications. For Type II, it was estimated that a C2 pedicular screw could not be applied safely. The complication risk of C2 pedicular screw application was predicted to increase in Type III and Type IV.

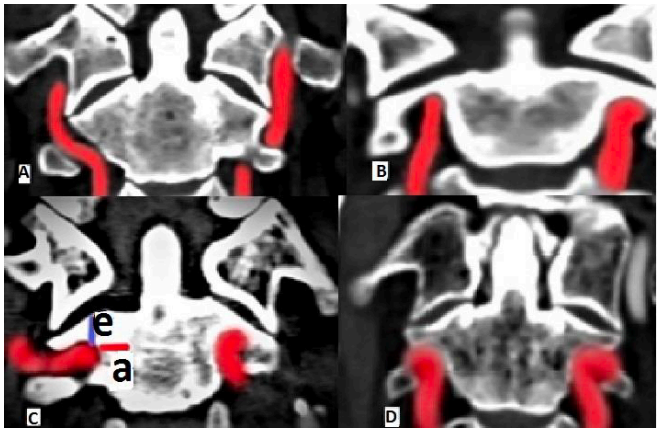

Fig. 2. Arteria vertebralis are coloured in 3D-CTA coronal section images. A) Bilateral Type I pedicle, male case. Right pedicle; e:8.32mm, a:8.15mm. Left pedicle e:8.51mm, a: 6.59mm B) Bilateral Type II pedicle, female patient. Right pedicle; e: 1.91mm, a: 0mm. Left pedicle e: 3.43mm, a: 3.92mm C) Bilateral Type III pedicle male case. Representative e and a parameters are shown. Right pedicle; e:5.86mm, a:3.7mm. Left pedicle e:5.55mm, a: 3.1mm D) Bilateral Type IV pedicle male case. Right pedicle; e:3.8mm, a: 6.11mm. Left pedicle e: 3.8mm, a:4.79mm.

2.1. Statistical Method

Data were evaluated using the IBM SPSS Statistics Standard Concurrent User V 26 statistical package program (IBM Corp., Armonk, New York, USA). Number of units (*n*), percent (%), mean ($\bar{x}$), standard deviation (*sd*), and standard error (*se*) were used to demonstrate descriptive statistics. The Shapiro-Wilk normality test was used to evaluate the normal distribution of numerical variable data. Levene's test was used to evaluate the homogeneity of the variances. For independent samples, the 'a' and 'e' values of male and female patients were compared using the *t*-test. Since male and female patients' ages differed statistically, age-adjusted 'a' and 'e' values were also compared using one-way covariance analysis. The Pearson chi-square test was used to compare the type distributions of males and females based on the measurement sides (right or left). In Pearson chi-square analyses, the Bonferroni (correction) two-ratio z-test was used to compare subgroups. A paired t-test was used to compare the a and e values obtained from both male and female patients' measurements from the right and left sides. The McNemar Bowker test was used for type comparisons for both left and right. A *p*-value of <0.05 was considered statistically significant.

3. Results

According to the incidence rates, 62 (62 %) Type I, 9 (9%) Type II, 6 (6%) Type III, 23 (23%) Type IV pedicle variations were found on the right side in 100 male patients, respectively. On the left side, 71 (71%) Type I, 1 (1%) Type II, 19 (19%) Type III, and 9 (9%) Type IV pedicle variations were found in 100 male patients. According to the incidence rates, 39 (39 %) Type I, 14 (14%) Type II, 6 (6%) Type III, 41(41%) Type IV pedicle variations were found on the right side in 100 female patients, respectively. On the left side, 57 (57%) Type I, 16 (16%) Type II, 10 (10%) Type III, and 17 (17%) Type IV pedicle variations were found in 100 female patients.

Type I was the most common variation in both males and females. While Type II was the least common variation in males, Type III was the least observed variation in females.

Table 1. Comparisons by male and female

Unadjusted values by age					Age adjusted values			
	Gender		**Test Statistics**		**Gender**		**Test Statistics**	
	Male (n:100) $\bar{x}\pm ss$	**Female (n:100)** $\bar{x}\pm ss$	*Test value*	*p*	**Male** $\bar{x}\pm sh$	**Female** $\bar{x}\pm sh$	*Test value*	*P value*
Age, (*yıl*)	59.8±15.8	53.6±15.2	**2.824**	**0.005**†	-	-	-	-
a Right	6.11±2.07	6.34±2.13	0.774	0.440†	6.12±0.21	6.31±0.21	0.388	0.534‡
e Right	5.11±1.33	4.34±1.30	**4.126**	**<0.001**†	5.12±0.13	4.31±0.13	**18.333**	**<0.001**‡
Type of pedicle on the right side *n* (%)								
I	62 (62.0)^a	39 (39.0)^b						
			11.387	**0.010**&				
II	9 (9.0)^a	14 (14.0)^a						
III	6 (6.0)^a	6 (6.0)^a						
IV	23 (23.0)^a	41 (41.0)^b						
a Left	6.16±2.10	5.73±2.22	1.409	0.161†	6.14±0.22	5.76±0.22	1.506	0.221‡
e Left	6.33±1.71	5.02±1.61	**5.571**	**<0.001**†	6.36±0.16	4.97±0.16	**33.390**	**<0.001**‡
Type of pedicle on the left side, *n* (%)								
I	71 (71.0)^a	57 (57.0)^b						
			20. 021	**<0.001**&				
II	1 (1.0)^a	16 (16.0)^b						
III	19 (19.0)^a	10 (10.0)^a						
IV	9 (9.0)^a	17 (17.0)^a						

n: Number of patients, %: Percentage of column, x̄:, ss: standard deviation, sh: standard error, †: Independent samples t test, ‡: One-way analysis of covariance, &: Pearson chi-square analysis&:, Superscripts a and b show the difference between genders in the same row. There is no statistical difference between genders with the same superscripts.

According to Table 1, male patients are statistically older than females (p=0.005). 'a' values obtained from the right side do not differ according to sex (p=0.440). Even when right side 'a' values were adjusted for age (p=0.534), no statistical difference was found between male and female patients. Right side 'e' values of male patients were statistically higher than females (p<0.001). When adjusted for age, the right side 'e' values of male patients were found to be statistically higher than female patients. The right-sided type variations differ statistically by sex (p=0.010). The number of Type I pedicles on the right side was 62 (62.0%) in males and 39 (39.0%) in females, which was showing that males having statistically more Type I pedicles than females. For the right side, there is no statistical difference between the distribution of males and females with a Type II pedicle. Female patients with Type IV pedicles on the right side out number males statistically.

Male and female patients' left side 'a' values were not statistically different (p=0.161). Even when left side 'a' values were adjusted for age (p=0.221), no statistical difference was found between male and female patients. Left side 'e' values of male patients were statistically higher than females (p<0.001). When adjusted for age, the left side 'e' values of male patients were found to be statistically higher than female patients (p<0.001). For the left side, the type distributions in males and females were statistically different (p<0.001). Males were statistically more than females in Type I for the left side, whereas females were statistically more than males in Type II.

No statistical difference was found in terms of the distribution of males and females for Type III and Type IV.

Table 2. Right and left side e-a comparisons in all patient group, male and female patients

Test Statistics				
	Right side $\bar{x}\pm ss$	**Left side** $\bar{x}\pm ss$	Test value	*P* value
All patients, *n*=200				
a	6.23±2.10	5.94±2.17	1.895	0.060[γ]
e	4.72±1.37	5.68±1.78	**8.044**	**<0.001**[γ]
Male patients *n*=100				
a	6.11±2.07	6.16±2.10	0.206	0.837[γ]
e	5.11±1.33	6.33±1.71	**6.873**	**<0.001**[γ]
Female patients, *n*=100				
a	6.34±2.12	5.73±2.22	**3.098**	**.003**[γ]
e	4.34±1.30	5.02±1.61	**4.476**	**0.001**[γ]

$\bar{x}$: mean, *ss*: standard deviation, [γ]: Paired samples t-test, n:number of patients

The differences in right and left 'a' values in all patients were not statistically significant (p=0.060), according to Table 2. In all patients, left side 'e' values were found to be statistically higher than right side 'e' values (p<0.001). No statistically significant difference was found between right and left 'a' values in males (p=0.837). In males, left side 'e' values were found to be statistically higher than right side 'e' values (p<0.001). In females, left side 'e' values were found to be statistically higher than right side 'e' values (p<0.001).

According to Table 3, the type distribution for the right and left pedicles was statistically different in all patients (p<0.001). Eighty-four (42.0%) of the patients had bilateral Type I pedicles, 6 (3.0%) had bilateral Type II pedicles, 9 (4.5%) had bilateral Type III pedicles, and 15 (7.5%) had bilateral Type IV pedicles. Thirty-four (17.0%) of the patients had a Type I pedicle on the left side and a Type IV pedicle on the right side.

Type distributions for the right and left sides in males were statistically different (p=0.003). Among male patients, 50 (50.0%) of them had bilateral Type I pedicles, 4 (4.0%) had bilateral Type III pedicles, and 4 (4.0%) had bilateral Type IV pedicles. No male patient had a bilateral Type II pedicle. Within the group of the males, 15 (15%) of them had Type I pedicle on the left side and Type IV pedicle on the right side.

Table 3. Right and left type comparisons in all patient group, male and female patients

Type of pedicle on the left side						Test Statistics	
	I	II	III	IV		Test value	*p* value
Type of pedicle on the right side							
All Patients n:200 *n* (%)							
I	84 (42.0)	3 (1.5)	8 (4.0)	6 (3.0)		**35.606**	**<0.001**[¥]
II	8 (4.0)	6 (3.0)	4 (2.0)	5 (2.5)			
III	2 (1.0)	1 (0.5)	9 (4.5)	0 (0.0)			
IV	34 (17.0)	7 (3.5)	8 (4.0)	15 (7.5)			
Male patients n:100 *n* (%)							
I	50 (50.0)	1 (1.0)	7 (7.0)	4 (4.0)		**19.946**	**0.003**[¥]
II	4 (4.0)	0 (0.0)	4 (4.0)	1 (1.0)			
III	2 (2.0)	0 (0.0)	4 (4.0)	0 (0.0)			
IV	15 (15.0)	0 (0.0)	4 (4.0)	4 (4.0)			
Female patients n:100 *n* (%)							
I	34 (34.0)	2 (2.0)	1 (1.0)	2 (2.0)		**21.002**	**0.002**[¥]
II	4 (4.0)	6 (6.0)	0 (0.0)	4 (4.0)			
III	0 (0.0)	1 (1.0)	5 (5.0)	0 (0.0)			
IV	19 (19.0)	7 (7.0)	4 (4.0)	11 (11.0)			

n: Number of patients, %: Percentage of the total number,[¥]: McNemar Bowker test

Type distributions for the right and left sides for females were statistically different (p=0.002). Within the females, 34 (34.0%) of them had bilateral Type I pedicles, 6 (6.0%) had bilateral Type II pedicles, 5 (5.0%) had bilateral Type III pedicles, and 11 (11.0%) had bilateral Type IV pedicles. Of the females, 19 (19.0%) of them had Type I pedicle on the left side and Type IV pedicle on the right side.

4. Discussion

The reason for the differences in cervical pedicle anatomical measurement may be because of sex or geographical race difference (3). In general, females have smaller pedicle diameters and heights than males in many races. According to cervical anatomical measurements, Asians have smaller pedicles than Europeans and Americans (4). Anatomical measurements obtained with sensitive imaging devices such as thin-section CTA will now more clearly reveal anatomical differences in different populations (5).

Upper cervical vertebral fractures, dislocations, transverse

ligament damage, congenital anomalies, rheumatic diseases, and upper cervical tumors may necessitate craniocervical stabilization. Çokluk and Aydın (6) reported that 17% of C2 fractures are upper cervical spine fractures.

The C2 pedicle is strong and has a well-vascularized structure (7,8). Therefore, pure C2 fractures are rare (7, 9). Biomechanical studies on cadavers show that C2 pedicle screws are quite stable (10). C2 pedicular screwing is easier to apply than transarticular screwing and the risk of complications is less (11,12). Although Yoshida et al. reported that both applications had the same surgical risk, Klepinowski et al. stated that C1 lateral mass and C2 screwing were the gold standards for craniocervical junction stabilization (13-14). Bicortical polyaxial screws are biomechanically strong and easier to apply in this narrow area. The facets are preserved, and when the fracture heals, motion can be preserved when the instrument is removed. Alternative surgical options can be preferred if a C2 pedicle screw is risky or impossible.

If the pedicle is narrow, pedicular screw insertion is commonly avoided. Maki et al. reported that a safe C2 screw can be applied when the C2 VAG is oriented towards the cranial, but if the C2 VAG is both cranial and medial, the C2 pedicular screw cannot be applied (15).

Lee et al. classified the intra-axial vertebral artery based on two anatomical parameters, namely in the coronal and lateral plane, and divided them into 9 types (16). They discovered an increase in intra-axial vertebral artery tortuosity in female and elderly patients.

Wang et al. used 74 transpedicular screws and 16 translaminar screws on the C2 pedicles, which they classified into four types. They reported that transpedicular screwing cannot be used if there is a Type II C2 pedicle. Pedicle screw placement is impossible in Type II because the "safe zone" of "*e* and a" is less than 4.5 x 4.5 mm (2). The C2 translaminar screw method may be recommended in such cases. The elimination of the potential risk of arterial injury is the most significant advantage of the C2 translaminar screw (17). Wang et al. observed vertebral canal breach in 2 pedicles with a rate of 2.7% in patients who underwent transpedicular screw application in their series (2). No complications were observed with translaminar screws. However, the outcomes of C2 translaminar screw surgery were often unsatisfactory, with significant rates of screw dislocation and reoperation (18).

Yeom et al. reported that a breach was observed in 8 of 39 C2 pedicle screws (21%) to the vertebral artery groove but none of them caused arterial injury (19). However, no breaches were detected on intraoperative and postoperative radiographs. They may have detected VAG violations at a high rate because they used 3D-CTA in both preoperative and postoperative measurements. Angiography is generally not preferred for routine postoperative contralateral angiography, even though 3D-CTA is sometimes used in preoperative preparations.

In our study, 57.2% Type I pedicles were observed in 200 patients. This rate is similar to the rate of 58.9% Type I C2 pedicles in the series of Wang et al. (2). In our study, Type I pedicle was observed in 66.5% of 100 male patients and 48% of 100 female patients.

In our study, 10% Type II pedicles were detected in 200 patients. Wang et al. reported a higher rate 17.8% of Type II pedicles in their series (2). Yusof et al. found that 3.5 mm diameter screws could not be used in 54.2% of males with only pedicle diameter measurement in their study on different levels of cervical vertebral pedicles in 40 Malaysian participants (5). Patwardhan et al. reported that 3.5 mm diameter screws could not penetrate approximately 30% of C2 pedicles, their study involved 27 participants from the Indian population (20).

However, in both studies, pedicle widths of less than 5 mm were taken into account. The prevalence of Type II pedicles in our study in males and females was 5% and 15%, respectively. It can be said that it is impossible to apply C2 pedicular screws to these pedicles. The rate of C2 pedicle Type II in females was found to be 3 times higher than that of males.

Wang et al. reported that of the C2 pedicles in their series, 14.4% of them were Type III and 8.9% were Type IV (2). In our study, of the 200 patients, 10.25% were Type III and 22.5% Type IV. Type III pedicle was observed more frequently in males (12.5%) than in females (8%). Type IV was more common in females (29%) than males (16%). The risk of C2 screw insertion in Type III and Type IV pedicles is higher than in Type I pedicles.

The limitations of this study include the fact that it was not based on clinical outcomes and was conducted retrospectively. Since it is a cross-sectional imaging study, it cannot reveal the frequency of vertebral artery injury. However, if surgery is required, preoperative pedicle classification based on C2 vertebral artery groove type may help with surgical planning. By avoiding potential complications, the risk of surgical morbidity and mortality can be reduced.

The classification of the type differences of the arteria vertebralis originating from the C2 vertebral artery groove variation can guide the surgeon in a practical manner in patients who are scheduled to undergo a C2 pedicular screw insertion. In order to prevent possible complications in C2 pedicle screw placement, it is necessary to determine the safe zone preoperatively. For this purpose, preoperative thin section 3D-CTA imaging of the upper cervical region is routinely recommended.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: D.A., Design: D.A., Data Collection or Processing: D.A., Analysis or Interpretation: D.A., Literature Search: D.A., Writing: D.A.

Ethical Statement

For this study, permission was obtained from the ethics committee University of Health Sciences İzmir Tepecik Training and Research Hospital, with Decision No: 2023/05-18.

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Retrospective study on hospitalized patients with epistaxis: Insights and implications

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Abstract

Epistaxis, commonly encountered in the Otolaryngology practice, refers to bleeding that occurs as a result of vascular pathologies and coagulation disorders. It can develop due to traumatic, iatrogenic, or spontaneous etiologies. In our study, we aim to retrospectively examine the epistaxis patients treated in our clinic to investigate the etiology causing epistaxis, associated diseases, and the use of anticoagulants-antiplatelets. Additionally, we plan to evaluate hemogram and coagulation parameters. The study included patients diagnosed with epistaxis and admitted for treatment at Samsun Education and Research Hospital ENT Clinic between 2019 and 2022. A retrospective analysis of the patients' medical records was conducted to gather data on demographic characteristics, comorbidities, medications used regularly, laboratory values, and clinical findings. A total of 130 patients were included in the study. No statistically significant difference was observed between the location of the bleeding focus and gender, presence of hypertension, use of antihypertensive medication, or anticoagulant use. When comparing patients with and without antiplatelet-anticoagulant use, a significantly longer nasal packing duration was found in patients who used these medications. In terms of the need for electrocauterization, statistically significant higher intervention rates were observed in patients who did not use anticoagulant medication. Our study identified a higher rate of surgical intervention in hospitalized epistaxis patients, potentially due to the inclusion of more severe cases requiring specialized care. Further research is needed to investigate the factors influencing the need for surgical management and to develop appropriate guidelines for the management of different severities of epistaxis.

Keywords: epistaxis, anticoagulant drug, nasal packing, electrocauterization

1. Introduction

Epistaxis, which can occur in the nasal cavity, paranasal sinuses, and nasopharynx, is characterized by bleeding resulting from mucosal damage, vascular pathologies, and coagulation disorders (1,2). It is one of the most commonly encountered emergencies in Otolaryngology practice. Although the exact prevalence is unknown, it is estimated to be around 60% in the general population throughout their lifetime, with approximately 10% of these cases requiring medical or surgical intervention (3). The incidence of epistaxis increases with age and is more commonly observed in males than females (1,4). The highest incidence is seen in the age group of 50-60 years (3,4).

The etiology of epistaxis can be traumatic (nasal fractures, nasal intubation, cocaine use, nasal foreign bodies, etc.), iatrogenic (following nasal and sinus surgeries), or spontaneous (5). Spontaneous epistaxis often occurs due to local factors such as dry air, infectious and allergic rhinitis, or due to systemic factors such as anticoagulant and antiplatelet medication use, hypertension, coagulation and platelet abnormalities, hereditary conditions (Osler-Weber-Rendu disease), and alcoholism(5).

Epistaxis can often resolve on its own or be controlled with conservative methods (4). The treatment of epistaxis begins with identifying the bleeding focus. Epistaxis is classified based on whether the bleeding focus is anterior or posterior (1,3). The bleeding can be diffuse or localized. In cases of localized epistaxis, the bleeding can be controlled by chemical or electrocauterization methods at the bleeding focus. If bleeding cannot be controlled or the bleeding focus cannot be identified, anterior or posterior nasal packing can be applied depending on the location of the bleeding (1,4). Epistaxis can be aggressive enough to require interventions such as repeated blood transfusions, arterial embolization, or surgical methods to control the bleeding (6).

Our study aims to retrospectively analyze the epistaxis patients treated in our clinic as inpatients to investigate the etiology of epistaxis, associated diseases, and the use of anticoagulant-antiplatelet medications. We also plan to evaluate the hemogram-coagulation parameters. Additionally, we will conduct a detailed assessment of the medications used by the patients and evaluate whether there are any differences in the severity of bleeding and response to treatment based on the use of anticoagulant-antiplatelet medications.

2. Materials and Methods

The research protocol, with the approval number SÜKAEK-2022/8/6, was obtained from the Ethics Committee of our hospital. The data of all patients aged 18 and over who were diagnosed with epistaxis and treated as inpatients at the

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Department of Otolaryngology of Samsun Education and Research Hospital between 2019 and 2022 were obtained retrospectively from the hospital automation system and archival files, ensuring that there were no missing records. The collected data included age, gender, previous systemic diseases, new systemic diseases diagnosed during this admission, use of antiplatelet and anticoagulant medications, active ingredients of the medications used, hemogram and coagulation parameters, methods applied to stop the bleeding (packing, cauterization), location of the bleeding focus, and duration of hospitalization.

IBM SPSS Statistics Version 21.0 software for Windows (Armonk, NY) was used to analyze outputs. The Kolmogorov-Smirnov test was performed to test the normal distribution of data. The Mann-Whitney U test (for two groups) and the Kruskal-Wallis H or one-way ANOVA tests (more than two groups) were used to compare groups. For categorical variables, the Chi-square test was performed. Statistical significance was defined as $p < 0.05$.

3. Results

The study included 130 patients, of whom 76 were male (58.5%) and 54 were female (41.5%). Their ages ranged from 19 to 93 years (mean: 58.4 ± 17.8). The duration of hospitalization for the patients was 3.34 ± 1.6 (min: 1; max: 9) days.

Out of the patients, 77 (59.2%) were diagnosed with hypertension, and 71 (92%) were using antihypertensive medications. Sixty-four patients (49.2%) were receiving anticoagulant therapy due to coronary artery disease. Among the patients receiving anticoagulation, 35 (26.9%) were using acetylsalicylic acid (ASA), 11 (8.5%) were using factor Xa inhibitors, 8 (6.2%) were using warfarin, 4 (3.1%) were using thrombin receptor inhibitors, 1 (0.8%) was using direct thrombin inhibitor, 1 (0.8%) was using low molecular weight heparin (enoxaparin), and 4 (3.1%) were using a combination of antiplatelet and anticoagulant drugs. Sixty-six patients (50.8%) were not using any anticoagulant medication. When comparing bleeding parameters and nasal packing durations between patients using the new generation drugs-NOACs (direct thrombin inhibitor, thrombin receptor antagonist, factor Xa inhibitor, and antiplatelet-ticagrelor) and the old generation drugs (ASA, warfarin, low molecular weight heparin), as well as patients not using any antiplatelet or anticoagulant drugs, significant differences were observed in platelet and INR values in favor of the new generation drug users. Additionally, patients using these medications exhibited significantly longer nasal packing durations. (Table 1).

Table 1. Bleeding Parameters and nasal packing duration in patients with and without antiplatelet-anticoagulant use

	Antiplatelet-Anticoagulant non-use(n=66) (mean ± SD)	New generation drugs-NOACs use(n=12) (mean ± SD)	Old generation drugs use(n=48) (mean ± SD)	p-value
Platelet (cells/mL)	258878 ± 77702	199833 ± 68568	245083 ± 75976	0.05 *0.04
INR	1.02 ± 0.1	1.44 ± 0.5	1.17 ± 0.4	<0.01 *<0.01
Nasal packing duration (day) (mean ± SD)	1.7 ± 0.9	2.08 ± 0.2	2.17 ± 0.4	0.03 *0.01

*Antiplatelet-anticoagulant non-use - New generation antiplatelet-anticoagulant use

When comparing the requirement for electrocauterization between patients with and without antiplatelet-anticoagulant use, it was found that non-users of these medications required statistically significantly more interventions. (Table 2).

Table 2. Requirement for electrocauterization in patients with and without antiplatelet-anticoagulant use

		Antiplatelet-Anticoagulant non-use(n=66) (mean ± SD)	New generation drugs-NOACs use(n=12) (mean ± SD)	Old generation drugs use(n=48) (mean ± SD)	p-value
Bipolar electrocauterization n (%)	yes	15 (22.7%)	3 (25%)	2 (4%)	0.018
	no	51 (77%)	9 (75%)	46 (96%)	*0.02

*Antiplatelet-anticoagulant non-use - Old generation antiplatelet-anticoagulant use

In 102 patients (78.5%), the bleeding focus could not be identified during hospitalization, while in 19 patients (14.6%), it was located in the anterior region, and in 9 patients (6.9%), it was located in the posterior region. There was no statistically significant difference between the location of the bleeding focus and gender, presence of hypertension, use of antihypertensive drugs, and anticoagulant use (p: 0.27, p: 0.44, p: 0.31, p: 0.69, respectively). Among 110 patients (84.6%), bleeding was controlled conservatively during follow-up, while in 20 patients (15.4%), bipolar electrocauterization was performed endoscopically in the operating room to achieve bleeding control. There was no statistically significant difference between the need for surgery and the location of bleeding (p: 0.20). Similarly, no statistically significant

difference was found between the need for surgery and the use of anticoagulants (p=0.21). A total of 115 patients (88.5%) were followed with anterior nasal packing during their hospital stay. No packing was applied in 15 patients (11.5%). The mean duration of packing removal was 2.1 days. Among the patients with packing, 92 (70.8%) had their packing removed on the second day, three patients (2.3%) on the first day, 19 patients (14.6%) on the third day, and one patient (0.8%) on the fourth day. There was no statistically significant difference between the packing duration and the bleeding location (p=0.54). Similarly, no statistically significant difference was found between the duration of packing and the anticoagulant groups (p=0.48).

During the hospitalization of patients with epistaxis, their hemoglobin levels were measured to be 12.5 ± 2.4 g/dL (min: 7.2, max: 18.8). Platelet counts were found to be in the range of 96,000 to 538,000 cells/mL (mean: 248,261 ± 76,531 cells/mL). INR values ranged from 0.83 to 3.16 (mean: 1.11 ± 0.34).

4. Discussion

Epistaxis is one of the most commonly encountered emergencies in otolaryngology clinics, and it can be associated with morbidity and rarely mortality. It is often self-limiting or controlled with conservative methods. Epistaxis is a symptom and can occur due to traumatic, iatrogenic, or spontaneous causes(3,5). In the literature, cardiovascular diseases and hypertension are among the leading systemic causes, but a direct relationship has not been established(1,3). In our study, 59.2% of the patients had a pre-existing diagnosis of hypertension before hospital admission for epistaxis, and 92% were receiving antihypertensive treatment.

ASA and clopidogrel are antiplatelet drugs with antithrombotic effects that prevent platelet aggregation during primary hemostasis. Among anticoagulant drugs, new oral anticoagulants(NOACs) have advantageous use and oral administration and do not require close monitoring of dosages. The most commonly used drugs are direct-acting factor Xa inhibitors (rivaroxaban, apixaban), direct thrombin inhibitors (dabigatran), and thrombin receptor antagonists. The risk of epistaxis associated with these drugs is lower than warfarin, another anticoagulant drug, and is generally dose-dependent(7,8). 49.2% of our patients were using anticoagulant-antiplatelet drugs, with 26.9% using ASA, which was the most commonly used drug among the 64 patients who were using anticoagulant-antiplatelet drugs out of the 130 patients included in the study. In our study, significantly longer nasal packing duration was observed in the group using these drugs when comparing patients with and without antiplatelet-anticoagulant use in terms of the need for electrocauterization. In terms of the need for electrocauterization, it was found that patients not using the drugs required significantly more interventions. These findings suggest that patients using antiplatelet-anticoagulant drugs may benefit from longer follow-up with nasal packing by temporarily discontinuing their medication, while in patients not using antiplatelet drugs, early consideration of electrocauterization may be warranted if bleeding continues despite nasal packing.

Epistaxis is more commonly seen in older people and males (1). A study conducted by Pollice et al. involving 249 patients found that 70% of epistaxis patients were 50 years and older (9). In a study by André et al. involving 205 patients, it was observed that bleeding was more common in males, and the average age of patients presenting with non-severe epistaxis was 67 (6). Consistent with the literature, our study also found a male-to-female ratio of 1.4 and an average age of 58.38.

Epistaxis can be divided into two groups based on its localization: anterior and posterior epistaxis. According to studies in the literature, approximately 90-95% of nosebleeds occur in the anterior region. The anterior region includes the front part of the nasal septum, known as the Little area, where the branches of the internal and external carotid arteries anastomose at the Kiesselbach plexus. This area is susceptible to trauma and can easily be exposed to hot and cold temperatures. It is also a low-humidity area (10). In our study, a single bleeding focus could not be identified upon admission in 78.5% of patients, while 14.6% had an anterior bleeding focus and 6.9% had a posterior bleeding focus. This could stem from the bleeding focus not being visible due to intense bleeding, patients arriving with pre-inserted packing, or the inability to assess the bleeding focus accurately after packing removal due to widespread mucosal damage.

Epistaxis is commonly managed with conservative treatment. After identifying the bleeding focus, chemical cauterization with silver nitrate or electrocauterization methods can be used. In active bleeding that cannot be controlled with pressure, nasal packing techniques can also be employed. Nasal packing should typically be left in the nasal passage for an average of 48 hours (11). In our study, nasal packing was applied to 115 patients (88.5%). The nasal pack was removed in an average of 2.1 days. If bleeding control cannot be achieved in epistaxis cases, various interventions can be performed under surgical conditions, including bipolar cautery of the sphenopalatine arteries, arterial embolization, ligation of the sphenopalatine artery, maxillary artery ligation, and external carotid artery ligation (1,11). Although the literature reports lower rates of bipolar cautery, our study achieved bleeding control in 20 patients (15.4%) using bipolar cautery with endoscopic electrocauterization in the operating room. However, no patient required ligation of the sphenopalatine, maxillary, or external carotid artery due to bleeding. This may be attributed to the fact that our hospital is a tertiary referral center, and patients with severe uncontrolled epistaxis were referred to us for treatment or included in the study after being admitted for treatment in our outpatient ENT clinic due to unsuccessful conservative management.

Based on our study, we evaluated the clinical characteristics and treatment approaches of patients admitted to our clinic due to epistaxis. Our findings were consistent with the literature, indicating that patients were mostly in the older age group and predominantly male. Conservative treatment methods were effective in the majority of epistaxis cases. However, surgical intervention may be required in some cases. We also examined the impact of anticoagulant and antiplatelet medication use on epistaxis. It was found that the new-generation anticoagulants did not pose a significantly higher risk of severe bleeding compared to other anticoagulant drugs.

In conclusion, epistaxis can generally be managed with conservative measures. However, surgical intervention may be necessary in more severe cases. The use of new anticoagulant medications is safer in terms of the risk of epistaxis compared to other drugs. These findings provide valuable insights for properly evaluating and selecting appropriate treatment modalities for patients with epistaxis.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: A.Ü., A.Ç. Design: H.A., Data Collection or Processing: A.Ü., H.A. S.N.C., Analysis or Interpretation: A.Ü., A.Ç., Literature Search: A.Ü., A.Ç., Writing: A.Ü., H.A. S.N.C, A.Ç.

Ethical Statement

Approval was obtained from Samsun University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 21/09/2022 and the number of ethical committee decisions is SÜKAEK-2022/8/6.

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Retrospective evaluation of lateral ventricular volume in Parkinson's patients and control group on magnetic resonance images

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Abstract

Due to its flexible and expandable nature, the lateral ventricle volume has the potential to change in response to neurodegenerative diseases. Therefore, our study aims to calculate the lateral ventricle volume and its ratios to both the intracranial and cerebrum volumes and compare these measurements between Parkinson's patients and controls. By examining these ratios, we aim to better understand the relationship between lateral ventricle volume and brain structure in the context of Parkinson's disease. Magnetic resonance imaging of 54 Parkinson's patients (13 female; 41 male) and 26 healthy controls (8 female; 18 male) were included in the study. Lateral ventricle and intracranial volumes were calculated using the Cavalieri method in the ImageJ program on magnetic resonance images. Cerebrum volume was calculated using the BrainSuite program. It was observed that female patients with Parkinson's had a higher right-sided lateral ventricle volume than female controls. The right-sided lateral ventricle volume ratio to cerebrum volume and intracranial volume was also higher ($p<0.05$). Changes in ventricle volume are of great importance in diseases due to their relationship with anatomical structures. As seen in the results of our study, we think that the gender factor should be taken into account when evaluating the ventricle volume in neurodegenerative diseases.

Keywords: BrainSuite, ImageJ, Horos, cerebrum volume, intracranial volume

1. Introduction

Parkinson's disease is a neurodegenerative disorder that arises from the degeneration of dopaminergic neurons in the substantia nigra pars compacta region. The disease can cause various changes in the cortical and subcortical areas of the brain (1-3).

The lateral ventricle is a flexible and expandable structure located between the cortical and subcortical structures in the brain, which contains cerebrospinal fluid. The volume of this ventricle can vary depending on age, brain size, and morphology. An increase in lateral ventricle volume may be an important sign during the progression of neurodegenerative diseases such as Parkinson's disease (4-6).

Therefore, it is important to calculate the lateral ventricle volume and the ratio of the lateral ventricle volume to cerebral and intracranial volume in Parkinson's patients and compare it to healthy controls (4, 7-9). This study was designed to understand better the relationship between morphological changes in the brain and lateral ventricle volume in Parkinson's disease.

In this study, the lateral ventricle volume and the ratio of ventricle volume to cerebral and intracranial volume in Parkinson's patients were compared with those of healthy controls. Thus, the aim is to determine the relationship between structural changes in the brain due to Parkinson's disease and lateral ventricle volume. Understanding the effects of neurodegenerative diseases such as Parkinson's disease on brain morphology is important for diagnosis, monitoring, and treatment. This study shows that the increase in lateral ventricle volume in Parkinson's patients may be an important marker in the progression of the disease.

2. Materials and Methods

2.1. Participants and Data Collection

This study was approved by the Clinical Research Ethics Committee of Tokat Gaziosmanpaşa University (Approval Date: 02.03.2023, Project No: 23-KAEK-047). We enrolled 54 patients with Parkinson's disease who were diagnosed based on neurological examination and underwent routine brain MRI (magnetic resonance images) for diagnostic purposes at the Gaziosmanpasa University Faculty of Medicine between January 1st, 2013, and January 1st, 2023. The control group included 26 patients who underwent brain MRI for diagnostic purposes for various reasons but had no history of trauma or pathology. All images were retrospectively reviewed through the medical faculty patient tracking system, and six MRI images from the patient group and four MRI images from the control group were excluded from the study due to imaging artifacts. The study participants were aged between 42 and 80 years.

2.2. Automatic Segmentation Software: BrainSuite

To calculate the volume of the cerebrum, we used the

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BrainSuite software (version 19a), an automatic segmentation tool. The MR images were saved in Digital Imaging and Communication in Medicine (DICOM) format using the Horos program (version 4.3.1.). The images were then imported into ImageJ and saved in Analyze 7.5 format to be used in BrainSuite software. After completing the analysis, the cerebrum volume was obtained from the "roiwise.stats" file, which contains brain structure volumes and cortical thicknesses.

2.3. Manual Measurements with ImageJ

We manually measured the total intracranial volume and lateral ventricle using the ImageJ program (version 1.52a). To analyze the lateral ventricle, stack using the "Convert Images to Stack" function in the "Stacks" submenu. The threshold value was set to ensure that the images were converted to binary display that matched the original image (Fig. 1).

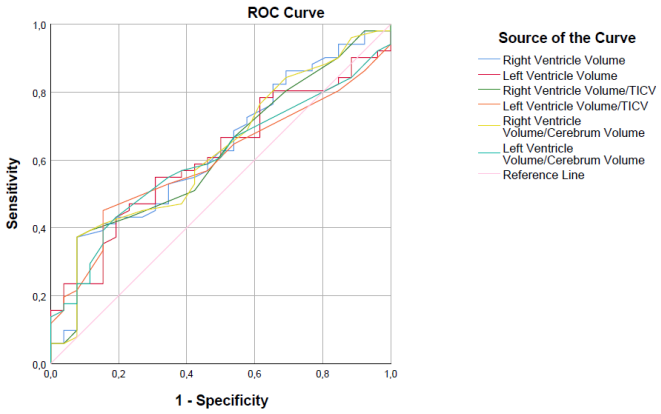

Fig. 1. Determination of lateral ventricle limits

We identified the lateral ventricle as the region of interest (ROI) for this study and outlined it manually on each lateral ventricle's boundaries using the "Polygon selection tool" in the "Analyze > Tools" menu. Each slice of the ROI was added to the ROI manager using the "Add" function. The area of each ROI was computed using the "Measure" function in the ROI manager menu.

The lateral ventricle was estimated by multiplying the section thickness with the total sectional surface area. The volume was calculated using the Cavalieri principle, based on the surface area multiplied by the cortical thickness. To compute the total volume of the lateral ventricle, we multiplied the sum of the areas (mm^2) of all the ROIs by the slice thickness (mm). This gave the total volume of the lateral ventricle (mm^3).

One of the first steps in the BrainSuite program was to define the skull borders, which were output as the "mask.nii.gz" file. This file, which contains the skull borders for each participant, was transferred to the ImageJ software. ImageJ measures the area inside the cranium using the Cavalieri principle. The area measurements of the intracranial cavity were calculated using the image series and the "adjust" and "threshold" tools in the "Image" menu, similar to the ventricle calculations.

2.4. Statistical Analysis

Statistical analysis was conducted using version 25.0 of SPSS (SPSS Inc., Chicago, IL). Data were analyzed per patient and on a normal and abnormal side basis. Categorical variables were described using frequency and percentage and numerical variables by the mean and standard deviation or median and minimum-maximum values. The independent sample median values were compared with the Mann-Whitney U Test. The study was performed at a 95% confidence level ($p < 0.05$ was considered statistically significant).

Receiver operating characteristic (ROC) analysis was also performed to evaluate the diagnostic performance of the variables. The area under the curve (AUC) was calculated for each variable, and the optimal cutoff point was determined based on the highest Youden index.

3. Results

Table 1 compares the measurements of right and left-sided lateral ventricle volumes, and lateral ventricle volume normalized to brain volume between the patients and control groups.

Table 1. Comparison of lateral ventricle volumes and volume ratios between patients and controls

	Patients (n=51)	Controls (n=26)	Test Ist.	p[1]
R Lateral Ventricle Volume (cm^3)	10.4 (1.9 - 37.3)/42.35	9.15 (3.1 - 24.7)/32.42	492.00	0.07
R Lateral Ventricle Volume/TICV	0.007 (0.001 - 0.024)/32.98	0.006 (0.002 -0.016)/42.07	506.50	0.09
R Lateral Ventricle Volume/Cerebrum Volume	0.013 (0.002 - 0.05)/42.26	0.011 (0.004 -0.033)/32.60	496.50	0.07
L Lateral Ventricle Volume (cm^3)	11.3 (1.9 - 36.5)/41.85	9 (4.3 - 23.4)/33.40	517.50	0.12
L Lateral Ventricle Volume /TICV	0.008 (0.001 - 0.024)/41.48	0.006 (0.003 -0.016)/34.13	536.50	0.17
L Lateral Ventricle Volume /Cerebrum Volume	0.015 (0.002 - 0.047)/41.71	0.012 (0.005 -0.031)/33.71	525.50	0.14

[1]Mann Whitney U testi, mean (min-mak)/mean rank, TICV: Total intracranial volume, R:Right-sided, L: Left-sided

Statistical analysis revealed that there were no significant differences between the two groups. However, compared to the controls, the patients had higher volumes of both right and left-sided lateral ventricles, as well as higher ratios of lateral ventricle volume to total intracranial volume (TICV) and cerebrum volume (Table 1).

Among female participants, the patients had significantly higher volumes of the right-sided lateral ventricle, as well as higher ratios of lateral ventricle volume to both total intracranial volume and cerebrum volume compared to the female controls ($p<0.05$) (Table 2).

Table 2. Comparison of patients and controls by gender for right-sided lateral ventricle volume normalized to brain volume and total intracranial volume

		Patients	Controls	Test Ist.	p[1]
R Lateral Ventricle Volume (cm^3)	Male	9.85(1.9 - 37.3)/29.50	9.5 (3.3 - 24.7)/26.39	304	0.505
	Female	13.8(4.4 - 18)/12.62	7.2 (3.1 - 10.5)/13.62	18	**0.013**
R Lateral Ventricle Volume /TICV	Male	0.006(0.001 - 0.024)/29.62	0.006 (0.002 - 0.016)/26.14	299.5	0.453
	Female	0.01 (0.003 - 0.015)/13.38	0.006 (0.002 - 0.008)/7.12	21	**0.025**
R Lateral Ventricle Volume /Cerebrum Volume	Male	0.012 (0.002 - 0.05)/29.79	0.011 (0.004 - 0.033)/25.78	293	0.389
	Female	0.02 (0.006 - 0.029)/13.35	0.011 (0.005 - 0.016)/7.19	21.5	**0.025**

[1]Mann Whitney U testi, mean (min-mak)/mean rank, TICV: Total intracranial volume, R:Right-sided, L: Left-sided

Regarding left-sided lateral ventricle volume and its ratios, no significant differences were found between female patients and female controls, as well as between male patients and male controls ($p>0.05$) (Table 3).

Table 3. Comparison of patients and controls by gender for left-sided lateral ventricle volume normalized to brain volume and total intracranial volume

		Patients	Controls	Test Ist.	p[1]
L Lateral Ventricle Volume (cm^3)	Male	11.3 (1.9 - 36.5)/29.38	10.5 (4.8 - 23.4)/26.64	308.5	0.557
	Female	12.5 (2.8 - 25.6)/13.08	6.75 (4.3 - 16.5)/7.62	25.0	0.053
L Lateral Ventricle Volume /TICV	Male	0.008 (0.001 - 0.024)/29.37	0.007 (0.003 - 0.016)/26.67	309.0	0.560
	Female	0.009 (0.002 - 0.02)/12.58	0.005 (0.003 - 0.012)/8.44	31.5	0.140
L Lateral Ventricle Volume /Cerebrum Volume	Male	0.015 (0.002 - 0.047)/29.61	0.013 (0.005 - 0.031)/26.17	300.0	0.461
	Female	0.019 (0.004 - 0.04)/15.58	0.01 (0.006 - 0.023)/8.44	31.5	0.140

[1]Mann Whitney U testi, mean (min-mak)/mean rank, TICV: Total intracranial volume, R:Right-sided, L: Left-sided

To evaluate the diagnostic accuracy of different measurements in distinguishing between patients and controls, we performed a receiver operating characteristic (ROC) analysis. This analysis included the measurements of both right and left-sided lateral ventricle volumes, lateral ventricle volume/total intracranial volume (TICV), and lateral ventricle volume/cerebrum volume ratios. The results are presented in a graph, where the true positive rate (sensitivity) is plotted on the y-axis, and the false positive rate (1-specificity) is plotted on the x-axis. The graph shows that the ROC curves for all the measurements are mainly concentrated in the upper left part of the graph, indicating good diagnostic accuracy. In other words, all the measurements have high sensitivity and specificity for distinguishing between patients and controls (Fig. 2).

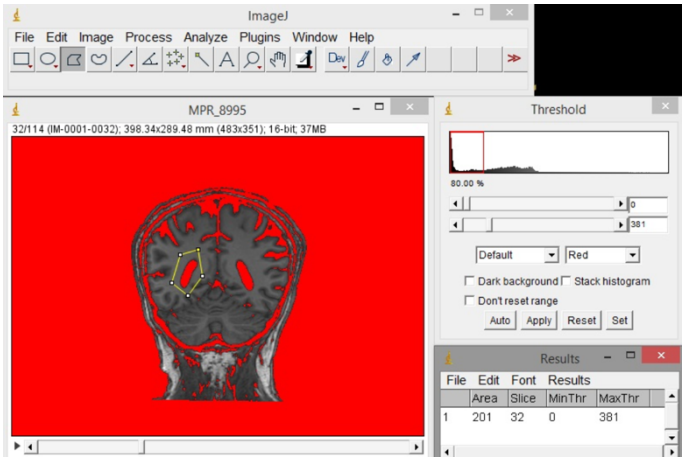

Fig. 2. ROC analysis graph

The diagnostic accuracy of different test result variables, including the right and left-sided lateral ventricle volumes, lateral ventricle volume/total intracranial volume (TICV), and

lateral ventricle volume/cerebrum volume ratios, were assessed using the area under the curve (AUC) values. The table presents the AUC values, along with their standard errors, asymptotic significance, and 95% confidence intervals. The AUC values range from 0.595 to 0.629, indicating fair to moderate diagnostic accuracy for all the measurements. In other words, these measurements can be used to distinguish between patients and controls, but the accuracy may not be high enough for clinical use (Table 4).

Table 4. Analysis test results

Area Under the Curve					
				Asymptotic 95% Confidence Interval	
Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Lower Bound	Upper Bound
R Lateral Ventricle Volume	0.629	0.066	0.065	0.5	0.758
R Lateral Ventricle Volume/TICV	0.61	0.065	0.117	0.483	0.737
R Lateral Ventricle Volume/Cerebrum Volume	0.618	0.066	0.092	0.489	0.747
L Lateral Ventricle Volume	0.595	0.064	0.173	0.469	0.721
L Lateral Ventricle Volume/TICV	0.626	0.066	0.073	0.496	0.755
L Lateral Ventricle Volume/Cerebrum Volume	0.604	0.064	0.139	0.478	0.73

The test result variable(s): R/L; lateral ventricle volume, lateral ventricle volume/TICV, lateral ventricle volume/cerebrum volume has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. a. Under the nonparametric assumption b. Null hypothesis: true area = 0.5

4. Discussion

The field of literature has extensively explored alterations in brain morphology associated with various diseases (10-13). These alterations may involve different structures, such as gray matter, white matter, and ventricle volume. In fact, changes in lateral ventricle volume can be due to changes in gray and white matter volume or can lead to alterations in these volumes (4, 9, 14-18). Furthermore, recent studies have discovered that changes in brain morphology are also linked to neurodegenerative (19-22) and psychiatric conditions (23-27). For example, Parkinson's disease is one of the neurodegenerative diseases that has been studied extensively in this context.

Our study compared the left and right-sided lateral ventricle volumes in patients with Parkinson's disease to those of a control group. We observed larger lateral ventricle volume in patients, but this difference was not statistically significant. However, we found that female patients had significantly higher right-side lateral ventricle volume, lateral ventricle volume/cerebrum volume, and lateral ventricle volume /TICV compared to the female controls. According to the ROC analysis, the range of AUC values was between 0.595 and 0.629, indicating an increase in this parameter, but it was not statistically significant.

Our study has yielded similar and different results compared to the literature. The study by Hikmet Kocaman et al. (2019) also compared lateral ventricle volume between Parkinson's patients and controls and reported no difference between the groups in total lateral ventricle volume. This finding is consistent with our study. However, when they analyzed the lateral ventricle by dividing it into sections, they observed that certain regions of the ventricle showed higher volume in Parkinson's patients. Although we did not segment the lateral ventricle in our study, we acknowledge this as a limitation, and future studies could benefit from a more specific segmentation analysis. The study highlights the importance of analyzing different regions of the brain, including the lateral ventricle, to understand the exact mechanism of Parkinson's disease. Hikmet Kocaman et al. found that although changes in certain regions of lateral ventricle volume may not cause a change in total lateral ventricle volume, they may be more significant. Therefore, a more comprehensive analysis of the brain may be necessary to fully understand the impact of Parkinson's disease on the brain (8). Other studies (15, 16, 28-31) have reported larger lateral ventricle volumes in Parkinson's patients compared to healthy controls. In a study by Liana et al., which is similar to ours, the lateral ventricle volume was compared between healthy controls and Parkinson's patients, and different results were obtained. The study was conducted on 35 patients (30). In the study by Richard et al., which was conducted on 50 patients, the Parkinson's group was further divided into subcategories (16). The difference in their findings compared to ours could be due to the smaller sample size used in their study. We believe that the results obtained from our study are more representative due to the larger sample size used in the analysis. Turi et al. reported a different result from our study, stating that the lateral ventricle volume of Parkinson's patients was higher than healthy controls. However, they used a

different method from ours, using FreeSurfer to calculate lateral ventricle volume. Our study determined the lateral ventricle areas by examining them in sections and calculated the volume accordingly. In this study, the lateral ventricle volume was calculated automatically, which could be a potential source of difference between the results. Therefore, the difference in findings between our study and Turi et al.'s study may be attributed to differences in methodology (29).

However, we believe these findings are insufficient, as brain and cranium sizes can vary between individuals, particularly regarding age and sex (6, 14, 17, 21, 32, 33). Therefore, we have sought to normalize ventricle volume by considering its ratio to brain and cranium volumes. Our aim here is to eliminate differences that may arise between individuals and obtain more objective results.

In a study by Tolga and colleagues, they compared ventricle volumes between patients with Alzheimer's disease and controls. The results showed higher volumes in the patient group, but no significant differences in total intracranial volume between the two groups were found (34). It should be noted that normalizing ventricle volume by considering its ratio to intracranial volume is necessary, even if there are no differences in intracranial volume between patient and control groups. Normalization is a method used to eliminate differences between individuals. Therefore, comparing groups individually without considering volume ratios is generally not appropriate.

A recent study suggested that there is more volume increase in the contralateral ventricles in Parkinson's patients on the symptomatic side (16). Although we did not differentiate between symptomatic and asymptomatic sides, our findings showed that the right ventricle volume was significantly larger than the left.

In conclusion, our study provides evidence that changes in ventricle volume may be linked to Parkinson's disease, particularly in female patients. However, our study's lack of statistically significant results could be due to the small sample size. Therefore, future studies with larger sample sizes and more advanced imaging techniques are needed to confirm our findings and further explore the role of ventricle volume changes in Parkinson's disease.

It is important to note that our study had some limitations. Firstly, the small sample size limited the generalizability of our findings. Secondly, we only examined ventricle volume without considering other brain structures or functions. A more comprehensive brain morphology and function analysis would provide a more detailed understanding of the disease mechanism. Thirdly, we only included patients with idiopathic Parkinson's disease and did not examine other types of Parkinsonism. Lastly, our study did not differentiate between symptomatic and asymptomatic sides, which may have affected our findings.

Despite these limitations, our study contributes to the existing literature by highlighting the importance of considering gender and normalizing ventricle volume in studies of Parkinson's disease.

Considering all of this, it seems that lateral ventricle volume can be affected by factors such as neurodegenerative diseases, mental illnesses, aging, and sex. Therefore, lateral ventricle volume can be used as an important biomarker in investigating brain health and diseases.

The changes in ventricle volume are a crucial factor in many neurodegenerative diseases, as they are closely related to the underlying anatomical structures. Our study aimed to investigate the effect of ventricle volume on Parkinson's disease, which is one of the neurodegenerative diseases. Specifically, our analysis revealed that in Parkinson's disease female patients, the right-sided lateral ventricle volume and the ratios of lateral ventricle volume to both total intracranial volume and cerebrum volume were significantly higher compared to female controls. However, the difference in lateral ventricle volume between male patients and male controls was not statistically significant.

The implications of these findings are important, as they suggest that gender should be considered as a critical factor when assessing lateral ventricle volume in neurodegenerative diseases. This information can be useful in developing more personalized treatment plans for patients with these conditions.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: M.N., B.D., Design: M.N., B.D., Data Collection or Processing: M.N., B.D., Analysis or Interpretation: M.N., B.D., Literature Search: M.N., B.D., Writing: M.N., B.D.,

Ethical Statement

The study was carried out in conformity with the Declaration of Helsinki after obtaining the approval of Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (Date: 02.03.2023, Project No: 23-KAEK-047).

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Reusable versus disposable surgical drapes: A cost-benefit analysis

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Abstract

Surgical drapes are frequently used in operating rooms and clinics where interventional procedures are performed. In hospitals, surgical drapes can be preferred as disposable or reusable. The choice of surgical drapes stands out as an important issue for hospitals, considering the costs. The objective of the study is to compare disposable and reusable surgical drapes in terms of their costs and benefits and choose the best alternative for the subject hospital. The study was carried out in a training and research hospital with more than 1000 patient beds in Ankara, Türkiye. Cost analysis of 27 reusable surgical drape sets was conducted using procurement, tailoring, sterilization, washing, and drying costs. Cost analysis of disposable surgical drape sets was conducted using purchase unit cost and waste disposal costs. The benefits of surgical drapes were compared using the Analytical Hierarchy Process method, weighted by field experts within the scope of some criteria. At last, the analysis was completed by taking the ratio of findings from both cost and benefit analysis for reusable and disposable surgical drapes. Reusable surgical drapes were found to be less costly and more beneficial when compared to disposables. However, it is not possible to reach a generally accepted conclusion as results may vary from hospital to hospital. Cost savings can be achieved by using reusables as primary surgical drapes in some, if not all surgeries. These results may be useful for decision-makers.

Keywords: analytical hierarchy process (AHP), cost-benefit analysis, disposable equipment, reusable equipment, surgical drapes

1. Introduction

The cost-effective use of resources in hospitals helps to reduce the overall cost of healthcare, which is beneficial for both patients and healthcare providers. By using resources efficiently, hospitals can save money on supplies, energy, and labor, which can be used to provide better care to patients or invest in other areas of the hospital. Additionally, cost-effective resource use can also improve patient outcomes by ensuring that hospitals have the necessary resources available to provide high-quality care. For example, having an adequate supply of medication and equipment can ensure that patients receive timely treatment, which can lead to better health outcomes. Cost-effective resource use can also improve the sustainability of healthcare systems by reducing waste and minimizing the environmental impact of hospitals. For instance, using reusable medical supplies sparingly and recycling the disposables whenever possible can help to reduce the amount of waste generated by hospitals.

Surgical drapes have been used by healthcare professionals in operating rooms and clinics where interventional procedures have been performed for more than a hundred years to eliminate or reduce the transfer of microorganisms to sterile environments during invasive interventions, to protect patients and healthcare professionals and to eliminate the risk of infection (1, 2). Surgical drapes are expected to be resistant to liquids with barrier properties, resistant to moisture and bacterial penetration, resistant to puncture, tearing, and abrasion, not leaving hair, air and dust, resistant to antistatic and flame, free from toxic substances, small porous and tightly woven, maintaining body temperature, compatible with sterilization processes, ergonomic, suitable for dimensions and positions, positive cost and benefit ratio (3, 4).

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Surgical drapes are used to protect the patient during the surgical operation. All these drapes are produced using nonwoven surfaces and woven fabrics for single-use (disposable) and multi-use (reusable). Reusable surgical drapes are made of woven fabric and are subjected to washing and sterilization between repeated uses. Disposable surgical drapes, on the other hand, are designed to be used once and are produced from nonwoven surface fabrics. Disposable surgical drapes are materials used on a patient during a single procedure and then disposed of according to certain rules and standards (5, 6).

Each of the two basic product types used in surgical drapes has its own advantages and disadvantages. In addition to the cost required for certain surgical procedures, there are significant differences in fabric features (breathability, moisture transmission, hardness/softness, comfort, noise level), functionality (protection and safety, readiness for use, continuity of quality), environmental factors (waste disposal, impact on natural resources). Therefore, it is important to compare the costs and benefits of different surgical drape alternatives in order to conclude an effective decision. Surgical drapes create a significant cost burden on hospitals (7). In this regard, it is thought that the implementation of cost-benefit analysis, which is a financial evaluation method that measures the costs and benefits of the service and shows the user whether the cost of the service provided is valuable, will guide decision-makers in the management of limited budget resources (1). Along with this information, the aim of the study is to create a cost-benefit analysis model based on the Analytical Hierarchy Process (AHP) for disposable and reusable surgical drapes and to demonstrate the application of this model with a case study.

2. Materials and Methods

This study was carried out in a tertiary hospital with more than a thousand hospital beds in Ankara, Türkiye. The use of both disposable and reusable surgical drapes in the hospital was an important factor in choosing this hospital. There are 27 different surgical drape sets used in different areas in the hospital, especially in operating rooms and examination rooms where interventional procedures are performed.

Ethical approval was obtained from the Ankara University Ethics Committee (Protocol Number: 56786525-050.04.04/82748) in the first stage of the study, and then administrative permissions were obtained from the hospital. After obtaining the necessary permissions, the literature was searched for the creation of data collection forms. In order to finalize the data collection forms created after the literature review, an expert team consisting of health and administrative personnel who used disposable and reusable surgical drape sets or worked on these sets was formed in the hospital. In line with the feedback of this team and a senior author who is an expert in the field, a data collection form to be used in the cost-benefit analysis was created. Both cost and benefit data were analyzed using Microsoft Excel.

2.1. Cost Analysis

27 different surgical drape sets were included in the cost analysis. Data for cost analysis were collected through face-to-face interviews with the administrative and financial affairs directorate, purchasing commission members, laundry and sterilization department supervisors, and operating room staff. Cost calculation of disposable and reusable surgical drapes has been made by considering different cost items, and details are given below. The data used to calculate the costs of reusable surgical drapes are listed in Table 1.

For calculating the costs of reusable surgical drapes; expenses incurred during the procurement phase, lighting, heating, and water expenses of business units, medical waste costs (the record of how much of the surgical drapes were recycled could not be reached), and storage and transportation costs are excluded. The steps followed for the analysis are as follows; firstly, the average cost per kg was calculated for each cost item using the relevant formulas. The total cost per kg was calculated by summing each cost item mentioned in Table 1. Subsequently, the actual weights of the surgical drape sets were multiplied by the total cost per kg; thereby, the cost of each set was calculated. The total cost per set and per usage was determined by taking into account the number of cycles determined. According to the literature, surgical drapes can be used between 40 to 75 cycles (1, 8-10). In order to calculate the cost per use within the scope of the hospital where the study was conducted, it was determined that the life cycle of the surgical drapes was approximately 40, and calculations were made accordingly. The data used to calculate the costs of disposable surgical drapes are listed in Table 1.

For calculating the costs of disposable surgical drapes; expenses incurred during the market research process during the procurement phase, labor costs within medical waste disposal and storage, and transportation costs are excluded. The steps followed for the analysis are as follows: firstly, the weights of each disposable drape set were multiplied by unit waste cost (per kg), and the total cost of the disposable surgical drapes was found by adding the purchase cost of each drape set. It was determined that the hospital management received bids from two different companies (company A and company B) during the procurement of disposable surgical drapes, and therefore cost calculations were made separately for both companies. A discount rate is used to convert expected future expenses to the present value but since the comparison is made within the same year this approach was not used.

2.2. Benefit Analysis - AHP

Some decision-making criteria were taken into account while determining the benefits of surgical drape sets. These decision-making criteria were determined based on the European standard EN 13795, publications prepared by INDA, literature review (2, 5, 8, 11) and face-to-face interviews with experts as mentioned earlier. The details about these criteria are given below and in Table 1 and Fig. 1.

Table 1. Cost-benefit analysis measures for reusable surgical drapes

Cost Analysis Measures	Description
Procurement cost	The procurement of green surgical drape material for the reusable surgical drapes is carried out as a result of a tender organized by the hospital procurement commission.
Tailoring costs	The reusable surgical drapes are sewn by the tailoring unit that provides services within the hospital. Tailoring costs consist of labor and raw material and supply costs.
Sterilization costs	These consist of labor, raw material and supply, depreciation, maintenance-repair, electricity and water costs.
Washing costs	These consist of labor, raw material and supply, depreciation, maintenance-repair, electricity and water costs.
Drying costs	These consist of labor, electricity and depreciation costs.
Benefit Analysis Measures	
c1. Fabric Features	This criterion covers the evaluation of the fabric features of surgical drapes. It contains five sub-criteria.
c1.1. Breathability	It refers to the fabric feature that will minimize the sweating of the user by allowing the passage of water vapor.
c1.2. Moisture transmission	It refers to the performance regarding the transmission of moisture through the gaps in the fabric.
c1.3. Hardness/softness	It refers to the hardness/softness level of the fabric.
c1.4. Comfort	It refers to the lightness of the fabric, ease of movement and fit the body.
c1.5. Noise level	It refers to the level of noise that occur during use, such as rustling.
c2. Functionality	Functionality consists of four sub-criteria: protection and safety, readiness for use and continuity of quality.
c2.1. Protection and safety	It refers to the barrier effect and the performance of protection from infection.
c2.2. Readiness for use	It refers to keeping surgical drape sets sterilized and bundled in case of need.
c2.3. Continuity of quality	It refers to the fact that the barrier effect of surgical drape sets is continuous means that the quality has been standardized without any decrease in the quality after use.
c3. Environmental Factors	There are two sub-criteria for environmental factors. These are waste disposal and its impact on natural resources.
c3.1. Waste disposal	Disposal of reusable and disposable surgical drapes can be different from each other and may affect nature in different ways.
c3.2. Impact on natural resources	The production of single and reusable packs and the sterilization of reusable packs can affect natural resources. For example; while disposable surgical drapes consume more energy and raw materials during the production phase, reusable surgical drapes consume more water and chemicals, causing more air and water pollution.

In order to determine which of these criteria is more important, the members of the expert team were asked to compare the criteria and sub-criteria. Afterward, they were asked to compare disposable and reusable surgical drapes within the scope of these criteria using AHP. AHP is a method that incorporates both rational and intuitive factors into the process to choose the best one among a series of alternatives evaluated according to various criteria (12) and provides a comprehensive framework to the decision maker in solving multi-criteria and multi-actor problems (13). Expert opinions are taken by using a scoring scale developed by Saaty to determine the relative importance levels for the criteria (13).

The use of AHP is a well-established approach in criteria weighting, therefore, in the evaluation of the benefits. Although there are several methods exist for criteria weighting, AHP is one of the most preferred methods, especially in healthcare institutions. Among the reasons why the AHP method is preferred more in applications in hospitals, and therefore it is preferred in this study, can be counted as the ease of application of the method, the inclusion of different decision makers or stakeholders in one model, evaluation of qualitative criteria as well as quantitative criteria and that it can be easily integrated into other methods (14).

The first step in AHP was forming the hierarchical structure. In the hierarchical structure of AHP, the purpose is at the top of the hierarchical structure. While the criteria are at the middle level of the hierarchical structure, there are alternatives at the lowest level of the hierarchical structure (Fig. 1). The hierarchical structure shows the relationships between criteria and purpose. The criteria in the present study were determined based on the literature review and expert opinions.

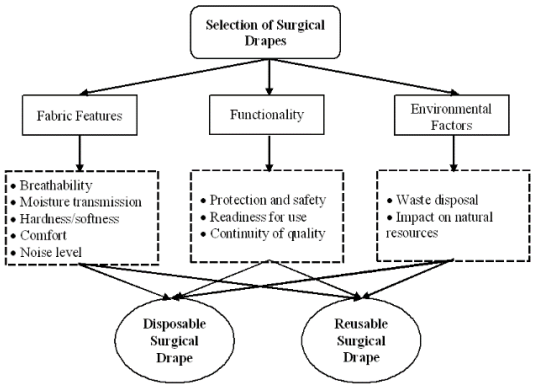

Fig. 1. Hierarchical decision structure. Fig 1 a three-level hierarchical decision structure showing the purpose (oval), criteria (rectangle), sub-criteria (dashed rectangle) and alternatives (circle)

After the hierarchical structure is established, in the second stage, the expert team consisting of health and administrative personnel evaluated the criteria at each level compared to each other. Pairwise comparisons start from the top of the hierarchical structure and are compared with the criteria at each level. In the AHP method, a scale of 1-9 developed by Saaty is used (Table 2). A brief description and set of examples of how to fill out the form were given in the AHP questionnaire. Of the 46 questionnaires, 4 were excluded because they were filled out incompletely or incorrectly, and as a result, a total of 42 of them were used for the analysis.

Table 2. An example of the AHP questionnaire

	9	8	7	6	5	4	3	2	1	2	3	4	5	6	7	8	9	
Fabric Features			X															Functionality
Breathability									X									Comfort
In terms of hardness/softness:																		
Disposable											X							Reusable

After the comparisons of the criteria at each level are made, in the third stage, the square matrices are normalized. The sum of each column is taken, and the row values are divided separately by the column totals. Relative importance weights are obtained by taking the average of each row in the normalized matrix.

In the last stage of the method, consistency analysis and sensitivity analysis are performed to confirm the decision made with the AHP method. For consistency analysis, the Consistency Index (CI) and Consistency Ratio (CR) were calculated. The CI is found to be 0.02, and if the value is less than 0.1, it indicates that the comparisons are correct. The CR of the present study is found as 3.06%, and if the value is smaller or equal to 10%, the inconsistency is acceptable. In the sensitivity analysis, the importance of the pairwise comparison matrices consists of the judgments given by the expert on the subject, and therefore, these judgments may differ from person to person, and since it is possible for people to change their thoughts over time, it is possible for different possible situations to occur for the resulting decision. Sensitivity analysis is a method developed to analyze the flexibility of the final decision based on these assumptions, and it is a guide to see how a change in the examined criteria affects the whole system. After some changes were applied to the comparisons and criteria weights, no major difference was found in the resulting decision. After the completion of cost and benefit analysis separately, data on both costs and benefits were normalized in order to calculate the cost-benefit ratio to compare disposable and reusable surgical drapes.

3. Results

3.1. Cost Analysis Results

The cost per kg of a set was calculated by considering different cost items. Table 3 shows the total cost per kg of reusable surgical drapes. The total cost of reusable surgical drapes per kg was 26.495 TRY (1.422 USD- As of 2022, 1 USD=18.63 TRY).

Table 3. Cost items for reusable surgical drapes (per kg)

Cost	Cost (kg/TRY)
Procurement	13.131
Tailoring	1.629
Sterilization	3.723
Washing	7.997
Drying	0.015
Total	**26.495**

Table 4 shows the unit and total costs of disposable and reusable surgical drapes. For reusable surgical drapes, information about the weight of each set was obtained from the hospital. The total cost per set and the actual weight of the set were multiplied, and the actual cost per set was calculated for 40 cycles. The average cost of reusable surgical drape sets to the hospital per use was calculated as 57.307 TRY. The total cost of each disposable surgical set was calculated by multiplying the weight of the set by the unit waste price and adding the result with the purchase unit cost. Accordingly, the average cost of disposable surgical drapes to the hospital was calculated as 261.009 TRY for company A and 378.242 TRY for company B. It was determined that in 26 of the 27 surgical drape sets the reusable surgical drape set was at a lower cost, and only the "head and neck" surgical drape set was at a lower cost when procured as a disposable from company B.

Table 4. Comparison of disposable and reusable surgical drapes costs

	Cost of Disposable Surgical Drape Sets							Cost of Reusable Surgical Drape Sets	
Surgical Drape Sets	**Purchase unit cost (company A) (a)**	**Purchase unit cost (company B) (b)**	**Weight (c)**	**Unit waste cost (d)**	**Total waste cost (e=c*d)**	**Total cost (company A) (a+e)**	**Total cost (company B) (b+e)**	**Unit cost (f)**	**Total cost (c*f)**
Minor Surgical Set	37.15	25.00	0.200	5.78	1.16	38.306	26.156	26.495	5.299
Pediatric Circumcision	125.00	390.00	0.890	5.78	5.14	130.144	395.144	26.495	23.581
Pediatric Cystoscopy	143.50	360.00	1.254	5.78	7.25	150.748	367.248	26.495	33.225
Pediatric Neurosurgery	160.00	540.00	2.120	5.78	12.25	172.254	552.254	26.495	56.169
Vertebra	177.00	550.00	1.620	5.78	9.36	186.364	559.364	26.495	42.922
T.U.R. (Urology)	191.00	410.00	1.450	5.78	8.38	199.381	418.381	26.495	38.418
Pediatric Laparotomy (Hernia-Appendicitis)	188.00	330.00	0.870	5.78	5.03	193.029	335.029	26.495	23.051
Pediatric Thyroid	255.00	360.00	1.304	5.78	7.54	262.537	367.537	26.495	34.549
General Surgical Set	185.75	290.00	3.230	5.78	18.67	204.419	308.669	26.495	85.579
Abdominal Cover Set	277.70	320.00	1.130	5.78	6.53	284.231	326.531	26.495	29.939
Abdominal Perinal	210.00	300.00	1.130	5.78	6.53	216.531	306.531	26.495	29.939
Thyroid	223.90	320.00	1.150	5.78	6.65	230.547	326.647	26.495	30.469
Breast Surgical Set	200.00	300.00	2.560	5.78	14.80	214.797	314.797	26.495	67.827
Cesarean	230.00	450.00	1.980	5.78	11.44	241.444	461.444	26.495	52.460
Limb	362.00	490.00	3.640	5.78	21.04	383.039	511.039	26.495	96.442
Percutaneous	272.00	450.00	2.150	5.78	12.43	284.427	462.427	26.495	56.964
Hip	309.60	490.00	4.120	5.78	23.81	333.414	513.814	26.495	109.159
Spinal Vertebra	332.00	600.00	3.430	5.78	19.83	351.825	619.825	26.495	90.878
Arthroscopy	375.00	490.00	3.540	5.78	20.46	395.461	510.461	26.495	93.792
Craniotomy	357.00	600.00	2.740	5.78	15.84	372.837	615.837	26.495	72.596
Shoulder Arthroscopy	384.00	450.00	4.520	5.78	26.13	410.126	476.126	26.495	119.757
Heart Valve	523.00	600.00	3.864	5.78	22.33	545.334	622.334	26.495	102.377
Coronary	792.00	600.00	4.862	5.78	28.10	820.102	628.102	26.495	128.819
Tools Table	42.10	30.00	0.254	5.78	1.47	43.568	31.468	26.495	6.730
Moon Table	48.00	45.00	0.263	5.78	1.52	49.521	46.521	26.495	6.968
Tools Table (cardiovascular surgery)	35.00	40.00	0.254	5.78	1.47	36.468	41.468	26.495	6.730
Head and Neck	274.00	45.00	3.874	5.78	22.39	296.392	67.392	26.495	102.642

* Decimal numbers have been rounded.

3.2. AHP Results

As a result of AHP analysis, weights of criterion and both local and global weights of sub-criteria were calculated. As a result of the evaluations of the expert team, the criteria are listed in order of importance as functionality, fabric features, and environmental factors. It was determined that the most important sub-criteria under the fabric features criterion was moisture transmission, the most important sub-criterion was protection and safety under the functionality criterion, and the most important sub-criteria under the environmental factors criterion was the effect on natural resources (local weights). When the global weights of the sub-criteria are examined, it has been determined that the first three most important sub-criteria are protection and safety, continuity of quality, and moisture transmission (Table 5).

The weights of each criterion and sub-criteria were used to compare the disposable and reusable surgical drapes. After the completion of cost and benefit analysis separately, data on both costs and benefits were normalized in order to calculate the cost-benefit ratio to compare disposable and reusable surgical drapes (Table 6). It is apparent from Table 6 that reusable surgical drapes were for company A approximately five times more, for company B approximately six times more cost beneficial when compared to disposable ones. The present study concludes that reusable surgical drapes outweigh disposable surgical drapes in terms of both benefit and cost.

For sensitivity analysis, different life cycles were used for the calculation to check for any major difference in the result. The calculations were repeated for five different life cycles (75, 60, 50, 40, 30) and reusable surgical drapes were found to be more cost-beneficial in all calculations and no major difference was spotted (Table 7). The sensitivity analysis reveals that a plausible increase or decrease in the life cycles would not have a significant effect on the outcome (9).

Table 5. Local and global weight rankings of criteria

Criterion	Weight Ranking	Sub-criteria	Local weight ranking of sub-criteria	Global weight ranking of sub-criteria
c.1. Fabric Features	2	c.1.1. Breathability	2	5
		c.1.2. Moisture transmission	1	3
		c.1.3. Hardness/softness	4	7
		c.1.4. Comfort	3	6
		c.1.5. Noise level	5	9
c.2. Functionality	1	c.2.1. Protection and safety	1	1
		c.2.2. Readiness for use	3	4
		c.2.3. Continuity of quality	2	2
c.3. Environmental Factors	3	c.3.1. Waste disposal	2	10
		c.3.2. Impact on natural resources	1	8

Table 6. Cost-benefit analysis result

	Normalized Benefits	Normalized Costs	Benefit/Cost
Reusable Drapes	0.831	0.180	4.613
Disposable Drapes (A)	0.169	0.820	0.207
Reusable Drapes	0.831	0.132	6.313
Disposable Drapes (B)	0.169	0.868	0.195

Table 7. Sensitivity analysis for cost-benefit analysis

Number of Cycles	Unit cost of reusables	Average cost per reusable sets	Average cost per disposable sets (company A)	Benefit/Cost (Reusable/Disposable-Company A)	Average cost per disposable sets (company B)	Benefit/Cost (Reusable/Disposable-Company B)
75	14.131	30.564	261.009	7.923 0.189	378.242	11.109 0.183
60	17.663	38.204	261.009	6.505 0.194	378.242	9.054 0.187
50	21.196	45.845	261.009	5.559 0.199	378.242	7.683 0.190
40	26.495	57.307	261.009	4.613 0.207	378.242	6.313 0.195
30	35.327	76.410	261.009	3.668 0.219	378.242	4.942 0.204

4. Discussion

Within the context of cost pressure on the healthcare system, it is necessary to identify and implement alternatives to increase the effectiveness and efficiency of health services and to ensure the sustainability of the healthcare system (15). In hospitals within the healthcare system, cost-benefit analysis on surgical drapes can be a guide in purchasing decisions. The main motivation for this purchase decision is to maximize savings by purchasing surgical drapes that offer the lowest cost and the most benefit and support more efficient use of limited resources. Accordingly, this study aimed to conduct a cost-benefit analysis to guide the decision-makers of the hospital to purchase surgical drapes. It was concluded that the cost-benefit ratio of reusable surgical drapes was better and that reusable surgical drapes should be preferred. As predicted prior to the study, it was determined that the total cost increased as the number of uses of reusable surgical drapes decreased. Surgical drapes should be cycled as much as possible to ensure efficient use. In order to ensure this, it is recommended to track the cycles of the surgical drapes. In order to track the cycle of surgical drapes more accurately and to manage the process correctly, it is recommended to use a barcode or RFID system. Considering that the costs to be incurred during the installation and use of the RFID system will be high, it is recommended to conduct a cost study for the use of the RFID system, as it will increase the costs of reusable surgical drapes. If financial resources cannot be allocated to these systems, manual control is recommended.

The main limitation of the study is the inability to include some cost items as mentioned in the method section in detail. A more comprehensive cost analysis can be made by including the missing cost items. Further studies, which take these variables such as waste disposal for reusable surgical drapes into account, will need to be undertaken. Another limitation of the study is that only one hospital was included in the analysis.

It has been determined that the majority of the cost of reusable surgical drapes consists of procurement and labor costs. Although it cannot be included in the cost analysis in this study, considering that medical waste costs constitute a significant part of the total cost, controlled separation of medical wastes related to reusable surgical drapes is recommended. It is recommended that all personnel using surgical drapes in the hospital be informed about the costs of

surgical drapes, especially medical waste costs and usage habits, and that awareness be raised.

It has been concluded that surgical drape sets used in more complicated surgeries are more costly than surgical drape sets used in interventional procedures. For instance, it has been determined that the top three surgical drape sets at the most cost are coronary, shoulder arthroscopy and hip drape sets. At this point, reusable surgical drapes can be preferred for higher-cost and frequent surgeries. The use of reusable surgical drapes for some surgeries and single-use surgical drapes for other surgeries in the same hospital will be able to provide more cost-beneficial results. In this respect, it is recommended to conduct more specific cost-benefit analyses based on surgical drape sets.

There is a limited number of studies on the cost-benefit analysis of surgical drapes and diverse results have been found when assessing it. A study by the University of Münster's CHM has shown that most hospital managers in Germany tended to make their procurement decisions based solely on the purchase price. As a result of the study, it is stated that reusables are preferable in terms of tensile strength, liquid absorption, and bacterial barrier protection while disposables are preferred mostly based on price alone (16). Two studies aimed to perform an AHP-based cost-benefit analysis on the selection of surgical gowns and drapes in a university hospital found that disposable surgical drapes and gowns provide higher benefits, but their costs are still high to replace reusable ones (7, 8, 10).

There are also some studies measuring and comparing only the costs or only benefits of disposable versus reusable surgical drapes or gowns. A recent study carried out in a medical center of a university hospital aimed to compare the costs of disposable and reusable surgical drapes and found that reusable surgical drapes were less costly than disposables, supporting our findings (1). Another cost analysis per use basis for 50 processing cycles conducted by the American Reusable Textile Association concluded that disposable surgical gowns were two times more expensive than reusables (17). A study comparing the costs of disposable and reusable surgical drapes found that the average cost of the minor disposable pack is less than the reusable one and concluded that using disposable drapes is not more expensive than using reusables (18). Another study conducted in a hospital found that the cost of reusable drapes was higher than disposables and preferring disposables would result in a cost-cutting of 9% per year (19). A cost analysis of surgical drapes used in 304 randomly selected surgeries in a training and research hospital was made and although the medical waste cost of disposable surgical drapes is higher than reusable surgical drapes it was determined that disposable surgical drapes were cost-effective since there is no cost of washing, sterilization, water and electricity (20).

Beyond their costs, surgical drapes are compared in terms of their benefits and effects on the environment. An environmental life cycle assessment revealed that reusable surgical drapes cause much less environmental pollution than disposables (21). According to a review study, compared to disposables, reusable surgical textiles are more beneficial in terms of energy, water, carbon footprint, volatile organics, solid wastes, and instrument recovery (22). Another study aimed to compare disposable and reusable surgical gowns in terms of their comfort and it was concluded that the comfort performance of disposable surgical gowns was lower. The study also stated that when surgeons wear disposable surgical gowns they perceive it as a 'papery' feeling (5). As a result of a study evaluating the bacterial permeability of disposable and reusable surgical drapes, it was recommended to use a disposable drape in surgeries lasting more than two hours. For surgeries lasting for two hours or less, it was recommended to use disposable drapes in surgeries where the surgical field is wet and infected, and reusable drapes for uninfected cases where the surgical field is not wet. In addition, it was suggested that the surgical drapes should be monitored and recorded after the washing, drying and sterilization processes, the number of uses should be marked, and each institution should monitor and control its own corrosion process (3).

Considering the findings of different studies, one clearly can state that there is no common knowledge about whether disposable or reusable surgical drapes are more cost beneficial. There are several reasons for this. In terms of cost calculations, there will be hidden or indirect costs such as the cost of lost hours for surgery due to the lack of an inadequate number of surgical drapes available which may affect the results of the cost analysis. Another issue is that costs and cost savings vary for not only each hospital but also for each surgical center within the same hospital since there are different procedures and usage and waste disposal habits for each surgery.

In terms of benefit calculations, since the contents and quality of disposable surgical drapes vary from provider to provider, it is not easy to compare the results. It should also be noted that surgeons' preferences play an important role in surgical drape selection. Also, the surgical experience of the operating room personnel, the attitude of the hospital managers, physicians and surgeons towards surgical drape use, whether the laundry and sterilization are outsourced, and whether the surgical drapes used are imported or domestically produced (affects the procurement cost), and organizational culture may have a significant impact on the result of cost-benefit analysis. Therefore, more comprehensive studies can be carried out by adding these out-of-scope cost items and considering some of these institutional factors.

Conflict of interest
The authors declared no conflict of interest.

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Ethical statement

Ethical approval was obtained from the Ankara University Ethics Committee (Protocol Number: 56786525-050.04.04/82748).

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Authors' contributions

Concept: E.E., G.Y.U., Ç.E.A., S.A., Design: E.E., G.Y.U., Ç.E.A., S.A., Data Collection or Processing: E.E., G.Y.U., Analysis or Interpretation: E.E., G.Y.U., Ç.E.A., Literature Search: E.E., G.Y.U., Writing: E.E., G.Y.U., Ç.E.A.

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The depression and anxiety levels and temperament characteristics of the mothers of children with asthma

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Abstract

In our study, we planned to compare the depression, anxiety levels, and dominant temperament characteristics of mothers of children with asthma with mothers of healthy children. The mothers of a total of 114 children who had asthma and mothers of 100 healthy children were included in the present study. The Sociodemographic Data Form, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), and TEMPS-A (Temperament Evaluation of Memphis, Pisa, Paris, San Diego Auto Questionnaire) were applied to the participants. The Asthma Quality of Life Questionnaire (PAQLQ) and Asthma Control Test (AKT) were also performed to have information on the disease for children with asthma. No significant differences were detected between the mean age of the children included in the study and their mothers ($p>0.05$). The participants' marital status, education status, income status, employment status, number of children, and the number of people living at home were similar ($p>0.05$). Although the smoking rate of the mothers of children who had asthma was 28.1%, the rate of smoking of the mothers in the control group was 12% ($p<0.05$). Irritable temperament, anxious temperament traits, and BAI scores were significantly higher in mothers of children with asthma ($p<0.05$). BAI scores were higher in mothers of children with multiple inhalant allergies and using high-level drugs according to the GINA (Global Initiative for Asthma) guideline ($p<0.05$). In our study, we found that irritable temperament, anxious temperament characteristics, and anxiety scores increased in mothers of children with asthma. Therefore, in addition to the asthma treatment of children, the application of psychological support programs to mothers should be considered.

Keywords: asthma, mother, depression, anxiety, temperament

1. Introduction

Asthma is a chronic respiratory disease that is very common worldwide, affecting people of all ages as one of the most common chronic inflammatory diseases in childhood, with a prevalence of 1-18%. According to the International Childhood Asthma and Allergy (ISAAC) study, its 12-month prevalence was reported to be between 1.6% and 36.8% (1). The rate of children and adolescents diagnosed with asthma varies between 0.7 and 17.8% in our country (2). In a previous study that was conducted by Topal et al. in Malatya in 2017, the prevalence of asthma was reported as 9% (3). Asthma is a chronic inflammatory disease causing reversible narrowing of the airway diameter. There are also personal and environmental risk factors in this regard. Although personal risk factors are genetics, epigenetics, atopy, gender, and obesity, environmental risk factors are allergens, microorganisms, air pollution, and exposure to cigarette smoke (2). Childhood asthma usually shows symptoms in the first years of life, with the most common symptoms being cough, wheezing, and shortness of breath. Sleep disturbance because of nighttime symptoms and poor school performance may also be observed. Also, in some patients, airway obstruction is partially reversible and may cause permanent lung damage. For this reason, it is important to follow up with the patients from the moment of the first symptoms and to diagnose them early. As well as family history and physical examination findings showing airway narrowing, allergy skin tests, respiratory function tests, radiological imaging, total or specific IgE measurement, and bronchial provocation tests are also used in the diagnosis (4).

The treatment is long-term because asthma is a chronic disease. The purpose of the treatment is to control bronchospasm during asthma attacks and to reduce mortality. Inhaled steroids, combinations of inhaled steroids and long-acting beta 2-agonists, leukotriene antagonists, long-acting anticholinergic (antimuscarinic) drugs, and oral beta 2-agonists are used. Among these, inhaled steroids are the most effective anti-inflammatory drugs (4). Informing the child about the

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characteristics of the disease, triggers, and ways of prevention in line with the family and age increases treatment compliance (5). Severe asthma in children also causes psychiatric morbidity, such as serious anxiety and difficulty coping with their disease. Increasing anxiety causes drug non-compliance and difficulty controlling the disease (6).

The management of this chronic disease, which progresses with attacks and has triggers, is very challenging, and especially the parents of asthmatic children are burdened with lifestyle changes to maintain treatment. Mothers take active roles in the care of their children and may even give up their favorite activities or jobs for this. For this reason, it was shown that they are under more stress with a higher risk of depression and anxiety when compared to fathers (7, 8). Although it is already known that mental diseases are more common in children with chronic diseases and their mothers, there are limited studies on this subject. This study aimed to evaluate depression and anxiety levels and dominant affective temperament characteristics in mothers of children with asthma. To develop a holistic approach to children with asthma, We think that together with the child's treatment, the data we will obtain in the study will provide useful information for psychiatric disorders that may develop in mothers over time.

2. Materials and Methods

2.1. Inclusion and exclusion criteria of the study

One hundred fourteen children with asthma aged 5-18 who applied to Fırat University Faculty of Medicine, Department of Pediatric Allergy-Immunology, and their mothers were included in the study. Children with asthma who were followed up in our clinic and regularly followed up with their mothers were included in the study. The diagnosis of asthma was made according to the Asthma Diagnostic Criteria in The Global Initiative for Asthma (GINA) Guideline (9) based on the severity and treatment of asthma (4). Children and mothers diagnosed with asthma at least one year ago, using regular prophylactic treatment according to the guidelines and followed in our clinic for at least one year, were included in the current study. The parents were informed about the study. One hundred healthy children were between the ages of 5-18, and their mothers were also included in the study as the control group.

The study did not include mothers with any chronic disease, psychiatric disease, drug use, inability to understand psychological scales, to answer survey questions, or to communicate.

2.2. The procedure

The pre-designed questionnaires were filled in with face-to-face interviews after the clinical examinations of the patients who met the inclusion criteria. The questionnaires of asthmatic patients were conducted face-to-face during routine follow-ups. The demographic characteristics of the patients, such as age, gender, asthma control status, presence of atopy, family income, education levels of the parents, asthma medications, and their use, were recorded in the study form.The pulmonary functions of the patients were measured by using a standard spirometer device (ZAN 100 Spiromed, Flow Handy, Germany), and FEV1, FVC, FEV1/FVC, PEF, and MEF 25-75 parameters were measured by making the patients fast and forceful expiration following rapid and forced inspiration. Also, standard allergen extracts of Alergopharma (Allergopharma JG Company) were used for skin prick testing with inhaled and food allergens in asthma patients.

2.3. Data collection tools

The demographic data of the children who had asthma were recorded during their examinations, and the Asthma Quality of Life Questionnaire (PAQLQ) and Asthma Control Test were filled in face-to-face interviews. Then, mothers were informed about the study, and those who agreed to participate were directed to the same psychiatrist. The same psychiatrist assessed mothers with asthmatic children and mothers with healthy children. The Sociodemographic Data Form, Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), and TEMPS-A (Temperament Evaluation of Memphis, Pisa, Paris, San Diego Auto Questionnaire) Scale were applied to the participants.

Sociodemographic and Clinical Data Form

The Sociodemographic and Clinical Data Form that was prepared by the researchers in line with the clinical experience and information obtained from the scanned sources and considering the purposes of the study was used in the cases. This semi-structured form included data such as the number of treatments and allergy types, sociodemographic information of the mother, such as age, gender, marital status, educational status, occupation, place of residence, economic status, family structure, and clinical data such as psychiatric support, and the child's age, gender, age of complaint onset, duration of diagnosis, status in the emergency department and intensive care unit, the status in the last one year in the clinic.

Asthma Quality of Life Questionnaire (PAQLQ)

The Asthma Quality of Life Questionnaire (PAQLQ), which was developed by Juniper et al. and translated into Turkish by Yüksel et al. to measure physical, mental, and social disorders, was used for the asthmatic patients who were included in the current study. The scale consists of 3 sub-units, including symptoms, activity limitation, and emotional functioning in 23 questions. Each question on the scale has equal weight. The recorded scores were analyzed directly, and the results were calculated as the total quality of life and the "mean score per question" for each dimension. The scoring varies between 1-7 for each item ("1: Always or extremely disturbed, 7: Never disturbed". The total quality of life score is calculated from the mean score of the questions. The total score is obtained by summing the three subscales. Although scores between 23 and 161 can be obtained from the scale, higher scores mean that the quality of life is good and less affected. There is no cut-off

score for the scale (10, 11).

Asthma Control Test

The 4-11-year-old childhood asthma control test, which was shown to be valid and reliable in Turkish for children who had asthma aged 5-12 years, was used in the study. This questionnaire consists of 7 questions, and the first four questions are made easier for children to understand by using shapes, and the child answers them himself (the child answers the questions using the answer scale ranging from a sad face to a smiling face). The last three questions consist of Likert-type questions answered by the parent. The responses to items range from 1 to 5, the responses to questions range from 0 to 3 points in children, and 0 to 5 points in parents. Low scores are consistent with poor asthma control. As the total score increases, it indicates better asthma control; ≤19 points suggest that asthma is not under adequate control, and 27-20 points suggest good asthma control (12,13).

The Asthma Control Test, developed by Juniper et al. and whose validity and reliability were demonstrated in Turkish by Uysal et al., was used for children aged ≥12 years. The test consists of 7 questions, and 5 of the questions are about asthma symptoms during the previous week, 1 is about inhaled bronchodilator use, and one is about forced expiratory volume (FEV1) level in 1 second. The highest point is 25, and the lowest is 5 points. A score between 25-20 is considered complete control, 16-19 is considered partial control, and ≤15 is considered uncontrolled (14, 15, 16).

The Clinical Version of the Structured Clinical Interview Scale for DSM-5 Disorders (SCID-5- CV)

The clinical version of SCID-5, adapted to Turkish and studied for reliability by Elbir et al., was used to determine clinical diagnoses (17).

Beck Depression Inventory (BDI)

Beck developed it in 1961 to measure the risk of depression in adults, the change in severity, and the level of depressive symptoms (18). Hisli conducted the Turkish validity and reliability study in 1989 (19). The cut-off point of the scale was found to be 17. It is frequently used in depression studies as a 21-item Likert-type self-assessment scale. Each item relates to a behavioral trait associated with depression. Items are scored between 0 and 3 according to the severity of depression. The total score ranges from 0 to 63. If the score is between 0 and 9, there are no depressive symptoms; 10-16 points indicate mild, 17-24 points moderate, and 25 and above indicate severe depressive symptoms.

Beck Anxiety Inventory (BAI)

It was developed by Beck et al. (20) as a self-assessment scale to determine the frequency of anxiety symptoms experienced by individuals. It consists of 21 items as a Likert-type scale scored between 0-3. Its validity and reliability for Turkey were performed by Ulusoy et al. (21).

TEMPS-A Scale (Temperament Evaluation of Memphis, Pisa, Paris, San Diego Auto Questionnaire)

It was designed to assess the dominant affective temperament. The original scale was 109 items for men and 110 items for women. The version adapted to Turkish consists of 99 items to determine depressive, hyperthymic, irritable, cyclothymic, and anxious temperaments. If the score obtained from each subtype is above the cut-off point calculated for that subtype, it is assumed that the person has that temperament dominantly. When more than one temperament cut-off point is exceeded, more than one dominant temperament is mentioned. The test-retest reliability of the Turkish translation was established (22). All tests were administered to the patients by the same doctor.

2.4. Statistical Analysis

Statistical analyses were performed in the "SPSS (Statistical Package For Social Sciences) 22.0 for Windows" statistical analysis package program. Descriptive statistics were expressed as numbers and percentages for categorical variables, as mean ± standard deviation value for continuous variables. The Independent Group t-test was used for the comparisons between the groups, the Mann-Whitney U Test was used when non-parametric conditions were met, the ANOVA was used when more than two groups were compared, the Kruskal-Wallis Test was used when non-parametric conditions were met, and the chi-square test was used for the comparisons of ratios between groups. $P<0.05$ was considered statistically significant in the study.

3. Results

The study included 114 children who had asthma, 114 mothers of children who had asthma, 100 healthy children, and mothers of 100 healthy children. The mean age of children with asthma was 11.12±3.74 years, and the mean age of healthy children was 9.68±3.05. The mean age of the mothers of children with asthma was 38.42±7.78 years, and the mean age of healthy children was 36.44±5.85. The age, marital status, educational status, income status, employment status, number of children, and number of people living at home were similar in both groups ($p>0.05$). The gender distribution of the children in the groups was similar ($p>0.05$). Although the smoking rate of the mothers of children who had asthma was 28.1%, the mothers' smoking rate was 12% in the control group ($p<0.05$). The sociodemographic characteristics of the participants are given in Table 1.

No significant differences between the two groups were detected regarding depressive temperament, cyclothymic temperament, hyperthymic temperament sub-scores, and BDI scores ($p>0.05$). Irritable temperament, Anxious temperament, and BAI scores were found to be significantly higher in mothers of children who had asthma ($p<0.05$) (Table 2). No significant differences were detected between the scales with seasonal or year-round persistence of asthma symptoms ($p>0.05$). No significant differences were detected between the scales with food allergy ($p>0.05$). A significant difference was

detected between multiple allergies and only BAI (p<0.024), and BAI was significantly higher in those with multiple inhaler allergies (p>0.045) (Fig. 1).

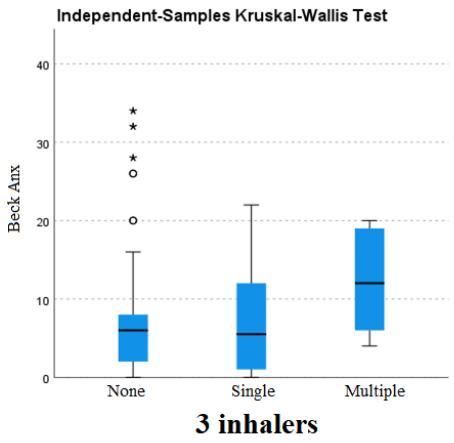

Fig. 1. The relationship between multiple inhaler use and anxiety levels

Depressive temperament scores were positively correlated with Cyclothymic temperament, Irritable temperament, Anxious temperament, BDI, BAI scores, and FEV1/FVC (p=0.000, p=0.000, p=0.000, p=0.001, p=0.031). Depressive temperament score was negatively correlated with FVC, FEV1, GINA guideline score, and Asthma QLS score (p=0.031, p=0.042, p=0.047, p=0.036). Cyclothymic temperament score was correlated negatively with Irritable temperament, Anxious temperament, BDI, and BAI scores and positively with FEV1/FVC (p=0.000, p=0.000, p=0.000, p=0.001), and GINA guideline score and Asthma QLS score (p=0.000, p=0.014). Hyperthymic temperament score was correlated positively with the duration of diagnosis and the number of visits to the clinic in the last year (p=0.037, p=0.009) and negatively with the Asthma QLS score (p=0.015). Irritable temperament scores were correlated positively with Depressive temperament, Cyclothymic temperament, BDI, BAI scores (p=0.000, p=0.000, p=0.000, p=0.009), and PEF and asthma were correlated negatively with QLS (p=0.029, p=0.026). Anxious temperament and Depressive temperament, Cyclothymic temperament, Irritable temperament, BDI, BAI, number of treatments in the intensive care unit in the last year, and FEV1/FVC ratio were positively correlated (p=0.000, p=0.000, p=0.021, p=0.024). A positive correlation was detected between BDI and Depressive temperament, Cyclothymic temperament, Irritable temperament, Anxious temperament, BAI scores, duration of diagnosis, and the number of treatments in the intensive care unit in the last year (p=0.000, p=0.000, p=0.000, p=0.000, p= 0.000, p=0.019, p=0.027). A negative correlation was detected with the number of emergency treatments in the last year (p=0.022). BAI and Depressive temperament, Cyclothymic temperament, Irritable temperament, Anxious temperament, and BDI scores were correlated positively (p=0.001, p=0.000, p=0.009, p=0.000, p=0.000), and the number of hospitalizations in the last year was correlated negatively (p=0.031). The age of onset of the complaints, the duration of diagnosis, and the number of patients treated in the emergency department, outpatient clinic, normal service, and intensive care unit for the last year were correlated negatively (p=0.012, p=0.038, p=0.001, p=0.009, p=0.002). MEF and FEV1/FVC were correlated positively with the age of onset of the complaints (p=0.019, p=0.030) (Table 3).

Table 1. The comparison of the sociodemographic characteristics of the participants

Features	Asthma	Control	Total	P value
Child's age (years)	11.12±3.74*	9.68±3.05		0.675
Mother age (years)	38.42±7.78	36.44±5.85		0.786
Marital status, n (%)				
Married	100 (87.7%)	90 (90%)	190 (88.8%)	
Divorced/Widowed	12 (10.5%)	10 (10.0%)	22 (10.35)	0.845
Living Separately	2 (1.8%)	0 (0%)	2 (0.9%)	
Educational Status, n (%)				
Primary school	42 (36.8%)	32 (32%)	74 (34.6%)	
Middle School	30 (26.3%)	0 (0%)	30 (14.0%)	
High school	28 (24.6%)	8 (8.0%)	36 (16.8%)	0.921
University	14 (12.3%)	58 (58.0%)	72 (33.6%)	
Still studying	0 (0%)	2 (2.0%)	2 (0.9%)	
Working status, n (%)				
Not working	52 (45.6%)	44 (44.0%)	96 (44.9%)	0.812
Working	62 (54.4%)	56 (56.0%)	118 (55.1%)	
Smoking, n (%)				
Yes	32 (28.1%)	12 (12.0%)	44 (20.6%)	
No	80 (70.2%)	88 (88.0%)	168 (78.5%)	0.03
Quit	2 (1.8%)	0 (0%)	2 (0.9%)	

*=mean±standard deviation

Table 2. The comparison of the psychiatric scales used

	Asthma	Control	Total	P value
Depressive temperament	5.21±3.657*	4.56±2.794	4.91±3.290	0.354
Cyclothymic temperament	6.93±4.323	6.46±3.888	6.71±4.123	0.564
Hyperthymic temperament	9.35±3.958	9.46±3.500	9.40±3.743	0.692
Irritable temperament	3.23±3.226	2.38±2.940	2.83±3.117	0.006
Anxious temperament	6.25±4.633	4.94±4.112	5.64±4.435	0.021
BDI	9.18±10.812	5.68±5.239	7.54±8.822	0.257
BAI	8.32±8.467	6.98±10.317	7.69±9.378	0.010

Abbreviations in the table: The first five rows of the table are TEMPS-a temperament characteristics; BDI: Beck Depression Inventory, BAI: Beck Anxiety Inventory. *P<0.05. *=mean±standard deviation

Table 3. The correlation analysis results of the mothers who had asthmatic children

Features	Depressive temperament	Cyclothymic temperament	Hyperthymic temperament	Irritable temperament	Anxious temperament	BDI	BAI
Depressive temperament		r= 0.636 ** P<0.0001	r= 0.051 P= 0.593	r= 0.363 ** P<0.0001	r= 0.560** P<0.0001	r= 0.423** P<0.0001	r= 0.320 ** P<0.0001
Cyclothymic temperament	r= 0.636 ** P<0.0001		r= -0.055 P= 0.564	r= 0.565 ** P<0.0001	r= 0.562** P<0.0001	r= 0.555 ** P<0.0001	r= 0.429 ** P<0.0001
Hyperthymic temperament	r= 0.051 P= 0.593	r= -0.055 P= 0.564		r= -0.053 P= 0.575	r= 0.046 P= 0.501	r= -0.080 P= 0.400	r= -0.068 P= 0.474
Irritable temperament	r= 0.363** P<0.0001	r= 0.565** P<0.0001	r= -0.053 P= 0.575		r= 0.370** P<0.0001	r= 0.349** P<0.0001	r= 0.244** P= 0.009
Anxious temperament	r= 0.560** P<0.0001	r= 0.562** P<0.0001	r= 0.046 P= 0.501	r= 0.370** P<0.0001		r= 0.473** P<0.0001	r= 0.387** P<0.0001
BDI	r= 0.423** P<0.0001	r= 0.555** P<0.0001	r= -0.080 P= 0.400	r= -0.349** P<0.0001	r= 0.473** P<0.0001		r= 0.497** P<0.0001
BAI	r= 0.320** P= 0.001	r= 0.429** P<0.0001	r= -0.068 P= 0.474	r= 0.244** P= 0.009	r= 0.387** P<0.0001	r= 0.497** P<0.0001	
Age of onset of complaints	r= 0.114 P= 0.227	r= 0.077 P= 0.416	r= 0.142 P= 0.132	r= 0.043 P= 0.647	r= 0.037 P= 0.697	r= 0.163 P= 0.083	r= 0.067 P= 0.480
Diagnosis time	r= 0.087 P= 0.357	r= 0.098 P= 0.300	r= 0.196* P= 0.037	r= 0.010 P= 0.917	r= 0.044 P= 0.641	r= 0.220* P= 0.019	r= 0.181 P= 0.054
In the last year treatment	r= 0.141 P= 0.134	r= 0.149 P= 0.114	r= 0.036 P= 0.706	r= 0.071 P= 0.452	r= 0.023 P= 0.811	r= 0.215* P= 0.022	r= 0.202* P= 0.031
Outpatient treatment in the last year	r= 0.076 P= 0.420	r= 0.090 P= 0.342	r= 0.243* P= 0.009	r= 0.074 P= 0.434	r= 0.181 P= 0.054	r= 0.124 P= 0.189	r= 0.004 P= 0.967
Last year hospitalized treatment	r= 0.063 P= 0.505	r= 0.027 P= 0.774	r= 0.009 P= 0.925	r= 0.090 P= 0.341	r= 0.131 P= 0.165	r= 0.050 P= 0.600	r= 0.156 P= 0.098
Last year ICU treatment	r= 0.029 P= 0.761	r= 0.062 P= 0.511	r= 0.098 P= 0.297	r= 0.087 P= 0.356	r= 0.216* P= 0.021	r= 0.207* P= 0.027	r= 0.091 P= 0.335
FVC	r= 0.202* P= 0.031	r= 0.007 P= 0.939	r= 0.071 P= 0.451	r= 0.007 P= 0.944	r= 0.098 P= 0.299	r= 0.079 P= 0.404	r= 0.103 P= 0.275
FEV1	r= 0.191* P= 0.042	r= 0.035 P= 0.713	r= 0.173 P= 0.065	r= 0.048 P= 0.609	r= 0.075* P= 0.429	r= 0.036 P= 0.705	r= 0.005 P= 0.957
FEV1/FVC	r= 0.202* P= 0.031	r= 0.311** P= 0.001	r= 0.058 P= 0.540	r= 0.020 P= 0.833	r= 0.211* P= 0.024	r= 0.102 P= 0.281	r= 0.179 P= 0.057
PEF	r= 0.001 P= 0.988	r= 0.001 P= 0.993	r= 0.034 P= 0.716	r= 0.204* P= 0.029	r= 0.019 P= 0.838	r= 0.013 P= 0.888	r= 0.098 P= 0.300
MEF25-75	r= 0.097 P= 0.305	r= 0.080 P= 0.398	r= 0.164 P= 0.081	r= 0.118 P= 0.212	r= 0.045 P= 0.631	r= 0.004 P= 0.966	r= 0.091 P= 0.334
GINA Directory	r= 0.186* P= 0.047	r= 0.417** P<0.0001	r= 0.061 P= 0.518	r= 0.062 P= 0.514	r= 0.019 P= 0.838	r= 0.061 P= 0.516	r= 0.038 P= 0.691
Asthma quality of life score	r= 0.197* P= 0.036	r= 0.229* P= 0.014	r= 0.228* P= 0.015	r= 0.208* P= 0.026	r= 0.135 P= 0.153	r= 0.065 P= 0.495	r= 0.064 P= 0.499
Asthma control test (5-12 years)	r= 0.152 P= 0.106	r= 0.009 P= 0.927	r= 0.025 P= 0.788	r= 0.183 P= 0.052	r= 0.007 P= 0.944	r= 0.161 P= 0.087	r= 0.008 P= 0.936
Asthma control test (≥12years)	r= 0.118 P= 0.211	r= 0.012 P= 0.898	r= 0.007 P= 0.937	r= 0.158 P= 0.093	r= 0.010 P= 0.918	r= 0.177 P= 0.060	r= 0.019 P= 0.840

**=Correlation is significant at the 0.01 level (2-tailed), *= Correlation is significant at the 0.05 level (2-tailed).
BAI= Beck Anxiety Inventory, BDI= Beck Depression Inventory, ICU= Intensive care units

4. Discussion

In the present study, the depression and anxiety levels and dominant temperament characteristics of the mothers of children with chronic diseases such as asthma and those of the mothers with healthy children were compared. In the results, anxiety scores and irritable temperament characteristics of the mothers with asthmatic children were higher than the other group. Also, mothers of asthmatic children had changes in the anxiety, depression levels, and dominant temperament characteristics of the children with the disease status. Although the temperament characteristics of the mothers whose children used multiple inhalers and had multiple allergies did not differ, their anxiety levels increased.

Asthma starts at an early age and progresses with attacks, and during some attacks, the patient must be brought to the emergency department or treated by an inpatient (2). Treatment is long-term in a chronic disease that progresses with attacks (4). Previous studies conducted with parents of children who had asthma in the literature showed that psychiatric complaints and sometimes mental diseases may occur (23-27). In a previous study conducted with a limited number of mothers (45 mothers) of asthmatic children, mothers had high levels of depression and anxiety (23). Another study conducted in our country in 2010 showed that the depression and anxiety levels of mothers with asthmatic children were higher than those of mothers with healthy children (28). It was reported in much older studies that the depression and anxiety levels of mothers with children who had asthma increased by 40% (29). A recent meta-analysis study reported that parents who had asthmatic children, not only mothers, had increased anxiety and depressive complaints. It was found that experiencing a serious fear of losing their children triggers the resulting anxiety and depressive complaints (24). In a compilation study, it was reported that the stress rate of families with children who had asthma was high, the increase in family stress increased the symptoms in the child, and the increase in the child's symptoms increased the anxiety of the family and created a vicious circle (30). In the present study, similar results were obtained from all these studies in the literature. Although the depression scores of the mothers with asthmatic children did not differ, their anxiety levels were higher than those of mothers with healthy children. Also, similar to the literature data, it was found that the anxiety levels of the group whose children used multiple drugs and had multiple allergies increased. As the management of the disease becomes more difficult, the need to use more drugs and pay attention to the situation can cause more allergies with multiple allergens, and it is considered an expected situation that the mothers' stress increases.

The dominant temperamental characteristics of the mothers with asthmatic children were not assessed in any previous study in the literature review. Our results found that the anxious and irritable temperament characteristics of these mothers were dominant. In the literature, studies conducted on personality traits were conducted with mothers of children/adolescents with psychiatric disorders. In the results, the existing mental disease of the child was associated with the personality structure of the mother (31-33). The mother's depressive temperament affected many areas, from language skills to school success (31). Similarly, it was reported that the temperament characteristics of the mothers of children with autism spectrum disorder may affect the severity of the symptoms in the child (32). In a previous study, the current situation in children with separation anxiety was associated with the mother's personality traits (34). In another study, the effect of the personality traits of the mothers of children with attention deficit and hyperactivity disorder on the occurrence and severity of symptoms was reported (35). Personality traits were not investigated in mothers of children with chronic diseases such as asthma that progressed with exacerbations. Although the mother's personality may not be effective in the formation of the disease in such chronic diseases, it is considered that it will be important in the child's anxiety management and intervention during the attacks.

In the results, the demographic data of the two groups (mothers with asthmatic children and mothers with healthy children) were similar in many respects. No difference was detected between demographic data such as mean age, marital status, educational status, economic level, and employment status of the mothers of the two groups. However, the smoking rate of the mothers of children who had asthma was calculated to be more than twice that of the other group (12% in one group, 28% in the other group). This difference was statistically significant. This result obtained was assessed in line with the literature data. In a previous study, parents with asthmatic children were divided into two groups: depressed and non-depressed. Smoking rates of depressed parents were found to be higher than the other group. Similarly, it was shown that asthma attacks are more common in the children of the depressed group (36). Another study found that the parents of asthmatic children have high rates of stressful life events, not only smoking but also alcohol/substance use (37). It was reported in a similar study that children who had asthma were exposed to more allergens and cigarette smoke (38). The smoking rate of the mothers of children with asthma was higher in our participants.

Finally, in the results of the correlation analysis, the duration of the children's asthma diagnosis and their depression scale scores were positively associated. Although the depression scores of the mothers were not different between the groups, a positive correlation was found with the duration of asthma diagnosis. Similarly, it was found that the higher the frequency of treatment in the intensive care unit, the higher the mother's depression scores, which was associated with the increase in the depressive complaints of the mothers as the duration of the child's disease prolonged or the deterioration in physiological functions such as sleep, appetite, and sexual functions, which the BDI questioned. Irritable temperament and anxious temperament characteristics of the mothers of

children who had asthma were found to be significant. In the correlation, it was found that as asthma quality of life scores of children decreased (quality of life decreased), irritability scores of the mothers increased as expected. This result is compatible with the literature data. In other words, it was found in the literature that the mothers' psychiatric symptoms increase as the children's limitations regarding the disease increase and the quality of life because of the disease becomes worse (37). Also, in line with the literature data, a similar result was obtained for the anxious temperament trait. It was found that the child's anxious temperament characteristics increased as the inpatient treatment in the intensive care unit increased in the last year. It was shown that as the caregiver's disease deteriorates or the symptoms of the disease progress, the anxiety and depression scores of the caregiver increase, and the burden of caregiving increases (39).

The present study must be assessed considering some limitations, the first of which was the relative inadequacy of the number of participants. Evaluation of only the mothers and not including the fathers, and finally, the evaluation of the participants with self-evaluation questionnaires can be considered, among other limitations. All these limitations prevent the generalization of the results. Large-scale studies involving both parents must be conducted in larger sample groups.

In conclusion, it was found that the anxiety scores of the mothers of children with chronic diseases such as asthma were higher than those of the mothers with healthy children, and irritable temperament characteristics were also dominant. The disease status, anxiety, depression levels, and dominant temperament characteristics also showed changes in the mothers of children with asthma. Although the temperament characteristics of the mothers whose children used multiple inhalers and who had multiple allergies did not differ, their anxiety levels increased. In light of all these findings, when examining and arranging the treatment of children with chronic diseases, it must be considered that their parents may also have psychiatric problems. It must also be kept in mind that the presence of psychiatric symptoms or diseases in the parents may also have possible negative effects on the treatment period of these children. It may also be recommended to provide routine psychiatric support to the parents of children with chronic diseases to provide psychiatric strengthening and well-being.

Conflict of interest

The authors report no conflicts of interest.

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Ethical statement

Ethics committee approval was obtained by Fırat University Clinical Research Evaluation Committee with the decision number 2021/09-33 dated 16.09.2021.

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None to declare.

Authors' contributions

Concept: N.K., M.K., Design: N.K., M.K., Data Collection or Processing: N.K., M.K., Analysis or Interpretation: N.K., M.K., Literature Search: N.K., M.K., Writing: N.K., M.K.

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Treatment results of patients with developmental dysplasia of the hip, who were treated with tubingen hip flexion splint

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Abstract

Developmental dysplasia of the hip (DDH) can be treated successfully with early diagnosis and conservative methods. In this study, we evaluated the results of patients with DDH who were treated with Tubingen hip flexion splint (THFS). The results of patients with DDH who were treated with a Tubingen hip flexion splint (THFS) were retrospectively evaluated. 75 hips of 50 patients were evaluated in the study. According to the Tönnis Stage system, 49 of the hips were dysplasia, 18 of the hips were subluxations, and 8 of the hips were dislocations. The mean age at the time of starting the THFS was 3.48 (1.5-5.5) months, and the duration of treatment was 5.5 (3-9) months. Ultrasonographically normal hip (Graf type 1) and/or sufficient acetabular index values with concentric hip reduction and hips without avascular necrosis of the femoral head were considered successful results. Treatment of four patients in this study failed. 3 of these patients were female. 3 of the failed patients had left hip dysplasia, and one had right hip dysplasia. There was no significant relation between hip side and treatment success ($p = 0.638$). None of the patients had avascular necrosis. The overall success rate was 92%. As a result of this study, we observed that THFS is an effective treatment option for DDH treatment.

Keywords: developmental dysplasia of the hip, treatment, tubingen splint, graf

1. Introduction

Developmental dysplasia of the hip (DDH) is a common orthopedic abnormality in infants and young children. DDH is a clinical entity that expresses developmental disorders that may occur before, during, or after birth. The incidence ranges between 1.6 and 28.5 per 1000 live births among different racial groups depending on the definition of the DDH (1).

Early diagnosis and treatment is critical for the DDH treatment. If the diagnosis is not made promptly, it can lead to severe consequences such as hip pain and limping in adulthood. Therefore, early hip reduction is necessary to achieve satisfactory results. Currently, many different methods are available for the treatment of DDH. Maintaining hip reduction is important for the conservative treatment of DDH and many orthoses such as the Frejka pad, the Pavlik harness, the von Rosen brace, the Ilfeld brace, and the Tubingen hip flexion brace can be used for that aim (2).

The Tubingen hip flexion splint (THFS) was first introduced in the 1990s as an alternative to Pavlik harness. The Tubingen splint has an adjustable abduction bar which allows adjustment to the desired abduction angle. THFS provides abduction. In addition to that it provides the advantages of preventing hip adduction and the knee and ankle joints remain free to move. Some studies report that THFS for the early treatment of DDH has the same or better results than the Pavlik harness and causes less avascular necrosis (AVN) (2). THFS has been reported to be an effective treatment modality in infants with DDH (3).

In this study, we aimed to report the results of our DDH patients who were treated with THFS.

2. Materials and Methods

The results of patients diagnosed with developmental hip dysplasia, who presented at the Orthopedics and Traumatology Department of Ondokuz University Faculty of Medicine between July 2014 and April 2017 and treated with Tubingen hip flexion splint were evaluated retrospectively. 75 hips of 50 patients were included.

The same research team examined all of the patients and made diagnosis. THFS was applied within 24 hours after diagnosis. At the diagnosis stage, physical examinations of all patients were performed, Ortolani test, Barlow test, and abduction limitation were evaluated and recorded.

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All patients underwent ultrasonography according to the Graf method for the diagnosis of the DDH. Patients with Graf type 2a and above hips were included in the study. The study did not include patients with myelomeningocele, neuromuscular and collagen connective tissue disease, and teratological hips. Due to the ossification of the femoral head epiphysis in the 4th and 6th months, direct anterior-posterior pelvic radiography was taken in addition to ultrasonography for the patients who were older than four months and whose follow-up exceeded four months. Measurements were made and recorded. On the radiographs, the Shenton-Menard line and the position of the femoral head relative to the Perkin line were carefully evaluated and noted. Acetabular index measurements were noted.

The patient's hips were considered dysplastic, subluxated and dislocated and classified according to the Tönnis staging system. Conversion to Graf 2b for Graf type D and Graf type 2c, 2b or 1 for type 3 and 4 was evaluated by hip ultrasonography at intervals of 2-4 weeks. The relationship of the femoral heads and the acetabulum were evaluated by the acetabular index angle in direct anteroposterior pelvis radiographs for the patients older than six months. Criterias for successful treatment were cases with normal hips detected in ultrasonography; reduction of the femoral heads concentrically to the acetabulum on direct anterior-posterior pelvis radiography (stage 1 according to the Tönnis staging system), sufficient acetabular index value and patients without avascular necrosis. Patients who did not meet this criterias were considered unsuccessful.

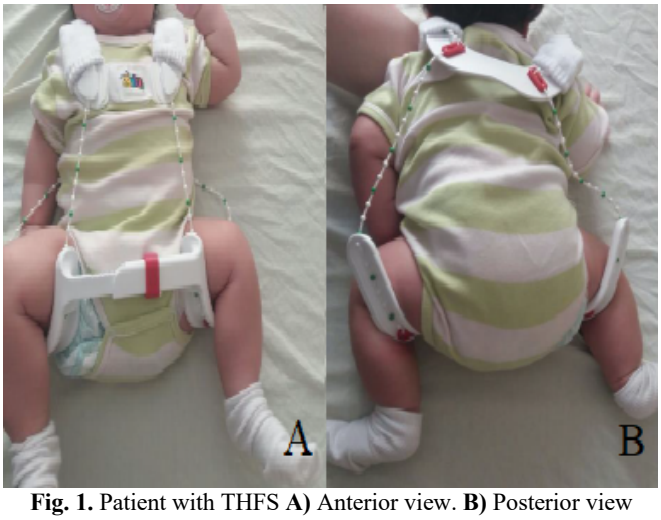

Fig. 1. Patient with THFS **A)** Anterior view. **B)** Posterior view

THFS was applied within 24 hours after obtaining family consent in all patients for whom treatment decision was made. Initially, flexion was set to 90-110 degrees and decreased to 70° as treatment progressed (Fig. 1). The child was allowed to perform 10-15 degrees of passive abduction.

Concentric reduction of the femoral head was confirmed by ultrasonography and/or direct anteroposterior pelvic radiography. Cases that concentric reduction could not be achieved were followed up for two weeks. If reduction was achieved at the end of this period, the treatment continued. In cases where the reduction was not achieved, other treatment methods were used. In patients with reduction achieved, flexion was gradually reduced to 70 degrees. THFS was applied 24 hours a day for at least six weeks in all patients. In cases with stable and adequate acetabular index values on ultrasonography and/or direct anterior-posterior pelvic X-ray, treatment was continued for 14 hours a day (during sleep) for 4-10 weeks, depending on the severity of dysplasia. Controls examination of the patients were planned with gradually increasing intervals until the skeletal maturity was completed.

The obtained data were statistically tested using the program (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp, 2012). Fisher's Exact test was used to evaluate success according to values such as gender, side, age at onset of treatment and examination findings. $p<0.05$ was taken as a criterion for statistical significance.

3. Results

In this study, 50 patients (40 female, 10 male; 75 hips) were evaluated. There was a family history for DDH in 8 of the cases, breech presentation in 2 and swaddling in 2 of the cases. 20 patients with 35 DDH hips were the first children of their parents. 49 hips of the patients were evaluated as dysplasia (65.3%), 18 hips were subluxated (24%), and 8 hips were dislocated (10.6%) (Fig 2). The mean duration of treatment with splint was 5.5 (3-9) months. 4 out of 50 patients, the treatment was unsuccessful. 3 of them were female and 1 was male. 3 of the failed patients had left hip dysplasi and 1 had right hip dysplasia. No significant correlation was found between hip side and success (p=0.638).

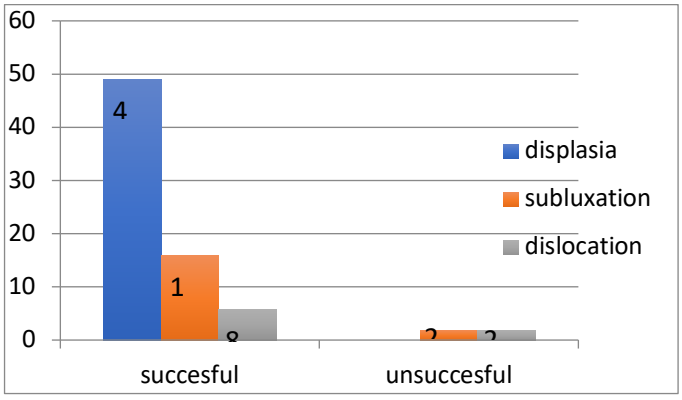

Fig. 2. Graph of success and failure according to hip type of patients

The overall success rate was determined as 92%, considering the number of patients. It was observed that 2 of the 4 unsuccessful patients had type 3 and 2 of them had type 4 irreducible hips according to the ultrasonography results. These patients were referred from another center and the mean age at presentation was 5 (4-5.5) months.

In our study, 25 patients had bilateral, 19 patients had isolated left hip, 6 patients had isolated right hip dysplasia. Successful results were obtained in all 25 patients with bilateral involvement. It was determined that 3 of the 4 failed patients

had left hip involvement and 1 had right hip involvement. There was no statistically difference between the right and left hips in terms of the success of the treatment ($p>0.05$). The success rate was 94.7% according to the number of hips treated. The failure rate was found to be higher in hips that were found to be dislocated and/or types 3 and 4 on ultrasonography compared to the others. Femoral head aseptic necrosis did not occur in any of the patients during the follow-up period. Closed reduction and pelvipedal cast were applied to 4 patients who failed reduction.

In the last controls for 3 of our treated patients, stage 1 according to Tönnis criteria, normal on ultrasound, but minimal acetabular dysplasia was present in the final control radiographs, while hip maturation was completely normal in all the remaining 43 patients. Case examples of this study are presented in Fig. 3.

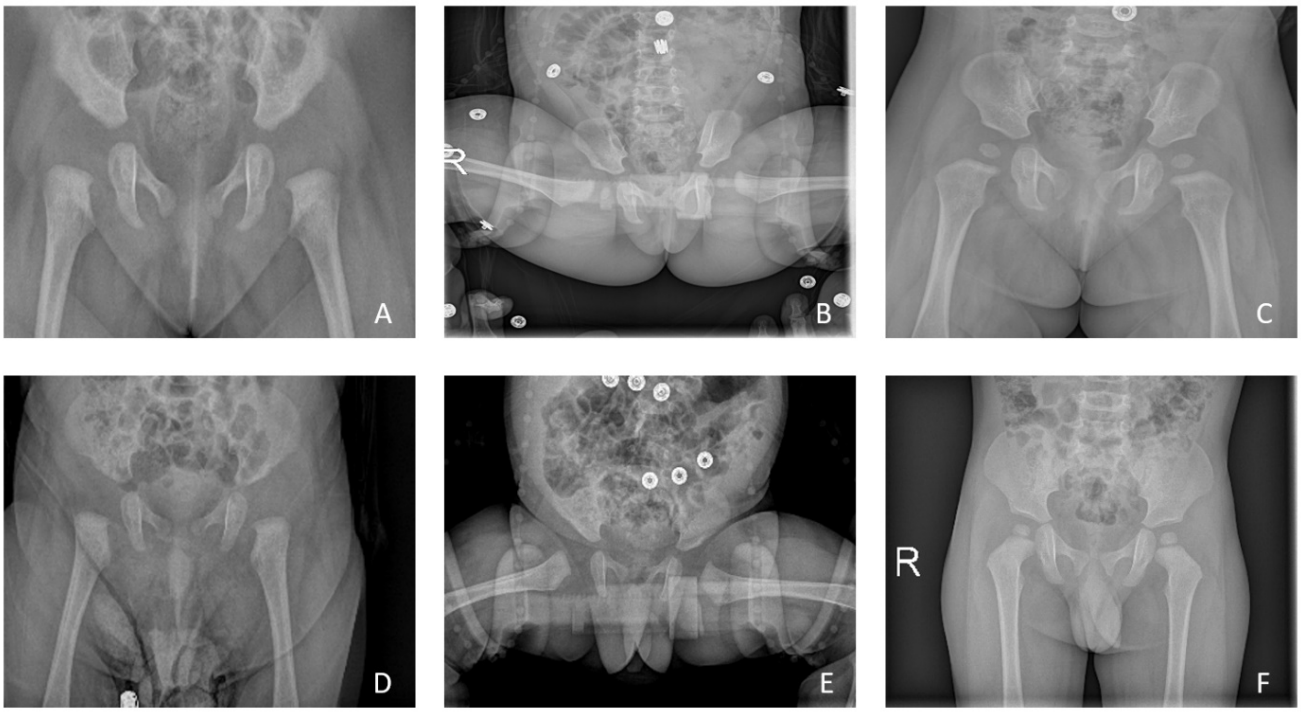

Fig. 3. Case examples of this study. A) First hospital admission (4 months) radiography. B) After applying the Tubingen hip flexion-abduction splint. C) 9th-month control radiography. D) Another patient, first hospital admission (4 months) radiography. E) After applying the Tubingen hip flexion-abduction splint. F) 19th-month control radiography

4. Discussion

Developmental dysplasia of the hip (DDH) is a disease which can be successfully treated with early diagnosis and conservative methods. The primary goal in treating developmental dysplasia of the hip is to achieve the concentric reduction by keeping the hip in flexion and abduction and maintaining this condition without avascular necrosis of the femoral head (4).

DDH is a disease with multifactorial etiology. Positive family history and breech presentation are the most common risk factors for DDH (5).

In the study by Pavone et al. (6) published in 2015, 10,274 hips of 5137 infants in the first three months of life in Italy were examined. As a risk factor, a breech presentation was found in 10.09% of patients, and positive family history was found in 7.12% of patients. In our study, five patients (10%) had a positive family history, and 2 (4%) patients had a breech presentation. Köse et al. (5) reported that positive family history was the most common risk factor. According to this study, DDH occurred in 1.34% of 4173 babies swaddled in Central Anatolia. Swaddling was detected in 2 (4%) of our patients. Despite many studies conducted over the years, the swaddling of babies is still common in our country, as seen in this study. Therefore, it continues as a risk factor.

In our study, the success rate was 94.7%. One of the factors affecting the treatment process is the Graf stage at the beginning of the treatment. Van de Sande et al. (7) reported 73% success rate for Graf type 3 and 30% success for Graf type 4. it is seen that the success rates are low especially in Graf type 4 patients. Sluijs et al. (8) stated that prolonged treatment was useless in Graf type 4. Walton et al. (9) reported that the patients they treated with the Pavlik bandage were unsuccessful in all of the dislocated and irreducible patients and that the Pavlik bandage was not an effective treatment method for this group. In the study of Seidl et al. (10) forty-nine of the fifty patients (98%) were successfully treated, and the only patient who failed was a child with type 4 hip. In this study, the failure rate was found to be higher in hips that were found to be Graf type 3 and 4. Similar to literature, we suggest that as the stage increases, the success rate decreases.

Among the researchers who started early treatment, Bin et al. (11) reported the mean age of starting treatment as five days, Atalar et al. (12) as eight weeks, and Uçar et al. (13) as 14.8

weeks. In some other studies, treatment was started at an average age of 4 months, similar to our research (4, 14). When we compare the studies performed with Tubingen hip flexion-abduction splint with our research, although we have a later onset age, similar success rates (92%) were observed, so we think there is no significant relationship between the age of onset of treatment.

Proximal femoral avascular necrosis rates have been reported between 2.38% (15) and 20% (16) in patients treated with the Pavlik bandage. It has been suggested that more than 110 degrees of flexion are the leading cause of avascular necrosis. The major factor for avascular necrosis in DDH treatment is forced abduction and flexion (17, 18). During treatment with THFS, abduction is adjustable with the rigid part of the splint between legs. In our study, we adjusted the splint so that 10° to 15° of passive abduction was possible.

In the literature, hip flexion was adjusted between 80 and 110 degrees in studies with a Pavlik bandage (11, 12, 19), and it is consistent with our study. In our study, flexion was set to 90-110 degrees and gradually decreased to 70° as treatment progressed to prevent avascular necrosis of the femoral head. Atalar et al. (12) stated that less than 90 degrees of flexion will direct the femoral head to the acetabulum's superior and is unsuitable for reduction.

It has been stated that parental compliance is essential for the treatment with Tubingen to be successful (10). In our study, attention was paid to family harmony, detailed information was given, and the appropriate use of the splint was ensured. And quadriceps muscle function were examined at every follow-up visit.

In our study, femoral nerve palsy and avascular necrosis of the femoral head was not reported during the Tubingen splint treatment, and no skin problems were encountered.

Grill et al. (15) noted that the straps preventing adduction caused a skin problem, negatively affecting the family and child harmony.

It has been stated that parental compliance is essential for the treatment with Tubingen to be successful (10). In our study, attention was paid to family harmony, detailed information was given, and the appropriate use of the splint was ensured.

THFS allows free movement of the knees and ankles and it is easy to use, practical, family-friendly, and easy to clean thanks to its plastic structure. As a result of this study, the Tubingen hip flexion-abduction splint is an effective and successful method for treating developmental dysplasia of the hip.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

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

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 20/07/2017 and the number of ethical committee decisions is 2017/260.

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Determination of the working conditions of emergency nurses

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Abstract

Working conditions are important in achieving patient and employee safety, quality care and favorable patient outcomes. The study was carried out in a descriptive design to determine the working conditions of emergency nurses in the context of personal rights. The sample of the study consisted of a total of 133 nurses working in nine private and one university hospital on the European side of Istanbul. The data were collected from January 2008 through October 2008. The data were analyzed using frequency, percentage distribution, mean and standard deviation. The majority of the emergency nurses were young, inexperienced and high school and associate degree graduates (72.2%), they were oriented to the institution and the department, they thought that they could not be promoted because they were poorly educated (59.4%), their performance was regularly evaluated (58.6%), they were worried that their salary was not sufficient (80.4%), they did not receive additional compensation because they were working in the emergency department (75.2%), they did not get enough leave because of long weekly hours and long daily hours; also they benefited from services of the department of occupational health and safety (96.3%), experienced health problems after starting to work in the emergency department (75.2%), were frequently exposed to verbal violence (79.7%), and experienced the thought of dismissal (75.2%). As a result, it can be claimed that emergency nurses are faced with negative situations in terms of working hours and breaks, working style, leaves, awareness about the leave rules and procedures, remuneration, career development, employee health and job security.

Keywords: emergency unit, emergency nurse, working conditions, personal rights, labor legistation

1. Introduction

Nursing is a profession that is responsible for the protection, development, treatment and rehabilitation services of the health of the individual, family and society (1). While providing these services, the working environment and working conditions have an important role in determining the quality of service (2). High-quality health care service also improves both patient satisfaction and personal satisfaction. Healthcare professionals need to work in a trouble-free environment in order to provide quality healthcare services (3).

While the conditions of the working environment affect not only the nurses but also the individuals they care for, it is stated that positive/favorable working conditions are important in achieving patient and employee safety, quality care and positive patient outcomes (4)

In the literature, there are studies showing that when a healthy working environment is provided for nurses, patient falls, pressure sores and mortality rates decrease, so it leads to better pain management of patients, increased quality of care and patient satisfaction (5-8). Therefore, considering its positive effect on nurse and patient outcomes, the importance of creating a healthy working environment for nurses is clear. Therefore, the importance of creating a healthy working environment for nurses is obvious when considering the positive effect on nurse and patient outcomes.

Although healthy work environments for nurses is a very important issue in terms of providing effective and efficient health services, it is also stated that a healthy and supportive work environment with basic standards cannot be provided for nurses (9). A negative work environment can lead to problems such as absenteeism, stress, stress-related illness and poor performance (4,10).

The emergency department, which is defined as the front door of hospitals, is the unit where the first care is applied especially to patients who require life-saving and emergency intervention, and emergency units provide uninterrupted service for individuals all over the world (11,12). Emergency nurses, on the other hand, work in these units, which are the most active, intense, stressful, and complex departments of hospitals, where life is aimed to be saved, patients requiring emergency intervention are evaluated, treated and cared for, and they are exposed to many unfavorable working conditions (13). This situation causes deterioration in the physical and psychological health of nurses, loss of workforce, increase in medical errors and decrease in the quality of care (13,14). Therefore, creating a healthy work environment is a necessary condition to deliver quality care. As a matter of fact, "Health Transformation Program in Turkey" which is reported by Ministry of Health in 2003, emphasized that work

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environments must first be improved in order to realize the quality and accreditation component for qualified and effective health services. (15).

In this context, it is thought to be important to determine the work conditions that can lead to and significantly affect situations such as stress, job satisfaction and exhaustion that may adversely affect the provision of quality health care services by emergency nurses. Accordingly, in the study, it was aimed to determine the working conditions of emergency nurses in the context of personal rights, especially on the basis of national and international labor legislation (Labour Act, Civil Servants' Act No. 657 (16,17) and the recommendations of the International Labor Organization (ILO) on nurses (18).

Research question: How are the working conditions of nurses working in emergency units in terms of personal rights?

2. Materials and Methods

2.1. Type of the study

This was a descriptive study. This study followed the STROBE reporting standard for cross-sectional studies. In this research, answers to the following questions were sought.

Q1. How are the working conditions of nurses working in emergency units in terms of personal rights?

2.2. Setting and participants

The population of the study consisted of nurses working in the emergency units of the Ministry of Health, university and private hospitals on the European side of Istanbul, and the sample of the study consisted of 170 nurses working in the emergency units of 9 private and 1 university hospitals where the necessary permissions for the study were obtained from hospitals with 100 beds or more (25 hospitals) by purposive sampling method. The study was completed with 133 nurses who agreed to participate in the study. The return rate of the study was 78.2%.

2.3. Characteristics of the hospitals included in the research

The university hospital where the research was conducted is a hospital affiliated with Istanbul University, with the highest bed capacity and providing services in all branches. In addition, nursing services have received the International Standard Organizations (ISO) quality certificate. Five of the private hospitals have been accredited by the international accreditation institution Joint Commission on Accreditation of Healthcare Organizations (JCHAO) and are among the most modern Group A hospitals in Istanbul. Another hospital is on its way to becoming a large healthcare group and continues its quality work. The other three hospitals are smaller and not certified in terms of service quality.

2.4. Data collection

The data of the study were hand-delivered to the nurses working in the emergency unit who voluntarily accepted to participate in the study by making the necessary explanations and then collected back by the researcher. The data were collected using as Personal and Professional Information Form and Personal Rights Information Form from January 2008 through October 2008. A total of 170 questionnaires were distributed and 133 completed forms were included in the statistical evaluation.

Personal and Professional Information Form: The form consists of five questions about age, educational status, institution where person staffs, professional experience, and emergency unit experience.

Personal Rights Information Form: In the form prepared by the researchers based on the national labor relations act, Labor Act, Civil Servants Law and International Labor Organization-ILO recommendations on nurses, the personal rights of nurses were questioned systematically. It consisted of a total of 36 questions to determine orientation to the institution and unit after starting work, continuous training (6 questions), working hours and leaves (8 questions), performance evaluation, remuneration, reward-punishment system, career development (11 questions), occupational health and safety and job security (11 questions).

2.5. Data Analysis

The data of the study were analyzed using frequency, percentage distribution, mean and standard deviation with SPSS 18 statistical program.

3. Results

It was determined that the emergency nurses participating in the study were in the age group of 23-25 (44.4%) ($\bar{X}$±SD: 24.51±3.76), graduated from high school and associate degree (72.7%), worked in a private hospital (83.5%), had 2-4 years of professional experience (42.1%) ($\bar{X}$±SD:4.24±3.65), an average of 2.57±2.73 years of institutional experience and 2-4 years (47.4%) ($\bar{X}$±SD: 3.05±3.2) of emergency unit experience (Table 1).

It was determined that the continuing education and orientation training received by emergency nurses adequately prepared them for the work environment (72.9%), that they received unit-specific training before starting to work in the emergency department (59.4%), that the emergency nurse had a job description (86.5%), and that they participated in institution/unit orientation and continuing education programs (85%). In addition, it was determined that the working hours of emergency nurses were harmonized so that they could participate in continuing education programs (72,2%), and the personnel rights of emergency nurses were also addressed in these training programs (67.7%) (Table 1).

Table 1. Distribution of personal and professional characteristics of emergency nurses (n:133)

Personal and professional variables	Sub-variables	n	%
Age X̄±SD: 24.51±3.76	≤ 22 years	27	20.3
	23-25 years	59	44.4
	≥ 26 years	47	35.3
Educational status	High school+associate degree	96	72.2
	Bachelor's degree	37	27.8
Institution of employment	University hospital	22	16.5
	Private hospital	111	83.5
Occupational experience X̄±SD: 4.24±3.65	1 year and below	26	19.6
	2-4 years	56	42.1
	5 years and more	51	38.3
Institutional experience X̄±SD: 2.57±2.73	1 year	52	39.1
	2-4 years	47	35.3
	5 years and more	34	25.6
Emergency unit experience X̄±SD: 3.05±3.29	1 year and below	46	34.6
	2-4 years	63	47.4
	5 years and more	24	18
Preparing status of the received nursing education for the profession	Yes	97	72.9
	No	36	27.1
Status of receiving unit-specific training before working in the emergency	Yes	79	59.4
	No	54	40.6
Existence of job description of emergency nurse	Existent	115	86.5
	Non-existent	18	13.5
Status of participating in in-service training programs	Yes	113	85
	No	20	15
Arrangements that facilitate participation in in-service training programs	Allowing	37	27.8
	Harmonization of working hours	96	72.2
The status of handling the personal rights of nurses in in-service training programs	Yes	90	67.7
	No	43	32.3

It was determined that the continuing education and orientation training received by emergency nurses adequately prepared them for the work environment (72.9%), that they received unit-specific training before starting to work in the emergency department (59.4%), that the emergency nurse had a job description (86.5%), and that they participated in institution/unit orientation and continuing education programs (85%). In addition, it was determined that the working hours of emergency nurses were harmonized so that they could participate in continuing education programs (72,2%), and the personnel rights of emergency nurses were also addressed in these training programs (67.7%) (Table 1).

Regarding the working hours and leaves of emergency nurses, it was determined that nurses worked in two shifts (85.7%), daily working hours were 12 hours during the day and 12 hours at night (57.9%), total working hours per month were 193-220 hours (51.1%), monthly overtime hours were 21-50 hours (46.6%), and daily rest periods ranged between 31-50 minutes (66.2%). It was determined that monthly overtime hours varied between 21-50 hours and daily rest periods varied between 31-50 minutes (66.2%). It was determined that emergency nurses' work lists were announced in less than a week (36.1%), they were able to use their weekly leaves (71.4%), and they were compensated for their work on duty, overtime, and public holidays (51.9%), while on-call, Nurses also stated that they receive normal wages (16.5%) for working overtime and on public holidays. In addition, they did not know whether they were able to use their right to legal leave before and after birth (60.1%), they could use unpaid leave after birth (52.6%), they could use maternity leave (57.9%), and whether they had legal leave before and after birth. It was determined that there were also nurses (36.1%) who expressed this (Table 2).

When the opinions of emergency nurses regarding performance evaluation, remuneration, reward-punishment system and career development were examined, it was found that the performance of nurses was regularly measured with criteria appropriate to job descriptions (58.6%), unsuccessful nurses were frequently warned (50.3%) and performance bonus was not paid (20.3%). When emergency nurses compared their wage/salary with the wages paid in other hospitals, it was determined that they did not earn enough salary (90.2%). It was determined that the level of education (76.7%), the unit worked in (66.2%), and the duration of experience in the institution (50.3%) were often taken into consideration when making salary adjustments, that no additional compensation was given to nurses working in the emergency unit (75.2%), and that there was no loss of position in cases such as interruption of work/change of department (71.4%). It has also been stated that emergency nurses do not think about working in the institution they work for many years and moving to higher positions (59,4%), the reason for this is that their training for these positions is not sufficient (46,8%), and there are also predetermined people for these positions (20,3%) and they do not receive the necessary support to be promoted (51,1%) (Table 3).

Table 2. Distribution of emergency nurses' characteristics regarding working hours and leaves (n:133)

		n	%
Working/shift pattern	Two shifts	114	85.7
	Daytime only	13	9.8
	Night only	6	4.5
Working hours per day	9 hours during the day 15 hours at night	6	4.5
	10 hours during the day 14 hours at night	50	37.6
	12 hours during the day 12 hours at night	77	57.9
Total working hours per month	≤ 192 hours	37	27.8
	193-220 hours	68	51.1
	≥ 221 hours	28	21.1
Monthly overtime hours	≤ 20 hours	26	19.6
	21-50 hours	62	46.6
	≥51 hours	8	6
	None	37	27.8
Total rest time per day	≤ 30 minutes	6	4.5
	31-50 minutes	88	66.2
	≥51 minutes	39	29.3
Pre-announcement period of work lists	Less than a week	48	36.1
	A week	43	32.3
	15 days	27	20.3
	One month	15	11.3
Weekly leave status	Yes	95	71.4
	No	25	18.8
	Sometimes	13	9.8
Remuneration for working on duty, overtime, and public holidays	I'm taking leave	69	51.9
	I get 50% increased salary	34	25.6
	I get 100% increased salary	8	6
	I get regular salary	22	16.5
Ability to take leave before and after birth	Yes	80	60.1
	No	5	3.8
	Don't know	48	36.1
Availability of breastfeeding leave in the unit	Yes	77	57.9
	No	3	2.3
	Don't know	53	39.8

Table 3. Distribution of emergency nurses' characteristics regarding performance evaluation, remuneration, reward-punishment system and career development (n:133)

		n	%
Regular measurement of performance with appropriate criteria	Yes	78	58.6
	No	55	41.4
Type of sanctions applied to unsuccessful nurses	No performance bonus is given	27	20.3
	Not being promoted	13	9.8
	Being laid off	13	9.8
	A warning is given	67	50.3
	The reason for the failure is being	13	9.8

	investigated		
Giving awards to successful nurses	Yes	48	36.1
	No	64	48.1
	Don't know	21	15.8
Type of award given to successful nurses	Plaquet	7	14.6
	Giving performance bonus	6	12.5
	Promoting	7	14.6
	Certificate of appreciation	28	58.3
Comparing one's own wage/salary with the wage paid to emergency nurses by other hospitals	Enough	13	9.8
	Not enough	120	90.2
Criteria taken into consideration when determining the salary level in the institution*	Education level	102	76.7
	Experience in the institution	67	50.3
	Working unit	88	66.2
	Professional experience	42	31.6
	Working/shift type	22	16.5
Additional compensation payment to employees in the emergency unit of the institution	Yes	26	19.6
	No	100	75.2
	Don't know	7	5.2
Job/department change or starting to work in a lower position after a break	Yes	21	15.8
	No	95	71.4
	Don't know	17	12.8
Thinking about working in the organization for many years and moving to higher positions (promotion)	Yes	54	40.6
	No	79	59.4
Reasons for not considering promotion	Presence of other persons considered for senior positions	53	20.3
	Requirement for higher education	26	46.8
Status of providing the necessary support for promotion	Yes	25	48.9
	No	108	51.1

Table 4. Distribution of emergency nurses' characteristics regarding occupational health, safety and security (n:133)

		n	%
The most frequently encountered risky situations in the emergency department*	Verbal assault	106	79.7
	Physical assault	18	13.5
	Infection	41	30.8
	Needlestick injury	61	45.9
	Falling	14	10.5
	Radiation	9	6.8
Presence of security guards in the emergency deparment	Yes	103	77.4
	No	30	22.6
Existence of occupational health unit in the institution	Yes	82	61.7
	No	51	38.3
Easy access to occupational health unit	Yes	79	96.3
	No	3	3.7
Health services provided in the health unit of the	Regular periodic examination	53	39.9

institution*	Periodic control and protective measures for risky situations	64	48.1
	Examination and treatment in cases of illness	105	78.9
Situation of being examined and treated in the institution in case of illness	Yes	104	78.2
	Emergencies only	29	21.8
Experiencing health problems after starting to work in the emergency unit	Yes	100	75.2
	No	33	24.8
Health problems experienced after starting to work in the emergency unit *	Extreme nervousness	49	49
	Digestive disorders	25	25
	Sleeping disorders	72	72
	Headache	37	37
	Stomachache	22	22
	Backache	37	37
The general approach of the institution in work accidents	No specific policy	19	14.2
	Compensation is paid	3	2.3
	Put on leave until recovery	61	45.9
	Precautions are taken to prevent recurrence	32	24.1
	Workplace accident procedures are implemented	50	13.5
Consideration of dismissal if the organization's expectations are not met	Yes	100	75.2
	No	33	24.8
Notification of reasons for dismissal to nurses in the institution	Yes	84	63.1
	No	32	24.1
	Don't know	17	12.8
Payment of compensation in case of dismissal	Yes	28	21.1
	No	28	21.1
	Don't know	77	57.8

*More than one option is selected.

Regarding occupational health and safety and job security, it was stated that the most common risky situations encountered by emergency nurses were being subjected to verbal attacks by patients and their relatives (79.7%), needle sticks (45.9%) and infection risk (30.8%) and that there were security guards in emergency units (77.4%). It was found that emergency nurses had an occupational health unit in the hospitals where they worked (61.7%) and that they could easily benefit from this unit (96.3%), that nurses benefited from this unit by being examined and treated in case of illness (78.9%), that interim control and protective measures were taken for risky situations in these units (48.1%), and that they could be examined and treated in the institution where they worked in case of illness (78.2%). It was found that the nurses encountered a health problem after they started working in the emergency department (75.2%), and the health problems were sleep disorders (72%), excessive irritability (49%), headache (37%), back pain (37%), digestive disorders (25%), and stomach pain (22%). In addition, it was determined that in cases of illness, injury, accident, etc. that occurred on the job, emergency nurses were given leave until they recovered (45.9%). Finally, it was stated that emergency nurses thought that they would be dismissed if they did not meet the expectations of the organization (75.2%), that the reasons for dismissal were reported in case of dismissal (63.1%), and that they did not know whether compensation would be given in case of dismissal (57.8%) (Table 4).

4. Discussion

In this article, the data were collected from a total of 133 nurses working in the emergency units of nine private and one university hospital. In this study, which aimed to determine the working conditions of nurses in the context of personal rights.

It has been revealed that emergency nurses are faced with negative situations in terms of working hours and breaks, working style, leaves, awareness about the leave rules and procedures, remuneration, career development, employee health and job security.

This study shows that the undergraduate education received by emergency nurses prepared them for the working environment and that they received unit-specific training before being assigned to the emergency unit. However,

Elçioğlu et al. (2021) found that 66.7% of the emergency nurses stated that they did not receive any training specific to the emergency unit after graduation (19). The finding obtained from the study can be evaluated as a very positive situation in terms of the quality of nurses' vocational training and patient and employee safety.

In the study, it was stated that emergency nurses participate in continuing education programs, and the institution regulates the working hours of nurses to facilitate participation in these trainings. Unlike the research finding, Yetik Aras (2019) revealed that half of the emergency nurses (50,2%) had not participated in any scientific training in the last year (20). One of the important criteria of quality and accreditation studies is to support the continuous training of employees. When the finding of our study is considered together with the information that the hospitals included in the sample are accredited or continue their quality studies, it can be considered as a situation that is expected to support and encourage continuous education by the hospital.

In the study, almost all the emergency nurses stated that they were aware of their job descriptions and responsibilities. Duran et al. (2013) supports the research finding that 57.4% of emergency workers have job descriptions (21).

Although it was determined in the study that emergency nurses were given training on their personal rights, when the findings related to working conditions (working hours, leaves, reward-punishment systems, etc.) are evaluated as a whole, it is contradictory to see that nurses do not know some of their rights. It can be claimed that the source of this contradiction is related to the fact that nurses are not sensitive enough about their personal rights, even though they are given training on issues that they do not have problems with.

According to another finding of this study, it was determined that emergency nurses mostly worked in 12-hour shifts during both day and night. The finding of Kebapçı and Akyolcu (2011) that emergency nurses work in shifts is parallel to the finding of our study (13). The finding of our study is in line with the finding of our study that Söyük and Arslan Kurtuluş (2017) stated on-call hours in emergency units are very long, and the night work is carried out under very harsh conditions compared to other employees (3). The finding obtained from the study is contrary to the recommendations of the International Labor Organization (ILO) regarding nurses. ILO states that nurses should not work more than eight hours a day and may work overtime for a maximum of four hours a day, but not frequently (ILO-R157-Nursing Personnel Recommendation-I33) (18). However, the fact that nurses should not work 14-16 hours, especially in night shifts, in a very busy and attention-requiring unit such as an emergency should be considered as an issue that needs to be examined.

When the monthly working hours of emergency nurses were considered in our study, it was found that nurses worked between 193-220 hours per month. In Kebapçı and Akyolcu's (2011) study, it was found that they worked between 150-200 hours per month (13), and in Özata et al.'s (2017) study, it was found that they worked 101-200 hours per month (22). While they are required to work 45 hours per week and 180 hours per month on average based on the Labor Act (16), the fact that their rest periods are very low suggests that emergency nurses work very intensely and do not get enough rest. The serious shortage of employees in the health system, especially in recent years, has caused many hospitals to try to provide services by overworking the few staff they have. As a result, nurses are forced to work long hours and provide services without adequate rest. This situation significantly increases the likelihood of medical errors. The finding that the nurse managers prepare the emergency work lists in a period of one week or less may make it difficult for emergency nurses to organize their social life and lead them to lead an unplanned life that is dependent on the unit they work in. As a matter of fact, in the recommendations of ILO, it is recommended that arrangements related to work shifts should be made before a period of time that will not prevent nurses from planning their social life (ILO-R157-Nursing Personnel Recommendation-I35) (18).

When the findings related to the leave of emergency nurses are examined; although emergency nurses mostly use weekly leave, the fact that 18.8% of emergency nurses stated that they could not use weekly rest leave and received normal wages for overwork (16.5%) is not a regulation in accordance with national labor legislation (Labor Act and DMK No. 657) and ILO decisions. The Labor Act and ILO resolutions specifically state that all nurses should use their weekly rest leave and be subject to additional remuneration for overtime work (Article 41 of the Labor Act, ILO R157-Nursing Personnel Recommendation-I37).

In the study, the fact that emergency nurses stated that they were using prenatal and postnatal leave, postnatal legal unpaid leave, and milk leave was found to be compatible with the national labor legislation; however, it is thought-provoking that emergency nurses do not know about the right to use prenatal and postnatal leave, the right to legal unpaid leave, and the right to use milk leave. Although the majority of emergency nurses received training on personal rights, the fact that they did not know about their legal rights related to pregnancy and motherhood can be considered as a situation related to their young age.

When the performance evaluations of emergency nurses were examined, the fact that the majority of the nurses stated that their performance was measured regularly with appropriate criteria can be interpreted as well-functioning performance evaluation systems since most of the sample consisted of private hospitals that carry out quality studies and are accredited, whereas the performance evaluations of nurses in public hospitals are not carried out regularly. The fact that

nurses who do not perform appropriately are given warnings and/or performance bonuses are not paid suggests that the results of the performance appraisal system are used to punish rather than to improve the individual and overcome deficiencies.

In our study, it was found that emergency nurses did not consider their wages sufficient when compared with the wages of emergency nurses working in other hospitals. In Kebapçı and Akyolcu's (2011) study, it was found that emergency nurses found their wages partially sufficient (13).

In the study, regarding the career development of emergency nurses, the finding that emergency nurses do not lose their position when their job/department is changed or when they take a break in the profession can be considered as a positive situation in terms of employee rights. In addition, it is stated in the recommendations of ILO that the career development of nurses should not be disrupted, and they should not lose their status due to interruptions in their professional life (ILO R157-Nursing Personnel Recommendation-I22-23). The fact that emergency nurses think that they cannot be promoted to higher positions in the institutions where they work and emphasize the lack of appropriate education as the reason for this is consistent with the findings related to the lack of adequate education and job security of emergency nurses. These findings can be considered as a result of the fact that licensed nurses are preferred for managerial positions in many private hospitals, especially in hospitals conducting accreditation studies, and education is included as a criterion in the appointment criteria. In addition, the fact that the emergency nurses who participated in the study stated that other people were considered for the positions to be promoted and that they did not receive sufficient support for promotion suggests that hospitals may consider different criteria for promotion other than qualifications such as education and merit.

It has been determined that emergency nurses are most frequently subjected to verbal attacks regarding occupational health and safety, and they also face risks such as needle sticks and infection. In studies conducted in the national and international literature, the determination that employees in emergency units are most exposed to verbal violence is parallel with the research findings (3, 23-31). In the studies of Özata et al. (2017), the most common risks faced by emergency service workers were verbal violence and infectious diseases (22), and Parlar Kılıç et al. (2016) study, the fact that needle sticks were the most common biological risk factors encountered by emergency nurses supports the research finding (28).

Although it was stated in the study that there are security guards in most emergency units, the fact that emergency workers are exposed to violence suggests that security measures are not taken at the desired level. In the studies of Duran et al. (2013) and Çoşkun and Karahan (2019), the finding that security measures were taken in the emergency units of most emergency workers, but were not sufficient, coincides with the findings of our research (21,26). In the studies of Söyük and Arslan Kurtuluş (2017), the failure to ensure the safety of employees in emergency units is expressed as a problem, which is parallel to the findings of our study (3).

The fact that nurses have an occupational health unit in the institution where they work, and that they can easily benefit from this unit can be associated with the obligation to have these units in private hospitals, which constitute the majority of the sample of the study, in accordance with the legislation (Labor Act).

The fact that most emergency nurses have complaints such as sleep disorders, extreme irritability, headache, and backache after they start working in the emergency department can be considered as a result of the negative working conditions. Similarly, in the study of Parlar Kılıç et al., (2016), it was observed that complaints such as stress, low back pain and insomnia were among the psychological and physiological disorders encountered by emergency nurses. In the study, it should be emphasized that despite the work accidents occurring in emergency units, the institution does not have a specific policy and the approach of giving the affected employee leave only until he recovers, but taking precautions to prevent recurrence is adopted at a low rate. In the study of Duran et al. (2013), they revealed that there is an infrastructure to prevent occupational accidents, the management takes corrective and preventive actions on this issue and solutions are produced, but they are not at a sufficient level (21). While it is expected that hospitals that receive quality certificates have adopted policies aimed at taking protective measures and preventing recurrence, research findings do not fully support this situation.

Finally, in the study, it was determined that emergency nurses often had the thought of being fired, and the reasons for their dismissal were often communicated to them, and they did not know whether they would receive compensation in case of dismissal. This situation can be considered as a result of the research being conducted in a sample predominantly of private hospitals. Although emergency nurses working in private hospitals work in accordance with the Labor Law, the findings suggest that private hospital managers do not act in accordance with the Labor Law. As a matter of fact, the Labor Law states the conditions for terminating the employment contract, mentions the rights of the employee, and emphasizes that the employment contract cannot be terminated unilaterally without a valid reason (Labor Act No.17, 18, 19, 20, 21, 22). In addition, this study found that nurses do not know their rights, suggesting that there may be problems in monitoring their personal rights.

4.1. Study limitations

The limitation of the study is that because private hospitals constitute the majority, the data collected from university hospitals are numerically small, and MoH Hospitals were

excluded from the sample due to lack of permission, comparative statistical analyzes could not be made for institutional differences, which are the determinants of working conditions, and the findings had to be examined in terms of percentage values. In addition, the research findings are limited to the sample and cannot be generalized to all nurses working in emergency units.

As a result of the findings obtained from the research, it was found that emergency nurses received training before being assigned to the emergency unit, they could participate in continuing education programs, their performance was regularly evaluated with appropriate criteria and low-performing nurses were warned, they mostly worked in two shifts, they could use their weekly leaves, their annual leaves were in accordance with the labor legislation, prenatal and postnatal leaves and milk leave were in compliance with the legal regulations related to working life, but a significant majority of nurses did not know their legal leave rights, they benefited from the occupational health units of their institutions, and they were generally given leave in case of work accidents; It was concluded that health problems such as sleep disturbance, excessive irritability, headache and low back pain increased after starting to work in the emergency unit, and although there were security guards in the emergency units, emergency nurses were frequently verbally assaulted, mostly emergency nurses had thoughts of dismissal, and they did not have information about compensation in case of dismissal.

In line with these results, since the nurses in the emergency units are young and inexperienced, new graduates should be started in these units, since it makes it easier for them to experience occupational burnout, the preferences of the employees should be taken into consideration in determining the unit where they will work, although emergency nurses have received training on personal rights, the fact that they do not have enough information about working hours, leaves, compensation payment, etc. should be emphasized and these issues should be addressed primarily in basic nursing education, nursing services managers should give more importance to rewarding while using the performance evaluation system, It can be suggested that the employees working in the emergency unit should not be overworked for 12 hours or more, care should be taken to ensure that they are given enough time off to rest, manpower planning should be done well to prevent health problems that may arise from working in the emergency unit, psychological support should be provided for the employees, rotation method should be used when necessary, security measures should be improved, both the physical structure and the number and quality of the officers should be increased, especially private hospital managers should create personnel policies that eliminate the fear of dismissal for employees, and managers should adopt a participatory management approach in open communication with their employees.

Conflict of interest
The authors declared no conflict of interest.

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Authors' contributions
Concept: N.U.B., U.B. Design: N.U.B., U.B. Data Collection or Processing: N.U.B. Analysis or Interpretation: N.U.B. Literature Search: N.U.B., U.B. Writing: N.U.B., U.B.

Ethical Statement
The study protocol was approved by the Cerrahpasa Faculty of Medicine Ethics Committee (date/no: 09.01.2007/1570). Before starting the research, written and verbal permissions were obtained from the administration of the relevant hospitals. The data collection tool was distributed to the nurses who voluntarily agreed to participate in the study. This study was conducted in accordance with the principles of the Declaration of Helsinki.

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Prescription habits to geriatric patients in psychiatry clinic-university hospital and training and research hospital comparison

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Abstract

This study aimed to examine the psychotropic drug prescribing habits of clinicians from different clinics for patients over the age of 65 who applied to the psychiatry outpatient clinic. Patients over 65 who applied to the psychiatry outpatient clinic in January 2020 were included in the study. As a result of the inclusion criteria, 523 patients, 241 from university and 282 from training and research hospitals, were included in the study. Age, gender, diagnoses, past psychiatric disease histories, and recommended treatments of patients were obtained from electronic files in the hospital automation system. Antidepressant treatment was used in 228 (94.6%) patients in the university hospital and in 232 (82.3%) patients in the training and research hospital ($p<0.001$). Clinicians preferred monotherapy for 71% (n: 171) of the patients in the university hospital and 56.4% (n: 159) in the training and research hospital ($p=0.001$). Selective serotonin reuptake inhibitors (SSRI) are the most commonly used antidepressant group in both the university hospital (80.3%) and the training and research hospital (71.5%) ($p=0.022$). Escitalopram was the most frequently used SSRI in both the university hospital (54.7%) and the training and research hospital (42.8%) ($p=0.027$). Atypical antipsychotics (96.5%) constituted most antipsychotic preferences in the university hospital, and quetiapine (90.9%) among atypicals. Among the antipsychotics, atypical antipsychotics (97.1%) were preferred most frequently in the training and research hospital, and quetiapine (59.4%) was the most common choice among them. The side effect profile is as important as the drug's effectiveness in selecting psychotropic medications in the geriatric period. For this reason, among antidepressants, serotonin reuptake inhibitors, and among antipsychotics, atypical antipsychotics are the first drug groups used.

Keywords: elderly, escitalopram, quetiapine, outpatient

1. Introduction

It is a symbolic approach to define the senior period as age from a certain cut-off point. There is no agreed-upon age. However, 65 years of age is the most emphasized age. With the increase in life expectancy worldwide, the proportion of the senior population in society is expected to increase. In our country, while the ratio of people over 65 to the general population was 5.6% in 2000 and 9.0% in 2019, this rate is expected to be 10.2% in 2023 and 16.3% in 2040 (1). In the United States (USA), this rate, which was 14% in 2019, is expected to exceed 20% in 2026. A demographic feature of this period is the higher proportion of women in this population due to their longer life expectancy (2). It can be expected that the number of geriatric patients that psychiatrists will encounter will increase, depending on the proportional and numerical increase in the number of geriatric patients.

Physiological changes brought about by ageing and increasing disease frequency make geriatric patients more susceptible to many factors. Physiological changes such as the decrease in body water ratio and lean tissue mass, increase in body fat amount, shift of stomach acidity to alkaline, reduction in liver and kidney functions, and decrease in drug metabolism with age can make people in the senior period more sensitive to the side effects of drugs. At the same time, common medical diseases in this period can cause drug-disease interactions (3). For these reasons, various guidelines are being developed to control drug use in the geriatric age. (4). According to STOPP (Screening Tool for Older Persons' Potentially Inappropriate Prescriptions) criteria, inappropriate drug use was observed in 25.6% of geriatric patients (5). It is known that 29.9% of individuals over 65 use five or more drugs. One of this group's most frequently used drug groups is antidepressants (6).

The use of antidepressants in the geriatric period can be in depressive disorders, anxiety disorders, alcohol substance use disorders, and other psychiatric diseases, as well as in conditions other than psychiatric diseases (7). The frequency varies between countries. In recent years, selective serotonin reuptake inhibitors (SSRI) have become the first preferred antidepressant group in all age groups due to their efficacy and side effects advantages (8). Compared with 20 years ago, the rate of antidepressant use in the geriatric population has increased 2.79 times (9). In a multinational study investigating antidepressants used for the first time in the senior period between 2009 and 2014, Tricyclic antidepressants (TCA) were found to be the most commonly used antidepressant group in the UK and Taiwan, and SSRIs in Canada and the USA. The drugs chosen within the groups also vary between countries (7). Antidepressants used in old age may cause a decrease in bone mineral density (especially for drugs in the SSRI group)

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and adverse effects on the cardiovascular system (especially for drugs in the TCA group) (10).

Antipsychotics can be prescribed for various reasons at an advanced age. Individuals in the geriatric period are susceptible to the side effects of antipsychotics. It has been reported that there is an increase in the side effects of cardiovascular disease, stroke, pneumonia, and extrapyramidal system side effects of antipsychotics in geriatric ages compared to young ages and that they may increase the risk of death. It is necessary to carry out the use of antipsychotics carefully in patients with dementia. Despite this information, insufficient attention is paid to the use of antipsychotics in elderly patients in the clinic (11). Antipsychotics can be used off-label at low doses in sleep-wake disorders (12). The side-effect potentials of antipsychotics differ between groups and drugs. It has been found that these risks may be higher in typical antipsychotic group drugs (13). Quetiapine has less stroke possibility, metabolic side effects and mortality, more falling than olanzapine; It has been shown to cause more metabolic side effects than risperidone (14).

This study aimed to examine the prescriptions written to patients over the age of 65 who applied to the psychiatry outpatient clinic. An evaluation will be made regarding the antidepressant and antipsychotic drugs used and their groups.

2. Material ve Methods

2.1. Participants and data collection

Patients over 65 who applied to the Ondokuz Mayıs University Faculty of Medicine and Kanuni Sultan Suleiman Training and Research Hospital psychiatry outpatient clinic in January 2020 were included in the study. The study did not include patients evaluated for consultation, medical board, or forensic reasons. The first application was evaluated in the case of two or more applications. During that period, 2341 applications to the university hospital and 3980 to the training and research hospital were received. Of these applications, 296 in the university hospital and 439 in the training and research hospital belong to the geriatric population. A total of 523 patients, 241 from the university hospital and 282 from the training and research hospital, who met the criteria for inclusion in the study, were included. Age, gender, past psychiatric disease history, diagnoses, and recommended treatments of the patients were obtained from electronic files in the hospital automation system.After the patient diagnoses were obtained, the diagnoses of the patients were divided into groups as anxiety disorders, depressive disorders, trauma-stress related disorders, sleep-wake disorders, neurocognitive disorders, and other disorders according to The Diagnostic and Statistical Manual of Mental Disorders-5 (DSM 5)

2.2. Statistical analysis

SPSS 15.0 package program was used for the statistical analysis of the study. The data of categorical variables are given as n (%). The age variable is given as mean±standard deviation. In the comparisons of categorical variables, the Chi-square test was applied. A value of $p<0.05$ was accepted as statistically significant.

3. Results

Of the geriatric patients who applied to the outpatient clinic, 162 (67.2%) in the university hospital and 181 (64.2%) in the training and research hospital were women. The mean age of the patients was 73.1±6.2 in the university hospital and 72.8±6.4 in the training and research hospital. The majority of geriatric patients who applied to the outpatient clinic, according to DSM 5 diagnosis categories, were composed of anxiety disorders and depressive disorders, respectively, both in the university hospital (48.5% and 22.4%) and in the training and research hospital (49.6% and 30.5%). It was determined that 33 (13.7%) patients in the university hospital and 8 (2.8%) patients in the training and research hospital had their first application to psychiatry ($p<0.001$). Monotherapy was preferred in 171 (71%) university hospital patients and 159 (56.4%) training and research hospital patients ($p=0.001$). Antidepressant treatment was used in 228 (94.6%) patients in the university hospital and in 232 (82.3%) patients in the training and research hospital ($p<0.001$). An antipsychotic drug was prescribed to 57 (23.6%) of the patients in the university hospital and 104 (36.9%) in the training and research hospital ($p=0.001$). The sociodemographic and clinical characteristics of the patients are given in Table 1.

Tablo 1. Sociodemographic and clinical characteristics of the patients

Variables		UH n (%)	TRH n (%)	X^2	t	p
Gender n (%)	**Male**	79 (32.8)	101 (35.8)	0.530		0.466
	Female	162 (67.2)	181 (64.2)			
Age Mean±Sd	**Male**	72.7±5.7	72.4±6.5		1.395	0.164
	Female	73.4±6.5	72.4±6.4			
	Total	73.1±6.2	72.8±6.4			
Diagnostic groups n (%)	**Anxiety disorder**	117 (48.5)	140 (49.6)	66.205		**** <0.001**
	Depressive disorder	54 (22.4)	86 (30.5)			
	Trauma-stressor-related disorder	27 (11.2)	0 (0)			
	Sleep-wake disorder	24 (10.0)	4 (1.4)			
	Neurocognitive disorder	8 (3.3)	10 (3.5)			
	Other	11 (4.6)	42 (14.9)			
First application n (%)	**Yes**	33 (13.7)	8 (2.8)	21.197		**** <0.001**
	No	208 (86.3)	274 (97.2)			

Form of treatment n (%)	**Monotherapy**	171 (71.0)	159 (56.4)	11.849		** **0.001**
	Combination therapy	70 (29.0)	123 (43.6)			
Preferred drug group n (%)	**Antidepressant**	228 (94.6)	232 (82.3)	18.666		** **<0.001**
	Antipsychotic	57 (23.6)	104 (36.9)	10.671		** **0.001**
	Benzodiazepine	6 (2.5)	5 (1.8)	0.324		0.569
	Mood stabilizer	3 (1.2)	15 (5.3)	6.491		* **0.011**

UH: University Hospital TRH: Training And Research Hospital Sd: Standard deviation. * p <0.05 ** p <0.01

Selective serotonin reuptake inhibitors (SSRI) are the most commonly used antidepressant group in both the university hospital (n: 183, 80.2%) and the training and research hospital (n: 166, 71.5%) (p=0.02). Mirtazapine was prescribed to 26 (11.4%) patients in the university hospital and 48 (20.7%) in the training and research hospital (p=0.500). Escitalopram was the most frequently used SSRI in both the university hospital (54.7%) and the training and research hospital (42.8%) (p=0.027). The second most frequently used SSRI was sertraline in the university hospital (26.3%) and the training and research hospital (36.7%) (p=0.034). While 55 (96.5%) of 57 patients using antipsychotics in the university hospital were prescribed atypical antipsychotics, and 2 (3.5%) were prescribed typical antipsychotics, 10 (9.6%) of 104 patients using antipsychotics in a training and research hospital were prescribed both typical and atypical antipsychotics, 4 (3.9%) were prescribed only typical antipsychotics, and 90 (86.5%) were prescribed only atypical antipsychotics. Atypical antipsychotics (96.5%) constituted most antipsychotic preferences in the university hospital, and quetiapine (90.9%) among atypicals. Among the antipsychotics, atypical antipsychotics (97.1%) were preferred most frequently in the training and research hospital, and quetiapine (59.4%) was the most common choice among them. However, a statistically significant difference was found between the university hospital and the training and research hospital regarding quetiapine preference (p<0.001). In addition, olanzapine was prescribed to 3 patients (5.4%) and risperidone to 2 patients (3.7%) as atypical antipsychotics in the university hospital. In the training and research hospital, 22 patients (21.8%) were prescribed aripiprazole. Detailed information on preferred antidepressants and antipsychotics is given in Table 2.

Tablo 2. Groups of antidepressants and antipsychotics used, and SSRI and atypical antipsychotic preferences

	UH n: (%)	TRH n (%)	X^2	p
Antidepressants used (n:460)	n:228	n:232		
SSRI	183 (80.3)	166 (71.5)	5.218	* **0.022**
Mirtazapine	26 (11.4)	48 (20.7)	0.454	0.500
Trazodone	20 (8.8)	44 (19.0)	0.070	0.791
SNRI	16 (7.0)	55 (23.7)	8.698	** **0.003**
Vortioxetine	5 (2.2)	19 (8.2)	3.557	0.059
TCA	4 (1.8)	7 (3.0)	0.163	0.722
Antipsychotics used (n:161)	n:57	n:104		
Typical antipsychotics	2 (3.5)	14 (13.5)	4.075	* **0.044**
Atypical antipsychotics	55 (96.5)	101 (97.1)	0.149	1.000
SSRI (n:349)	n:183	n:166		
Escitalopram	100 (54.7)	71 (42.8)	4.911	* **0.027**
Sertraline	48 (26.3)	61 (36.7)	4.483	* **0.034**
Paroxetine	14 (7.6)	14 (8.4)	0.072	0.788
Citalopram	10 (5.4)	11 (6.7)	0.208	0.648
Fluoxetine	10 (5.4)	9 (5.4)	0.000	0.986
Fluvoxamine	1 (0.6)	0 (0)	0.910	1.000
Atypical antipsychotics (n:156)	n:55	n:101		
Quetiapine	50 (90.9)	60 (59.4)	16.997	** **<0.001**
Olanzapine	3 (5.4)	14 (13.9)	2.592	0.107
Risperidone	2 (3.7)	6 (5.9)	0.389	0.713
Aripiprazole	0 (0)	22 (21.8)	13.947	** **<0.001**
Paliperidone	0 (0)	15 (14.9)	9.037	** **0.003**
Sulpiride	0 (0)	8 (7.9)	4.592	0.051
Amisulpride	0 (0)	3 (3)	1.666	0.552
Clozapine	0 (0)	2 (2)	1.103	0.541

SSRI: Selective serotonin reuptake inhibitors SNRI: Serotonin noradrenaline reuptake inhibitors TCA: Tricyclic antidepressants UH: University

Hospital TRH: Training And Research Hospital * p <0.05 ** p <0.01

4. Discussion

The current study found that most of the patients who applied to the psychiatry outpatient clinic were women; anxiety and depressive disorders were the most common diagnoses, and most patients had applied to psychiatry before. Monotherapy was preferred as the main treatment method. Antidepressants were preferred more frequently as the drug group used. SSRIs among antidepressants and atypical antipsychotics among antipsychotics were the most selected groups. Escitalopram and sertraline, among antidepressants, and quetiapine, among antipsychotics, are the most commonly used drugs.

Biological, social, and psychological differences brought about by advanced age can have various undesirable consequences on the mental health of individuals. Depending on this, there may be applications to the psychiatry outpatient clinic. In general, depressive and anxiety disorders are the most common reasons for referral to psychiatry outpatient clinics and emergencies in the geriatric period (15, 16).

Experiencing side effects related to antidepressants in the geriatric period increases the possibility of drug change or discontinuation (17). In recent years, SSRIs have been the first line of antidepressant treatment. Having a better profile than other antidepressant groups regarding side effects, having similar results to other antidepressant groups in terms of efficacy, and less possibility of drug interaction are seen as the most important reasons for being a first-line treatment (18). In the senior period, SSRIs are the first-line treatment as antidepressant treatment (19). Therefore, most antidepressants used in the senior period are SSRIs (20). However, there are differences in the use of SSRIs in the geriatric period. However, there are differences in the use of SSRIs in the geriatric period. Among the SSRIs in use, paroxetine is one of the drugs that inhibit CYP enzymes with the highest affinity and has the most increased anticholinergic side effects (21). According to the Beers criteria published in 2019, paroxetine is not recommended for use in senior periods like TCAs due to its anticholinergic properties. However, it is stated that the clinician should make the final decision on a case-by-case basis (22). It is recommended that fluvoxamine should not be preferred in the first place in the senior period due to drug interactions and fluoxetine due to its long half-life. Less binding of escitalopram and citalopram to plasma proteins than other SSRIs may have positive aspects for them to be preferred in old age (23). When the side effect profile and efficacy of escitalopram are evaluated together, it has been shown that it may be a better option than other antidepressants (24). Sertraline is an SSRI that has been found to be safe in the geriatric period in terms of efficacy and side effects (25). For these reasons, as seen in our study, escitalopram, citalopram, and sertraline may be considered more prominent than others in the case of SSRI use at an advanced age (26). However, the person's previous treatment experiences should also be considered when choosing drug therapy.

Benzodiazepines and TCAs have side effects that can have dangerous consequences in old age. Depending on the pharmacokinetic and pharmacodynamic changes that develop due to the regular physiological changes that occur with ageing, there may be various changes in the effects of these drugs. A comparison study conducted between 2004 and 2011 showed that benzodiazepine use decreased by 10% and TCA use by 55% (27).

Mirtazapine is an antidepressant treatment that can be an alternative to SSRIs in the geriatric period. In addition to its antidepressant effect, it is a drug that can be preferred by people who have difficulty falling and staying asleep due to its sedation effect and people who have weight loss or decreased appetite due to increased appetite (28). Trazodone is a relatively safe drug in the geriatric population, primarily preferred in cases with difficulty falling and staying asleep (29).

Quetiapine is an atypical antipsychotic approved for schizophrenia, bipolar affective disorder, and major depressive disorder as adjuvant therapy. In addition, it can be used frequently for posttraumatic stress disorder, anxiety disorders, dementia, and behavioural symptoms of Parkinson's disease and insomnia. Compared with other antipsychotics, there is no increased risk of side effects in advanced age (14). For these reasons, it is an expected finding that it is the most commonly used antipsychotic in geriatric patients.

There were some limitations to this study. First, our study is cross-sectional. Secondly, since the data were obtained from the electronic files of the patients in the hospital automation system, some patient data could not be included in the study because some information was not recorded in the system by the physicians, and the existing data could not be verified by interviewing the patients.

As a result, SSRIs are preferred in the first place due to their safe profile when antidepressant treatment is needed in senior age. Among the antipsychotics, atypical antipsychotics are more preferred than typical antipsychotics. In addition, it is understood that combined treatments are preferred more frequently in geriatric patients in the training and research hospital compared to the university hospital. Research to understand the reasons for this situation will be beneficial.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: K.L., S.Ö., Design: K.L., S.Ö., Data Collection or

Processing: K.L., S.Ö., Analysis or Interpretation: K.L., S.Ö., Literature Search: K.L., S.Ö., Writing: K.L., S.Ö.

Ethical Statement

Approval was obtained from Ondokuz Mayıs University Clinical Research Ethics Committee, the study started. The ethics committee decision date is 13/05/2020 and the number of ethical committee decisions is 2020/347.

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Association between subfatin level and preeclampsia

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Abstract

Preeclampsia is a progressive and pregnancy-specific disorder and it affects multiorgan systems. Although the pathophysiology of preeclampsia is still unknown, it involves both maternal and fetal/placental factors. Endothelial cell damage and impaired endothelial cell function play an important role in the development of preeclampsia. Adipokines have role in many pathophysiological processes in the body. Subfatin is a newly discovered adipokine, if dysfunctional may cause endothelial damage. This study investigated whether subfatin levels can be used as a predictive marker for possible pathophysiology. This study was designed as prospective case-control study. Fifty-six pregnant women who had delivered between gestational age of 37th and 41th weeks with singleton pregnancies in a tertiary reference hospital were included. Pregnant women with a diagnosis of preeclampsia were defined as the case group and normotensive pregnants without a diagnosis of preeclampsia were defined as the control group. There was no statistically significant difference in terms of age, BMI, gravidity and parity between the groups. Mean systolic and diastolic blood pressures were observed higher in case group than control group. Subfatin level of control group was found higher than case group and this difference was statistically significant. Subfatin level ≤ 49.32 ng/mL with 78.6% sensitivity and 71.4% specificity was found significant for case group. Subfatin may have a role in endothelial dysfunction and take part in pathophysiology of preeclampsia. According to this study we suggest that as a newly diagnosed adipokine, subfatin may be helpful in predicting preeclampsia development in pregnant women..

Keywords: adipokine, preeclampsia, pregnancy, subfatin

1. Introduction

Preeclampsia is a progressive and pregnancy-specific disorder and it affects multiorgan systems. New onset of hypertension and proteinuria are the main findings and end-organ dysfunction can be seen. It typically presents in second half of gestation or in postpartum period (1,2). Although the pathophysiology of preeclampsia is still unknown, it involves both maternal and fetal/placental factors (3). Abnormal placental vasculature in early pregnancy may result in relative placental under perfusion which leads to hypoxia and ischemia. Subsequent activation of the intravascular coagulation system, vasospasm, increased platelet activation and consumption are important features (4,5).

In preeclampsia, a significant decrease in uteroplacental blood flow is observed compared with normal pregnancy, and this ischemia is thought to trigger pathophysiological changes (6). The excessive migration and outflow of trophoblasts as a result of placental ischemia probably cause this endothelial cell dysfunction. Endothelial cell damage and impaired endothelial cell function play an important role in the development of preeclampsia. Accumulation of antiangiogenic factors into the maternal circulation may cause hypertension and other systemic dysfunctions of the disease (hematologic, neurologic, cardiac, pulmonary, renal, and hepatic) (7,8).

Adipose tissue secretes bioactive molecules which are called adipokines. These have role in many pathophysiological processes in the body (9,10). The relationship between adiponectin and preeclampsia remains controversial. Studies have shown both increased and decreased concentration of adiponectin in preeclampsia (11-15).

Subfatin is a newly discovered adipokine. In a study investigating the effect of this adipokine, it was suggested that anti-inflammatory genes in adipose tissue was regulated by subfatin and if dysfunctional may cause endothelial damage (16,17).

In consistent with this data from literature, by comparing

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blood subfatin levels of preeclamptic and normotensive pregnant women, we aimed to investigate whether subfatin level can be used as a predictive marker for a possible pathophysiology.

2. Materials and Methods

This study was designed as prospective case-control study. G*Power was used for calculating the sample size (power, 80%; p=0.05 and an α-value of 0.05). 20 women for case group and 20 women for control group, total of 40 women were calculated for the sample size. Fifty-six women who had delivered between gestational age of 37th and 41th weeks with singleton pregnancies in a tertiary reference hospital between March 2022 and June 2022 were included in study. Pregnant women with a diagnosis of preeclampsia were defined as the case group and normotensive pregnants without a diagnosis of preeclampsia were defined as the control group. Exclusion criteria included patients with multiple pregnancies, comorbidities (cardiovascular disease, Diabetes Mellitus), preterm premature rupture of membranes, inflammatory bowel disease, maternal infections (TORCH), uterine malformations, fetal malformations, trisomies, collagen-vascular diseases, polyhydramnios, maternal teratogenous drug use, sickle cell anemia, hereditary thrombophilia and age of <18 or >40 years. This study has been approved by the Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital Local Ethics Committee (2022/26-16/02/2022) and it was conducted in accordance with the Declaration of Helsinki. Written and signed informed consent was obtained from all participants before study. The study group included 28 women with term pregnancies and with diagnosis of preeclampsia. The control group included 28 normotensive term pregnancies without diagnosis of preeclampsia.

Demographic data including age, gravidity, parity, Body Mass Index (BMI) and gestational age were noted. The gestational age estimation was calculated according to the last menstural period and first trimester ultrasonography (11th-14th weeks). The maternal and neonatal data were recorded and ultrasonographic measurements were made at the date of hospitalization and labor. After sit down and relaxation for 10 minutes blood pressure measurement was taken from the left upper arm. Venous blood samples were taken when patients were admitted for delivery. Samples for Subfatin centrifuged at 1000xg for five minutes, stored in -80 oC and analyzed within three months. Serum level of subfatin was measured by the ELISA technique.

2.1. Statistical Analyses

Statistical analyzes was done with the SPSS version 26. Histogram and Shapiro-Wilk normality tests were used to determine the distribution of variables. Descriptive statistics were presented as mean ± standard deviation. X2 tests were used to compare categorical variables. For categorical variables number and percentage (n, %) was used. The independent-samples T test was used for comparison of nominal data. Receiver Operating Characteristic (ROC) analysis was used to for calculating the predictive value of Subfatin for preeclampsia. Area under the curve (AUC), cut-off value, sensitivity and specificity were calculated according to ROC analysis. 95% confidence interval (CI) and p value of < 0.05 were considered significant.

3. Results

Fifty-six pregnant women was included in study as 28 in case group and 28 in control group. Table 1 shows the comparison of demographic features and perinatal outcomes of two groups. In terms of age, BMI, gravidity and parity there was no statistically significant difference between groups (Table 1). Mean systolic (157.14±15.36 vs 113.21±6.55 mm/Hg, $p < 0.001$) and diastolic blood pressures (95.71±8.35 vs 73.21±5.47 mm/Hg, $p < 0.001$) were observed higher in case group than control group (Table 2).

All laboratory results were statistically similar except for platelet count and BUN levels. Platelet count (265.75±80.54 vs 225.25±66.77 x103/L, $p = 0.045$) and BUN level (35.42±33.81 vs 57.60±31.32 mg/dl, $p = 0.014$) were higher in control group (Table 2).

There was no difference between groups in terms of gestational age (39 vs 38 w, $p = 0.234$) and birth weight (3339.82±424.23 vs 3082.32±563.91 g, $p = 0.059$).

Subfatin level of control group was found higher than case group and this difference was statistically significant (58.89±17.66 vs 31.85±16.58 ng/mL, $p < 0.001$).

According to ROC analyzes; cutoff value of Subfatin level ≤ 49.32 ng/mL with 78.6% sensitivity and 71.4% specificity was found significant for case group. AUC was calculated as 0.872 ($p < 0.001$) (Fig.1).

Table 1. Comparison of demographic features and perinatal outcomes of case and control groups

	Control (n=28)	Case (n=28)	p value
Age (years)	27.28±4.25	30.28±7.76	0.080
BMI (kg/m^2)	29.05±3.52	29.7±3.49	0.492
Gravidity (n, range)	2 (1-6)	3 (0-6)	0.721
Parity (n, range)	1 (0-5)	1 (0-4)	0.462
Gestational age (week)	39 (37-40)	38 (37-39)	0.234
Birth weight (gram)	3339.82±424.23	3082.32±563.91	0.059

Table 2. Comparison of clinical and laboratory results of case and control groups

	Control (n=28)	Case (n=28)	p value
Systolic Blood Pressure (mm/Hg)	113.21±6.55	157.14±15.36	**<0.001**
Diastolic Blood Pressure (mm/Hg)	73.21±5.47	95.71±8.35	**<0.001**
Hb (mg/dl)	10.96±3.66	11.13±3.87	0.860
Platelet (x10^3/L)	265.75±80.54	225.25±66.77	**0.045**
BUN (mg/dl)	57.60±31.32	35.42±33.81	**0.014**
Creatinine (mg/dl)	4.28±1.11	3.41±3.6	0.229
ALT (U/L)	12.96±11.98	20.82±29.61	0.199
AST (U/L)	20.39±6.28	27.46±31.84	0.254
Subfatin level (ng/mL)	58.89±17.66	31.85±16.58	**<0.001**

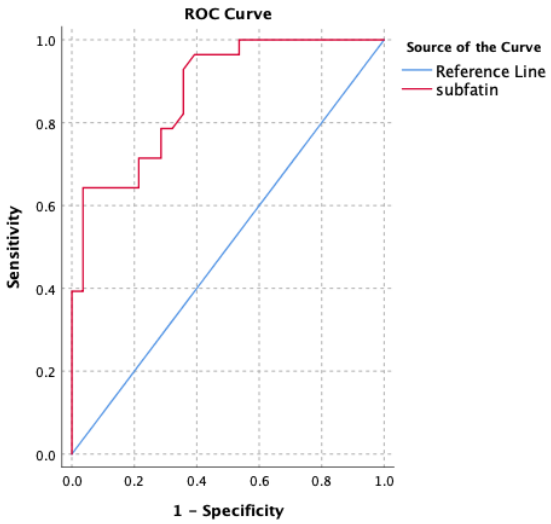

Fig. 1. ROC curve for Subfatin (AUC=0.872, an optimal cutoff ≤ 49.32, 78.6% sensitivity, 71.4%speficity)

4. Discussion

The mechanisms involved in the development of preeclampsia are not yet clearly understood. For this reason, studies are currently being conducted on numerous biomarkers for the detection and treatment of preeclampsia. In recent years, studies on the coexistence of plasma adipokines and preeclampsia have been conducted and have gained popularity (11-15).

Adiponectin has a role in trophoblast proliferation, differentiation and invasion of the decidua and decidual angiogenesis. These are the major phases of placentation. Adu-Gyamfi et al. found that while pregnancy get term adiponectin level physiologically decreases. According to this study, adiponectin takes role in placentation and protect from preeclampsia (18). Another study by Poston et al. relationship between adipokine, leptin, and preeclampsia were evaluated. Elevated levels of leptin in the blood have been shown to cause preeclampsia by increasing sympathetic nervous system activity (19). Similarly, Bawah et al, found that low adiponectin level and high levels of leptin, resistin and visfatin were found to be significant predictors of preeclampsia (20). Rao et al. compared the maternal serum adiponectin and leptin levels and their ratio between preeclamptic and normotensive women. They found that adiponectin-leptin ratio could be considered as a biomarker for preeclampsia (21). Based on these studies in the literature, in this study it was aimed to evaluate the relationship between subfatin, new adipokine, and preeclampsia. In preeclamptic women subfatin levels were found significantly lower than normotensive women and low levels of subfatin was found independent risk factor for preeclampsia. Low levels of subfatin which is effective in endothelial dysfunction may have a contribution to development of preeclampsia.

Lee et al. found that in nondiabetic women subfatin levels were higher than newly diagnosed diabetic women (22). In a study by Dadmanesh et al. serum Subfatin levels were found lower in patients with coronary artery disease (CAD) and type 2 diabetes compared to the control group (23). El-Ashmawy et al. found subfatin levels were low in Type 2 diabetic patients. (24). Contrastly, Chug et al.and AlKhairi et al. found that in subjects with type 2 DM blood Subfatin levels are increased compared to non-diabetic patients (25-26). In a recently published meta-analysis, there was no significant correlation between serum subfatin level and type 2 DM and CAD (27). AlKhairi et al. mentioned that impaired endothelial function and atherosclerosis may be related to subfatin level. Subfatin level may be the independent risk factor of Type 2 DM and also can be used as a marker for endothelial dysfunction.

Fadai et al found that serum subfatin level was low and serum adhesion molecules level was high in diabetic women. In diabetic patients subfatin and vascular adhesion molecules showed a negative correlation. This reduction in subfatin level and its negative correlation with vascular adhesion molecules may suggest endothelial dysfunction in diabetic women (28).

In patients with CAD, subfatin level was found lower than control group (23, 29). Yilmaz et al. evaluates the relation between subfatin level and acute myocardial infarction (AMI), subfatin levels were found significantly lower in non-ST elevated myocardial infarction than control group (30). This may be the result of relatively prolonged and permanent cellular injury in non-ST elevated myocardial infarction.

There have been conflicting results about BMI and subfatin

level. Pellitero et al. showed that subfatin levels were found low in obese women (31). But in another study no correlation was found between subfatin level and BMI (22). In our study, groups were similar in terms of age and BMI and there was no relation between subfatin and BMI.

One of the strengths of the study is subfatin level was investigated for the first time in preeclamptic women. Homogeneity of case and control groups in terms of influencing factors such as age and BMI is another strength. The main limitation of this study is that the number of patients in the kit study was kept to a minimum for economic reasons. Future studies which compare the first and second-third trimester subfatin levels and investigates its predictivity for preeclampsia and other diseases will shed light on the literature.

Subfatin may play a role in endothelial dysfunction and may be involved in the pathophysiology of preeclampsia. According to this study, we suggest that subfatin, as a newly diagnosed adipokine, may be useful in predicting the development of preeclampsia in pregnant women. These data should be supported by studies with a large number of cases.

Conflict of interest

Authors declared no conflict of interest.

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Ethical Statement

This study has been approved by the Ankara Etlik Zubeyde Hanım Women's Health Training and Research Hospital Local Ethics Committee (2022/26-16/02/2022) and it was conducted in accordance with the Declaration of Helsinki.

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None.

Authors' contributions

Concept: C.K., B.K., F.B.F., Y.A.R., G.B., B.Ş., Y.E.Ü. Design: C.K.,B.K., F.B.F., Y.A.R., G.B., B.Ş., Y.E.Ü. Data Collection or Processing: C.K.,B.K., F.B.F., Y.A.R., G.B., B.Ş., Analysis or Interpretation: C.K.,B.K., Literature Search: C.K.,B.K., F.B.F., Writing: C.K.,B.K., F.B.F, Editing and Supervison: Y.E.Ü.

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Research Article

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Primary malignant bone tumours of hematopoietic origin: A single-centre, restrospective study

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Abstract

Tumors of hematopoietic origin are rare tumors that can be confused with benign and malignant tumors of the bone with their clinical and morphological features. Plasma cell myeloma, solitary plasmacytoma, Non-Hodgkin Lymphoma, acute lymphoblastic leukemia/lymphoma, and Langerhans cell histiocytosis are hematological neoplasms that mainly involve the bone. Plasma cell myeloma is the most common primary malignant bone neoplasm, and diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma arising in bone. In this case report, we will retrospectively investigate the diagnosis and treatment management and postoperative clinical outcomes of patients diagnosed with tumors of hematopoietic origin, evaluated by the Bone and Soft Tissue Tumor Council between 2005 and 2021, and emphasize the importance of the approach to these tumors.

Keywords: hematopoetic myelodysplasia, multiple myeloma, bone neoplasms, leukemia

1. Introduction

Neoplasias of hematopoietic origin are seen extremely rarely in bones. The most widely seen hematological neoplasias primarily involving the bone are plasma cell myeloma, solitary plasmacytoma, non-Hodgkin's lymphoma, acute lymphoblastic leukemia/lymphoma, and Langerhans cell histiocytosis (1). Although tumors of hematopoietic system origin are rarely seen, the clinical and morphological characteristics may be confused with malignant bone tumors and may also be confused with benign proliferations of the bone. This should be kept in mind in the differential diagnosis.

In this study, a retrospective investigation was made of the demographic data obtained from patients presented to the Bone and Soft Tissue Tumour Council,the diagnosis and treatment management of these tumors and the importance of the approach to these tumors are emphasized.

2. Material and Method

A retrospective examination was made of patients who presented at the Orthopaedics and Traumatology Polyclinic between January 2005 and December 2021, underwent advanced examination and were diagnosed with bone metastasis of hematopoietic origin. A record was made of the patient's age, gender, complaints, and definitive diagnosis. The clinical findings, radiological images, and pathology results, if present, were evaluated. All the patients included in the study were diagnosed radiologically and /or pathologically with bone tumors of hematopoietic origin.

3. Results

The evaluation was made of a total of 40 patients with bone tumors of hematopoietic origin diagnosed following bone biopsy or postoperatively from operation material, who presented at the Orthopaedics and Traumatology Polyclinic between 2005 and 2021.

The patients comprised 22 females(%55) and 18 males(%45) with a mean age of 57.45 years. The patients were classified as 18 multiple myelomas, 11 plasmacytoma, 9 non-Hodgkin's lymphoma, 1 Langerhans cell histiocytosis, and 1 amyloidosis (Table 1).

Table 1. Distribution of hematopoietic origin tumours

Hematopoietic origin tumours	n=40	%
Multiple Myeloma	18	45
Plasmacytoma	11	27.5
Non-Hodgkin's lymphoma	9	22.5
Langerhan's cell histiocytosis	1	2.5
Amyloidosis	1	2.5

Tumour localization was determined as mostly (42.5%) in the proximal femur. Involvement was seen in the femur in 20 patients, the humerus in 5, the tibia in 1, the pelvis in 6, the vertebra corpus in 1, the clavicle in 1, and the radius in 1. There was seen to be extramedullary involvement in the proximal thigh in 2 patients, in the arm in 1, in the anteromedial thigh in 1, and in the groin in 1. The diagnosis was made following a pathological fracture in 14 patients (37.5%).

Treatment was planned according to the patient's age,

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clinical condition, and tumor localization. The most frequently selected treatment was wide resection and tumor resection prosthesis (n=27), followed by curettage, cementation, and intramedullary nailing (n=6). Conservative follow-up with a splint was applied to 1 patient. Vertebroplasty was performed on 1 patient with vertebral involvement. The remaining 5 patients were referred to the medical oncology unit, with the decision to conservative orthopedic follow-up. Surgical treatment was performed on 2 patients diagnosed with multiple myeloma due to multiple involvement (Fig. 1 and 2).

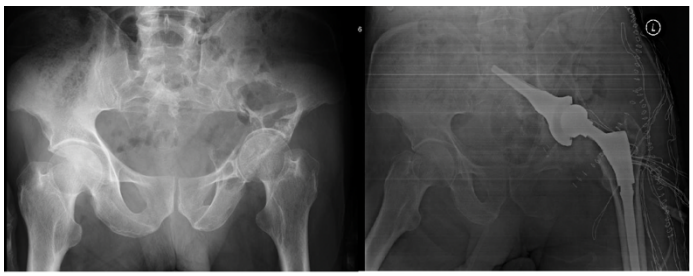

Fig. 1. In a 69-year-old male patient with multiple myeloma metastasis in the left iliac wing, a modular tumor prosthesis was applied following iliac wing resection

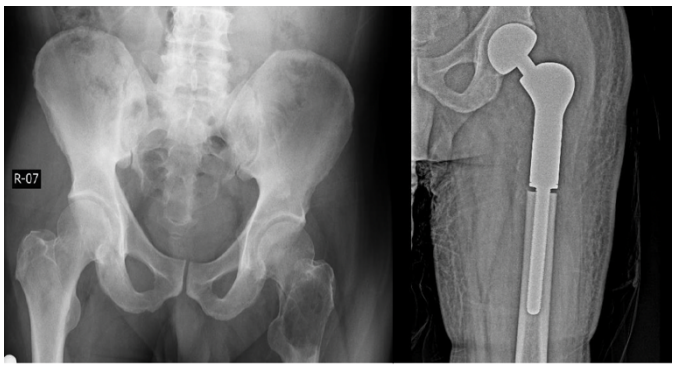

Fig. 2. A 53-year-old male patient with multiple myeloma metastasis in the left proximal femur was treated with proximal femur resection prosthesis.

4. Discussion

The skeletal system is the organ most frequently affected by metastatic cancers, and tumors originating from the breast, prostate, thyroid, lungs, and kidneys have a tendency to spread to the bones (2, 3). Neoplasias of hematopoietic origin are seen extremely rarely in bones. The hematopoietic-origin neoplasias primarily involving the bone are plasma cell myeloma, solitary plasmacytoma, non-Hodgkin's lymphoma, acute lymphoblastic leukemia/lymphoma, and Langerhans cell histiocytosis.

Multiple myeloma is the most frequently seen primary malignant bone neoplasm, and the complication seen most often in patients with multiple myeloma is bone involvement. Osteoclast over-activation is a process that disrupts the balance of bone remodeling, including the inhibition of osteoblasts, osteocytes, and bone marrow stromal cells (4). Of the 40 patients in this study with metastasis of hematopoietic origin, 18 (45%) were diagnosed with multiple myeloma. Due to multiple involvements in 1 patient diagnosed with multiple myeloma, a bilateral humerus shaft and femur proximal third fracture developed, and these were treated surgically (Fig. 3).

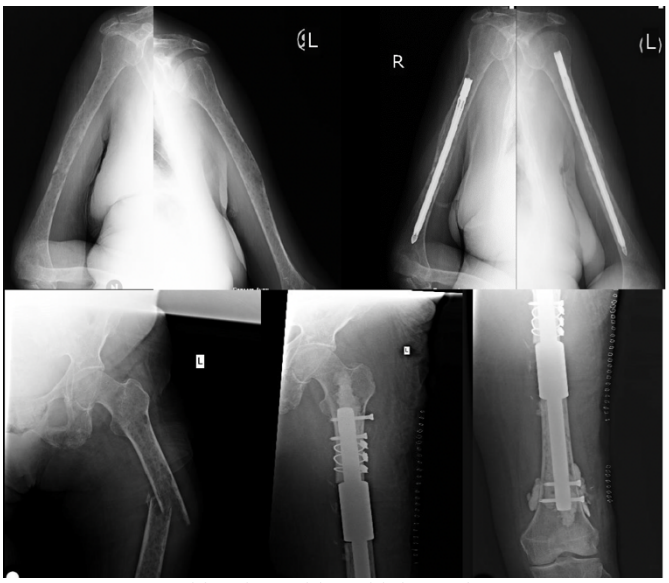

Fig. 3. A 67-year-old patient with multiple myeloma metastases was treated with bilateral humerus intramedullar nailing, and an intercalar tumour prosthesis for a left femur shaft fracture

Solitary plasmacytomas are rare and constitute <5% of plasma cell neoplasms. Solitary bone plasmacytomas generally form in the spine and skull, almost always emerge in the head and neck, and are more common than extramedullary plasmacytomas, which can spread to regional lymph nodes (5). In the current study, 11 (27.5%) patients were diagnosed with plasmacytoma, and involvement was determined in the proximal femur in 6 of these, in the distal femur in 1, in the clavicle in 1, in the proximal humerus in 1, and in the pelvic region in 2.

The most common subtype of malignant lymphoma is diffuse large B-cell lymphoma. Primary bone lymphoma (PBL) is rare and constitutes <1% of all non-Hodgkin's lymphoma. PBL is approximately 3% of all malignant and primary bone tumors (6). All bones in the skeletal system can be involved, but the long bones are most often affected, and the most commonly affected bone is the femur (7). In the current study, 9 (22.5%) patients had non-Hodgkin's lymphoma involvement, and of these 9, a diagnosis of extramedullary involvement was made in 6 cases.

Langerhans cell histiocytosis is originating from the uncontrolled proliferation and accumulation of immature myeloid dendritic cells of bone marrow origin and is an uncommon but serious inflammatory neoplasia. Any bone may be affected, but >50% of bone lesions are in the skull, ribs, and pelvis. Clinical presentation may be with pain, swelling, a soft tissue mass, sometimes bone deformity, and bone fracture (8). In the current study, an 11-year-old male patient was applied with curettage and grafting because of a lesion in the acetabulum, and the pathological diagnosis of the cyst material was consistent with Langerhans cell histiocytosis.

Bone metastases cause severe morbidity. Pain, restricted movement, hypercalcemia, pathological fracture, spinal cord or nerve root compression, and bone marrow infiltrations are

among the main complications (9). Metastatic destruction of the bone reduces the weight-bearing capacity of the bone and initially causes deterioration of the trabecular structure and microfractures, followed by loss of bone integrity. In the current study, 14 (37.5%) patients with bone involvement presented with complaints of pathological fracture.

Radiographs are generally the first step in the evaluation of bone metastases. Osteolytic and osteoblastic lesions may be determined. Scintigraphy is a highly sensitive method in screening bone metastases. In the early period, an increase in osteogenic activity is helpful in the determination of bone metastasis. Computed tomography (CT) and magnetic resonance imaging (MRI) are expensive but very sensitive and specific methods (9, 10). Prophylactic surgical procedures such as curative resection and reconstructive prostheses lead to better survival rates than osteosynthesis of pathological fractures in some patients (11). Arthroplasty, intramedullary nailing, and bone cement injections are other surgical procedures for the treatment of bone metastases.

Limitations of the current study were primarily the retrospective design and relatively low number of patients. A reason for the low number of patients was that only patients with bone metastases of hematopoietic origin who presented at the Orthopaedics and Traumatology Clinic were included. Finally, the treatment section was not given in detail in the study. Therefore, there is a need for further studies to examine larger patient populations with a primary malignant bone tumor of hematopoietic origin and discuss surgical and non-surgical treatments in more detail.

Tumors of hematopoietic origin are rarely seen, but the clinical and morphological characteristics may be confused with malignant bone tumors, so this must be kept in mind in the differential diagnosis. Patients of advanced age, in particular, presenting with atraumatic fractures, must be screened in respect of malignancy, must be examined in detail in respect of pathological fracture, and a treatment plan must be made. With the appropriate diagnostic tools and a multidisciplinary approach, adjuvant treatment selections, and the selection of extremity-sparing surgery as a result, it is possible to obtain successful treatment results.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: İ.B., Design: T.C., O.M, Data Collection or Processing: Ş.Ş., T.C. Analysis or Interpretation: N.D., H.S.C., Literature Search: Ş.Ş., O.M, Writing: H.S.C

Ethical Statement

Approval for the study was granted by the Institutional Ethics Committee (decision no: B.30.2.ODM.0.20.08/342-459 dated:27.07.2022).

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Research Article

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The relationship between the severity of the disease and burden on the caregivers

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Abstract

This study aimed to evaluate the relationship between the severity of the disease and the burden on the caregiver in rehabilitation patients. The study included 45 caregivers and 45 neurological rehabilitation patients (hemiplegia, paraplegia, and tetraplegia). Caregiver Strain Index (CSI) was applied to participants for caregiver burnout, Nottingham Health Profile (NHP) was applied for patients' quality of life, To assess sleep quality, the Pittsburgh Sleep Quality Index (PSQI) was applied and Beck Depression Inventory (BDI) was applied for symptoms of depression. The Barthel Index was used to measure patients' performance in daily life activities. The average motor functional independence score of our patients was 44.29 ± 20.70. In the correlations of CSI with NHP, BDE, PSQI, and Barthel, CSI-PSQI (r: 0.443 p: 0.002), CSI-BDE (r: 0.382 P: 0.01), NHP2-CSI (r: 0.417 p:0.004), and NHP social isolation-CSI were positively correlated (r:353 p: 0.017). BARHEL-CSI was negatively correlated (r:-0.332 p: 0.026). According to the regression analysis, PSQI, BDE, NHP social isolation, and NHP2 values (p: 0.002, p: 0.014, p: 0.017, p: 0.004) had a significant positive effect on CSI. Barthel ındex had a negative effect. The higher the level of addiction in rehabilitation patients, the greater the stress experienced by caregivers. Exhaustion of those who are interested in people with disabilities will also put a burden on the patient and society.

Keywords: caregiver strain ındex, burden, caregiver, quality of life

1. Introduction

Stroke, which is the most common neurological disease in the world, is one of the leading causes of disability in the adult population (1). Cerebrovascular events (CVEs), paraplegia and tetraplegia in addition to causing motor function disorders, also significantly affect patients' daily life activities with emotional changes. Neurological, functional, and cognitive disabilities may leave a patient psychologically shattered and physically dependent on others (2).

Caregivers are defined as individuals who provide basic support and medical care to people with cancer, disabilities, injuries, or chronic illnesses in home and community-based settings. In dependent patients, caregivers are given significant responsibilities for the patient's ongoing care and rehabilitation (3). Caregivers may sometimes have to provide care beyond their personal resources or capacities. As the burden on caregivers increases, so do the risks in terms of psychological consequences (4).

Epidemiological studies have shown that the survival rate increases following diseases that cause motor function disorders. Especially in the prevention of complications, rehabilitation, and ensuring good care, the role of caregivers becomes even more important (5).

Neurological diseases have negative psychological effects on both families and patients.

Depression, sleep disorders, and deterioration in quality of life are commonly observed in individuals who take care of the patient during the care process, treatment, prevention of complications, and recovery at home (6-8). While there are many publications in the international literature on the incidence and severity of these problems in individuals, data is limited in our country (9,10).

Based on this, we aimed to evaluate the relationship between the severity of the disease and the burden on the caregiver, as well as the effects on the caregiver in our study.

2. Materials and Methods

In the study, 45 individuals who care for patients with neurological diseases (hemiplegia, paraplegia, tetraplegia...) aged 18 and over who applied to our hospital's Physical Medicine and Rehabilitation clinics, and 45 neurological rehabilitation patients with whom communication could be established were included.

The inclusion criteria for the study were determined as being 18 years of age or older, providing care for the patient

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for at least 3 months, providing care for 8-10 hours per day, accepting to participate in the study, and being reachable. Caregivers of patients with transient ischemic attack, professional caregivers, non-continuous caregivers, caregivers who did not understand or speak Turkish were not included in the study.

The Caregiver Strain Index (CSI) was applied to the participants for caregiver burnout, the Nottingham Health Profile (NHP) was applied for the patients' quality of life, the Pittsburgh Sleep Quality Index (PSQI) was applied to evaluate sleep quality, and the Beck Depression Inventory (BDI) was applied for depression symptoms. The Barthel Index was used to measure the patients' performance in daily life activities.

The scales were applied to the individuals by the researcher. The interviews were conducted in a separate room and in private.

The individuals were informed that their names would not be written on the scales and that the information obtained would only be used by the researcher. During the application, any unclear points were explained individually to the person without any guidance. The application of the scales took an average of 30-35 minutes for each individual.

To conduct the research, approval was obtained from the local Ethics Committee (Ethics Committee approval number: 2022-16/24). In accordance with the principles of the Helsinki Declaration, participants were informed about the research, and written and verbal consents were obtained.

Caregiver Strain Index (CSI): Covers issues such as emotional stress, physical needs, and time constraints. It consists of 13 items. Patients respond with yes (1) or no (0). The score is calculated by adding the 0 and 1 answers, giving a score range between 0 and 13 (11). Turkish validity was made by Uğur and friends (12).

Nottingham Health Profile (NHP): Consists of sub-dimensions such as Pain, Emotional Reactions, Energy, Physical Mobility, Social Isolation, and Sleep. Scoring is between 0 and 100. Low scores indicate less impact from the disease, while high scores indicate more impact. Turkish validity was made by Küçükdeveci and friends (13).

Pittsburg Quality Index (PSQI): The scale was defined in 1989 to identify good and poor sleep and to determine sleep quality. Scoring is between 0 and 21. A high score indicates poor sleep quality (14).

Beck Depression Inventory (BDI): It was developed by Aron T. Beck in 1961. It is a 21-item self-assessment scale that measures attitudes and symptoms of depression. It is an easy-to-score scale. 0-9: indicates normal level, 10-18: indicates mild symptoms of depression, 19-29: indicates moderate level of depression, 30-63: indicates severe symptoms of depression.

Barthel Index: It is an assessment scale used to measure an individual's performance in daily life activities. Daily life activities in 10 areas are evaluated. According to the scoring, 0-20 is considered totally dependent, 21-61 is considered severely dependent, 62-90 is considered moderately dependent, 91-99 is considered mildly dependent, and 100 is considered independent(15).

3. Results

The study included 45 patients and 45 individuals who undertook patient care. 71.1% of the caregivers were female, while 28.1% were male. The average motor functional independence score of our patients was 44.29 ± 20.70. The demographic characteristics and clinical information of the patients and caregivers included in the study are presented in Table 1. Table 2 includes the demographic and clinical characteristics of the patients included in the study. One-way ANOVA test was performed among the diagnostic groups. Depression, quality of life scales, mean ± SD, and p-values are shown in Table 3.

Table 1. Distribution of caregivers' socio-demographic characteristics (n=45)

Socio-demographic characteristics	Number of caregivers	Number of patients
Age		
18-24	2	4
25-34	6	0
35-44	15	4
45-54	9	6
>55	13	31
Gender		
Female	32	21
Male	13	24

Table 2. Demographic and clinical characteristics of patients

Parameters	
Age (year) Avg ±SD	63.51±12.05
Gender (%)	
Male	52
Female	58
Duration of illness (month)	9.37±8.06
Etiology (%)	
Thromboembolic	80.6
Hemorrhagic	19.4
Diagnosis n (%)	
Hemiplegia	37 (82.7)
Paraplegia	5 (11.1)
Other	3 (6.7)
Barthel index (patient)	
Motor function Avg±SD	44.29±20.70
Cognitive AVG±SD	23.47±10.95
Total AVG±SD	67.06±28.01

Table 3. Quality of life scales by patient groups and CSI results of caregivers

	Hemiplegia (AVG±SD)	Paraplegia (AVG±SD)	Other (AVG±SD)	p
PSQI	8.2 ±3.4	9±2.3	3.6±4.7	0.07
BDI	19.4± 13.05	24.8 ±14.2	26.3± 7.02	0.505
NHP (Pain)	70.3±36.9	60± 54.7	20.8± 23.6	0.108
NHP (Emotional)	57.5± 34.2	64.1± 34.1	55.2± 41.6	0.911
NHP (Sİ.)	50.3±43.6	92.3±13.7	47.2 ±45.6	0.117
NHP (P.A.)	56.3±34.5	26.2±39.3	64.4±21.5	0.117
NHP1	364.2±166.1	381.2±170.2	249.4±120.5	0.49
NHP2	4.8±2.6	2.8± 3.03	3.3± 0.5	0.19
CSI	8.2±4.3	11±2.2	7±4.3	0.3

NHP: Nottingham Health Profile; BDI: Beck Depression Inventory; PSQI: Pittsburg Quality Index; CSI: Caregiver Strain Index; S.İ: Social İsolation; P.A: physical activation

In the correlations of CSI with NHP, BDE, PSQI, and Barthel, CSI-PSQI (r: 0.443 p: 0.002), CSI-BDE (r: 0.382 P: 0.01), NHP2-CSI (r: 0.417 p:0.004), and NHP social isolation-CSI were positively correlated (r:353 p: 0.017). BARHEL-CSI was negatively correlated (r:-0.332 p: 0.026). There was no significant relationship between CSI and caregiver age (p>0.005).

According to the regression analysis, PSQI, BDE, NHP social isolation, and NHP2 values (p: 0.002, p: 0.014, p: 0.017, p: 0.004) had a significant positive effect on CSI. Barthel ındex had a negative effect.

The results of the regression analysis of patients' NHP, BDE, PSQI, Barthel scales, and caregivers' degree of strain are given in Table 4.

Table 4. The results of regression analysis on the relationship between patients' NHP, BDE, PSQI, Barthel scales and caregivers' level of distress.

	B	B(Beta)	p	R^2
PSQI	0.52	0.443	0.002	0.19
BDI	0.11	0.365	0.014	0.13
Barthel	-0.05	-0.33	0.026	0.11
NHP (Pain)	0.02	0.23	0.119	0.05
NHP (Emotion)	0.014	0.112	0.46	0.012
NHP (S.İ.)	0.03	0.353	0.017	0.12
NHP (P.A.)	-0.012	-0.09	0.52	0.009
NHP (Sleep)	0.03	0.26	0.07	0.07
NHP1	0.006	0.22	0.13	0.05
NHP2	0.66	0.41	0.004	0.17

NHP: Nottingham Sağlık Profili; BDI: Beck Depression Inventory; PSQI: Pittsburg Quality Index; S.İ: Social İsolation; P.A: Physical activation

4. Discussion

The aim of this study was to evaluate the relationship between the severity of illness in dependent patients and the burden on caregivers, as well as the effects on the caregiver. We found that as limitations in daily life activities, depression, and sleep quality in patients increased, the burden on caregivers also significantly increased. Linear regression analysis revealed a strong association between the Barthel index and caregiver distress index.

Our findings, which showed a significant decrease in the caregiver's burden on CSI as the patient's dependence on the caregiver decreased, are in line with the literature (16,17). Our results indicate the potential value of targeting not only the patient but also the caregiver in dependent patients.

In situations that cause disability, women usually take on the caregiver role, and two-thirds of all caregivers are women (18). In our study, 32 (71.1%) of the caregivers were female. Rivera and colleagues found that caregivers for women with spinal cord injuries were mostly mothers, sisters, or daughters, indicating that psychological problems may be more prevalent (19). The average CSI for male and female caregivers was 8±4.96 and 9.5±2.7, respectively, with no significant difference (p=0.27). These statistics indicate that the increase in CSI is not dependent on the gender of the caregiver. Our findings contradict other studies that have observed higher levels of stress in female caregivers (20). The age group of the caregivers and the relatively short average duration of illness (9.37±8.06 months) may explain the discrepancy.

In the regression analysis results for the patients' impact evaluation, PSQI, BDE, NHP social isolation, and NHP2 values showed a significant positive effect on CSI (p: 0.002, p: 0.014, p: 0.017, p: 0.004, respectively). Sleep disorders are commonly seen in dependent patients. Poor sleep quality can worsen the patient's clinical condition and cause functional impairment. A study conducted on American adults at the national level found that patients with disabilities had shorter sleep duration compared to non-disabled individuals (21,22). Consistent with our evaluations, it was concluded that disrupted sleep quality in patients increased caregiver burden. NHP is an easy and quick test to measure the quality of life in care patients. In dependent patients, feelings of emotional and social isolation have been reported to be worse than in same-aged individuals. Additionally, dependent patients have higher levels of pain and worse sleep scores (23,24). In our study, it was also found that an increase in NHP social isolation values resulted in a significant burden on the caregiver.

We found that an increase in patients' BDI also increased caregiver burden. Depression related to disability is a condition that leads to unsatisfactory rehabilitation, low quality of life, and increased mortality (25). Our clear conclusion from evaluating all measures is that any condition that increases the burden of the disease also increases the strain on the caregiver.

Ultimately, dependency affects not only the lives of patients, but also their caregivers. The higher the level of dependency in stroke patients, the greater the stress experienced by caregivers. The exhaustion of those who take care of people with disabilities will also burden the patient and society. Minimizing exhaustion is a necessity. First, professional support should be provided instead of selecting caregivers from family members or relatives. Programs aimed at reducing caregiver burden should include practices that increase the patient's self-sufficiency and reduce psychological burden.

Limitations: It is our limit that we have a low number of patients included in the study, the degree of addiction of our patients and the more detailed grouping according to their rehabilitation status.

Conflict of interest

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in this article.

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Ethical statement

To conduct the research, approval was obtained from the local Ethics Committee (Ethics Committee approval number: 2022-16/24). In accordance with the principles of the Helsinki Declaration, participants were informed about the research, and written and verbal consents were obtained.

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Authors' contributions

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

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The herbal supplement induces G2 arrest and apoptosis in A549 cells in vitro

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Abstract

In treating cancer, an important health problem, alternative treatment approaches with herbal content continue to be investigated. The study was carried out to determine an herbal supplement's cytotoxic effect and anticancer potential on lung cancer cells. Human lung adenocarcinoma A549 cells were treated with the herbal supplement and cisplatin. The supplement's growth inhibitory and anticancer potential were determined by MTT assay, Annexin V–PI apoptosis assay, cell cycle analysis, and real-time PCR analysis of the apoptosis and cell cycle-related genes. The herbal supplement was determined to have significant growth-inhibitory effects on A549 cells. Results of the apoptosis and cell cycle analyses showed that the supplement caused a significant increase in the rate of late apoptotic cells and induced G2 arrest. Gene expression analysis results showed that pro-apoptotic BAK1, BAX, APAF1, and cell cycle inhibitor CDKN1A significantly increased mRNA levels without altering BCL2 and TP53 expressions in A549 cells. Our experiments have shown that the herbal supplement has a potential anticancer effect on the lung cancer A549 cell line through growth inhibition and apoptosis. However, more in vitro studies and in vivo experiments are needed.

Keywords: herbal supplement, cancer, apoptosis, cell cycle, antiproliferative

1. Introduction

Cancer continues to be an important health problem worldwide. Although there has been an increase in the survival rate for most cancer types in recent years, it has been reported that this rate is lower for lung cancer than for other cancers due to its advanced-stage diagnosis (1). Lung cancer is the second leading cause of cancer-related mortality worldwide, with more than 1.6 million patients diagnosed with lung cancer each year. Most lung cancer patients are diagnosed at an advanced stage and have been associated with an overall survival of about five years. Despite advances in lung cancer treatment, no effective treatment has yet been developed (2). Although the chemotherapy regimen is an effective therapeutic approach in cancer treatment (3, 4), it is known to cause serious side effects such as neutropenia (5, 6). It has also been reported that chemotherapy agents have cytotoxic effects on cancer cells as well as similar toxic effects on healthy cells (7). This negatively affects the quality of life of cancer patients, causes severe side effects, and may even cause non-cancer deaths. However, alternative approaches to cancer treatment are also being developed, and various studies have been reported on the effect of different herbal products on cancer (8-10).

Using supportive herbal formulas is quite common among cancer patients. It is believed that herbal therapies will generally cause fewer side effects for individuals and are effective in obtaining more successful results in the treatment (11). A natural nutrient mixture containing lysine, proline, arginine, ascorbic acid, and green tea extract has been shown to suppress lung carcinoma xenografts in mice (9). Similarly, it has been reported that a rich nutritional supplement containing lysine, proline, arginine, ascorbic acid, green tea extract, N-acetyl cysteine, selenium, copper, and manganese has preventive potential on the development of ureteral lung cancer in mice (12). With this, the search for alternative treatments for cancer treatment, especially lung cancer, continues. In this study, the cytotoxic effect of a commercially available herbal supplement on lung cancer cell lines was investigated. The commercially available product that we refer to as the Diverse Mixture of Herbs (DMH) has been reported that various plants such as Commipharis myrha (Myrrh), Curcuma zedoaria (Cedvar), Elettaria cardamomum (Cardamom), Olea europaea (Olive), Ernyngium (Bullthorn), Illicuim verum (Star anise), Cinnamomum verum (Tarcin), Mysristica and Crocus sativus (Saffron)are prepared by soaking in ethyl alcohol. In addition, as a result of the chemical analysis, it was determined that the product contains YM-53601, L-Arginine, Betaine, Benzanthrone, Lupanine, Germanaism B, Sarpangine, 4-Hydroxycoumarin, Amigdalin, Phenylacetic Acid, Derrustone, Ichtynone, a-Asaron, Visnadin, 16-Fenoxy-o -tetranor PGE2; Osthol, Anthralin, n-Pentadecylamine, 2-Methoxyxanthone, 13-Docosenamide, Sparfloxacin and Triphenylphospha (13). The anti-tumor potentials of YM-53601 and osthol in the content of the product have been supported by various studies (14, 15). In addition, it has been reported that this herbal mixture contains a high concentration of amygdalin compared to other

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components, and amygdalin, a natural glycoside product, inhibits the proliferation of some cancer cells and is important in alternative cancer treatment (16-18). Again, the results of the study performed on lung cancer cell lines revealed the potential therapeutic efficacy of amygdalin for lung cancer by inhibiting the proliferation and migration of cancer cells (19).

2. Materials and Methods

2.1. Cell culture

A549 human non-small cell lung cancer cells, a generous gift from Yeditepe University Regenerative Biology Research Group, were cultured in Dulbecco's Modified Eagle Medium - High Glucose (Sigma-Aldrich, USA) (DMEM-H) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin-100 µg/mL streptomycin (Sigma Aldrich, USA) and 2.5 µg/ml amphotericin B (Sigma Aldrich, USA) at 37°C in a humidified atmosphere of 5% CO_2.

2.2. Cell viability assay

A549 cells were seeded in 96-well plates in at least three technical replicates at a density of $1x10^4$ per well. After seeding, the cells were incubated in a humid 37°C incubator containing 5% CO_2 to adhere to the surface. Cells were exposed to 4%, 8%, and 32% (v/v) DMH herbal mixture prepared in DMEM-H for 24 hours. The herbal mixture was filtered through a 0.22 µm filter before being prepared in the medium. For vehicle control, ethanol 2% was used as in the herbal mixture. The medium of the control group was also refreshed. After 24 hours of incubation, 10 µl MTT reagent (5 mg/ml stock solution) was added to each well, and cells were incubated in the incubator for 4 hours. Then 100 µl of solubilization solution (0.01 N HCL containing 10% SDS) was added to the wells. After 16 hours, absorbances were measured at 570 nm in a microplate reader.

2.3. Apoptosis assay

To determine the apoptotic effect of the herbal mixture at the concentration selected (8% (v/v)) as a result of the cell viability assay, A549 cells were seeded in 6-well plates at a density of $15x10^4$ per well and incubated for 24h. After the incubation, cells were treated with the herbal mixture for 24 h. Ethanol 2% was used as vehicle control. 20 µM of cisplatin (CDDP), which is close to the therapeutic upper limit cisplatin concentration (approximately 16.6 µM) (20) was used as a positive control. An unstained control was also included to set the acquisition gate for the population of interest. Then, cells were harvested and labeled following the BD Pharmingen™ FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, USA) protocol guidelines. All samples were run on a BD FACSCalibur™ (BD Biosciences, USA) flow cytometer. The results were analyzed using a free online tool Floreada.io (USA).

2.4. Cell cycle analysis

A549 cells were plated in 6-well plates at a seeding density of $15x10^4$ cells/well and incubated for cells to adhere to the surface. Then, the culture medium was aspirated from wells, and cells were washed with 1x PBS. After washing, the starvation medium was added to wells, followed by 24 hours of incubation. Then, the media were replaced with respect to the experimental groups of control, CDDP 20 µM, and DMH %8. Following incubation of the cells for 24 h, A549 cells were stained according to the Cell Cycle Analysis Kit (PromoCell, Germany) instructions. Flow cytometry was performed on BD FACSCalibur™ (BD Biosciences, USA). Output files were analyzed using a third-party software.

2.5. Gene expression analysis

A549 cells were treated with the herbal mixture at a concentration of 8% (v/v) in a 6-well plate for 24 h. 20 µM cisplatin was used as a positive control, and the medium of the control group was renewed. At the end of 24 hours of incubation, cellular RNA isolation was performed by the Quick-RNA™ MiniPrep Kit (Zymo Research, USA) according to the kit's protocol. 5 µg of isolated RNAs were transformed to cDNA using the Protoscript First Strand cDNA Synthesis Kit (New England Biolabs, USA) according to the manufacturer's instructions for use. 120 ng of cDNAs were utilized for each real-time PCR (qPCR) reaction.

HNRNPL was used as the housekeeping gene, and the whole primer sequences are shown in Table 1. qPCR reaction mixes were prepared using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA). The reactions were run in a qPCR instrument (Applied Biosystems™ 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA)). Cq values were analyzed using DataAssist™ v3.01 software (Thermo Fisher Scientific, USA). Then, the gene expression data were log2 transformed (Log2RQ) for normalization.

Table1. Human primer sequences used for qPCR

Gene Symbol	Primer Sequences (5'-3')	Reference
APAF1	F: GCCAAGCAGGAGGTCGATAATG R: GACCATCCTCAGAAAAGCAGGC	(25)
BAK1	F: TTACCGCCATCAGCAGGAACAG R: GGAACTCTGAGTCATAGCGTCG	(26)
BAX	F: TCAGGATGCGTCCACCAAGAAG R: TGTGTCCACGGCGGCAATCATC	(25)
BCL2	F: ATCGCCCTGTGGATGACTGAGT R: GCCAGGAGAAATCAAACAGAGGC	(27)
CDKN1A	F: AGGTGGACCTGGAGACTCTCAG R: TCCTCTTGGAGAAGATCAGCCG	(25)
CYCS	F: ACCTTCCATCTTGGCTAGTTGTG R: ATCGCTTGAGCCTGGGAAATAG	(28)
HNRNPL	F: GTGTGGTGGAAGCAGACCTTGT R: CAAACTCCACCAGTGCTTGTCTC	(29)
TP53	F: CCTCAGCATCTTATCCGAGTGG R: TGGATGGTGGTACAGTCAGAGC	(25)

2.6. Statistical analysis

All statistical calculations were conducted using Prism software, v7 (GraphPad Software Inc., USA). While one-way ANOVA, with Tukey test, was performed in the cell viability and apoptosis analyses, two-way ANOVA followed by Tukey test was used for the gene expression analysis and p values were given in GraphPad Style. The graphical representations of the results were prepared using GraphPad Prism v7 ModFit LT™ v5 was also used to visualize the cell cycle analysis results in addition to GraphPad Prism v7

3. Results

3.1. Herbal mixture causes the cytotoxic effect on A549 cells

The cytotoxic effect of the herbal supplement mixture on A549 cells was determined by an MTT assay. Cells were exposed to DMH at different concentrations (4%, 8%, and 32%) for 24 hours. The effective and relatively low concentration of the applied product was determined. Although no statistically significant change was observed in cell viability at the DMH concentration of 4%, cell viability was significantly decreased by DMH treatment at concentrations of 8% and 32% after 24 hours ($p < 0.0001$(****)) (Fig. 1A). The relatively low dose DMH concentration of 8% was further compared with the vehicle control, and approximately 55% inhibition in cell viability was observed ($p < 0.0001$(****)) (Fig. 1).

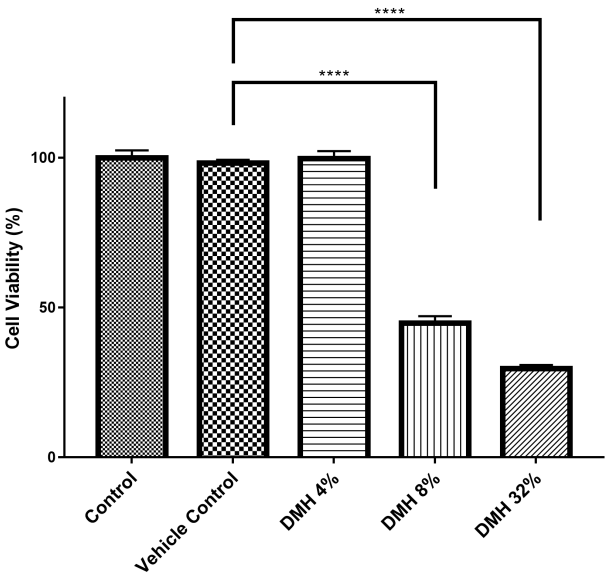

Fig. 1. MTT assay results of cytotoxic effect of DMH (the Diverse Mixture of Herbs) on A549 cells at different concentrations ($p < 0.0001$(****))

3.2. The apoptotic induction of the herbal mixture on A549 cells is greater than that of cisplatin.

Annexin V-PI staining followed by flow cytometry was performed to investigate the apoptotic effect of DMH on A549 cells. Flow cytometric dot plot diagrams of one replicate of every sample are represented in Figure 2A. In the A549 vehicle control group, the proportion of mean viable cells was detected at 89.33%, mean early apoptotic cells at 1.36%, mean late apoptotic cells at 4.22%, and mean necrotic cells at 5.09%. The percentage of mean viable, early apoptotic, late apoptotic, and necrotic A549 cells in the DMH 8% treatment group were 38.34%, 11.01%, 36.18%, and 14.48%, respectively. Compared to vehicle control, DMH induced a significant increase in early, late apoptotic, and necrotic cells ($p = 0.0004$ (***), $p < 0.0001$ (****), and $p = 0.0005$ (***), respectively) (Fig. 2.).

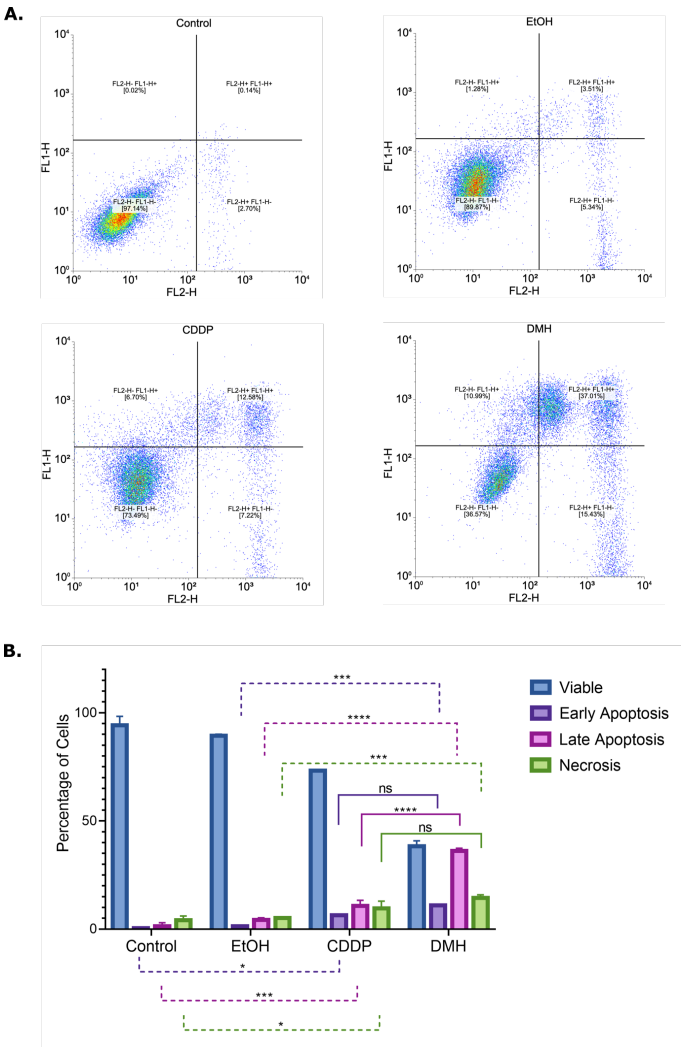

Fig. 2. Flow cytometric apoptosis analysis in A549 cells treated with EtOH 2%, CCDP 20 µM, or DMH 8% for 24 hours. (A) Representative dot plots of Annexin V (y-axis) vs PI (x-axis). Viable cells are shown in the lower left quadrant, cells in the upper left quadrant represent early apoptotic cells, late apoptotic cells are shown in the upper right quadrant, and cells in the lower right quadrant represent necrotic cells. (B) Percentage of viable, early apoptotic, late apoptotic, and necrotic cells. Asterisks show statistically significant differences between the vehicle control (EtOH) and DMH treatment group. ***, ****, *** indicate Tukey's corrected p values of 0.0004, 0.0001, and $p = 0.0005$, respectively

3.3. DMH induces G2 phase cell cycle arrest

The effect of DMH on the cell cycle was analyzed using flow cytometry. While cisplatin at 20 µM, which we used as a positive control in the study, caused the cells to accumulate in the G1 phase (Figure 3B), DMH at an 8% concentration induced G2 phase arrest in A549 cells dramatically after 24 hours (Fig. 3).

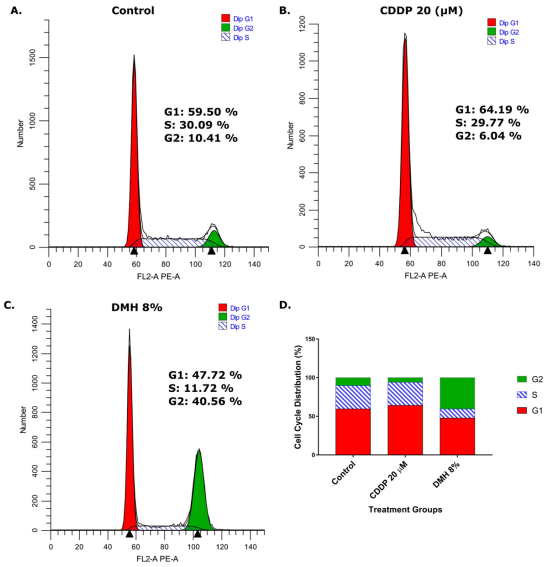

Fig. 3. Cell cycle analysis by flow cytometry in A549 cells. (A-C) Cell cycle histograms of A549 cells in untreated and treated groups. (A) control group; (B) cells treated with CDDP 20 µM or (C) DMH 8% for 24 hours. (D) Cell cycle distributions of the control and experimental groups were summarized in the bar graph

3.4. DMH increases the transcription of pro-apoptotic genes without significant effect on *BCL2* and *TP53* mRNA levels

Quantitative PCR results demonstrated that DMH 8% significantly elevated mRNA levels of pro-apoptotic *BAK1* ($p < 0.0001$), *BAX* ($p = 0.0076$), *APAF1* ($p = 0.0075$), and cell cycle inhibitor *CDKN1A* ($p < 0.0001$) without leading to significant changes in *BCL2* and *TP53* expressions in A549 cells. On the other hand, as expected, 20 µM of cisplatin upregulated the expressions of pro-apoptotic *BAK1*, *BAX*, *APAF1*, and cell cycle-related genes *CDKN1A* and *TP53* while downregulating anti-apoptotic *BCL2* expression significantly (Fig. 4).

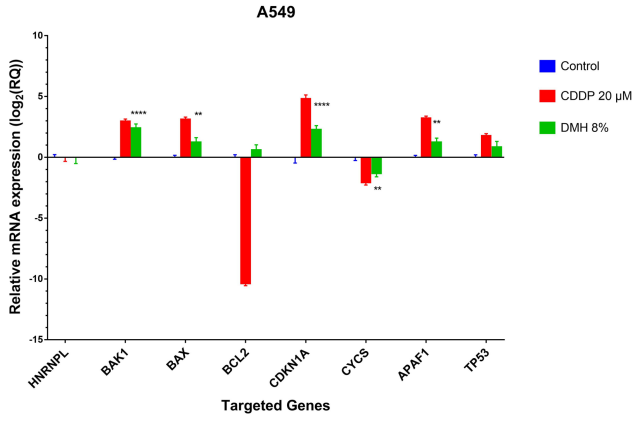

Fig. 4. Mean log2 relative quantity (log2(RQ)) values of gene expressions of apoptosis- and cell cycle-related biomarkers. Relative expression levels of the genes were normalized to *HNRNPL*. Asterisks above the boxplots indicate significant differences between the control and DMH-treated groups. More asterisks imply a higher significance level. Error bars represent standard deviations

4. Discussion

The main treatment approaches for lung cancer are chemotherapy, radiotherapy, and surgery. In addition, immunotherapy and targeted therapies have gained importance in recent years. Although radiotherapy, chemotherapy, and surgery are important in treating early-stage lung cancer patients, there is a risk of cancer recurrence after treatment. Thus, searching for more effective and tolerable treatment alternatives has been going on for many years (21). Numerous studies have shown the anticancer activities of different herbal products used on cancer cells (22, 23). In our study, the cytotoxic effect of the commercially available herbal mixture on A549 cells was observed.

The apoptotic mechanism is important in the control of cell number in healthy cells and is known to be impaired in cancer cells (24). Apoptosis induction is one of the most significant hallmarks of cytotoxic antitumor agents. Many natural herbal compounds act by inducing apoptotic pathways in cancer cells that escape apoptosis through various mechanisms in cancer cells. Various studies have shown that many patients struggling with cancer often use herbs or herbal products as supportive treatments. It is also known that some chemotherapeutic drugs, such as paclitaxel are used as plant-derived anticancer agents (25). In this study, it was observed that the herbal mixture DMH caused a significant increase, especially in the rate of late apoptotic cells, inducing cell death. Important regulatory mechanisms of the cell cycle are checkpoints. The occurrence of cell cycle arrest during cell division represents the difficulty in repairing damage and error during cell division and is a targeted outcome in cancer therapy. Cell cycle arrest in the G2/M phase indicates that intracellular DNA damage is challenging to repair, and many studies have shown that different herbal products induce G2/M arrest in cancer cells (26-28). Our cell cycle analysis results, which we carried out within the scope of the study, showed that DMH induced G2 arrest in A549 cells in conformity with the literature.

The gene expression analysis results demonstrated that DMH significantly increased the mRNA levels of pro-apoptotic *BAK1*, *BAX*, *APAF1*, and cell cycle inhibitor *CDKN1A* without changing *BCL2* and *TP53* expressions in A549 cells. Induction of p21 (*CDKN1A*) in the absence of p53 has been demonstrated in the studies of distinct p53-mutant mouse and human cancer types (29, 30). There has been no reported decrease in Bax and Apaf-1 expression levels compared to wild-type in p53-null hematopoietic cancer cells. Furthermore, apoptosis can be induced independently of p53-dependent transcription, as shown in studies on some human and mouse cancer cell lines (31). In another study, it has been demonstrated in a gastric cancer cell line that Bak overexpression, independent of p53, promotes apoptosis (32). One of the important mechanisms in the apoptotic pathway is the formation of apoptosomes associated with the intrinsic pathway. In the intrinsic pathway, Cytochrome c binds to and activates Apaf-1. Then, with the addition of ATP, a complex called the apoptosome is formed (33). In the mechanism of apoptosis, it has been reported that Cytochrome c will induce apoptosis in any cell to which it is delivered (34). In our study, a statistically significant increase in the expression level of

CYCS was observed in the DMH administered group, in line with the increase in the rate of apoptosis.

Considering all these data, we concluded that DMH treatment on A549 cells caused G2 cell cycle arrest by increasing *CDKN1A* expression and subsequently elevated *BAX*, *BAK1*, and *APAF1* expressions, leading to an anti-apoptotic *BCL2* threshold resulting from *TP53* stabilization to be exceeded, thus induced late apoptosis.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: M.Y., Design: M.Y., C.G., Data Collection or Processing: M.Y., C.G., Analysis or Interpretation: C.G., Literature Search: M.Y., C.G., Writing: M.Y., C.G.

Ethical Statement

Ethics committee permission is not required fort his study.

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Is there a correlation between dominant extremity and cervical disc herniation using machine learning methods?

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Abstract

We aimed to investigate the possibility of determining the relationship between the dominant extremity and cervical disc herniation using a machine learning approach. A total of 561 patients diagnosed with cervical disc herniation were examined for dominant extremity, level and side of cervical disc herniation, and the nature of the herniation (calcified/soft). These patients formed the basis for a two-step machine learning system creation. The first step (included the data of 80% of the patients) focused on determining the type of cervical disc herniation by selecting the top five performing classification models out of 15 different models and tuning the hyperparameters. In the second step, the machine learning system was validated using data from a randomly selected subset of patients (20% of the patients). The study results showed that while most models performed well, the gradient boosting classifier was the most accurate (89.38%) for determining the herniated disc nature. However, for classifying the disc herniation direction, the models did not exhibit strong performance. Thus, machine learning can accurately identify the relationship between cervical disc herniation and dominant extremity with a high degree of accuracy.

Keywords: machine learning, artificial intelligence, disc degeneration, spinal disease

1. Introduction

Machine learning (ML) is rapidly being established in every aspect of modern life. Its ability to simultaneously process larger amounts of data and quickly perform multiple comparisons make it an integral part of our future (1). In the medical field, research and studies on ML are becoming increasingly prevalent. Due to the awareness generated by these efforts, the widespread adoption of ML in areas such as healthcare data systems and medical education is inevitable (2).

Because the cervical vertebrae bears a relatively lesser load than the lumbar vertebrae, experiences fewer traumas, and is less affected by environmental factors, they can be evaluated more easily using ML methods. The nature and determination of the direction of cervical disc herniation (CDH) has been a relatively underexplored area in artificial intelligence (AI) studies. To shed light on future studies, we aimed to approach this issue by incorporating AI (3).

2. Material and Methods

Between January 2020 and June 2023, a total of 561 patients who were diagnosed with CDH and had presented at the neurosurgery clinic of a tertiary university hospital were included in the study. The present study was performed in accordance with the framework of the Declaration of Helsinki and approved by the Alanya Alaaddin Keykubat University Ethics Committee (No: 11/06; approval date: 14.06.2023). The dominant extremity, level and side of CDH, and nature analysis of the radiological data (calcified (Fig. 1) or soft (Fig. 2), were documented for each patient. From these patients, 80% were randomly selected. The data of these selected patients were integrated into an AI module to create the main data table. Subsequently, the accuracy of different methods in generating data was investigated using the data of the remaining 20% of the patients. The suitability of the system was also explored in this context.

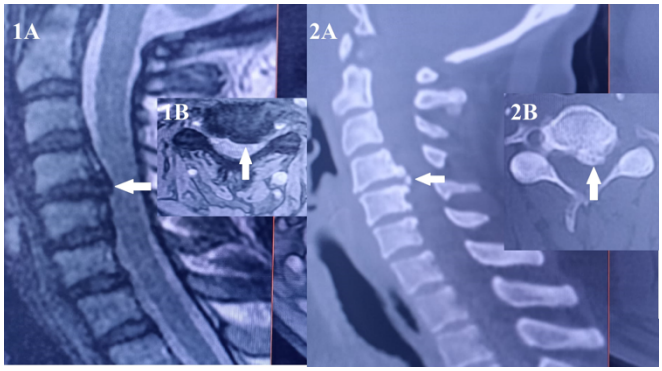

Fig. 1. Computed tomography (1 A, B) and magnetic resonance imaging (2 A, B) of CDH calcification

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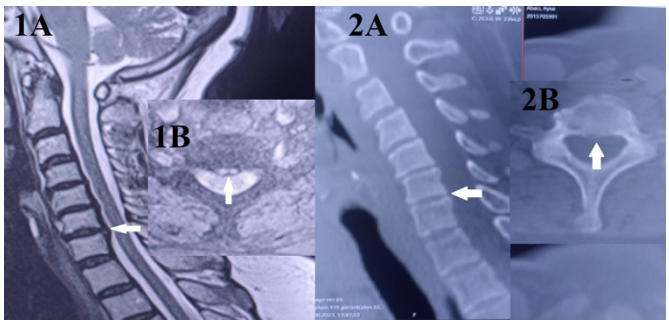

Fig. 2. Magnetic resonance imaging (1 A and B) and computed tomography (1 A and B) images of soft herniated disc

2.1. Statistical analysis

The normally distributed continuous variables are expressed as mean ± Standard Deviation (SD) and the non-normally distributed continuous variables are expressed as median (min-max). The Shapiro–Wilk test was used to determine the normality of the data. The categorical variables are expressed as frequencies (n) and percentages (%). The Pearson chi-square and Fisher's exact tests were used to determine the relationship between the categorical variables. The Kruskal–Wallis test was used for non-parametric comparisons of continuous data, and the independent t-test and one-way ANOVA were used for parametric comparisons. A post-hoc analysis was performed using the Bonferroni correction. All statistical analyses were carried out using IBM SPSS Statistics for Windows (version 23.0; IBM Corp., Armonk, NY). A two-sided $p < 0.05$ was considered statistically significant.

2.2. Methodology of machine learning

To be able to use classification models for determining the direction and nature of disc herniation, a custom dataset was created by collecting information from 561 patients treated clinically and surgically. The dataset included the following data: age, gender, dominant hand, CDH level and its direction and nature, and direction of arm pain (4).

Using this dataset, 15 different classification models were evaluated. The top five models that performed the best for each of the two outcomes (direction and nature) were selected, and their hyperparameters were tuned accordingly. Subsequently, the models were evaluated using metrics such as accuracy, precision, specificity, recall, F1-score, negative predictive value (NPV), and false positive rate (FPR).

The models used for classifying the nature of CDH were decision tree (DT) classifier, random forest classifier, gradient boosting (GB) classifier, multi-layer perceptron (MLP) classifier, and eXtreme gradient boosting (XGB) classifier. The models that produced the best results for determining the CDH direction were K-nearest neighbors (KNN) classifier, GB classifier, DT classifier, random forest classifier, and XGB classifier.

2.3. Models used

GB classifier

Boosting algorithms progressively combine weak learners, which perform marginally better than random guessing, to create strong learners. GB is a regression technique that shares similarities with boosting. It determines an estimate of the function that maps input samples to their corresponding output values by minimizing the error function's estimated value using the training data.

DT classifier

DTs are supervised learning models capable of handling classification and regression tasks, although they are predominantly used for solving classification problems. They have a tree-like structure, where each node corresponds to a feature value check, branches represent test outcomes, and leaf nodes represent the final classifications. DTs can efficiently generate interpretable rules and classify data with minimal computation.

Random forest classifier

The random forest classifier is a popular ML technique that leverages multiple DTs built on various subsets of the main dataset to make predictions. It functions as both a regression and classification model. As a regression model, it computes the mean of all the DT outcomes. As a classification model, it combines the votes from multiple DTs to obtain the final prediction.

KNN classifier

The KNN classifier prediction algorithm follows a lazy learning technique, generating predictions based on the KNN input. When predictions for any instance are requested, the entire prediction process is carried out. The Euclidean distance method is commonly used to determine the proximity between instances.

MLP classifier

The MLP classifier is a prediction algorithm based on an artificial neural network (MLP). When predictions for any instance are required, the neural network processes the input through its layers to generate the output. The MLP classifier is particularly effective for solving complex classification problems and can determine both linear and non-linear relationships in the data.

XGB classifier

The XGB classifier is a speedy and robust implementation of GB used for classification purposes. It leverages decision trees to make precise predictions, and the final outcome is determined through the combined voting of multiple trees.

2.4. Metrics used

Classification accuracy

The overall accuracy of the classifiers indicates the percentage of correct predictions among all predictions. The accuracy was calculated using the following equation: $Accuracy = (TP + TN) / (TP + TN + FP + FN)$, where TP is True Positives, TN is True Negatives, FP is False Positives, and FN is False

Negatives.

Precision

Precision is a crucial metric used to assess the classifier performance. It represents the ratio of true positives to the sum of true positives and false positives. The precision was calculated using the follow equation: $Precision = (TP) / (TP + FP)$, where TP is true positives and FP is false positives.

Recall/Sensitivity/True positive rate

True positive recall, commonly referred to as recall, is a metric defined as the ratio of true positive results to the sum of true positive and false negative results. Recall was calculated using the following equation: $Sensitivity = (TP) / (TP + FN)$, where TP is true positives and FN is false negatives.

FPR

The FPR is the ratio of false positive values to the sum of false positive values and true negative values. FPR was calculated using the following equation: $FPR = (FP) / (FP + TN)$, where FP is false positives and TN is true negatives.

NPV

The NPV is another significant metric used to assess the classifier performance. It represents the ratio of true negative values to the sum of true negative and false negative values. NPV was calculated using the following equation: $NPV = (TN) / (TN + FN)$, where TN is true negatives and FN is false negatives.

F1-score

The F-measure, also known as the F1-score, is determined by obtaining the harmonic mean of accuracy and recall. A value of 0 indicates the worst performance, while a value of 1 indicates the best performance. The F1-score was calculated using the following formula: $F1\text{-}score = 2 * precision * recall / (precision + recall)$

2.5. Experimental setup

All ML algorithms and classification and regression models used in this study were evaluated using the same dataset with the same split ratios in Google Colab (Global Al Hub, Matterhorn, Switzerland). The top five models that yielded the best results were selected, and their hyperparameters were tuned. Google Colab is a free platform that offers various tools for working with AI, allowing us to utilize ML effectively.

While deep learning and clustering models were utilized, their outcomes were not assessed due to their lower performance compared to the described classification models.

2.6. Machine learning dataset

This was a novel study we conducted in this field of study. Hence, there was no existing dataset to be used. To create our own custom dataset, we collected the following information from the 561 admitted symptomatic patients after obtaining their consent: age, gender, dominant hand, CDH level and its direction and nature, and direction of arm pain. The following eight features were used as inputs: age, gender, dominant hand, pain direction and CDH at C3-4, C4-5, C5-6, and C6-7). The remaining factors, herniation nature and CDH direction, were used as outputs.

Within our dataset, 443 patients had a soft disc and 118 patients had a hard disc. A total of 197 patients had a right-sided CDH, 222 patients had a left-sided CHD, and 142 patients had bilateral CDH. The training set comprised of 80% of the dataset, while the test set comprised of 20% of the dataset (Table 1).

Table 1. Detailed information about the dataset

No	Attribute Name	Abbreviation	Values	Explonation
1	Gender	Gender	0-1	Male-Female
2	Age	Age	21-81	21-81
3	Dominant Hand	dHand	0-2	Right-Left-Both
4	CDH Level C3-4	hLevel_C3-4	0-1	Yes-No
5	CDH Level C4-5	hLevel_C4-5	0-1	Yes-No
6	CDH Level C5-6	hLevel_C5-6	0-1	Yes-No
7	CDH Level C6-7	hLevel_C6-7	0-1	Yes-No
8	CDH Direction	hDirection	0-2	Right-Left-Both
9	Hernia Type	hType	0-1	Soft-Hard
10	Pain Direction	pDirection	0-2	Right-Left-Both

3. Results

The mean age of the patients in the outpatient clinic and surgical groups (48.97 ± 11.69 and 48.98 ± 10.15; p = 0.995) and the gender distributions (p = 0.875) were statistically similar. No significant differences were observed in the dominant hand (p = 0.639), CDH level (p = 0.792), and CDH nature (p = 0.871) between the two groups. A left-sided CDH was more commonly seen than a right-sided CDH in the outpatient clinic (40.9% vs. 26%). In the surgical group, a central CDH was seem more commonly than a right- or left-sided CDH (38% vs. 24.1%; p = 0.048). The proportion of patients with bilateral arm pain was higher in the surgical group than in the outpatient clinic group (24% vs. 6.7%) (p = 0.001) (Table 2).

Independent t-test, Pearson chi-square test, Fisher's exact test. Statistical analysis indicated no significant differences between the groups within the same column of lowercase letters.

Table 2. Demographics information of patients'

Variables	Clinic patients (n=511)	Surgical patients (n=50)	
Age (year)			
Mean±SD	48.97±11.69	48.98±10.15	0.995
Min-maks	21-81	33-73	
Gender, *n(%)*			
Male	200(39.1)	19(38.0)	0.875
Female	311(60.9)	31(62)	
Hand preference, *n(%)*			
Right	394(77.1)	37(74.0)	0.639
Left	50(9.8)	7(14.0)	
Both hands	67(13.1)	6(12.0)	
Level of CDH. *n(%)*			
C3-4	9(1.8)	1(2.0)	0.792
C4-5	59(11.5)	8(16.0)	
C5-6	284(55.8)	25(50.0)	
C6-7	159(31.1)	16(32.0)	
Direction of CDH, *n(%)*			
Right	179(35) [a]	18(36)[a]	**0.048**
Left	209(40.9) [b]	13(26) [a]	
Central	123(24.1) [b]	19(38)[a]	
Nature of CDH, *n(%)*			
Soft	402(79)	39(78)	0.871
Hard	107(21)	11(22)	
Direction of Arm Pain, *n(%)*			
Right	265(51.9)[a]	20(40)[a]	**0.001**
Left	212(41.5)[a]	18(36)[a]	
Both Sides	34(6.7)[b]	12(24)[a]	

Table 3. Patients' ages according to disease-related characteristics

	age			
Characteristics	**X̄±SD**	**Min-Maks**	**p**	**Post-Hoc (Adj P)**
Dominant hand				
Right	49.35±11.10	21-81	0.369	
Left	46.98±13.5	27-74		
Both Hands	48.26±12.85	29-75		
Level of CDH				
1.C3-4 (low count)	50.90±10.78	39-66	**<0.001**	1>2 (NS) 1>3(NS)
2.C4-5	45.03±10.31	21-68		1<4(NS) 2<3(NS)
3.C5-6	47.55±10.31	22-75		2<4 (<0.001) 3<4(<0.001)
4.C6-7	52.88±13.04	23-81		
Direction of CDH				
1. Right	48.56±11.64	22-81	**<0.001**	**1>2 (NS)**
2. Left	46.55±11.93	21-75		2<3(<0.001)
3.Central	53.32±11.24	27-76		1<3(0.001)
Nature of CDH				
Soft	45.55±9.25	21-68	**<0.001**	
Hard	61.60±10.55	28-81		
Direction of Arm Pain				
Right	48.50±10.72	22-77	0.147	
Left	48.95±12.69	21-81		
Both Sides	51.98±10.28	34-73		

The dominant hand ($p = 0.369$) and direction of arm pain ($p = 0.147$) were not associated with the patient's age. The mean age of the patients with CDH at the C6-7 level was higher than those with CDH at the C4-5 and C5-6 levels ($p < 0.001$). Patients with a central CDH or hard disc had higher mean ages than those with right- or left-sided CDH or soft disc ($p < 0.001$) (Table 3).

Independent t-test, one-way ANOVA, Kruskal–Wallis test. The same lowercase letters within a column indicate no significant difference between the groups. Bonferroni, NS: non-significant

No significant differences were observed in the distribution of dominant hand and direction of arm pain across age groups ($p = 0.228$). CDH was more prevalent at the C5-6 level in patients aged < 50 years than in those aged ≥ 50 years (60.1% vs. 48.6%). CDH was more prevalent at the C6-7 level in patients aged ≥ 50 than in those aged < 50 years (39.5% vs. 25.1%; $p = 0.001$). A left-sided CDH was more common in patients aged < 50 years old than in those aged ≥ 50 years (46.5% vs. 30.5%). A central CDH was more common in patients aged ≥ 50 than in those aged < 50 years (35.8% vs. 17.3%; $p < 0.001$). Furthermore, the prevalence of a hard disc was significantly higher in patients aged ≥ 50 than in those aged < 50 years (42.7% vs. 4.7%; $p < 0.001$) (Table 4).

Pearson chi-square test, Fisher's exact test. The same lowercase letters within a row indicate no significant difference between the groups.

There is no significant difference in the CDH level ($p = 0.156$), herniation direction ($p = 0.095$), and CDH nature ($p = 0.318$) according to the patient's dominant hand. Left-sided arm pain was higher in patients predominantly using the left hand than in those using both hands (54.4% vs. 32.9%). In the group using both hands, pain occurred more frequent on both sides than in the group predominantly using the left hand

(24.7% vs. 1.8%, p < 0.001). In the group using both hands, more patients had right-sided dominance than left-sided dominance (24.7% vs. 6.3%) (Table 5).

Table 4. Disease-Related Characteristics According to Age Groups of Patients

	Age		
Characteristics, n(%)	**<50 (n=318)**	**≥50 (n=243)**	**p**
Dominant hand			
Right	236(74.2)	195(80.2)	0.228
Left	37(11.6)	20(8.2)	
Both Hands	45(14.2)	28(11.5)	
Level of CDH			
C3-4	4(1.3)[a]	6(2.5)[a]	**0.001**
C4-5	44(13.8)[a]	23(9.5)[a]	
C5-6	191(60.1)[a]	118(48.6)[b]	
C6-7	79(25.1)[a]	96(39.5)[b]	
Direction of CDH			
Right	115(36.2)[a]	82(33.7)[a]	**<0.001**
Left	148(46.5)[a]	74(30.5)[b]	
Central	55(17.3)[a]	87(35.8)[b]	
Nature of CDH			
Soft	303(95.3)[a]	138(57.3)[b]	**<0.001**
Hard	15(4.7)[a]	103(42.7)[b]	
Direction of Arm Pain			
Right	160(50.3)	125(51.4)	0.213
Left	137(43.1)	93(38.3)	
Both Sides	21(6.6)	25(10.3)	

Pearson chi-square test, Fisher's exact test. The same lowercase letters within a row indicate no significant difference between the groups.

3.1. Machine learning analysis

The experiment used the herniation nature and direction data, which was split into the training (80%) and testing (20%) datasets. A confusion matrix measured the performance of the classifier models (Tables 2 and 4). The results of the study classifiers are listed in Tables 3 and 5.

Table 5. Other Characteristics According to Patients' Dominant Hand

	Dominant hand			
Characteristics, n(%)	**Right hand**	**Left hand**	**Both hands**	**p**
Level of CDH				
C3-4	10(2.3)	0(0)	0(0)	0.156
C4-5	57(12.3)	6(10.5)	4(5.5)	
C5-6	239(55.5)	29(50.9)	41(56.2)	
C6-7	125(29.0)	22(38.6)	28(38.4)	
Direction of CDH				
Right	156(3.2)	13(22.8)	28(38.4)	0.095
Left	162(37.6)	32(56.1)	28(38.4)	
Central	113(26.2)	12(21.1)	17(23.3)	
Nature of CDH				
Soft	337(78.6)	49(86.0)	55(75.3)	0.318
Hard	92(21.4)	8(14.0)	18(24.7)	
Direction of Arm Pain				
Right	229(53.1)[a]	25(43.9)[a]	31(42.5)[a]	**<0.001**
Left	175(40.6)[a.b]	31(54.4)[b]	24(32.9)[a]	
Both Hands	27(6.3)[a]	1(1.8)[a]	18(24.7)[b]	

The determination of herniation nature yielded the most successful classification model (Table 3), with the GB classifier achieving a performance of 89.3%. Although the other models were not as successful as GB, their performances were still quite impressive. The DT and random forest classifiers both achieved the same percentage result (87.6%), the XGB classifier achieved a performance of 86.7%, and the MLP classifier yielded a performance of 85.8% (Table 3).

The models of herniation direction determination did not achieve the same level of success as that of herniation nature classification models. The classification models could not establish sufficient correlations between the data or adequately generalize data. Thus, the RF was the most successful (62.8%), followed closely by GB (59.2%). The XGB, MLP, and KNN models achieved results of 52.2%, 51.3%, and 50.4%, respectively (Tables 6, 7, 8, and 9)

Table 6. Summarised Depiction of Confusion Matrices for Herniation Nature for All Classifiers

	Decision Tree Classifier	Random Forest Classifier	Gradient Boosting Classifier	MLP Classifier	XGB Classifier
TP	17	16	16	19	17
TN	81	81	82	67	81
FP	3	3	2	17	3
FN	12	13	13	10	12

Three different results were obtained regarding herniation direction. This is because the CDH could occur on the right, left, or bilaterally within our dataset. The models we used provided separate results for each scenario (Table 3).

The F1-score (Table 9) provided us with an idea of the model's classification capability; the higher the value, the higher the model's classification ability. The classification model with the highest accuracy also had the highest F1-score. For herniation nature determination, the GB classification model yielded the highest F1-score (0.854), while the MLP model yielded the lowest F1-score (0.788).

Table 7. Performance Statistics of All Classifiers for Herniation Nature

Model	Accuracy (%)	Precision	Sensitivity	Recall	F1-score	NPV (%)	FPR (%)
Decision Tree Classifier	87.6	0.883	0.894	0.781	0.814	87.2	2.3
Random Forest Classifier	87.6	0.883	0.894	0.781	0.814	87.2	2.3
Gradient Boost	89.3	0.898	0.904	0.815	0.845	89.1	2.3

Classifier							
MLP Classifier	85.8	0.851	0.842	0.758	0.788	86.1	3.5
XGB Classifier	86.7	0.860	0.850	0.775	0.804	87.0	3.5

Table 8. Summarised Depiction of Confusion Matrices for Herniation Direction for All Classifiers

	K-Neighbors Classifier	Random Forest Classifier	Gradient Boosting Classifier	MLP Classifier	XGB Classifier
TN-Right	49	50	50	69	51
TN-Left	53	57	58	0	54
TN-Both	68	74	71	83	67
FP- Right	20	19	19	0	18
FP- Left	21	17	16	74	20
FP- Both	15	9	12	0	16
TP- Right	25	32	30	0	24
TP- Left	24	26	26	39	25
TP- Both	8	10	10	0	10
FN- Right	19	12	14	44	20
FN- Left	15	13	13	0	14
FN- Both	22	20	20	30	20
Sensitivity- Right	0.555	62.7	61.2	Nan	57.1
Sensitivity- Left	0.533	60.4	61.9	34.5	55.5
Sensitivity- Both	0.347	52.6	45.4	Nan	34.8
NPV- Right (%)	72.0	80.6	78.1	61.0	71.8
NPV- Left (%)	77.9	81.4	81.6	Nan	79.4
NPV- Both (%)	77.5	78.7	78.0	74.3	77.0
FPR- Right (%)	28.9	27.5	27.5	0	26.0
FPR- Left (%)	28.3	22.9	21.6	1	27.0
FPR- Both (%)	18.0	10.8	14.4	0	19.2

Table 9. Performance statistics of All Classifiers for Herniation Direction

Model	Accuracy (%)	Precision	Recall	F1-score
K-Neighbors Classifier	50.4	0.478	0.483	0.478
Random Forest Classifier	62.8	0.612	0.602	0.599
Gradient Boosting Classifier	59.2	0.580	0.571	0.570
MLP Classifier	51.3	0.466	0.473	0.443
XGB Classifier	52.2	0.503	0.506	0.503

The NPV and FPR indicate the accuracy of detecting negative cases and the rate of incorrectly detecting negative values, respectively. Thus, a higher NPV and lower FPR indicate better results. Sensitivity shows how successful we are in achieving a true positive rate in correct outcomes. Finally, the recall value is necessary for calculating some key metrics, as mentioned before.

Thus, our classification models exhibit a performance of 89.3% for determination of herniation nature and a performance of 62.8% for hernia direction determination. This issue arises from the inability of the models to adequately generalize the data used because of the insufficient data or a lack of correlation between the available data and desired outcomes.

4. Discussion

In the current era, ML is becoming increasingly prominent in our lives. ML is expected to enable software-powered robots to perform tasks that were once carried out by humans. Moreover, the utilization of ML in fields such as healthcare and education are steadily on the rise (1, 2). Thus, software programs capable of simultaneously comparing multiple parameters and producing results will replace humans who obtain experience and skills gained through formal education. This could make it possible for a general practitioner triaging in an emergency room or an individual overseeing healthcare insurance expenses to be replaced by such programs in the near future. Thus, we need to determine the usability of ML within the realm of healthcare and how it should be employed. Our study significantly contributes to literature by investigating the potential contribution of ML in assessing spinal pathologies and shedding light on how effective it can be in this context.

Cervical trauma is less commonly encountered compared to trauma at other spinal regions. Factors that could lead to spinal pathologies, such as obesity, pregnancy, and heavy lifting, have a lesser impact on the cervical region than on the other spinal regions (5). Due to its simpler dynamic function, the cervical spine is more amenable to investigation for disc degeneration and herniation using ML systems than the other spinal regions (3). The most influential factors in this regard include the age, dominant extremity, nature and side of the disc herniation, and level of disc herniation. In our study, we initially statistically compared these data in patients and assessed their comparability with those in literature. Subsequently, we compared these findings with the results obtained via ML.

Takahashi et al. reported a higher prevalence of CDH, especially at the C6-7 level, in individuals with left-hand dominance (6). Kang et al. reported that factors such as

dominant extremity, age, disc level, and nature play a crucial role in disc herniation and emphasized the need for further research to support these findings. They attributed this to patients unknowingly placing their non-dominant arm higher while writing with their dominant hand and more frequently bearing loads on the dominant hand (7). However, in our study, we did not find a significant correlation between dominant extremity and disc herniation, both during statistical analyses and within the ML system.

As people age, an increase in spinal degeneration, calcification, and the presence of pathologies such as osteophytes often become inevitable (8). This standard knowledge for medical practitioners is easily predictable due to its frequent occurrence in educational and practical applications. In our statistical study, there was a statistically significant correlation between aging and the occurrence of calcified CDH. However, in ML, achieving a high level of accuracy, often close to 90%, is crucial. For instance, health insurance companies aim to streamline expenses by eliminating unnecessary tests for their customers. In such a scenario, a computer programmer without basic medical knowledge could monitor the expenditures of insured customers. Similarly, medical students lacking sufficient clinical experience could benefit from similar support programs.

Our AI-supported study results indicate that age and gender heavily impact disc nature (8). In the test models, a significant decrease did not occur when only age and gender parameters were used. Thus, our original intention, to establish a correlation between the arm pain direction and hand dominance and the direction of CDH, might not hold true based on the study findings. This insight offers valuable guidance for further exploration and analysis of spinal pathologies.

This study focused on CDH. AI-supported applications do not yet appear to be suitable for practical use in this field due to the lack of sufficient data and correlations as well as inadequate diversity in the data utilized. However, AI-supported applications hold promise for the future. The appropriate identification, collection, and processing of relevant data will pave the way for more accurate results. This study has identified important foundation points to be considered in future AI-supported studies on the detection of CDH nature and direction.

Furthermore, changes in the diversity and quantity of collected data can lead to different results in other classification models. Models developed using deep learning algorithms could yield entirely different outcomes for parameters that are considered independent.

This study serves as a valuable reference for forthcoming AI-supported research on the detection of CDH nature and direction. Adjustments in the diversity and quantity of collected data, as well as the application of deep learning algorithms, could potentially yield diverse and impactful outcomes.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: E.Y., Design: E.Y., Data Collection or Processing: E.Y., Analysis or Interpretation: Y.A., Literature Search: Y.A., Writing: E.Y., Y.A.,

Ethical Statement

The study was approved by the Alanya Alaaddin Keykubat University Ethics Committee (No: 11/06; approval date: 14.06.2023)

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The effects of systemic inflammatory indices, lactate, and blood gas parameters on drug-resistant and drug-nonresistant epilepsy

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Abstract

Epilepsy is one of the neurological diseases that affects a significant number of individuals in the world. In addition to having important effects on the social lives of the patients, this disease is a significant cause of disability. Neurological mechanisms underlying the disease are still being investigated. The patients' gender, age, hemogram parameters (white blood cells, hemoglobin, erythrocyte, neutrophil, lymphocyte, monocyte, eosinophil, platelet), blood gas values, drugs used, presence of drug-resistant epilepsy, and the duration of the disease were analyzed. The patients were divided into two groups of similar ages and genders as DRE (drug-resistant epilepsy) and DNRE (drug-nonresistant epilepsy) patients. This grouping was made according to the ILAE (International League Against Epilepsy) classification by considering the patients' anamnesis, clinical history, seizure frequency, and antiepileptic drugs currently used. Seventy-seven drug-resistant and 129 drug-nonresistant epilepsy patients, 206 in total, were included in the study. 64.9% of the drug-resistant epilepsy group were male, and the remaining 35.1% were female, while 65.9% of the drug-nonresistant epilepsy group were male and 34.1% were female. No significant difference was found between the groups in terms of systemic inflammatory indices. The disease duration of the patients in the drug-resistant epilepsy group was significantly higher than those in the drug-nonresistant epilepsy group. A negative and significant correlation was found between pH and pO2, pCO2, and lactate. In addition, a positive and significant correlation was determined between pO2 and lactate, and a negative and significant correlation was found between pO2 and pCO2. It was determined that there was no significant difference between drug-resistant epilepsy patients and drug-nonresistant epilepsy patients in terms of parameters used as a systemic inflammatory biomarker in epilepsy patients. New biomarkers that would significantly affect these patients should be investigated.

Keywords: epilepsy, drug-resistant epilepsy, NLR, PLR, MLR, lactate, blood gas

1. Introduction

Epilepsy is a neurological disease affecting nearly 50 million individuals worldwide with 16-53 new onset cases in 100,000 individuals yearly (1). Epilepsy is one the diseases that frequently leads to disability and can affect individuals of all ages, races, social classes, and geographical regions (2, 3). The WHO (World Health Organization) has reported that DRE (drug-resistant epilepsy) develops in approximately one-third of these patients (4-6).

DRE refers to situations in which a seizure-free period could not be achieved despite using two or more tolerable antiepileptic drugs (monotherapy or polytherapy) (7). These patients experience significant socioeconomic and psychological restrictions, such as decreased quality of life and increased mortality risk (8, 9).

Basic neuronal mechanisms that underly epileptogenicity have long been investigated. It has been argued that the blood-brain barrier (BBB) is disrupted, especially in epileptogenic foci, and neuroinflammation has a significant role in pathogenesis (2). Finding the biomarkers for epileptogenesis can offer important opportunities for diagnosing and treating the disease. It is known that systemic inflammatory indices (SII) [(Neutrophil/Lymphocyte ratio (NLR), Platelet/Lymphocyte ratio (PLR), Monocyte/Lymphocyte ratio (MLR)] can display differences in epilepsy patients similar to many systemic and neurological diseases (10-18). Hence, this study aimed to examine the changes in SII and blood gas parameters in patients grouped as drug-resistant epilepsy (DRE) and drug-nonresistant epilepsy (DNRE) according to their clinical history.

2. Materials and Methods

2.1. Data Collection

Ethical approval for the study was obtained from Malatya Turgut Ozal University Non-Interventional Clinical Research Ethics Committee with the decision numbered 2022/92 and dated 10.05.2022. The study had a retrospective design. The patients' gender, age, hemogram parameters (white blood cells, hemoglobin, erythrocyte, neutrophil, lymphocyte, monocyte,

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eosinophil, platelet), arterial bold gas values, drugs used, presence of drug-resistant epilepsy, and the duration of the disease were examined. The patients included in the study were divided into two groups of similar ages and genders as DRE (whose seizures continue despite treatment with two or more antiepileptic drugs) and drug-nonresistant epilepsy patients according to ILAE classification by considering the patients' anamnesis, clinical history, seizure frequency, and antiepileptic drugs currently used. The patients were included in the study according to the following criteria:

A. Inclusion criteria

Being 18 years old and above, presenting to the emergency service with an epileptic seizure within the last 60 minutes, being a patient diagnosed with epilepsy by a neurologist and treatment being started, having whole blood count and blood gas examination at presentation to the emergency service are our inclusion criteria.

B. Exclusion criteria

Being younger than 18 years old, presenting to the emergency service with an epileptic seizure prior to the last 60 minutes, presenting with seizure-like clinical conditions and symptomatic seizure (trauma, deep anemia (Hb value being 7 g/dl and below), hypoglycemia, syncope, etc.), presenting with the first seizure, having no laboratory examinations performed at presentation to the emergency service, having coagulation result in hemogram and blood gas, being suspected with experiencing a nonepileptic psychogenic seizure, having undergone vagal nerve stimulation (VNS) or epilepsy surgery are our exclusion criteria.

2.2. Statistical Analysis

Statistical analyses were performed through SPSS (Statistical Packagefor the social sciences; SPSS Inc., Chicago, IL) 22 package software. Descriptive data were expressed as numbers and percentages for categorical variables and mean±standard deviation (Mean±SD) for continuous variables. Chi-square analysis (Pearson Chi-square) was used in the intergroup comparison of categorical variables. Compliance of continuous variables with normal distribution was evaluated with the Kolmogorov-Smirnov test. The Mann-Whitney U test was employed in the pairwise comparison of the groups. The spearman correlation test was used to examine the relationship between continuous variables. The statistical significance level in the analyses was set at $p<0.05$.

3. Results

Two hundred six epilepsy patients in total, 77 drug-resistant and 129 drug-nonresistant epilepsy patients, were included in the study. Of the patients in the DRE group, 64.9% were male, and 35.1% were female. 65.9% of the patients in the DNRE group were male, while 34.1% were female. No significant difference was found between the groups in terms of gender ($p=0.889$). The mean age of the patients in the DRE group was determined to be 40.00±12.96 years, while it was 38.67±14.72 years in the DNRE group. There was no statistically significant difference between the groups in terms of age ($p=0.248$). The mean disease duration of the patients in the DRE group was found to be higher than that of the patients in the DRNE group ($p<0.001$) (Table 1).

Table 1. Comparison of the groups in terms of demographic characteristics and disease duration

		DRE (n=77)		DNRE (n=129)		p
		Number	%	Number	%	
Gender	Male	50	64.9	85	65.9	0.889*
	Female	27	35.1	44	34.1	
Age Mean±SD		40.00±12.96		38.67±14.72		0.248**
Disease Duration Mean±SD		12.47±4.08		10.04±434		**<0.001****

*Chi-square analysis, **Mann-Whitney U testwas applied.
DRE: Drug-resistant epilepsy, DNRE: Drug-nonresistant epilepsy

No significant difference was observed between the groups in terms of leukocyte ($p=0.729$), platelet ($p=0.132$), neutrophil ($p=0.676$), lymphocyte ($p=0.398$), monocyte ($p=0.658$), NLR ($p=0.355$), PLR ($p=0.846$), MLR ($p=0.064$), pH ($p=0198$), pO2 ($p=0.288$), pCO2 ($p=0.349$) and lactate ($p=0.358$) (Table 2).

A negative and significant correlation was found between age and platelet count in the correlation analysis. A positive and significant correlation was found between leukocyte and platelet, neutrophil, lymphocyte, monocyte, NLR, pO2, and lactate; a negative and significant correlation was determined between leukocyte and PLR and pH. Platelet was found to have a positive and significant correlation with neutrophil, lymphocyte, monocyte, PLR, and lactate and a negative and significant correlation with pH. Also, a positive and significant correlation existed between neutrophil and monocyte, NLR, MLR, and lactate. A positive and significant correlation between lymphocyte and monocyte, pO2, and lactate, and a negative and significant correlation between lymphocyte and NLR, PLR, MLR, and pH were determined. There was a positive and significant correlation between monocyte and MLR, pO2, and lactate, while a negative and significant correlation was found between monocyte and PLR. NLR correlated positively and significantly with PLR, MLR, and pH and negatively and significantly with lactate. It was found that PLR has a positive and significant correlation with MLR and pH and a negative and significant correlation with lactate. MLR was found to correlate positively with pH and negatively

with lactate significantly. In addition, a negative and significant correlation was determined between pH, pO2, and lactate. Finally, pO2 correlated positively with lactate and negatively with pCO2 (Table 3).

Table 2: Comparison of blood parameters of the groups

	DRE (n=77)	DNRE (n=129)	p*
	Mean±SD	Mean±SD	
WBC	9.55±3.72	9.57±3.55	0.729
PLT	232.16±82.57	252.88±78.89	0.132
N	5.83±2.99	5.62±2.83	0.676
L	2.86±1.49	3.07±1.55	0.398
M	0.74±0.38	0.69±0.29	0.658
NLR	2.66±2.35	2.38±2.13	0.355
PLR	101.80±62.46	102.41±57.19	0.846
MLR	0.30±0.19	0.26±0.13	0.064
pH	7.32±0.10	7.33±0.11	0.198
pO2	44.90±17.18	107.04±683.03	0.288
pCO2	42.40±7.22	71.45±334.29	0.349
Lactate	4.67±4.20	5.16±4.56	0.358

*Mann-Whitney U test was applied.
WBC: White blood cell, PLT: Platelet, N: Neutrophil, L: Lymphocyte, M: Monocyte
NLR: Neutrophil/Lymphocyte ratio, PLR: Platelet/Lymphocyte ratio, MLR: Monocyte/Lymphocyte ratio
DRE: Drug-resistant epilepsy, DNRE: Drug-nonresistant epilepsy

Table 3. Correlation between age, disease duration, and blood parameters

		Age	Disease duration	WBC	PLT	N	L	M	NLR	PLR	MLR	pH	pO2	pCO2
Disease duration	r	-.002												
	p	.982												
WBC	r	.035	-.126											
	p	.616	.071											
PLT	r	**-.143**	-.065	**.337**										
	p	**.041**	.352	**.000**										
N	r	.042	-.114	**.835**	**.302**									
	p	.552	.102	**.000**	**.000**									
L	r	-.066	-.005	**.551**	**.230**	.093								
	p	.344	.942	**.000**	**.001**	.185								
M	r	-.066	-.043	**.704**	**.309**	**.474**	**.551**							
	p	.347	.541	**.000**	**.000**	**.000**	**.000**							
NLR	r	.086	-.074	**.139**	.034	**.614**	**-.685**	-.105						
	p	.219	.289	**.047**	.627	**.000**	**.000**	.132						
PLR	r	-.017	-.041	**-.328**	**.302**	.087	**-.820**	**-.355**	**.678**					
	p	.804	.559	**.000**	**.000**	.214	**.000**	**.000**	**.000**					
MLR	r	.028	-.039	-.021	.034	**.326**	**-.637**	**.228**	**.711**	**.622**				
	p	.693	.582	.763	.626	**.000**	**.000**	**.001**	**.000**	**.000**				
pH	r	-.006	-.029	**-.242**	**-.138**	-.105	**-.297**	-.137	**.152**	**.199**	**.155**			
	p	.931	.681	**.000**	**.047**	.133	**.000**	.050	**.029**	**.004**	**.026**			
pO2	r	-.045	-.056	**.206**	.073	.128	**.172**	**.267**	-.049	-.133	.068	**-.163**		
	p	.520	.422	**.003**	.296	.067	**.013**	**.000**	.488	.056	.334	**.019**		
pCO2	r	.000	.071	-.090	-.015	-.094	.015	-.081	-.024	-.008	-.067	**-.218**	**-.347**	
	p	.999	.309	.198	.835	.177	.832	.248	.729	.905	.337	**.002**	**.000**	
Lactate	r	.099	-.056	**.374**	**.225**	**.198**	**.407**	**.343**	**-.163**	**-.272**	**-.089**	**-.667**	**.298**	-.062
	p	.155	.423	**.000**	**.001**	**.004**	**.000**	**.000**	**.019**	**.000**	**.205**	**.000**	**.000**	.376

WBC: White blood cell, PLT: Platelet, N:Neutrophil, L:Lymphocyte, M:Monocyte
NLR: Neutrophil/Lymphocyte ratio, PLR: Platelet/Lymphocyte ratio, MLR: Monocyte/Lymphocyte ratio

4. Discussion

According to WHO's Global Burden of Disease study, epilepsy is a neurological disease with the second most frequent economic burden and a cause of disability (19). Many mechanisms, such as genetic predisposition, developmental dysfunctions, neuronal death, dysfunctional synaptic changes, and hyperexcitable neuronal transmission, are held responsible for the pathogenesis of epilepsy (20). In many situations that especially lead to brain damage, an acute neuroinflammatory response occurs in which proinflammatory molecules increase and the blood-brain barrier (BBB) is disrupted (21, 22). In preclinic epilepsy model studies recently conducted, it has been emphasized that the increased inflammation in the brain regions where the seizure starts and spreads leads to neuronal hyperexcitability, which has a role in seizure generation (23, 24). The contribution of local and systemic inflammatory response in epileptogenesis has been clarified by demonstrating the increase in chemokine and cytokine values (20).

Approximately 30% of epilepsy patients have been classified as DRE, and despite effective therapy, seizure control could not be achieved (5). The ILAE (International League Against Epilepsy) defined DRE as a disease in which freedom from seizure could not be achieved despite combining two or more tolerable and appropriately chosen drugs (7). In these patients, difficulty in seizure control, psychological dysfunction, decreased quality of life, and increased mortality risk are more frequently seen (22). In addition, patients who present to emergency services with frequent epileptic seizures continue to create a significant economic and work burden (25). In our study, we aimed to examine systemic inflammatory indices and blood gas parameters in order to evaluate clinical progression in patients who presented to our emergency service with epileptic seizures and were grouped according to their anamnesis, clinical history, and medication history.

Systemic inflammatory indices such as neutrophil/lymphocyte, platelet/lymphocyte, and monocyte/lymphocyte ratios are applied in many diseases as cheap and available biomarkers (26-30). Systemic inflammatory indices are one of the important parameters that can be used in acute neurological diseases, as in many diseases (10-12, 26). Stredny et al. emphasized the importance of determining a peripheral biomarker instead of invasive methods for reliable predictability of epilepsy seizures (15). In many studies, the usability of these biomarkers in the differentiation of epileptic seizures from nonepileptic conditions has been investigated (13, 16-18, 31).

In a case-control study comparing epilepsy patients with healthy control group patients, the mean NLR ratio was significantly higher in epilepsy patients (p=0.026) (13). Our study found NLR and MLR levels in DRE patients to be statistically insignificant but relatively high. In another study, a significant relationship was found between retrospective NLR levels of 116 patients followed up in intensive care with status epilepticus and their intensive care unit (ICU) requirement and hospital stay durations (p=0,046, p=0,020, respectively) (14). In our study, in DRE patients whose NLR levels were higher, disease duration was significantly longer than in DNRE patients ($p<0.01$).

In a study conducted by Gunes et al. comparing patients presenting with generalized tonic-clonic seizure and healthy control group patients, NLR and PLR levels were found to be statistically significant in the first 60 minutes and subacute (in hour 72 of epileptic seizure) periods ($p<0.01$, $p<0.05$, respectively). In that study, it was shown that a one-unit increase in NLR caused an increase in seizure risk by 1.95 times and was associated with epileptic seizure and neutrophil-mediated inflammation (16). In our study, the NLR ratio rather than the PLR value was higher in DRE patients, which suggests that neutrophil-mediated inflammations could be more prominent.

Moreover, studies were conducted on the diagnostic property of changes in serum lactate levels during seizures (17, 18). In a study by Magnusson et al., increased serum lactate levels were demonstrated to be a valuable biomarker for predicting epileptic seizures in individuals who experience transient loss of consciousness (17). In another study, serum lactate levels (suggested cut-off value 2.43 nmol/l) in the first two hours were found to be significantly higher in generalized tonic-clonic seizures compared to psychogenic nonepileptic seizures (PNES) and syncope patients ($p<0.001$, $p<0.001$, respectively) (18). In our study, serum lactate levels were increased in both groups, but no significant difference was observed between the groups.

Although there are studies which examined systemic inflammatory indices and blood gas comparing epilepsy patients and non-epilepsy patients, studies on the differences within epilepsy patients are few. Our study examined these parameters between DRE and DNRE patients, but we could not determine a statistically significant difference.

In order to understand treatment options for epilepsy, it is necessary to examine the underlying mechanisms in depth. Determining certain biomarkers is important in terms of estimating the course of the disease in order to especially predict the progression of the disease in DRE patients, who constitute nearly 30% of all epilepsy patients. As a result of the study, it was seen that there was no significant difference between DRE patients and DNRE patients in terms of the parameters used as systemic inflammatory biomarkers in epilepsy patients.

Limitations of Study

Our study has a few limitations. The first of these is the small number of patients. Our second limitation is that the study is retrospective. Another limitation of our study is that it is single-centered.

Conflict of interest
The author has no conflicts of interest to declare.

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Ethical statement
Ethical approval for the study was obtained from Malatya Turgut Ozal University Non-Interventional Clinical Research Ethics Committee with the decision numbered 2022/92 and dated 10.05.2022.

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None to declare.

Authors' contributions
Concept: T.E., Design: T.E., Data Collection or Processing: T.E., Analysis or Interpretation: T.E., Literature Search: T.E., Writing: T.E.

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Investigating the role of microRNAs, inflammation, and *Helicobacter pylori* in Epstein-Barr virus associated gastric cancer

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Abstract

Epstein-Barr virus-associated gastric carcinoma (EBVaGC) is a distinct subtype that accounts for nearly 10% of gastric carcinomas. This type of gastric cancer has no relation to any mutation in chromosomal genes, and EBV causes cancer by affecting the epigenetics of host cells through methylation and inactivation of the promoter of tumor suppressor genes. This suggests that EBV infection precedes the clonal growth of EBV-infected cells and subsequently develops carcinoma. Chronic gastritis in the background of EBVaGC might enhance the chance of interaction between gastric epithelial cells and B lymphocytes, and cytokines produced by inflammatory cells might support the growth of EBV-infected gastric epithelial cells. Numerous modifiable risk factors have been identified for gastric cancer (GC). Inflammation is a complicated host immune response to biological, chemical, and physical invasions. Chronic inflammation, which is caused by genetic mutations, autoimmune diseases, constant exposure to environmental factors, and viral infections, can significantly increase the risk of cancer. According to epidemiologic studies, chronic infection and inflammation are considered the main risk factors for different types of cancer. Furthermore, although oncogenic viruses stimulate inflammation by dint of different mechanisms, they generally activate certain signaling pathways, including NF-κB and STAT3, in charge of cancer development. The role of EBV in chronic gastric inflammation has received little attention. However, several studies have indicated that EBV as well as *Helicobacter pylori* is initially involved in the oncogenic process of GC by increasing chronic inflammation and tissue damage. Moreover, other risk factors, including lifestyle and HPV infection, play a role in the progression of GC.

Keywords: Gastric cancer, Epstein Barr virus, *Helicobacter pylori*, inflammation, microRNA

1. Introduction

Gastric cancer (GC) has been indicated as one of the cancers with a high mortal rate particularly among older males. GLOBOCAN 2018 data reports GC as the 5th most common cancer and the 3rd leading cause of cancer-related mortality; and estimates 783,000 deaths in 2018 (1). GC is one of the most often diagnosed malignancies globally, with a highly dismal prognosis (2). Considering the type of cell involved, GC is classified into the following 4 types: 1) Adenocarcinoma: in the gastric inner lining cells (mucous surface); 2) Lymphoma: immune system cancer in the gastric lymphoid tissue, which is extremely rare; 3) Gastrointestinal stromal tumors (GIST): the gastrointestinal epithelial tumors in the interstitial Cajal cells, which occur rarely; 4) Carcinoid tumors are generally formed in the cells secreting the gastrointestinal hormone (3). Over 90% of gastric tumors are adenocarcinomas, and etiological factors such as socioeconomic conditions, diet, hereditary factors, *Helicobacter pylori* (*H. pylori*) infections, and the Epstein Barr Virus (EBV) are significantly reported to be involved in their pathogenesis (4, 5). GC manifests in various symptoms; however, patients are often diagnosed in the advanced stages. Hence, the identification of the risk factors along with their early treatment can be helpful in the prevention of GC development (6).

EBV infection is related to certain types of cancer, including Hodgkin's lymphoma (HL), Burkitt's lymphoma (BL), lymphoma in diarrhea patients, and some other carcinomas. This infection was first discovered in 1964 by Anthony Epstein and Yvonne Barr, who used electron microscopy to identify the herpes simplex virus in a subpopulation of BL cells in African patients (7, 8). EBV is a herpes virus that infects more than 90% of the world population

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before adolescence. This virus has been observed in epithelial malignancies including GC (9). EBV-associated gastric carcinoma (EBVaGC) accounts for 8 to 10% of cases, and it is estimated to infect more than 90,000 individuals yearly (10). Following the onset of the infection, EBV remains latent in the B-lymphocytes at a rate of 1/106. EBV is hard to diagnose since the expression of very few numbers of viral proteins provides the preservation, control, and proliferation of the cells (11, 12). In a pediatric study, infection with EBV was reported to be the main cause of severe gastritis and chronic inflammation in comparison to the separate infections of the related pathogen (13).

Furthermore, H. pylori infects about 50% of people worldwide which induces gastric inflammation and optimizes the necessities for EBV tumorigenesis. It has been demonstrated that overexpression of inflammatory markers and epigenetic alterations such as hypermethylation associated with EBVaGC are due to H. pylori infection (14).

Inflammation is defined by the attempt of the immune system to fight against infections, injuries, and toxins, and is characterized by the infiltration of mononuclear cells, especially macrophages in the damaged tissue. Due to the presence of inflammatory cells in tumor tissues, it was suggested that chronic inflammation may play a key role during carcinogenesis through persistent activation, leading to continuous tissue damage. Later, it was determined that about 25% of all cancer types including GC are associated with chronic inflammation. H. pylori causes chronic gastritis and there is a well-known correlation between H. pylori and chronic inflammation, which together result in gastric adenocarcinoma (15, 16).

Randomized clinical studies show that individuals with gastroesophageal adenocarcinoma and nonmetastatic GC benefit from combined treatment. Although postoperative chemotherapy following an appropriate lymph node dissection is a treatment choice, current recommendations identify perioperative chemotherapy or postoperative chemotherapy with chemoradiation as preferable treatments (17).

MicroRNAs (miRNAs) are a class of non-coding RNAs (ncRNAs) that modulate gene expression by suppressing translation. It is noteworthy that the expression of these RNA molecules can be regulated by further DNA methylation and chemical modifications of the histones. It has been extensively reported that mutations or the wrong expressions of miRNAs are associated with various human cancers, indicating their ability to inhibit tumorigenic and oncogenic agents (18, 19). EBV can encode miRNAs in its own DNA sequences. Various genomic profiling studies have claimed EBV-encoded miRNAs play a crucial role in EBVaGC (20).

In the current study, we reviewed the role of chronic inflammation, H. pylori infection, HPV infection, viral miRNAs and lifestyle during the development and progression of EBVaGC (Fig.1).

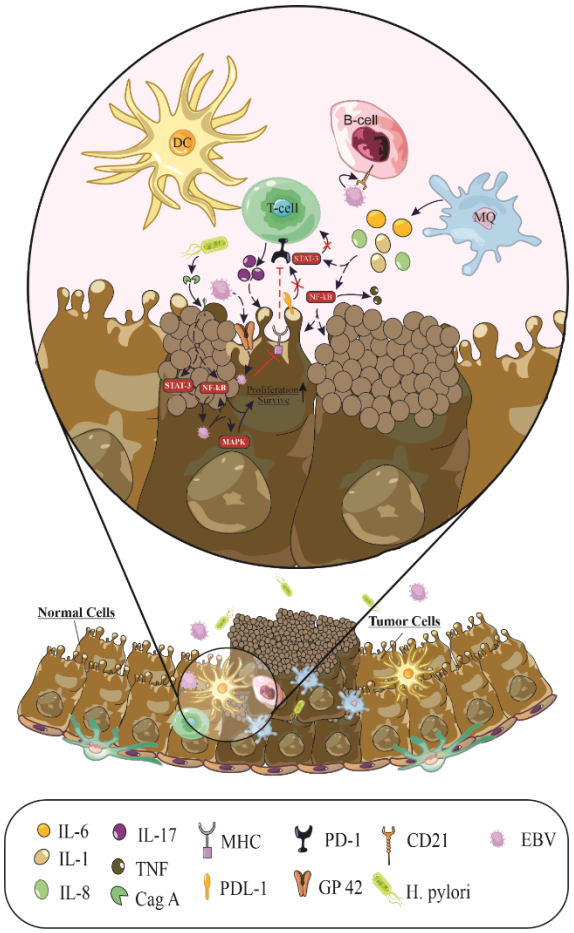

Fig. 1. Co-infection of H. pylori infection and EBV

Infection with *H. pylori* leads to the activation of a series of intracellular signaling pathways, followed by the reactivation of latent EBV infection. Oncogenes expressed by EBV cause disturbances in the identification of tumor cells and subsequently reduce the response of immune cells during infection and thus increase the development of malignancy.

2. Chronic Infection-related inflammation, and carcinogenesis

It has been reported that chronic inflammation is associated with tumorigenesis as well as increased cell proliferation, cell survival, invasion, angiogenesis, and metastasis (21). According to the estimates, infectious diseases and chronic inflammation make up approximately 25% of carcinogens (22). Research findings also indicate that inflammation suppresses the DNA repair system (23). On the other hand, macrophages (MQ) play a key role in chronic inflammation and can trigger a pro-inflammatory response by secreting inflammatory factors and several prototypical pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8 (24, 25). In this regard, interleukin-10 (IL-10) is an important anti-inflammatory cytokine capable of suppressing DNA damage (26). Persistent infection can result in an over-inflammatory response that is generally harmful to the host by exerting mutagenic effects on the host genome (27). The onset of cancer in the course of a viral infection is classified into two categories

considering the capacity of virus for direct or indirect involvement in the stimulation of cell proliferation and/or interfere with apoptosis, playing a direct role in carcinogenesis (28). Inflammatory excitation and the direct effects of the virus also activate the signaling pathways responsible for carcinogenesis including Nuclear factor-kappa B (NF-κB) and signal transducer and activator of transcription3 (STAT3) (29, 30).

NF-κB and STAT3 are two major factors linking inflammation to cancer. NF-κB is considered as pro-inflammatory signaling pathway due to its activation by proinflammatory cytokines and tumor necrosis factor α (TNFα). NF-κB controls several cellular processes including proliferation, apoptosis, and angiogenesis. The NF-κB signaling pathway is considered as an apparent mediator between inflammation and development of various cell populations in the stomach to cancer. Activation of NF-κB is crucial for regulating immune responses. It has been reported that aberrant expression of NF-κB can lead to inflammation and therefore cancer-associated inflammation, especially in human gastrointestinal cancer (29, 31).

On the other hand, the STAT family proteins, especially STAT3, play crucial roles in the induction of a pro-carcinogenic inflammatory microenvironment. STAT3 can be activated by various genes, including cytokines and oncogenes. It has been reported that continuous activation of STAT3 suppresses anti-cancer immunity and therefore induces tumor cell survival proliferation, and invasion. Recently, what has been investigated? GC? has been investigated the role of NF-κB and STAT3 in a few cancer types including GC. Also, the activation and interaction between STAT3 and NF-κB play a key role in the connection between cancer cells and inflammatory cells (29, 32).

In chronic inflammations, ROS/RNS are released by inflammatory cells as well as the epithelial cells, stimulating DNA damage in the affected organs and instigating the risk of carcinogenesis (33, 34). Epigenetic gene silencing also significantly contributes to carcinogenesis by reducing the expression of the tumor suppressor genes and microRNAs. In inflammatory microenvironments, exposure to ROS/RNS or pro-inflammatory cytokines such as IL-6 influences the expression of DNA methyltransferase 1 (*DNMT1)*, increasing DNA methylation in the tumor suppressor genes and miRNAs (35). Malignant human tumors are characterized by the alterations in the DNA methylation patterns. This process involves the general hypomethylation of cancer cells and hypermethylation of some CpG islands, most of which are within gene promoters (36). DNA methylation in GC rich regions is an important stimulus for the oncogenic process and is linked to *H. pylori* and EBV infections (37, 38).

3. The Role of EBV in gastric carcinogenesis

It was initially proposed that EBV infects B cells only, later it was reported in the nasopharynx epithelial cells (39), liver cells (40), gastric epithelial cells (41), and brain cells (42). According to the previous studies, EBV may be transmitted into the cells through the oral epithelium by the EBV+IgA combination (43). The EBV transmission potentially results in the early penetration of the virus into the B cells, triggering a systemic infection. In patients infected with EBV, the virus can also be secreted in the saliva (44, 45). The B-cell surface receptor for EBV is CR2, or CD21. The EBV envelope glycoprotein, gp350/220, is a ligand that binds to the cell's CD21 surface marker (46). The second EBV receptor on the B-lymphocytes is the class II HLA molecules, where the virus binds them through the GP42 glycoprotein. The class II HLA molecules are not present on the epithelial cells, and thus it can be proposed that the glycoprotein GP42 is not necessary for the EBV infection of epithelial cells. Nevertheless, not only is GP42 not regulated in epithelial cell infections, but its presence can also impose inhibitory effects (47, 48). Herpes viruses are suggested to have two different life cycles: lytic replication and latent replication. EBV can either lead to latent infection or enter a lytic cycle in the host cells; however, expressing some viral genes, including ncRNAs, prefers to remain latent. The phase change from latent infection to the lytic cycle is done through two genes, BZLF*1* and BRLF*1,* which encode the two transcription factors Zta and Rta, respectively (49). In particular, EBV can infect the host gastric epithelial cells by employing direct and indirect mechanisms. In direct infection, viral glycoproteins bind to the cell receptors, modifying the viral proteins with constructive changes and culminating in the consequent increase in the fusion of viral envelopes and epithelial cellular membranes (50). EBV is most probably not a passive carrier but rather the oncogene of an active virus contributing to the development of GC in the early stages (51). In recent years, it has been increasingly reported that EBV may contribute to GC through the expression of viral proteins and miRNAs as well as by inducing aberrant DNA methylation in CpG islands and posttranslational histone modification (52). These alterations are suggested by the existing scientific evidence to be involved in the development of EBVaGC (53).

4. EBV gene expression in infected cells and their functions

Considering the subset of the expressed viral genes, the herpes virus-related tumors are classified into the following four categories: latency Ia, latency Ib, latency II, and latency III. EBVaGC belongs to the latency type I category, which includes Epstein-Barr virus latent membrane protein 2A (LMP2A), Bam-HI A rightward transcripts (BARTs), and Epstein-Barr virus (EBV)-encoded EBV-encoded small ribonucleic acid 1/2 (EBER1/2) (54, 55). LMP1 and LMP2 activate the well-known NF-κB and MAP kinase signaling pathways, which are responsible for cell survival and proliferation (56, 57). The viral LMP2A protein also influences the NF-κB pathway and increases the production of survival genes. Therefore, it increases apoptosis inhibition as well as cancer stem cells induction in the EBV-associated epithelial cancers (58). Several cases of the involvement of BamHI-A

rightward frame 1 (*BARF-1*) in GC have also been reported. Almost every case of EBVaGC has the BARF-1 gene expressed (59). BARF-1 is demonstrated to enhance cell proliferation by regulating the NF-κB and cyclin-D1 expressions in EBV-infected gastric carcinoma cells. Besides, BARF-1 reduces expression by inhibiting p21 (60). EBNA1 is an EBV-encoded sequence-specific DNA-binding protein that is consistently expressed in EBV-associated tumors and required for stable maintenance of the viral genome in proliferating cells. EBNA1 is also thought to play a role in cell survival in latently infected cells (61). Inhibition of EBNA1 through compound inhibitors diminished the particular EBV genome copy number in Raji Burkitt lymphoma cells (62). EBERs are the most abundant genes among the latent ones in the infected cell (63). So, EBER1-*in situ* hybridization (ISH) is considered as the gold standard method to detect EBV. There are some findings around the role of EBER1 in the EBVaGC. As it is obvious, downregulation of E-cadherin is necessary for tumorgenicity. It has been reported that EBER-1 can dysregulate the cellular miRNA expression levels to suppress E-cadherin, and therefore induce epithelial-to-mesenchymal transition (EMT) in gastric carcinoma cells (64). Interestingly, Banerjee *et al.* proved that EBERs can upregulate pro-metastatic markers such as pFAK and pPAK1, and suppress anti-metastatic factors, which accounts for cell migration. Further, EBERs could promote chemoresistance by indirect downregulation of the p21 and p27 cell cycle inhibitors (65).

4.1. Immune responses in EBVaGC

During the infection of epithelial cells, the EBV encoded regulatory viral RNAs might modulate the host's innate immune responses (66). Moreover, BARF1, BART miRNA, EBER1/2, and EBNA-1 inhibit the interferon response. EBNA-1 and BNLF2 interfere with the antigen presentation by MHC molecules and the identification via CTL. Tumor cells also express the programmed death-ligand 1 (PD-L1), which binds to the PD-1 receptor in CTLs and other immune cells and inhibits the effective immune response. Similarly, BART miRNA inhibits the expression of major histocompatibility complex class 1-related chain B (MICB) to prevent NK cell and CTL recognition (67, 68). The different forms of CD44, which is a cell surface glycoprotein and functions as an adhesion molecule, are especially expressed in EBVaGC (69). EBVaGC lymphocytes are primarily CD8-positive cytotoxic T cells (70), which improve antitumor immunity (71). However, during tumor growth, the exact mechanism of the carcinoma cells preventing the host immune response is not fully understood. In this regard, IL-1β is the only cytokine that is considerably expressed on a large scale in EBVaGCs as compared to EBVnGCs. IL-1β may use a large number of nonspecific lymphocytes to protect EBV-specific cytotoxic T cells and tumor cells from direct exposure (72, 73). It also inhibits the secretion of gastric acid, allowing for the growth of EBVaGC (74). Infiltrating protective cells at a minimum degree aids antitumor defenses by increasing the complete destruction of EBV-positive cancerous cells (70, 71, 75). Finally, the expression of EBER1/2 and BART miRNAs improves the immune suppressor level, i.e. IL-10 expression (76, 77).

4.2. The Role of MicroRNAs in EBV-Associated Gastric Carcinogenesis

It has been highlighted in EBVaGCs that microRNAs, as well as long noncoding RNAs (lncRNAs), play significant roles in the regulation of gene expression following transcription (78, 79). This process involves approximately 25 viral miRNA precursors and 44 mature microRNAs, which are classified into two large clusters: miR-BART and miR-BARF-1. The targetome of EBV miRNAs are associated with signal transduction, oncogenesis, cell adhesion, and apoptosis, all of which contribute essentially to carcinogenesis (80). For instance, BART reduces BID (BH3 interacting-domain death agonist) expression, which is an apoptotic molecule. Furthermore, miR-BARTs are more abundant in NPC and EBVaGC than in EBV-positive B lymphoma (79, 81). It was also found that miR-BART20-5p suppressed lytic replication by directly targeting *BRLF1* and *BZLF1* (49). Further, it improves the discrimination of the apoptosis factor BAD (BCL2 associated agonist of cell death) and stimulates the proliferation of GC cells through BAD suppression (82). Besides, it has been reported that miR-BART20-5p could target BID, which belongs to the Bc1-2 gene family and suppresses cell death in GC cells. Regarding the targeting of BAD and BID, it was indicated that miR-BARTs suppress cell death by targeting different genes (80, 83). When EBVaGC is induced by latent EBV infection, it expresses low amounts of viral antigen to enable the virus to escape the immune system, maintaining a certain degree of infection. MiRBART6 also stimulates latent EBV infections. The host cellular miRNAs can be dysregulated by the latent EBV genes, resulting in epithelial-mesenchymal transition (EMT). Moreover, the levels of two host miRNAs, miR-200a and miR-200b, are decreased in EBVaGC. MiR-200a and miR-200b target two transcription repressors, ZEB1 and ZEB2, which control E-cadherin expression levels. Therefore, downregulation of the aforementioned miRNAs can lead to EMT and promote tumorigenesis. The EBV-derived miRNAs prevent the translation of viral and host mRNAs. For instance, Shinozaki *et al.* reported that EBV-encoded LMP2A suppresses the expression of pri-miR-200 as a receptor of EMT (64, 79, 80, 84). These reports also suggest that EBV infections change the attributes of the host cells, which may increase the metastatic activity of tumor cells infected with EBV.

4.3. *H. pylori* in GC

Marshall and Warren earned the Nobel Prize for Medicine in 2005 for proving that *H. pylori* contributes to peptic ulcer disease (85). *H. pylori* is a spiral-shaped, gram-negative, urease-positive bacteria (86) that resides in the human gastric and duodenum, which is present in the body of half of the world's population. Peptic ulcers, GC, MALT lymphoma, and

other extra-gastrointestinal disorders have all been linked to *H. pylori* as a cause (87, 88). *H. pylori* has been categorized as a Group 1 carcinogenic pathogen, according to the International Agency for Research on Cancer (IARC). In both Western and Eastern nations, *H. pylori* infection is regarded as a significant risk factor for GC (89).

H. pylori encodes a wide range of genes that are involved in its pathogenicity and microenvironment modification including urease, carbonic anhydrase, Lewis antigen, VecA, CagA and outer proteins (BabA2) (90). The virulence factors produced by *H. pylori* can alter the signaling pathways in the host cell. *H. pylori*'s ability to survive for decades in the stomach environment due to the host's inability to eradicate the infection makes this trait particularly significant. Due to the pathogen's urease, which converts urea to ammonia and creates a neutral environment around the bacteria, it may colonize the stomach's extremely acidic environment. *H. pylori* is capable of eluding host immune responses while interacting with gastric cells and surviving in the severe environment of the gastric corpus (85). In order to create an environment that is immunosuppressive and supports chronic infection, *H. pylori* aggressively manipulates host tissues. *H. pylori* inhibits the effector activities of CD4+ T cells, dendritic cells, and macrophages while promoting the production of regulatory T cells and myeloid-derived suppressor cells (91). In conclusion, Lewis antigen is a crucial protein that supports *H. pylori* survival in difficult habitat circumstances, followed by CagA, Bab, and VacA that cause *H. pylori* to inhabit the gastric epithelium and cause inflammation. The evolution of *H. pylori* is being served by all of these genes and the proteins that are produced as a result of them (90).

5. EBV co-infection with *H. pylori* in GC

Several reports have revealed the synergistic effects of *H. pylori* and EBV in the development of GC. In the initial phases, patients suffering from *H. pylori* and EBV demonstrate severe inflammatory signs compared to patients suffering from *H. pylori* alone (13). Another study on co-infections suggests that EBV accompanied by *H. pylori* induces inflammatory responses in patients, increasing the risk of gastrointestinal cancer progression (92). It was also indicated that *H. pylori* infection is associated with EBV reactivation in patients showing gastric signs (93). Moreover, the activation of EBV in the latent cycle of the infected gastric epithelial cells is incited by monochloramine, which is produced by *H. pylori* (94). According to this evidence, the co-infection of these pathogens can probably increase the risk of GC (95, 96). Furthermore, in the *H. pylori*-positive patients, the level of EBV DNA is often evidently higher, suggesting the role of *H. pylori* in the transformation of the lytic EBV cycle (94). Two possible mechanisms are involved in this process. The first mechanism induces an additional inflammatory response in the co-infection, where both the EBV and *H. pylori* can increase tissue damage (13, 97). Therefore, a significant increase in IL-1β (98), TNFα (99), and IL-8 (100), levels is observed. The second mechanism involves the interactions among the gene products, which mainly happen between EBV and *H. pylori*. The findings from an in vitro study indicated that the EBV reaction takes place via the PLC_{γ} signaling pathway, while an *H. pylori* toxin known as CagA severely activates PLC_{γ}, as well as several other kinases (101, 102). CagA in *H. pylori* and LMP1 and LMP2 in EBV activate the MAP (mitogen-activated protein) kinase and NF-κB pathways, which are the well-known pathways for the proliferation and survival of the cells during carcinogenesis (56, 57). *H. pylori*, using CagA oncoprotein, triggers the unusual activation of the WNT signaling pathway, resulting in the activation of CDX1 as a downstream gene (103, 104). Both pathogens share several pathways and activate the transformer factors in the gastric epithelial cells via the β-catenin signaling pathway (13, 105). Some studies have also indicated that *H. pylori* reduces the expression of TGF-β, which reactivates the lytic phase of EBV. Hence, it may be involved in the prevention of the reactivation of the lytic phase of EBV and prevention of GC (106). Therefore, more studies must be carried out on the co-infection of *H. pylori* and EBV to unveil the potential roles of both pathogens.

6. HPV infection and GC

Another factor associated with GC is human papilloma virus (HPV) infection (107). HPVs are part of the family of DNA viruses named Papillomaviridiae family, and new species are continually being identified. The epithelia of the upper respiratory tract, genitalia, and skin are where this virus exhibits the highest rate of tropism (108-110). According to findings, HPV is one of the key infectious agents contributing to prostate, cervical, anal, and colorectal cancer as well. The majority of studies contend that co-occurring HPV and *H. pylori* can cause cancer, while some research indicates no such association (111-114). According to studies, high-risk HPV types 16 and 18 are closely associated with GC specimens, which may serve as a warning to those in the control group who tested positive for these HPV subtypes (107, 115). Moreover, immunization against this virus (HPV) has to be pursued more aggressively in order to hinder malignancies linked to it (107). HPV is thought to increase the chance of developing neoplasms. Neoplastic transformation is typically a protracted, extremely complicated, and multi-step process and is brought on by numerous genetic and epigenetic variations (116). However, there is some debate on the connection between GC and HPV infection and more study is required to be sure (108).

7. Epigenetic modifications in EBVaGC: DNA methylation

Several aberrantly methylated genes have been observed in gastric adenocarcinomas caused by EBV and *H. pylori* co-infection. The most common hypermethylated genes are *CDK2A*, *CDH1*, *DAPK*, *COX2*, and *MLH1*. These genes are often altered in different cancers including GC (81). Methylation of tumor suppressor genes is the main cause of the unusual state of EBVaGC. In EBVaGC tumor cells, methylation of different CpG regions in the tumor-associated

promoters has been repeatedly observed and substantial roles have been attributed to them in the development of GC (117, 118). CpG island methylation is an epigenetic process in gene presentation, influencing all cellular pathways (119). Higher than half of genes hold the CpG site in the promoter section which is found as CpG islands (120). Methylation in CpG sites inside of a promoter region could suppress binding in transcription conditions to the succession of neoplasm suppressor genes (121). The repeated methylation of the tumor suppressor genes (such as *RASSF1A*, *PTEN*, and *APC*) and the adhesion genes (e.g., *THBS1* and E-cadherin) in EBVaGC are evidently higher as compared to EBV-negative samples (122, 123). Moreover, the EBV infection is associated with an increase in the expression of *DNMT1* in gastric carcinomas (124). The high frequency of methylation in the genome has been reported in EBVaGC. Besides, several methylated genes including *RUNX3, p73, p16, DAPK1, PTEN, RASSF1A*, and *GSTP1* are often observed in EBVaGC as well as EBV-negative GC although with lower methylation levels (125, 126). EBV-related LMP2A activates several cellular signaling pathways including the PI3K/AKT and JAK/STAT3 that carry out most *DNMT* regulations as well as other epigenetic modifiers during the EBVaGC pathogenesis. LMP2A can increase *DNMT3b, DNMT1*, and the expression of B lymphoma Mo-MLV insertion region 1 homolog (*BMI1)* on the transcription and translation levels. Besides, it increases the expression of *DNMT1* by inducing STAT3 phosphorylation, which is independent of the stimulation of IL-6. It increases PTEN methylation, suppressing it in EBVaGC (127, 128). CpG island methylation in the promoter regions (CIMP) of the tumor suppressor genes such as *PTEN* and *CDKN2A* also occurs. This process entails the structural activation of the PI3K pathway and the inactivation of the cell cycle checkpoints. The Akt, PI3K, and mTOR inhibitors, beta-catenin, and notch can also act against the EMT, and stemming resistance mechanisms (129, 130). On the other hand, LMP1 is rarely expressed and this protein is not generally expressed in EBV-associated gastric carcinoma. The transfer of BARF1 to a GC cell makes considerable changes to the host gene expression. It particularly changes the expression of genes associated with proliferation and apoptosis. BARF1-transfected cells demonstrate chemical resistance and higher expression of Bcl-2 in comparison to Bax (131, 132). The various expression patterns connected to the three stages of the life cycle of the EBV are controlled by epigenetic principles (133). All in all, the methylation of both viral and host DNA strands is one of the major mechanisms involved in the development of EBVaGC. The EBV infection of epithelial cells can result in DNA methylation. Viral DNA methylation prevents recessive EBV genes. Finally, the DNA methylation of the host cells inactivates the tumor suppressor genes and the associated antigens (134).

8. Lifestyle

Primary cancer prevention through lifestyle and dietary modifications is still a top focus since it is an essential technique for lowering the population burden of many cancers. Physical exercise and lifestyle variables like relative body mass are thought to be major modifiable factors in cancer prevention (2). Several dietary exposures have been related to GC; however, the connections may be influenced by intrinsic biases. Tobacco usage, alcohol drinking, salt-preserved foods, age, gender, medical history and industrial and chemical pollutants are also known to be connected with an elevated GC risk (135, 136). Tobacco smoking has been linked to 11% of GC cases globally. Similarly, alcohol use has been linked to the development of GC. A big pooled investigation discovered a link between high alcohol use and the chance of developing GC (137). Moreover, fruit consumption may be beneficial to both genders (137). Certain risk factors include *H. pylori* infection as well (137).

As estimated, half of the people worldwide are thought to have *H. pylori* infection though its frequency varies geographically (89). Improvements in living conditions have led to a decline in *H. pylori* infection rates in some nations, although the prevalence is still high in the majority of underdeveloped nations (89), that's the reason why infections with *H. pylori* are less common in developed countries than in developing ones (87). Depending on socioeconomic condition and the quality of hygiene, the prevalence of *H. pylori* infection has been estimated to range from 41.5% to 72.3% in China (89).

9. Other factors influencing the progress of GC

There are other factors influencing the progress of GC P53 mutation and overexpression are common during the development of GC and are therefore recognized in cancer areas as well as precancerous dysplasia and metaplasia (138). This approach points out of that p53 mutation may be an earlier occurrence for GC. EBVaGC usually shows more varieties of p53 in contrast to EBV-negative GC (139). Mainly because GC malignancy is connected to *H. pylori*, some sort of influencing factor with severe gastritis, abdominal metaplasia, and cancer malignancy located primarily on the antrum. These kinds of pathogenic agents happen to be a reason for influencing GC malignancy with an independent process (140). In addition, regular prognosis with both EBV and *H. pylori* in the mucous membrane having reasonable for chronic atrophic gastritis with instigative cell infiltration (95).

Factors affecting the development of GC are summarized in Table1.

Table1. Factors affecting the development of gastric cancer

Factor	Component	Function/Mechanism	References
Inflammation and immune responses	Macrophages	Triggering a pro-inflammatory response by secreting inflammatory factors and several prototypical pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8	(24, 25)
	Over-inflammatory responses	Mutagenic effects on the host genome	(27)
		Suppressing DNA repair system	(23)
	NF-κB	Activated by proinflammatory cytokines and tumor necrosis factor α (TNFα)	(29, 31)
		Controlling several cellular processes including proliferation, apoptosis, and angiogenesis	
		Mediator between inflammation and development of various cell populations	
	STAT3	Activated by various genes including cytokines and oncogenes	(29, 32)
		Induction of a pro carcinogenic inflammatory microenvironment	
		Continuous activation of STAT3 suppresses anti-cancer immunity and therefore induces tumor cell survival proliferation, and invasion	
	ROS/RNS	Stimulating DNA damages	(33, 34)
	PD-L1	Binding to the PD-1 receptor in CTLs and other immune cells and inhibiting the effective immune response	(67, 68)
	IL-1β	Inhibiting the secretion of gastric acid, allowing for the growth of EBVaGC	(74)
Epigenetic gene silencing	MicroRNAs	Reducing the expression of the tumor suppressor genes and microRNAs	(35)
	DNA methylation	Influencing the expression of *DNMT1*, increasing DNA methylation in the tumor suppressor genes and miRNAs	
		DNA methylation in GC rich regions is a stimulus for the oncogenic process	(37, 38)
		Viral DNA methylation prevents recessive EBV genes. Finally, the DNA methylation of the host cells inactivates the tumor suppressor genes and the associated antigens	(134)
Epstein-Barr virus	BZLF*1* gene and BRLF*1* gene	Encoding two transcription factors named Zta and Rta	(49)
	Virus itself	Inducing aberrant DNA methylation in CpG islands	(52)
		Posttranslational histone modification	
	LMP1 and LMP2	Activating NF-κB and MAP kinase signaling pathways, which are responsible for cell survival and proliferation	(56, 57)
	BARF-1	Enhancing cell proliferation by regulating NF-κB and cyclin-D1 expressions in the EBV-infected gastric carcinoma cells	(60)
		Reducing cell expression by inhibiting p21	
		Inhibiting interferon response	(67, 68)
	EBNA1	Causing stable maintenance of viral genome in proliferating cells	(61)
		Providing cell survival function in latently infected cells	
		Interfering with the antigen presentation by MHC molecules and the identification via CTL	(67, 68)
	EBER1	Dysregulating the cellular miRNA expression levels to suppress E-cadherin (which is necessary for tumorgenicity) and therefore inducing epithelial-to-mesenchymal transition	(64)
		Upregulating pro-metastatic markers such as pFAK and pPAK1	(65)
		Suppressing anti-metastatic factors	
		Promoting chemoresistance by indirectly downregulation of the p21 and p27 cell cycle inhibitors	
		Inhibiting interferon response	(67, 68)
		Up regulation of interleukin-10	(77)
MicroRNAs	miR-BART (miR-BART20-5p)	Suppressing lytic replication by directly targeting *BRLF1* and *BZLF1*	(49)
		Improving the discrimination of the apoptosis factor BAD	(82)
		Stimulating the proliferation of gastric cancer cells through BAD suppression	
	miR-BARF-1	Having a role in gastric cancer development	(82)

Table1. Factors affecting the development of gastric cancer (continue)

Factor	Component	Function/Mechanism	References
Other infections	H. pylori	Producing monochloramine by *H. pylori* and cause EBV reactivation	(94)
		Having a role in the transformation of the lytic EBV cycle	
		Inducing an additional inflammatory response and increasing tissue damage	(13, 97)
		Interactions among the gene products e.g. EBV reaction takes place via the PLC_{γ} signaling pathway, while an *H. pylori* toxin known as CagA severely activates PLC_{γ},	(101, 102)
		Activating the transformer factors in the gastric epithelial cells via β-catenin signaling pathway	(13, 105)
	HPV	High-risk HPV types 16 and 18 are closely associated with stomach cancer specimens	(107)
Oncogenes	P53	P53 mutation is recognized in cancer areas and additionally for sectors of precancerous dysplasia together with metaplasia	(138)
Lifestyle	Smoking	Known as a risk factor for gastric cancer	(135, 136, 141)
	Body mass		
	Alcohol drinking		
	Diet		

10. Treatment of EBVaGC

Several studies have determined the resistance of EBVaGC to some chemotherapy drugs, including docetaxel and 5-FU. Recently, some researches have shifted forward to test the clinical response to anti-PD1 inhibitors in EBVaGC (14). It has been reported that PD-L1 is overexpressed in EBVaGC. Sho Sasaki1 *et al*. found that in EBVaGC cell lines with highly expressed PD-L1, the proliferation of T-cells was suppressed by PD-L1 overexpression. Further, using PD-L1 antibody treatment led to a moderately lost G0/G1 arrest of the T-cells in EBVaGC (142). In another study, it was determined that in GC patients who are resistant to chemotherapy, the anti-PD-1 antibody nivolumab has a better prognosis and prolonged survival relative to conventional chemotherapy (143).

On the other hand, as hypermethylation is considered as one of the mechanisms underlying EBVaGC, some preclinical studies showed that demethylating agents such as 5-Aza cytidine could be considered as a potential treatment by restoring the expression pattern of methylated genes and stimulating lytic infection, which results in cell lysis (144).

Another approach is using small-molecule EBNA1 inhibitors. EBNA1 plays a key role in EBV-associated cancers. It was determined that applying EBNA1 inhibitor treatment in Raji cells decreases the EVB copy number in a dose-dependent manner in affected cells (145).

The development of EBV vaccines is another strategy for preventive and also clinical use. The particular gp350 glycoprotein is actually for the majority of vaccines being used as an antigen. Moreover, EBNA1, as well as LMP2A, are also utilized for antigens (146).

11. Conclusion

Worldwide, about 90% of people are infected with EBV before adolescence. We reviewed the highlighted risk factors in developing EBVaGC. Chronic inflammation is an important risk factor for EBVaGC which develops tumorigenesis. Infectious disease along with chronic inflammation results in about 25% of malignancies. The findings from several studies suggest that H. pylori helps EBV remain in the latent phase. Infection with EBV also changes the miRNA-related activities of the host cells, and these modifications may increase the metastatic activity of the EBV-infected tumor cells. It triggers the considerable LMP2-induced methylation of the host genome, leading to hypermethylation of several unique methylated genes in EBVaGC. Furthermore, several cellular pathways in EBVaGC are dysregulated, contributing to tumorigenesis. These pathways improve the proliferation. Many modifiable risk factors have been identified for GC including lifestyle, HPV infection, H. pylori infection, and immune response and DNA methylation. Recently, advances in the PD-L1 inhibitors approach makes better prognosis in GC patients. Taken tougher, a better understanding of the molecular mechanisms of EBVaGC may account for finding novel treatment approaches for GC patients.

Conflict of interest

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Authors' contributions

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Review

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Autologous hematopoietic stemcell transplantation in multiple myeloma

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Abstract

Treating multiple myeloma with high-level dose chemotherapy supported by transplanted autologous stem cells is still the primary and appropriate therapeutic strategy in affected patients. Today, although the responses have improved considerably, especially during the treatment with the induction of the combination of immunomodulatory medication and proteasome blockers, no treatment approach has taken autologous stem cell transplantation into the background. In this review, we will share the recent place of autologous stem cell transplantation in myeloma will be discussed from pretransplant treatment to posttransplant.

Keywords: autologous stem cell transplantation, indication, multiple myeloma

1. Introduction

Plasma cell myeloma represents one per cent of all cancers and approximately 10% of all hematological malignancies. Multiple myeloma is slightly less familiar in women than in men. At diagnosis, the average age is around 65 years (1). MM is rough to heal because of the disease and patient-associated heterogeneities. Unfortunately, multiple myeloma persists as an incurable condition. Also, most patients experience one or more recurrences (2). For over two decades, an excessive-concentration dose of chemotherapy followed by autologous stem cell transplantation has been the standard care for newly investigated multiple myeloma in individuals over 65 years. Still, today it is not correct to talk about a specific chronological age limit (3). We know that HCT after high-dose chemotherapy is not a proper therapeutic procedure. Still, overall survival and event-free survival are extended and opposed to treatment with standard myeloma treatments. Age is not enough of a criterion for evaluating autologous stem cell transplantation suitability. Age, cardiac, urinary, pulmonary functions and performance status all must be assessed. Today, especially the developments in supportive treatments allow high-dose chemotherapy supported by autologous stem cell transplantation to be successfully applied up to the age of 80. In this review, initial therapy, stem cell mobilization and storage, the timing of autologous stem cell transplantation, conditioning regimen, care during transplantation, single or tandem transplantation and posttransplant follow-up will be discussed in patients eligible for autologous transplantation.

2. Treatment before autologous stem cell transplant

Even before autologous stem cell transplantation therapy, patients are classified as either standard or high risk based on risk rating. The risk classification in multiple myeloma is shown in Table 1 (4). For patients with standard risk MM, three-drug administrations are preferable to two-drug regimens, as randomized studies suggest that they improve OS (5). The use of four drug regimens is evolving. The triple-drug regimen can be selected according to the patient's comorbid conditions. Nowadays, the VRD regimen is preferred, but if the patient has acute renal failure or thromboembolism, VCD may be selected. If possible high-risk patients should be referred to clinical trials. In the absence of clinical studies, the VRD regimen or another regimen with daratumumab may be preferred. Drugs such as melphalan that damage the hematopoietic stem cell compartment should not be used in the initial treatment; also, more than four cycles should not be used in regimens containing lenalidomide.

Table 1. Risk stratification of multiple myeloma

High Risk	Standard Risk
t(4;14)	Trisomies
t(14;16)	t(11;14)
t(14;20)	t(6;14)
Del 17p	
p53 mutation	
Gain 1q	
R-ISS Stage 3	
High Plasma Cell S-phase	
GEP: High-risk signature	

t: Translocation; R-ISS: Revised International Staging System; GEP: Gene Expression Profiling

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3. Stem cell mobilization and storage

Hematopoietic stem cell (HSC) collection, also called mobilization, is a prerequisite for ASCT. Several variables affect the CD34+ cells collection target value, including the planned number of transplants and mobilization strategies (6). Most studies have shown that transplantation with Peripheral blood stem cells (PBSC) leads to a great harvest of stem cells (7). The optimal CD34+ cell harvest is above 5.106 CD34+/kg; a minimum threshold of 2.106 CD34+/kg is required to achieve engraftment. So, how is stem cell harvesting done? It should be noted that hematopoietic progenitor stem cells must be harvested before the patient is subjected to alkylating medications (8). Hematopoietic stem cells can be collected by apheresis from the peripheral bloodstream after excitation with granulocyte colony-stimulating factor (G-CSF) or with additional chemotherapy. They may be gathered directly from the bone marrow. Peripheral blood progenitor cells (PBPCs) are the appropriate type of bone marrow cells for the transplantation process in MM. Usually, G-CSF is the principal critical agent used in hematopoietic stem cell transplantation (9). The maximum standard dose of G-CSF used was 10 μg/kg/day. Daily administration for one time by G-CSF resulted in mobilization; these results similar to double daily administration. In contrast, the total amount of stem cells that come to the periphery is highest on days 5 and 7. Generally, G-CSF is administrated for four days continuously, whereas apheresis onsets on the 5th day. (10). G-CSF biosimilar drugs have similarities to the original agent, such as safety, quality, pharmacokinetic properties of bioequivalent and effectiveness (11).

Another option for mobilization is chemotherapy-based mobilization. Chemotherapy used for mobilization can be applied separately from the standard treatment or included in the induction or rescue therapy regimen, depending on the centre's experience. Several studies have reported increased efficacy by combining hematopoietic growth factors and chemotherapy (6). The most commonly used chemo mobilization agents are high-dose cyclophosphamide and etoposide (12).

Plerixafor is a reversible and selective inhibitor of CXCR4, approved by the FDA for use in combination with G-CSF to facilitate the mobilization of HPC in MM patients. As a result of the disruption of the interactivity among CXCR4 and its ligand with plerixafor, HPCs emerge from the bone marrow microenvironment into the circulation, resulting in an increased number of HPCs (13). In addition, the plerixafor inhibitor utilized with G-CSF to promote stem cell mobilization in patients severely from significant risk of mobilization malfunction seems reasonable based on the available evidence. Inadequately mobilized patients were frequently known as patients with a peripheral blood CD34+ stem cell count below 20 x 10^6/L at maximum stimulation or with a collection yield was less than 2 x 10^6 CD34+ cells/kg with a maximum of four apheresis methods (14). Mobilization failure develops in around fifteen per cent of mobilized patients. The main predictive risk factors regarding mobilize loss are listed in table 2 (6). Among patients affected with mobilization failure, remobilization or bone marrow stem cell collection can also be carried out through a more intensive system or novel regiment. In addition, cytokines can also be used in association with plerixafor and/or chemotherapy, but not alone. In all patients receiving G-CSF only as a mobilizing therapy, a 2 to 4 weeks rest period followed by remobilization can be performed using plerixafor, and G-CSF is combined with chemotherapy (15). It is essential to know patients at risk for failed mobilization. Lenalidomide is a medication which is more effective and widely used for induction medicine of MM patients and is known to harm HPC mobilization. Early collection and mobilization of stem cells during 4-6 cycles after the first therapy is recommended for patients with MM who are eligible for ASCT (16).

Table 2. Factors related to risk of mobilization failure

Treatment-related
High numbers of previous chemotherapy (≥ two lines of chemotherapy)
Exposure to alkylating agents, purine analogues, or lenalidomide
Extended field radiotherapy to bone marrow-containing sites
Patient-related
Older age (>65 y)
Female sex
Diagnosis of non-Hodgkin lymphoma
Diabetes and smoking
At mobilization
The longer interval from the last chemotherapy to mobilization initiation
Bone marrow infiltration by primary disease (cellularity < 30%) at mobilization
Pre-apheresis peripheral blood $CD34^+$ cell number (<20 × 10^6/μL)
Low day-one apheresis yield
Collection procedure
Timing of apheresis,
Type of cell separator used
Rate and volume of whole blood processed

4. Timing of ASCT

It remains unclear how the improvement in survival after MM diagnosis is related to the timing of ASCT. Although it predates the era of new agents, the first phase of the third RCT to address the timing of ASCT demonstrated that, even though ASCT was previously associated with superior PFS, no difference was observed in OS, delaying ASCT until the time of relapse. In a recent IFM 2009 study, early and delayed autologous stem cell transplantation was compared in patients who underwent VRD induction. It was shown that the rate of minimal residual disease and progression-free survival was higher in patients who underwent early transplantation. Although there is no variation in overall survival with the help of effective salvage therapies and delayed autologous stem cell transplantation, autologous stem cell transplantation is considered a standard treatment after 4-6 cycles of induction therapy in suitable patients (3).

5. Conditioning Regimen

Currently, the standard conditioning regimen for patients with MM scheduled for ASCT is melphalan 200 mg/m^2. In a phase III RCT, patients receiving melphalan 200 mg/m^2 had superior OS at 45 months (65.8% vs 45.5%; P = .05) and less toxicity observed compared to patients receiving 140 mg/m^2 of melphalan with 8 Gy total body irradiation. In comparison, Patients with impaired renal function before ASCT are at higher risk of ASCT-related morbidity and mortality. Therefore, 140 mg/m^2 melphalan is recommended for individuals with creatinine clearance below 60 mL/min and undergoing ASCT (17, 18).

6. Care During Transplantation

After 24 hours of the conditioning regimen, peripheral blood progenitor cells (PBPCs) are reinfused. In addition, the pancytopenia period begins. So during this period, attention should be paid to avoiding infections and gastrointestinal toxicity, and hematological support should be provided. Depending on their level of cytopenia and immunodeficiency, patients are at risk of bacterial, viral and fungal infections in the post-transplant period. Febrile neutropenia is observed in approximately 40 per cent of patients with MM undergoing autologous HCT (19). Prophylactic treatments to avoid infection contain antifungal and antiviral drugs, which are recommended during increased risk.

Consequently, the most significant adverse event of high-dose chemotherapy is gastrointestinal toxicity. Nausea, mucositis, and diarrhea are common. Mucositis may occur in more than half of patients and may limit oral intake. Hematopoietic colony-stimulating factors like G-CSF can be accelerated to engulf the neutrophil. Usually, neutrophil engraftment occurs between days 12 to 14, and platelet engraftment is generally expected on days 14 to 16. According to the patient and transfusion policy of the centres, red blood cells and platelet transfusions are applied when necessary.

7. Single or Tandem transplantation

A Tandem (double) stem cell transplant is the second one scheduled within six months of the first. This approach idea was first explored in newly diagnosed patients by Barlogie et al. in 1999, and it is performance encouraging results with an average of EFS and OS of 43 and 68 months, respectively (20). Subsequently, two randomized trials confirmed the benefit of tandem transplantation with EFS but not OS (21, 22). In one meta-analysis, tandem ASCT was associated with improving response rates, but OS did not show an advantage (23). In some studies, the survival benefit of tandem ASCT was observed only in patients who did not achieve CR or perfect partial response (VGPR) after initial transplantation. In other words, patients who already received VGPR after their first transplant did not benefit significantly from the second ASCT (21, 22). NCCN guidelines (2023, version 3) recommended that sufficient stem cells be harvested to do two transplants in all transplant-eligible patients and that a second ASCT may be considered for patients who achieve a little under VGPR following the first HDT. In the STaMINA study, 758 patients undergoing a first autologous HCT were randomized 1:1:1 to receive a second autologous HCT followed by lenalidomide maintenance; four courses of bortezomib, lenalidomide and dexamethasone (VRd) followed by lenalidomide maintenance or lenalidomide maintenance alone. All three arms showed similar progression-free survival (PFS) and OS at 38 months (24). Currently, most patients with MM routinely undergo tandem autologous HCT. Since high-risk patients have worse outcomes than standard-risk MM, tandem HCT may be considered in selected patients with high-risk MM.

8. Posttransplant Follow

All autologous stem cell transplant patients should receive antifungal, antiviral and anti-pneumocystis jiroveci pneumonia prophylaxis and should be included in the vaccination program after six months (25). Patients should be evaluated for response to treatment after autologous stem cell transplantation. Patients are assessed on day 100 following HCT and should be reassessed every three to four months. High-risk patients may be evaluated earlier for treatment decisions such as tandem transplantation, consolidation, or dual drug maintenance. A second HCT at relapse is recommended for patients who tolerate the first transplant and experience sustained remission after the first HCT. This includes patients with progression-free survival (PFS) of at least three years after maintenance HCT or at least two years of PFS after HCT without maintenance (26). Two randomized studies showed an improved PFS with second autologous HCT versus therapy with chemotherapy only in patients with late relapse after the first stem cell transplant (27, 28).

9. Conclusion

ASCT remains integral to managing previously untreated patients of multiple myeloma and has value in the direction of patients with relapsed MM. Studies have shown that adding new agents before and after ASCT can lead to an improvement

in CR rate as well as delayed progression and prolonged OS. The complete response is that following ASCT is associated with beneficial long-term outcomes. Alternative treatment strategies are required to enhance results in patients who do not achieve post-transplant CR, especially those who are influenced by high-risk diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors' contributions

Concept: A.N.G., E.K., Design: A.N.G., E.K., Data Collection or Processing: A.N.G., E.K., Analysis or Interpretation: A.N.G., E.K., Literature Search: A.N.G., E.K., Writing: A.N.G., E.K.

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Case Report

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Exploring pearls and pitfalls in the diagnosis of rickettsia among children: Mini-review and case report

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Abstract

Rickets, a condition causing softening of bones in children, is prevalent in developing regions like the Middle East, Africa, and Asia. Diagnosis of rickets can be challenging due to their non-specific symptoms, often resembling other infectious or inflammatory diseases. Nevertheless, early detection and treatment of rickets remain a global priority. Hence, gaining valuable insights into its clinical presentation, diagnostic complexities, treatment options, geographical distribution, and preventive measures is essential to ensure improved healthcare for affected children.We present a case of a 10-year-old female patient who was brought to the hospital with a history of frequent falls while playing and experiencing leg pain. The patient had poor eating habits, disliked snacking, and was not exclusively breastfed. Physical examination revealed her legs being shaped like the letter "X," with hyperlaxity and a genu valgum posture. Laboratory results indicated low levels of inorganic phosphorus and total vitamin D 25-OH. The X-ray examination showed Erlenmeyer Flask Deformity, bilateral genu valgum, bilateral distal femur, and proximal tibia metaphyseal widening deformities, and no fractures or other deformities. The patient was diagnosed with rickets and promptly treated with calcium, vit D3, multivitamin syrup, and a high protein, high-calorie diet. After one month of treatment, the patient reported reduced pain, improved balance, and fewer falls.Rickets can be prevented through effective education of parents and pregnant women about calcium and vitamin D-rich food sources and the significance of sun exposure. Pregnant women should be advised to receive a daily intake of 600 IU of vitamin D. For breastfed infants and those consuming less than 500 mL of fortified formula daily in their first year of life, oral vitamin D supplementation of 200-400 IU per day is recommended.

Keywords: Rickettsia, children, diagnosis, risk, case report

1. Introduction

The disease *rickets*, also known as rachitis, is common in developing nations, particularly in the Middle East, Africa, and Asia. Between 3 and 18 months of age is when rickets is most prevalent (1,2). Rachitis is a condition of pediatric bone softening and osteopenia with irregular calcification, resulting in a higher proportion of osteoid tissue before epiphyseal closure. The most frequent causes include poor dietary vitamin D intake and sun exposure, as well as other factors including celiac disease and hereditary disorders (3–5). It is certainly influenced by several risk factors that can increase the incidence of rickets in children (6,7).

Although clinical databases show that rickets is an uncommon disease, it is likely that clinical underdiagnosis of the condition occurs because studies meant to check healthy children for radiographic signs of rickets revealed an unexpectedly much higher frequency (8). Due to poor nutritional intake and environmental circumstances, such as avoiding sunlight, subclinical rickets is a common issue among adolescent students, particularly in girls (9). In Australia, where it is a rare disease but still affects some communities, including Indigenous Australians and immigrants, the epidemiology of rickets has also been researched (10).

Bone pain, simple fractures, early bone abnormalities, delayed fontanel closure, and softening of the skull's bones (craniotabes) are all signs and symptoms of this condition (11,12). Radiological findings, biochemical tests, the patient's medical history, and a physical examination can all be used to diagnose rickets. The most prevalent type of rickets among youngsters in Saudi Arabia is nutritional rickets, which is brought on by a vitamin D deficit (13). Osteoblasts and osteoclasts are two types of cells that have different functions specifically in the process of bone production. Calcium salts are required for osteoid, the organic part of the bone matrix, to mineralize in order for bone to mature. This process is hindered in rickets, which leads to a buildup of osteoid behind the growth plate and, over time, softening of the bone (14,15).

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Pediatricians and other medical practitioners should make an effort to ensure that kids and teens get the daily amounts of vitamin D they need based on their risk factors, cultural practices, and other variables (16,17). Starting in the first two months of life, it is advised that all newborns—including those who are exclusively breastfed—receive at least 200 IU of vitamin D daily. Additionally, it is advised that vitamin D consumption be maintained throughout infancy and adolescence because it can be difficult for some people to decide how much sun exposure is enough for them. (17-19). Although significant, ultraviolet exposure is not the sole significant factor contributing to vitamin D insufficiency. Studies indicate that due to their bigger surface area to volume ratio and stronger and better capacity to generate vitamin D, children, especially babies, may require less sun exposure than adults. Skin vitamin D synthesis depends on the exposed skin surface and duration of sun exposure. The use of topical sunscreens may hinder effective skin synthesis. SPF 30 sunscreens can cut the generation of vitamin D by 95%. Further limiting factors for solar exposure include air pollution and cloud cover. Higher elevations and sunny locations have higher UVB levels (19,20).

Lastly,hereditary factors might raise the risk of hypovitaminosis D. There have been several reports linking hypovitaminosis D to single nucleotide polymorphisms in genes linked to vitamin D. This might account for the significant inter-individual variation in vitamin D sensitivity that could affect the risk of disease. On chromosome 12q13–14, the VDR gene exhibits a number of polymorphism areas. Circulating 25(OH)D levels are also impacted by two frequent variants in the VDBP gene on chromosome 4q12-q13 (21,22).

A first period of intense therapy is followed by a second phase of maintenance therapy. For rickets, which is caused by a vitamin D shortage in the diet, there are several treatment plans available. All of them include the injection of vitamin D, either vitamin D2 (ergocalciferol) or vitamin D3 (cholecalciferol), followed by follow-up checks to see if the condition has improved. Children who don't get enough calcium from their diets are given the intense phase of vitamin D treatment for two to three months combined with 500 mg of calcium supplementation through food or supplements (23). Monitoring 25-OHD levels is necessary to confirm sufficient vitamin D replacement (24). Once rickets has been controlled, bone deformities tendto regress. This improvement can often be enhanced with the use of appropriate splints. Despite adequate medical therapy and nonoperative orthopaedic measures, severe rickets deformity persists, and then operative treatment with osteotomy is indicated. Under these circumstances, vitamin D therapy should be discontinued 1 month before surgery to avoid the risk of severe hypercalcemia that would normally occur during the postoperative immobilization period (24).

When administered properly, rickets treatment is often safe and successful. Nevertheless, depending on the method of treatment and the underlying cause of the disease, there can be certain difficulties with rickets treatment. To repair skeletal deformities brought on by rickets, orthopedic care may occasionally be required. According to a study on patients with X-linked hypophosphatemic rickets, conventional orthopedic treatment can have unintended consequences include not achieving treatment objectives, leaving behind permanent sequelae, and developing new pathologies. However, rickets-related bone abnormalities may require surgery to be corrected. Although surgery is normally safe, there is a chance of complications like infections, bleeding, and problems with the anesthesia (25,26).

Rickets is a disease that may be prevented. The best strategy to avoid nutritional rickets is to inform pregnant women and parents about the value of getting enough sun exposure as well as appropriate dietary sources for calcium and vitamin D. Ideally, pregnant women should consume 600 IU of vitamin D daily together with other minerals. Additionally, infants who are breastfed and newborns who consume <500 mL of fortified formula per day in the first year of life can both get a universal 200–400 IU oral vitamin D supplement daily to help avoid rickets. High-risk populations for deficiency in vitamin D beyond infancy (children with a history of rickets and at high risk of vitamin D deficiency in their diet) should consume 600 IU of vitamin D daily, either through food or supplements (23).

2. Case Report

A 10-year-old female patient, Ms. A, residing in Jakarta, the capital city of Indonesia. Came with her parents with complaints of falling downfrequently while playing with her friends due to imbalance that had been felt since the patient was 4 - 5 years old. Other accompanying complaints included intermittent pain in the legs, especially inthe calves. When walking, the patient usually seemed to drag his leg because of the pain felt in theleft leg, especially when running. The sports teacher at the patient's school told the patient's parentsthat he could not achieve the target of running sports activities. The patient was never taken to thehospital. Since the complaints became more frequent and the patient's leg was shaped like the letter "X", the patient's parents brought him to the hospital.

The patient's mother explained that Ms. A's delivery history was at term with a birth weightof 2500 grams. A was at term with a birth weight of 2500 grams. Ms. Ms. A was known to be a poor eater since childhood, ate little rice, and did not like snacking. The patient was never exclusively breastfed and only drank formula milk (sufor). The patient was able to sit at 6 months,stand at 9 months, and walk at 11 months. Ms. A is

the fourth of four children. The first, third, and fourth child complained of the same thing and her legs were also shaped like the letter "X", exceptfor the second child. The patient's parents had no similar history. During the pregnancy of Ms. A'smother admitted that she rarely paid attention to nutrition during pregnancy, especially vitamin Dand calcium.

When measured, her height and weight were 125 cm and 20 kg. Physical examination of the patient revealed hyperlaxity and genu valgum posture. On the right leg, *true length* 71 cm and*appearance length* 73 cm were obtained, while the left leg measured *true length* 67 cm and *appearance length* 73 cm. Supporting examinations performed were laboratory examinations andradiology x-rays. Laboratory results showed that the inorganic phosphorus value level was 4.4 mg/dL and total vitamin D 25-OH was 18.5 ng/mL. The results of X-ray examination of the bilateral *long leg* region and bilateral genu as shown in Figure 1 and 2, showed that there was a picture of *Erlenmeyer Flask Deformity,* bilateral genu valgum, no significant *leg length discrepancy*, bilateral distal femur and proximal tibia metaphyseal widening deformities, and no fractures or deformities of bilateral genu bones.

Fig. 1. Physical examination results on the patient's feet. Description (1) front view inspection, (2) back view inspection, (3) measurement on the right lower limb with application, (4) measurement on the left lower limb with the application (Source: personal documentation)

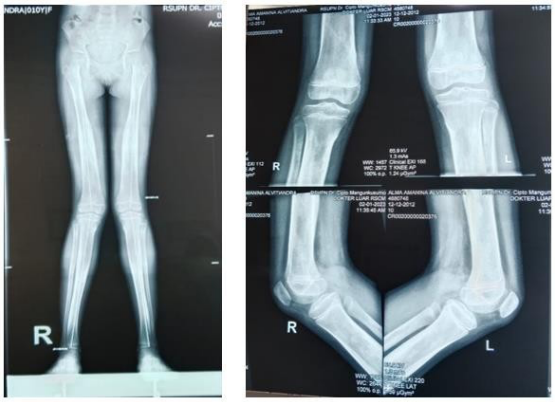

Fig.2. X-ray examination results of the patient (Source: personal documentation)

Finally, patient was given treatment of supplement tablet 1x1 tablet/day which consists of 500 mg calcium hydrogen phosphate and cholecalciferol 133 IU, Multivitamin syrup which consists of multivitamin and mineral supplements, accompanied by high calorie and high protein diets. After one month of treatment, patient developed a huge improvement in terms of reduced leg pain as well as reduced imbalance and fall frequency. Furthermore, there is also improvement in terms of laboratory findings, such as increased vitamin D and calcium level, which are 26.6 ng/mL and 9.9 mg/dL, respectively.

3. Discussion

Rickets may be divided into two primary groups. These groups consists of phosphopenic and calcipenic.(27,28) (Fig. 3.)

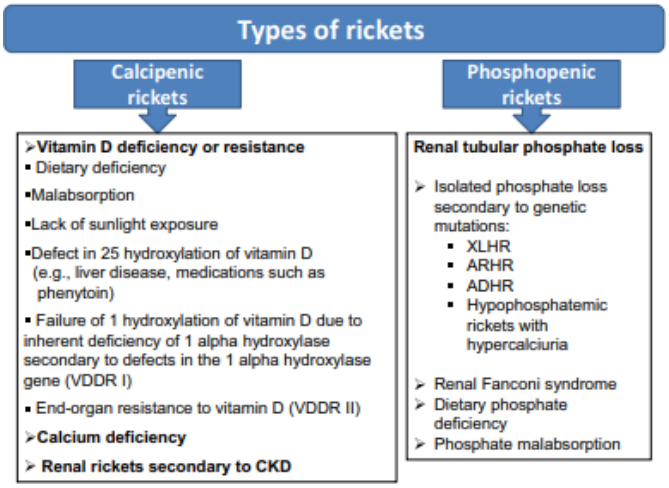

Fig. 3. Type of *rickets* (Source: Chanchlani R et al., 2020) (27).

Risk factors for rickets are described in Table 1(29). During pregnancy, the patient's mother explained that she tended to eat anything without *paying* attention to the nutrients in each food, especially vitamin D. This was also a factor of the patient being a poor eater since childhood. An increasing amount of research demonstrates that maternal hypovitaminosis D affects mother and child health. Fetal vitamin D stores throughout pregnancy are solely reliant on maternal vitamin D status (30,31). Infants only receive a maximum of roughly 40 IU of vitamin D per 750 ml of breast milk, whether or not vitamin D levels are adequate in the mother (27).

Although our patient received sufforus as a kid, she was never exclusively breastfed. According to several studies, vitamin D is abundantly consumed by formula-fed newborns since the milk has been fortified with minerals. Therefore, deficiency of vitamin D is rare in newborns who are fed formula milk (29,32). However, since the patient was not very fondof eating or snacking as a child, it is possible to make the patient's condition vitamin D deficient.

Other factors include lack of sun exposure due to the use of clothes that cover the whole body or inadequate lighting at home. It can be seen that the patient also wears a hijab when goingout. Even though Saudi Arabia has enough of sunshine and ultraviolet radiation, 100% of healthy young women there are vitamin D deficient (33-35). All of the individuals in a research with 465 female participants had low levels of vitamin D (25-hydroxyvitamin 75 nmol/l), with an average level of 18.34 8.2 nmol/l. This contradiction may result from Saudi Arabian Muslim women being expected to cover themselves completely in accordance with local customs, preventing sun exposure (35)

Clinical manifestations in pediatric patients with rickets may present with tetany or seizures due to hypocalcemia. Parents may notice that there is failure to thrive, lethargy, and muscle flaccidity. Early skeletal changes are skull deformities (craniotabes) and thickening of the knees, ankles and wrists from overgrowth plates. Enlargement of the costochondral junction ('rickety rosary') and lateral curve of the chest (Harrison's sulcus) may also occur. Limb deformities such as coxa vara and bowing. The femur and tibia bones may develop after weight bearing, while overall growth may be retarded (24).

Biochemical testing and radiographic pictures are the following steps to confirm the diagnosis if rickets is clinically suspected. Serum *alkaline phosphatase* (ALP), which is often elevated due to rickets being a condition of aberrant mineralization and enhanced osteoblastic activity, is the most crucial laboratory marker for diagnosing rickets. In rickets, phosphate shortage causes ALP activity. ALP levels are often found between 400 and 800 IU/L in cases of phosphopenic rickets, whereas values up to or exceeding 2000 IU/L are frequently seen in cases of calciphenic rickets.

Another laboratory indicator that aids in the diagnosis of rickets is the amount of serum 25-hydroxyvitamin D, particularly in cases of deficiency in nutritional vitamin D. 1,25-Dihydroxyvitamin D, which is the active form of the vitamin, has a short half-life of 5 to 10 hours. The primary circulating form of vitamin D, serum 25-hydroxyvitamin D levels, are frequently used to determine vitamin D status. Serum 25-hydroxyvitamin D levels in children with nutritional rickets brought on by vitamin D insufficiency are typically less than 10 ng/mL. Deficiency in vitamin D is defined as 25-hydroxyvitamin D levels of less than 30 ng/mL, inadequate levels of 30 to 50 ng/mL, and adequate levels of >50 ng/mL (according to the global consensus recommendations on the prevention and treatment of nutritional rickets) (23).

Serum ALP levels are elevated in most types of rickets, but in one hereditary type of hypophosphatasia rickets it is normal. However, differentiation between different types of rickets necessitates the use of a number of standard diagnostic methods. For example, elevated blood creatinine and serum inorganic phosphorus levels indicate a renal lesion, whereas abnormal blood creatinine and decreased serum inorganic phosphorus levels (hypophosphatetnia), in the absence of vitamin D deficiency, indicate a renal tubular defect (31).

The patient's condition showed clinical manifestations of *calcipenic rickets*. Supporting laboratory results were inorganic phosphorus values within normal limits (4.4 mg/dL), while total vitamin D 25-OH levels were less than normal,at 18.5 ng/mL (normal 30-100 ng/mL). X-rays revealed *Erlenmeyer flask deformity*, genu valgum, and bilateral distal femur and proximal tibia metaphyseal widening deformities.

Typical radiographic examination of changes at the ends of growing long bones, which show widened radiolucent zones at the epiphyseal plate (resulting in uncalcified preosseous cartilage) and also by the general rough appearance of trabeculations resulting from mineralizationdefects of all bony areas. Rachis modifications also include trabecular metaphysis development, *cupping*, *fraying*, and *splaying*. A *rachitic rosary* and enlargement of the costo-costal junction are visible on the chest X-ray. In more severe phases, angular abnormalities and pathological fractures of the bones in the upper and lower limbs may be seen (23).

Rickets inhibits the mineralization of osteoid, the organic component of the bone matrix, resulting in softness in the bone over time. There are two main types of rickets, which are phosphopenic and calcipenic. The patient's clinical manifestations were consistent with calcipenic rickets. Supporting laboratory results showed inorganic phosphorus values within normal limits, while total vitamin D 25-OH levels were below normal. X-rays revealed Erlenmeyer flask deformity, genu valgum, and bilateral distal femur and proximal tibia metaphyseal widening deformities

The study implies that rickets continue to be a prevalent condition in developing regions. The diagnosis of rickets can be challenging due to its non-specific symptoms. Early detection and treatment of rickets are crucial to improve healthcare for affected children. Overall, the study emphasizes the importance of raising awareness about rickets, improving diagnostic capabilities, and implementing preventive measures to combat this condition effectively, particularly in regions with a high prevalence of rickets.

Rickets is confirmed as the cause by a combination of risk factors in the patient, positive clinical signs, significant laboratory results (high ALP, hypocalcemia, or hypophosphatemia), and indicative radiographic features. Even if the serum calcium and phosphate levels are normal, the diagnosis may still be made. Similar to this, early clinical indications are not identified. The distal ends of the upper and lower extremities' quickly expanding bones, as well as pictures of the ribs, should all be visible on radiologic imaging. Although nowadays people are educated about the importance of sun exposure and vitamin D for bones, in fact rickets can still occur in people living in urban areas.

Conflict of interest
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Authors' contributions
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Ethical Statement

Ethics committee permission is not required for this study.

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Case Report

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Minimally invasive transthoracic repaired Morgagni hernia: A case report

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Abstract

Morgagni hernia (MH) is a congenital diaphragmatic hernia that is rarely seen, comprising 2-4% of diaphragmatic hernia cases. While it can remain asymptomatic, it has the potential to give rise to symptoms like cough, difficulty breathing and retrosternal pain. A chest X-ray and a computed tomography (CT) scan of the chest are sufficient for diagnosis. The only option in treatment is surgery. The objective of this study was to propose the treatment of a patient who sought our clinic due to right-sided chest pain and was diagnosed with MH, utilizing a minimally invasive surgical technique.

Keywords: Congenital Diaphragmatic Defect, Morgagni Hernia, VATS

1. Introduction

Congenital diaphragmatic hernias account for 8% of congenital malformations and occur due to a defect in the diaphragm between the abdominal and thoracic cavities. MH is a congenital hernia type situated within the anteromedial region of the diaphragm, comprising 2-4% diaphragmatic hernia cases. MH involves the herniation of intra-abdominal organs into the thoracic cavity. The condition is often asymptomatic, with the majority of cases being incidentally identified through radiological imaging. The most common symptoms are cough, difficulty breathing, and retrosternal pain (1). From the defect, typically colon and omentum, rarely stomach, small intestine, and a part of the liver herniate into the thoracic cavity (2). In MH, where surgical intervention is the sole treatment method, the approach can be either a transthoracic or transabdominal, although minimally invasive methods have been increasingly preferred in recent times (3).

In this article, we aimed to present a case diagnosed with MH and treated with minimally invasive video-assisted thoracic surgery (VATS) in light of the literature.

2. Case Presentation

A female patient, aged 48, visited our clinic with a complaint of stabbing-like right-sided chest pain that started approximately two weeks ago. In this patient, who does not have any gastrointestinal complaints, an opacity was detected int the right substernal area at the cardio-phrenic angle on the chest radiograph (Fig. 1a a, b). Contrast-enhanced thoracic computed tomography (CT) reported a well-defined hypodense lesion with dimensions of 60x44x62 mm in the right paracardiac area, indicative of fat density (lipoma?) (Fig. 1a c, d). Additionally, a consolidation appearance consistent with subsegmental band atelectasis was observed in the right lung's middle lobe adjacent to the lesion.

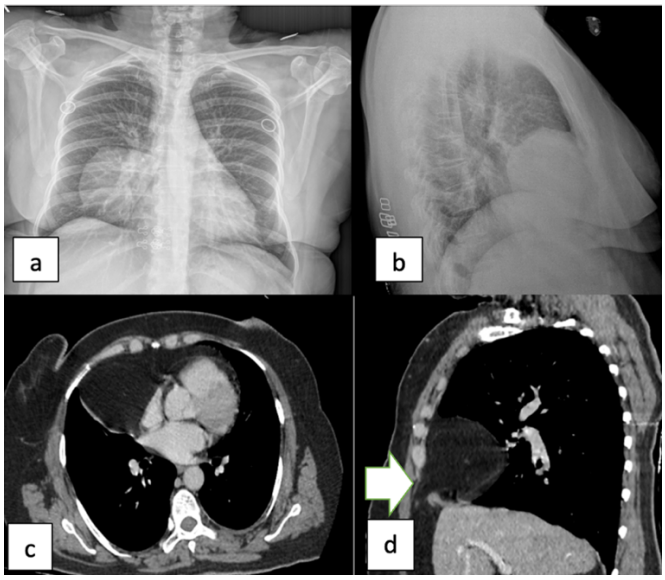

Fig. 1a. (a). Posteroanterior chest X-ray in a 48-year-old woman with Morgagni's hernia, shows a round right pericardiophrenic density. (b) Lateral chest radiograph localizes the opacity to the retrosternal area. (c) Axial computed tomographic scan showing a right retrosternal hernia containing omentum in the anterior cardiophrenic angle. (d) Sagittal computed tomographic scan showing the diaphragmatic defect (arrow).

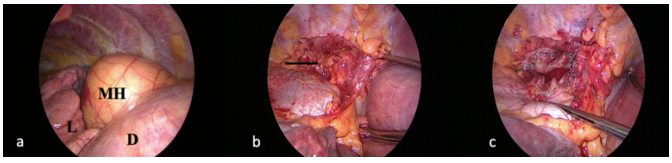

Fig. 1b. (a). Thoracoscopic view of hernia sac containing the omentum. L: Lung, D: Diaphragm, MH: Morgagni Hernia. (b)

*Correspondence: drcanerisevi@gmail.com

Thoracoscopic view of defect of the diaphragm (arrow). (c) Repair of diaphragmatic defect with polymesh® and proTack™.

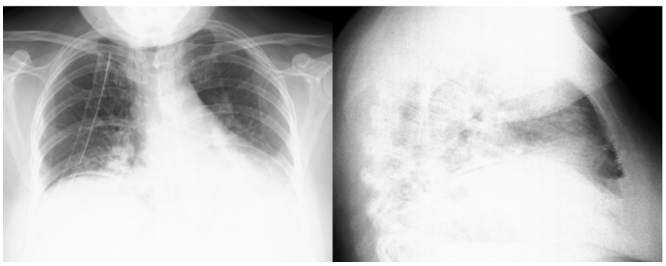

Fig. 1c. Postoperative posteroanterior and lateral chest X-ray of the patient.

The patient does not have any additional comorbidities and her physical examination revealed arterial blood pressure: 120/70 mmHg, pulse rate: 72 beat/min , and respiratory rate: 16 min. During auscultation, it was determined that breath sounds had decreased within the lower right zone. Laboratory findings showed a white blood cell count of 7670 / uL, hemoglobin level of 13 g/dL, erythrocyte sedimentation rate of 31 mm/h, and C-reactive protein level of 35 mg/L.

VATS exploration was planned for the patient with suspected MH. Double-lumen intubation was performed by the anesthesia team, and after skin incision at the 7th intercostal space (ICS) along the right mid-axillary line, thoracic entry was achieved through scissor dissection. A thoracoscopic port was placed, and a 30° video camera was directed into the right hemithorax. An approximately 3 cm utility incision was made at the 5th ICS along the right anterior axillary line. During VATS exploration, a hernia sac containing omentum and jejunum with the sizes of approximately 8x6 cm was detected in the substernal region at the cardiophrenic angle. The hernia sac was completely excised by blunt dissection and ligasure, and the omentum and jejunum were pushed back into the abdomen. The defect in the diaphragm, measuring 10x10 cm, was closed using polymesh® (Betatech, Turkey) and proTack™ (Medtronic, United Kingdom). After controlling for bleeding and leakage, a 32 French chest tube was placed in the right hemithorax, and the right hemithorax was closed according to the standard procedure. The patient, whose postoperative follow-ups were conducted in the room on the ward, had the chest tube removed on the 2nd day postoperatively due to the absence of active drainage and no pathological findings on the chest X-ray (Fig 1c). The patient, who had no active complaints, was discharged on the 3rd postoperative day.

3. Discussion

While classic Bochdalek hernia (BH) accounts for about 80% of all congenital diaphragmatic hernias, MH constitutes approximately 3-4% of both pediatric and adult diaphragmatic hernias. MH is rarely encountered as a congenital diagnosis during infancy. It is more prevalent in females and is typically diagnosed after the age of 50 (4). In MH, the defect is generally small, and the symptoms vary based on the size of the hernia and the organ involved in the herniation. Herniated organs most commonly include omentum and segments of the colon; however, the stomach, liver, and small intestines can also become herniated (3). Radiologically, the presence of air-fluid levels from the stomach and intestines in the thorax is characteristic, but in cases where only omentum is present, an intrathoracic extrapleural mass appearance can also be observed. Unlike BH, MH generally has a hernia sac and in 90% of cases, it occurs on the right side, in 8% on the left side, and in 2% on both sides (3). In our case, the hernia was on the right side, and the omentum and jejunum were herniated into the thorax, accompanied by an intrathoracic extrapleural mass appearance and a hernia sac formed by both the thoracic and abdominal membranes.

In MH cases, predisposing factors such as pregnancy, trauma, obesity, chronic constipation, and chronic cough are present in 41% of cases. Difficulty in breathing, chest pain, and abdominal discomfort are commonly reported complaints. Nevertheless, gastrointestinal symptoms and respiratory symptoms like recurrent lung infections and acute respiratory distress syndrome can also be observed. During chest examination, the most notable indicators for diagnosis are the detection of decreased breath sounds or bowel sounds (4). In our case, the patient presented with a complaint of stabbing-like right-sided chest pain, and during the physical examination, only decreased breath sounds were noted in the lower right zone. Additionally, there was no presence of gastrointestinal symptoms.

Chest radiography, thoracic CT, and contrast-enhanced radiography of the gastrointestinal system are used in the diagnosis of Morgagni hernia. The absence of any tissue within the hernia sac or its containing solid organs like the liver can complicate the diagnosis (5). It has been documented that thoracic magnetic resonance imaging has been utilized for this purpose in cases where diagnosis is challenging (6). In our case, an opacity area was detected in the right paracardiac region on the chest radiograph, and contrast-enhanced thoracic CT revealed a fat pad in the paracardiac area.

Minimal invasive surgical techniques offer advantages such as reduced postoperative pain, shorter hospitalization duration, earlier return to daily activities, and better aesthetic outcomes. Thoracoscopic or laparoscopic repair of Morgagni hernia has been increasingly preferred in recent years. Pfannschmidt et al. reported that thoracic approach is superior in right-sided hernias due to better visualization of the diaphragmatic foramen and pericardial and pleural adhesions (5). Ambrogi et al. also recommended transthoracic repair of MH (7). In their study presenting their experiences with VATS repair of MH, Nakashima et al. advocate for a thoracoscopic approach as the first choice instead of a laparoscopic approach for patients with BH or MH and severe adhesions (8). We recommend an abdominal approach in cases where the diagnosis is uncertain, or when there is peritonitis and bilateral hernia. In other situations, VATS should be the first choice.

In MH cases, it is essential to excise the hernia sac and

thoroughly examine the diaphragmatic defect. If the defect is small and can be sutured without tension, primary suturing may be the preferred approach (9). Otherwise, the diaphragmatic defect must be sutured using mesh (such as polytetrafluoroethylene, polypropylene, etc.), which will help prevent potential recurrence. In our case, after the hernia sac was completely excised, it was deemed that the defect could not be closed primarily, so it was closed using a polypropylene mesh and proTack™.

As a result, MH cases, even if asymptomatic, should be treated surgically. Whenever possible, minimally invasive procedures should be preferred, especially when distinguishing them from mediastinal masses is challenging, VATS should be considered. If the defect in the diaphragm cannot be repaired with primary sutures, mesh should be used to prevent recurrence.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: B.Ç., C.İ., Design: C.İ., Data Collection or Processing: C.İ., B.C.Ö., Analysis or Interpretation: M.G.P., B.Ç., Literature Search: M.G.P., Writing: C.İ., M.G.P.

Ethical Statement

Ethic commitee approval is not required for this study.

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An entire book: Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Web page:

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