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Aims and Scope

Cyprus Journal of Medical Sciences (Cyprus J Med Sci) is the scientific, peer reviewed, open access international publication organ of Cyprus Turkish Medical Association. The journal is published three times a year, in April, August, and December. As of 2020, the journal has become a quarterly publication, publishing in March, June, September, and December. The journal's publication language is English.

The aim of the journal is to publish original research papers of the highest scientific and clinical value in all medical fields. Cyprus Journal of Medical Sciences also publishes reviews, rare case report and letters to the editors.

The target audience of the journal includes healthcare professionals physicians, and researchers who are interested or working in in all fields of medicine.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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based upon the originality and importance of the investigation. The main text of original articles should be structured with Introduction, Material and Methods, Results, and Discussion subheadings. An original article can be signed by maximum 6 authors unless it is a multi-center study or that it required extensive labour. Please check Table I for the limitations for Original Articles.

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Cyprus Journal of Medical Sciences adopts the ICMJE's clinical trial registration policy, which requires that clinical trials must be registered in a publicly accessible registry that is a primary register of the WHO International Trials Registry Platform (ICTRP) or in ClinicalTrials.gov.

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- Authors are required to state a data sharing plan for the clinical trial registration. Please see details under "Data Sharing" section.
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- Whether individual deidentified participant data will be shared
- What data in particular will be shared
- Whether additional, related documents will be available
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- By what access criteria will be shared

Authors are recommended to check the ICMJE data sharing examples at http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html

While submitting a clinical trial to Cyprus Journal of Medical Sciences;

- Authors are required to make registration to a publicly accessible registry according to ICMJE recommendations and the instructions above.
- The name of the registry and the registration number should be provided in the Title Page during the initial submission.
- Data sharing statement should also be stated in the Title Page even the authors do not plan to share it.

Clinical trial and data sharing policy of the journal will be valid for the articles submitted from I February 2020.

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Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	4000	250 (Structured)	35	6	5 or total of 10 images
Review Article	5000	250	50	6	10 or total of 15 images
Case Report	1200	200	15	No tables	4 or total of 8 images
Letter to the Editor	400	No abstract	5	No tables	No media

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Units should be prepared in accordance with the International System of Units (SI).

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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

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Books with a Single Author: Sweetman SC. Martindale the complete drug reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92.

Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

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Original Article

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Renal Cell Carcinoma: A 10-Year Retrospective Study

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Cite this article as: Zahir ST, Heidarymeybodi Z, Herman M. Renal Cell Carcinoma: A 10-Year Retrospective Study. Cyprus J Med Sci 2021; 6(2): 107-111.

BACKGROUND/AIMS
This study was conducted to investigate survival and its associated factors in patients with clear cell renal cell carcinoma (CCRCC).

MATERIAL and METHODS
This retrospective study recruited patients diagnosed with CCRCC in Shahid Rahnemoon Hospital, Yazd, Iran, followed them up from their presentation until their death or end of the study and examined their demographic information and clinical and tumor characteristics. Continuous variables were expressed as mean±SD, and univariate analyses of survival were conducted using the Kaplan–Meier method and log-rank test and multivariate analyses using the Cox regression model.

RESULTS
The study recruited 206 patients, including 132 males, with CCRCC and a mean age of 57.9 ± 13.6 years. During the follow up, 53.9% (n = 111) of the patients survived and data regarding the survival status of 24.3% (n = 50) patients were missing. The mean survival duration was obtained as 59.9 ± 2.7 months. The independent survival indicators were grade 4 (HR: 2.4, 95% CI: 0.4-5.7, $P = .02$), older age (HR: 4.14, 95% CI: 1.7-8.4, $P = .02$), and treatment method includes post-operative chemotherapy (HR: 4.9, 95% CI: 1.7-10.8, $P = .004$) and post-operative radiochemotherapy (HR: 8.4, 95% CI: 1.9-16.2, $P = .03$).

CONCLUSION
This study found survival to be negatively correlated with grade 4, older age, and treatment method, i.e. post-operative chemotherapy and post-operative radiochemotherapy.

Keywords: Renal cell carcinoma, clear cell renal cell carcinoma, prognostic factor, cancer, survival

INTRODUCTION

As the 13th most fatal cancer worldwide,[1] renal cell carcinoma (RCC) accounts for 90% of all kidney cancers.[2] In recent years, advanced abdominal imaging has shown increases in the incidence of RCC, especially in developed countries.[3]

The survival rate has been differently reported depending on the tumor characteristics. The prognosis of tumors that are limited to the renal parenchyma is excellent, resulting in a 5-year survival rate of up to 90%; nevertheless, the survival rate is below 10% in metastases despite using multimodal treatments.[4,5] The survival rate is affected by the histological subtypes of RCC, including clear cell renal cell carcinoma (CCRCC) as the most common with the lowest survival rate,[6] papillary RCC, and chromophobe RCC.[5]

Although the prognostic roles of demographic and tumor characteristics have been addressed in literature, effective factors in the survival of patients with CCRCC are to be determined.[7–14] Given the reported significantly-high mortality of this cancer,[1] the present research aimed at investigating the overall survival and its contributing factors in the patients.

MATERIAL and METHODS

Study Population

This observational retrospective study focused on RCC patients in Shahid Rahnemoon Hospital, Yazd, Iran. An experienced pathologist reexamined all the RCC samples referred to the pathology department from 2008 to 2018. This study

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included all the samples positive for CCRCC based on the 2016 WHO classification and diagnostic criteria.[15] Patients with metastatic CCRCC were excluded from the study. All the patients were followed up from the date of diagnosing their cancer until their death or the end of the study. This study was performed after receiving the approval of the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (SSU. 1398.3738).

Data Collection

The study variables included patient age, clinical presentation of cancer, tumor stage, tumor grade, tumor site (right or left kidney), tumor size (below 4 cm, and above 4 cm), perinephric fat invasion (PFI), treatment method, survival status, and time of death (if applicable). All of the data were collected from the hospital records. Tumor stage was evaluated based on American Joint Committee on cancer 8th edition,[16] and tumor grade was categorized according to Furman Grading System. Tumor size was evaluated based on pathologic findings.

Statistical Analysis

The continuous variables were expressed as their mean values. The Kaplan–Meier method was used to calculate the survival duration and the log-rank test to compare survival curves for the individual categorical variables. The significant variables determined using the log-rank test were inserted into the Cox proportional hazard model to determine their correlations with survival. The statistical analyses were performed in Statistical Package for the Social Sciences (SPSS) version 22 (IBM SPSS Corp.; Armonk, NY, USA), and $P < .05$ was set as the level of statistical significance.

RESULTS

General Characteristics

This study included 206 consecutive patients (64% male and 36% female) with CCRCC and a mean age of 57.9 ± 13.6 years. The most frequent symptoms included abdominal pain and hematuria. The tumor lay on the right side of the kidney in 49.55% of the patients, its size exceeded 4 cm in 68%, PFI was observed in 21.85% at the time of diagnosis, and grade I tumor and stage I cancer were, respectively, reported in 32% and 29%. Table I summarizes the clinical and tumor characteristics of the patients.

Survival

During the follow-up, 111 out of the 206 patients survived and the survival status of 50 was missed. The mean survival duration was obtained as 59.9 ± 2.7 months (95% CI, 51.5-62.2, Figure 1). According to the univariate analysis, the relationships of survival with the patients' age (95% CI, 52.6-63.2, $P = .02$, Figure 2), treatment method (95% CI, 52.4-63.1, $P < .001$, Figure 3), and cancer grade (95% CI, 53.5-64.7, $P < .001$, Figure 4) were statistically significant. The multivariate analysis showed the significant and negative correlations of survival with grade 4, age, and treatment method, i.e. post-operative chemotherapy and post-operative radiochemotherapy (Table 2).

In contrast to the multivariate analysis (Table 2), the univariate analysis showed statistically significant differences in the survival duration between genders (95% CI, 50.3-61.4, $P = .03$), tumor sizes (95% CI, 51.4-62.7, $P = .04$), cancer stages (95% CI, 51.0-62.1, $P < .001$), and PFI (95% CI, 53.4-64.2, $P = .01$).

Main Points

- The classical triad of presentation for Renal Cell Carcinoma includes gross hematuria, flank mass, and flank pain found in almost 20% of Iranian patients which is 2 times higher than developed countries.
- The mean survival of the patients with nonmetastatic Renal Cell Carcinoma in Iranian population is 59.9 months.
- Patents' age and tumor's grade are independent indicators for survival in patients with Renal Cell Carcinoma.
- Tumor's size and perinephric fat invasion may not independently affect survival of patients with Renal Cell Carcinoma.

TABLE I. Clinical Information and Tumor Characteristics

Factor	Number	Percent
Clinical manifestation		
Hematuria	53	26
Flank pain	46	22
Flank mass	18	9
Hematuria and flank pain and mass	46	22
Without symptom	43	21
Stage		
1	60	29
2	68	33
3	55	27
Missing	23	11
Grade		
1	66	32
2	60	29
3	23	11
4	16	8
Missing	41	20
Type of treatment		
Surgery	140	68
Surgery and chemotherapy	59	29
Surgery and chemotherapy and radiotherapy	7	3

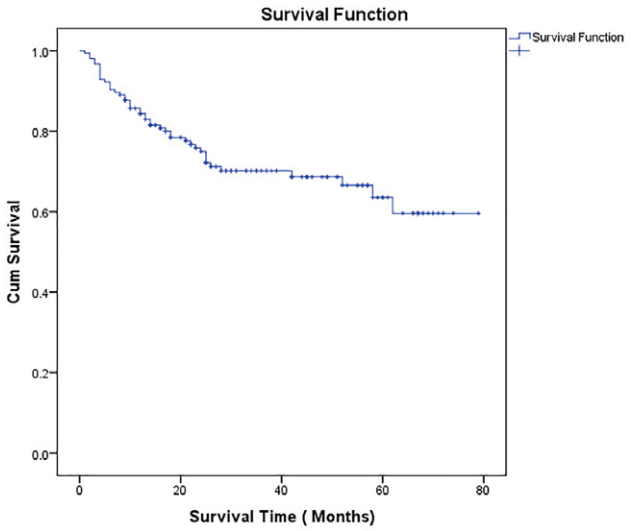

Figure 1. Curves of overall survival duration (month)

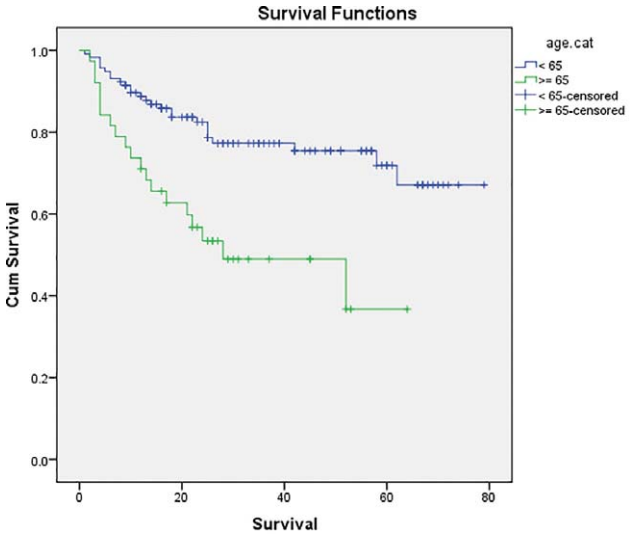

Figure 2. Survival duration (month) versus cancer grade

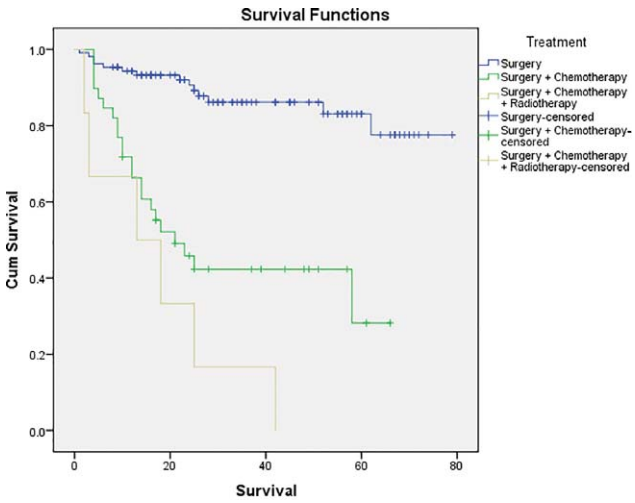

Figure 3. Survival duration (month) versus patient age

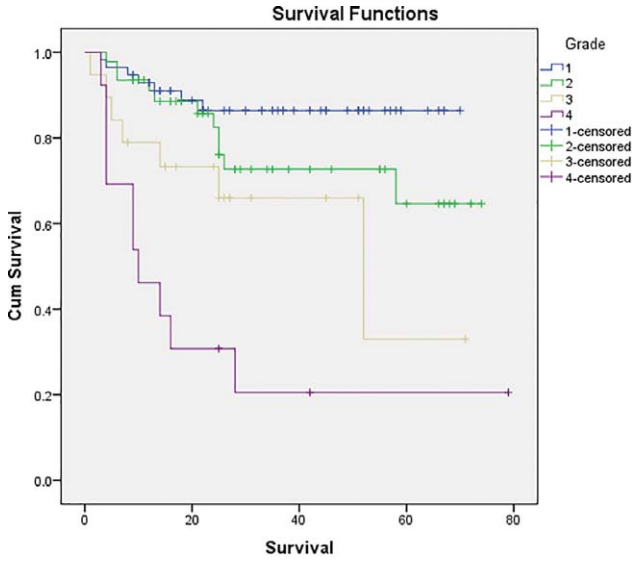

Figure 4. Survival duration (month) versus treatment method

TABLE 2. Survival Multivariate Analysis

Factors	HR (95% CI)	*P* value
Age cat		
<65	Reference	
>65	4.14 (1.7-8.3)	.02
Sex		
Male	Reference	
Female	0.6 (0.2-1.5)	.29
PFI		
No	Reference	
Yes	1.4 (0.5-3.6)	.47
Stage		
I	Reference	
2	1.1 (0.3-5.2)	.94
3	2.4 (0.4-6.8)	.68
Grade		
I	Reference	
2	1.5 (0.5-4.5)	.45
3	0.7 (0.1-3.6)	.12
4	2.4 (0.4-5.7)	.02
Tumor size		
≤4 cm	Reference	
>4 cm	1.7 (0.4-2.9)	.24
Treatment		
Surgery	Reference	
Surgery and chemotherapy	4.9 (1.7-10.8)	.004
Surgery and chemotherapy and radiotherapy	8.4 (1.9-16.2)	.03

DISCUSSION

RCC is the 6th and 10th most prevalent cancer in men and women, respectively.[2] Despite the improvements in screening methods and increased survival from localized cancers, a high mortality risk has been reported for this malignancy.[3,17] The histological subtypes and genetic characteristics of RCC are different. CCRCC is the most common subtype with the lowest survival rate compared to that of the other subtypes.[6,18] The present study was conducted to determine survival indicators in patients diagnosed with CCRCC.

The prognostic indicators of RCC were found to include the cancer stage, the tumor grade, and histological type of tumor.[19] The univariate model suggested the grade and stage significantly affect survival, and the multivariate model showed grade-4 tumors to constitute the determinant of survival. Investigating the TIN0M0 RCC prognostic factor in an Asian population, Zhang et al.[20] found the Fuhrman grade and tumor size to affect patient survival. Only nonmetastatic RCC included in this study, and this may be the probable cause for nonprognostic value of tumor stage in this study.

Research suggests significant correlations between tumor size and survival.[21–23] Cheville et al.[21] reported the mortality of tumors exceeding 5 cm in size to be 4.7 times higher. Bhindi et al.[23] reported positive correlations between tumor size and the risk of aggressive histology. The statistically significant correlations between tumor size and survival reported using the univariate model were also found to be insignificant based on

the multivariate model. Given the indirect effect of tumor size on survival through tumor grade, it was not an independent indicator of survival after adjusting tumor grade. Similarly, Thompson et al.[22] reported an increase of 25% in the risk of high-grade tumors with a 1-cm increase in tumor size.

The role of PFI in the survival of patients with RCC is still controversial. Despite the prognostic role of PFI reported in some studies,[24,25] its correlation with survival was found not to be independent after adjusting the tumor size.[26–28] Classical survival indicators do not include factors such as renal vein invasion and sinus fat invasion, which are often associated with PFI.[25] Kume et al.[25] found PFI to be correlated with aggressive tumor features and age. Cancer-induced mortality was higher in patients with PFI and even with small tumors. Hedgire et al.[4] found PFI to be an independent risk factor for cancer-specific survival even after tumor size adjustment. In contrast to the multivariate model, the univariate model of the present study showed PFI to constitute a risk factor for survival. Similarly, Ornellas et al.[29] found PFI to constitute a significant index for disease-free survival as per the univariate rather than multivariate model.

The potentially significant effect of age on survival has been addressed in literature in recent years.[30] The present study found age to constitute an independent indicator of survival in the patients. Scoll et al.[30] reported a negative relationship between the survival and tumor size (below 4 cm versus larger than 7 cm) in all age groups and found age to be a prognostic factor in medium-sized tumors (4-7 cm).

The classical triad of presentation for RCC includes gross hematuria, flank mass, and flank pain.[31] Advances in imaging and screening methods help with the earlier diagnosis of RCC even with asymptomatic tumors.[3,32] A 5-year survival was reported in 93% of patients with asymptomatic tumors and 59% with symptomatic tumors.[33] Research in western communities suggests the incidental diagnosis of almost 60% of patients with asymptomatic RCC, and that only 10% of the patients present with the classical triad.[5] Diagnosing the majority of the present study, patients with symptomatic tumors exceeding 4 cm in size can explain the lower mean survival of the patients with nonmetastatic RCC (59.9 months) compared to that in western populations (175.7 months).[34] It is therefore recommended that screening and clinical accuracy be improved in routine clinical practice to increase the survival rate in Iranian patients with RCC, and cancer studies be conducted at a national scale to acquire a broader perspective of RCC in Iran.

Treatment of patients is considered according to the stage of the disease and patients' age, so that patients in stages 1-3 are given priority with surgery. Surgery can be performed partially or radically so that patients in stage 1 and tumor size less than 7 cm are candidates for partial surgery. In tumors larger than 7 cm in size, radical nephrectomy is the treatment of choice.[35] In this study, our patients underwent partial and radical nephrectomy in stages 1-3. In patients with stage 3, based on patients' age and other factors influencing treatment choice, including cardiopulmonary status and comorbidities, radical nephrectomy with systemic therapy as well as radiation therapy was considered. Patients in advanced stage received radiation therapy, immunotherapy, and chemotherapy along with surgery if indicated. In this study, patients who received chemotherapy, radiotherapy, and surgery or patients received chemotherapy and surgery compare to patients who underwent surgery had a shorter lifespan. The probable explanation for this shorter lifespan could be other comorbidities rather than treatment itself. In the study of Goebell et al.,[36] they evaluate 1,085 patients with CCRCC. It is determined that high risk patients who usually excluded from clinical trials (ineligible-trial patients) because had significantly lower survival compare to trial-eligible patients and the type of treatment could not increase life expectancy.

The present study limitations included its unicenter and retrospective design, small sample and missing data, as well as failure to evaluate RCC subtypes other than CCRCC.

RCC was diagnosed mainly at its symptomatic stage, and its most prevalent clinical symptoms included abdominal pain and hematuria. The tumors identified mostly exceeded 4 cm in size. The present research found grade 4, age, and treatment method (post-operative chemotherapy and post-operative radiochemotherapy) to be independently and negatively correlated with survival.

Ethics Committee Approval: Ethical committee approval was received from the Shahid Sadoughi University of Medical Sciences, Yazd, Iran (SSU. 1398.3738).

Informed Consent: N/A

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - S.T.Z., M.H.; Design - S.T.Z., M.H.; Supervision - S.T.Z., ; Resources - S.T.Z.; Materials - M.H., Z.H.; Data Collection and/or Processing - Z.H., M.H.; Analysis and/or Interpretation - Z.H.; Literature Search - Z.H., S.T.Z.; Writing Manuscript - Z.H., S.T.Z.; Critical Reviews - S.T.Z., Z.H.

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Original Article

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Bone Marrow Aspiration: The Indications and the Diagnostic Value

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BACKGROUND/AIMS
Bone marrow aspiration (BMA) is a common and useful investigation tool in clinical practice that helps gather information about both hematological and nonhematological disorders. Against this backdrop, the aim of this work was to identify the main indications for BMA tertiary hospital and to determine the common diagnoses encountered.

MATERIAL and METHODS
This was a prospective laboratory-based study conducted in a tertiary hospital, from December 2015 to August 2017. BMA was carried out in 204 cases of suspected hematologic disorders. The information extracted was inclusive of the main indications for performing the procedure, the age groups involved, as well as the most common diagnoses established. In addition, a specially designed form was used for this purpose, after which the data were analyzed using the Statistical Package for the Social Sciences computer program.

RESULTS
Two hundred and four cases of BMA were performed and analyzed. The most patients (30.4%) were aged 15-30 years, of which 62.7% were males and 37.3% were females. This resulted in a male-to-female ratio of 1.7:1.0. The most frequent indications were found to be pancytopenia (48.1%), suspected leukemia (26.5%), and unexplained splenomegaly (8.3%). Hypersplenism was the most common diagnosis (20.1%) followed by visceral leishmaniasis (15.2%) of the cases. 16 (7.8%) of the aspirates revealed a normally functioning marrow.

CONCLUSION
BMA assumes importance in the context of establishing the diagnosis in several medical conditions. The most common indication for this procedure was pancytopenia, while the most common finding was hypersplenism.

Keywords: Bone marrow aspiration, diagnostic role, hematological disorders

INTRODUCTION

Bone marrow aspiration (BMA) is a cytologic preparation of bone marrow cells obtained by aspirating of marrow mostly from the posterior iliac crest. Notably, it is indicated in patients with suspected hematological diseases whose diagnosis remains unclear after examination of the peripheral blood with complete blood count (CBC) and peripheral smear examination. This procedure may be necessary for the diagnosis and management of hematological as well as, to some extent, nonhematological disorders, for staging, prognostication, and evaluation of therapeutic response.[1]

BMA is one of the most commonly-used, cost-effective, and safe invasive procedures; it is associated with minimal complications after precautions are taken into consideration.[2] Megaloblastic anemia and acute myeloid leukemia were the most common hematological disorders reported in the study conducted by Atla et al.[3] Correspondingly, the study carried out by Sreedevi et al.,[4] the most common hematological disorder was erythroid hyperplasia, followed by idiopathic thrombocytopenic purpura. Meanwhile Pudasaini et al.[5] stated in their study that the most common hematological disorder was erythroid hyperplasia, followed by megaloblastic anemia and acute leukemia.

The aim of the current study was to identify the spectrum of diseases diagnosed by BMA at Referral Teaching Hospital, Asmara, Eritrea.

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MATERIAL and METHODS

This prospective laboratory-based study was carried out in a tertiary hospital, over a period of 21 months from December 2015 to August 2017.

All the patients came for BMA with results of CBC performed as a routine test. A brief medical history was taken from all of them. Along with revised CBC and indication, the general condition of the patients (including examination of liver, spleen, and lymph node groups) were checked, patient consent was taken, and his/her comfort was ensured; patients were instructed to lie down on their left side flexing the right knee joint and extend the left leg, whereas BMA was performed from the posterior superior iliac spine under aseptic condition using Klima BMA needle. In order to avoid dilution of the yield by peripheral blood, only approximately 0.5 mL bone marrow was aspirated from each patient and deliberately kept in ethylenediamine tetra-acetic acid anticoagulated.

For the purpose of assessing the adequacy of the sample, smears were made for staining and examination, it was concluded. Four cases out of 208 cases were accounted for by "dry tap," which represents about 1.9% of the total; these samples were excluded from this study.

Data Collection and Analysis

The information obtained included the demographic data, the primary indications for performing bone marrow examination as well as the most common diagnoses established. A special form was designed for this purpose in which data were displayed.

Thereafter, data generated were coded, validated, and analyzed using Statistical Package for the Social Sciences (SPSS) version 22 (IBM SPSS Corp.; Armonk, NY, USA). A Pearson chi-squared test was used to assess the significance between proportions; to that end, *P*-value below .05 was considered to be statistically significant. The main variables evaluated included age, sex, an indication of BMA, as well as final conclusion/diagnosis.

Main Points

- Bone marrow aspiration assumes important in the context of revealing the diagnosis in several medical conditions.
- In males, the most common diagnosis was hypersplenism followed by Visceral Leishmaniasis. the most common hematological neoplasm was acute leukemia and chronic lymphoid leukemia followed by chronic myeloid leukemia.
- In females, acute leukemia was the common diagnosis followed by hypersplenism.
- Pancytopenia was observed to be the most common clinical indication encountered.
- Visceral Leishmaniasis was the common cause of pancytopenia in children, followed by hypersplenism. In adults, this was hypersplenism, followed by Megaloblastic anemia.
- The second common indication was suspected leukemia.
- The need to rule out the underlying causes of hypersplenism by conducting further investigations to detect the root cause of hyperactive spleen.

TABLE I. Age and Sex Distribution of BMA Cases

Age-group	Sex = n (%)		N = 204 (%)
	Male	Female	
<15	34 (60.8)	22 (39.2)	56 (27.45)
15-30	34 (54.8)	28 (45.2)	62 (30.39)
31-45	26 (70.3)	11 (29.7)	37 (18.14)
>45	34 (69.4)	15 (30.6)	49 (24.02)
Total	128 (62.7)	76 (37.3)	204 (100)

Bone Marrow Aspiration Films Preparation and Staining Method

BMA smears are made by placing a drop of bone marrow on a slide and using a smear preparation technique. The film is then air dried and flooded with the Leishman's stain. After 2 minutes, the volume of water is doubled and the film stained for 5-7 minutes. Afterwards, it is washed in a stream of buffered water until it acquires a pinkish tinge (up to 2 minutes).

After the back of the slide has been wiped clean, it is then left (set upright) to dry.[6] The slides were observed for the quality, fragments, and stain. Preparation of smears and staining was undertaken by the same person in order to avoid errors caused by a change in performer. The slides were examined by the author, a hematopathologist under a light microscope.

Bone Marrow Aspiration Examination Findings

The bone marrow fragments were first examined on a lower power to determine the cellularity and megakaryoblasts/cytes. Then on high power, an examination is undertaken for red blood cells precursors, white blood cells precursors, the myeloid/erythroid ration (ME ratio), and the maturity of cells were examined: for example, blasts may make equal or more than 20% of the type of white blood cells in cases of acute lymphocytic leukemia and acute myelocytic leukemia, plasma cells, parasites, as well as any other types of cells. Write report and suggest further investigations if needed.[2]

Ethical Approval

The research proposal was reviewed by the Ethical Committee of the research unit in the ministry of health, and ethical clearance was approved by the National Health Laboratory, Asmara, Eritrea (registration number: 34/19 and the approval date is 08/06/2019).

RESULTS

A total of 204 cases were included in this study. The age of the patients ranged from 9 months to 80 years with a mean age of 29.5 years, and males being the most common gender (1.6:1.0) are shown in Table I. Of the 204 cases, (30.4%) were between 15 and 30 years. The smallest patient was a 9-month-old baby as shown in Table I.

Hypercellular marrow was reported in 175 (85.8%) of the cases with 10 (4.9%) cases of hypocellular marrow are shown in Table 2.

The clinical indications for the BMA included pancytopenia (48.1%), suspected leukemia (26.5%), unexplained splenomegaly

TABLE 2. The Cellularity of the Bone Marrow Aspiration

Cellularity of the marrow	No. of patients	Percentage (%)
Hypercellular	175	85.8
Normocellular	20	9.8
Hypocellular	9	4.4
Total	204	100

TABLE 3. Clinical Indications of Bone Marrow Aspiration

Indication	No. of patients	Percentage (%)
Pancytopenia	98	48.1
Suspected leukemia	54	26.5
Unexplained splenomegaly	17	8.3
Unexplained fever	14	6.9
Thrombocytopenia	9	4.4
Anemia	6	2.9
Suspected malignancy	6	2.9
Total	204	100

(08.3%), unexplained fever (06.9%), and suspected malignancy (2.9%) are shown in Table 3.

In 188 (92.2%) of these cases, BMA provided either the diagnosis or diagnostic clues concerning the disease process, while 16 (7.8%) of the aspirates revealed a normally functioning marrow.

The most frequent diagnoses made included hypersplenism 20.1%, visceral leishmaniasis 9.8%, acute leukemia 9.8%, megaloblastic anemia 8.3%, chronic myeloid leukemia 8.3%, chronic lymphoid leukemia 7.4%, bone marrow hypoplasia 4.9%, idiopathic thrombocytopenic purpura 4.4%, hemolytic anemia 3.9%, iron deficiency anemia 1.5%, multiple myeloma 1.0%, and polycythemia 1.0%. Meanwhile there was one case (0.5%) for each of the following conditions: Gaucher's disease, secondary malignancy, and essential thrombocythemia are shown in Table 4.

DISCUSSION

In this study, we described briefly the basic concepts of BMA, in addition to our findings of the clinical indications and the final diagnoses within a 21-month period in Asmara city.

Two hundred and four cases were involved in this study. This number is lower than the expectations, particularly when considering population of 896,000 living in the Asmara locality. However, since BMA service was not previously available in this city, the few numbers of requests are not unexpected, due to the fact that the physicians need some time to be aware of the services available upon their request.

Some authors are of the view that BMA has deteriorated as a diagnostic tool in clinical practice.[7] This might be true in certain settings where other alternative diagnostic procedures exist. However, BMA is still essential and of great importance in most developing countries for establishing the diagnosis of many conditions. A difference was observed between males and females who underwent BMA during the study period. Notably, 128 (62.7%) were male and 76 (37.3%) were female; the male to female ratio was 1:1.6.

In our study, in males, the most common diagnosis was hypersplenism 40 cases (31.3%), followed by visceral leishmaniasis 23 cases (17.9%). Meanwhile the most common hematological neoplasm was acute leukemia and chronic lymphoid leukemia nine cases (7%) for each, followed by chronic myeloid leukemia eight cases (6.3%).

In our study, in females, the most common diagnosis was acute leukemia 11 cases (14.5%), followed by hypersplenism 10 cases (13.2%). Males are affected by hypersplenism more than females in many studies.[8,9] The exact cause can't be identified. In our society, females may be presented less than males to tertiary care facilities, and usually after serious illnesses like leukemia, as shown in our study.

Importantly, the most common age group that underwent the procedure was in the 15-30 age group. This finding suggests that the rate of suspected hematological disorders is high in the youngest population. Pancytopenia was observed to be the most common clinical indication encountered (48%). This is similar to the study conducted by Ahmed et al.[10] However, pancytopenia was the third most common indication in a study done by Bashawri.[11]

The study found visceral leishmaniasis to be the most common cause of pancytopenia in children, followed by hypersplenism. In adults, this was hypersplenism, followed by megaloblastic anemia. According to our study, the second common indication was suspected leukemia (26.5%), which is consistent with the finding of Pudasaini et al.[5] and Bashawri.[11]

We found that most of the BMA was hypercellular (60.17%), which is comparable to the observations of Marwah et al.[12] It is attributed to compensatory trilineage hyperplasia seen in the BMA due to peripheral pooling of blood cells within the enlarged spleen. Of importance is also the fact that hypersplenism was found to be the most common diagnosis in our study, which is similar to the results of Sreedevi et al.[4]

Erythroid hyperplasia emerges as the most common BMA finding in the study conducted by Pudasaini et al.[5] In our laboratory, visceral leishmaniasis (kalazar) was the second most common diagnosis encountered, as evidenced in 31 (15.2%) of the cases. However, all of these cases, when traced back, were found to be from endemic areas of the disease in the west, Eritrea.

Acute leukemia was seen in 20 (9.8%) of the cases, which is lesser than the findings of Pudasaini et al. (12.3%). Acute leukemia cases were correlated with the clinical picture as well as the presence of more than 20% of blasts in the peripheral blood smear findings. The diagnosis was later confirmed by flowcytometry.

Megaloblastic anemia meanwhile was observed in 17 (8.3%) of the cases as compared to other studies of Pudasaini et al.[5] and Atla et al.[3]—(12.3%) and (40%), respectively. The BMA findings in megaloblastic anemia were erythroid hyperplasia with megaloblatic changes and delay in maturation of myeloid series. The diagnosed confirmed later by biochemical methods.

In Eritrea, such nutritional deficiencies are a common phenomenon. Chronic myeloid leukemia was seen in 17 (8.3%) of all

TABLE 4. Bone Marrow Examination Findings

Broad category (%)	Diagnosis	No. of patients	Percentage (%)
Nutritional anemia (13.7%)	Iron deficiency anemia	3	1.5
	Megaloblastic anemia	17	8.3
	Hemolytic anemia	8	3.9
Hypoplastic anemia (4.9%)	Aplastic anemia	9	4.4
	Pure red cell aplasia	1	0.5
Hematological malignancy (28%)	Acute leukemia	20	9.8
	Chronic myeloid leukemia	17	8.3
	Chronic lymphoid leukemia	15	7.4
	Multiple myeloma	2	1.0
	Polycythemia vera	2	1.0
	Essential thrombocythemia	1	0.5
Idiopathic thrombocytopenia purpura (4.4%)	Idiopathic thrombocytopenia purpura	9	4.4
Others (42.7%)	Hypersplenism	41	20.1
	Visceral leishmanasis	31	15.2
	Infection	10	4.9
	Gaucher's disease	1	0.5
	Secondary malignancy	1	0.5
Normal bone marrow (7.8%)	Normal bone marrow	16	7.8
Total		204	100

cases and correlated with the presence of all stages of maturation in the peripheral blood smear findings and high total white blood cells in the CBC, which was confirmed by positive Philadelphia chromosome testing. Chronic lymphoid leukemia was seen in 15 (7.4%) of the cases, was correlated with the high lymphocytes cells in the CBC, and confirmed by flowcytometry; 10 (4.9%) of these cases were found to have infectious etiology other than leishmaniasis.

The rarity of the cases diagnosed in this study may be attributed to the fact that there is a long list of differential diagnoses for pyrexia of unknown origin (PUO), thus implying that BMA can hardly settle the diagnosis. The role of BMA in settling the diagnosis in PUO has been well documented by some authors,[13] as BMA diagnostic was seen in only 16.5%. Hypoplastic anemia was observed in 10 (4.9%) of the cases, which is similar to the findings of Pudasaini et al.[5] (5.3%).

In all cases of hypoplastic anemia, the marrow was hypocellular and all three lineages of the cell were suppressed. The diagnosis was based on the examination of fragments and clot section examination due to the unavailability of bone marrow biopsy. Both aspiration and trephine biopsy are recommended to be conducted simultaneously in cases of pancytopenia, especially if hypoplastic or aplastic anemia is suspected, though aspiration smears are known to be superior for morphological details. Bone marrow biopsy provides a more reliable index of cellularity, revealing bone marrow infiltration, fibrosis, and granulomas.[14]

Disorders of the platelet were observed in 4.4% of all cases. These were mostly accounted for by males in the age group of 15-30 years and diagnosed as immune thrombocytopenic purpura. Meanwhile other studies showed (6.21%), (14.5%), and (6.8%) cases of immune thrombocytopenic purpura, respectively, in their studies.[12,15] Hemolytic anemia, which showed erythroid hyperplasia in BMA, was evidenced in 8 (3.9%) of these cases, and this diagnosis confirmed later by biochemical methods and genetic disorders. This finding is comparable to that of Jha et al.[14] (19.6%).

Microcytic anemia seems to be an uncommon finding as we came across only three such cases (1.5%). The BMA finding in microcytic anemia was erythroid hyperplasias that were subsequently labeled as iron deficiency anemia after conducting an iron profile study. We encountered two cases (1.0%) of multiple myeloma which showed 22% and 12% plasma cells and correlated with the biochemical, radiological, and clinical findings compared to that of Kibria et al.[15] (9.04%).

Two cases (4.6%) of polycythemia Vera showed trilineage hyperplasia and correlated with CBC and peripheral blood findings along with the Janus kinase 2 (JAK2) mutation study. One case of Gaucher's disease was diagnosed by a bone marrow examination. As per our finding, bone marrow examination is helpful in making the primary diagnosis of storage disease.

In children, age less than 15 years (56 cases), the most common non-neoplastic disorders were found to be visceral leishmaniasis with 17 cases (30.4%), followed by hypersplenism with eight cases (14.3%). On the other hand, the most common neoplastic hematological disorder was acute leukemia 11 cases (19.6%). In adults whose age ranged from 15 to 45 years (99 cases), hypersplenism was the most common non-neoplastic disorders with 28 cases (28.3%), followed by megaloblastic anemia with 14 cases (14.1%) and visceral leishmaniasis with 13 cases (13.1%). Similarly, the most common neoplastic hematological disorder was chronic myeloid leukemia nine cases (9.1%), followed by acute leukemia eight cases (8.1%).

Among the elderly, in cases of age more than 45 years (49 cases), the most common non-neoplastic disorder was hypersplenism with nine cases (18.4%). Meanwhile, the most common neoplastic hematological disorders were chronic lymphoid leukemia with 13 cases (26.5%), followed by chronic myeloid leukemia with 10 cases (20.4%). The rate of neoplastic hematological disorders was also found to be considerable (28%), although it is lower than that found in a study done in Saudi Arabia.[11]

The most common hematological malignancy was acute leukemia 20 cases (9.8%), followed by chronic myeloid leukemia with

17 cases (8.3) and chronic lymphoid leukemia with 15 cases (7.4%). The rate of non-neoplastic hematological disorders was seen in (63.6%) of cases. On the other hand, the most common non-neoplastic hematological disorders were hypersplenism with 41 cases (20.1%), followed by visceral leishmaniasis with 31 cases (15.1%) and megaloblastic anemia with 17 cases (8.3%). Among all cases, it was found that 7.9% of BMA was absolutely normal without any pathology.

In this study, we observed that the most common BMA finding was hypersplenism diagnosed by trilineage hyperplasia on BMA and enlarged spleen. This necessitates further investigation in order to detect the underlying causes of splenomegaly. We also noticed 41 (20.1%) of the cases with visceral leishmaniasis and infectious etiology other than leishmaniasis.

There is a strong need for collaboration between physicians, hematologist, pathologists, oncologists, and technicians in order to improve the diagnostic yield of bone marrow examination. Although BMA and biopsy are an uncomfortable procedure for the patient and should only be performed when there is a clear clinical indication.[16] It is a useful technique to diagnose blood disorders for various systemic illnesses.

Limitations of our study include the small size of sample and time constraints that may not allow for generalization. Despite this limitation, this study is novel in that it is the first of its kind in Asmara, Eritrea and is expected to constitute a database for future studies.

BMA is a useful investigation tool in clinical practice. It is a safe and cost-effective procedure, particularly in a resource-scarce country like Eritrea. In this regard, the study provides valuable insights into the most common hematological malignancy and non-neoplastic hematological disorders in Eritrea.

In our study, the most common indication for this procedure was pancytopenia, whereas the most common finding was hypersplenism. This study also focused on the need to rule out the underlying causes of hypersplenism by conducting further investigations to detect the root cause of hyperactive spleen. It is important to note that there were considerable percentages of cases diagnosed with visceral leishmaniasis.

Ethics Committee Approval: Ethics committee approval was received for this study from the National Health Laboratory, Asmara, Eritrea (registration number: 34/19 and the approval date is 08/06/2019).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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Bloodstream Yeast Species and In Vitro Antifungal Susceptibility Profiles

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BACKGROUND/AIMS
Bloodstream yeast infections have increased substantially in many countries worldwide and pose a significant threat. This study investigated the distribution of bloodstream yeast species and determined the antifungal susceptibility profiles.

MATERIAL and METHODS
A total of 52 yeast isolates were collected from the microbiology laboratory of the University Hospital. These isolates were collected from blood specimens that were submitted for culture test from in-patients, who have high body temperature. Of these isolates, 41 isolates of *Candida* spp. were identified using Brilliance™ *Candida* Agar, germ tube test, and tested for the chitin synthase 1 (*CHS1*) gene. Selected isolates were tested against seven antifungal agents by disk diffusion method, and the minimum inhibitory concentration (MIC) was determined by MIC Test Strips for the mutidrug resistant isolates. The study protocol was reviewed and approved by the Institutional Review Board.

RESULTS
Candida albicans accounted for 52%, *C. glabrata* (26.9%), *C. krusei* (9.6%), and *C. tropicalis* (6.7%). The newly emerging yeasts included *C. parapsilosis*, *C. zeylanoides*, *C. apicola*, *Blastoschizomyces capitatus*, *Cryptococcus neoformans*, *Kluyveromyces*, and *Rhodotorula mucilaginosa*. Most Candida species were highly susceptible to amphotericin B and caspofungin. Between 38 and 69% of *C. albicans*, isolates were resistant to the azoles. The non-*Candida albicans* (NCA) species showed different susceptibility patterns. Anidulafungin had the lowest MIC_{90} at 0.047 µg/mL. The CHS1 gene was detected in *C. albicans* and in three NCA spp.

CONCLUSION
Identifying the isolated species and determining their susceptibility pattern are essential to alert physicians to infections with rare and uncommon fungal species to optimize the antifungal therapy.

Keywords: Bloodstream infections, *Candida* spp., chitin synthase 1 (*CHS1*) gene, *Cryptococcus* species, newly emerging yeasts

INTRODUCTION

Many *Candida* species pose no threat to humans as they live as harmless commensals in the gut flora, on the human skin and mucosa, and they are controlled by the host innate immune responses. However, *Candida* species can invade and cause disease when the immune system is compromised or mucosal barriers are disrupted.[1] In addition, overgrowths of some species have transformed these commensals into medically important agents causing infections in hospitals.[2]

There has been a steady increase in the incidence of invasive candidiasis since 1980s, which is largely due to the increasing population of immunocompromised individuals,[3] with *Candida albicans* as one of the most common opportunistic pathogens. It colonizes and invades various tissues and organs causing systemic fungal infections (eg, hematogenous disseminated candidiasis). The non-*Candida albicans* (NCA) isolates such as *C. krusei*, *C. tropicalis*, and *C. parapsilosis* also have emerged as clinically significant opportunistic pathogens.[4]

The intrinsic resistance to antifungal therapy seen in some *Candida* species, and the development of acquired resistance during treatment is a major challenge for clinicians.[5]

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Chitin is an integral structural polysaccharide in fungal cell wall that is required for cell shape and morphogenesis. The hyphal cell walls of *C. albicans* have been reported to have higher chitin content than other yeasts,[6] and the hyphae chitin synthase activity has been estimated to be twice that of yeast cells. Although chitin synthase 2 (CaChs2p) is the most abundant protein among the three *C. albicans* chitin synthases, it is not necessary for hyphal growth, viability, or virulence, whereas CaChsI is expressed in both yeast and hyphae at a lower level[7] and maintains the integrity of the lateral cell wall. Chitin has also recently emerged as a significant player in the activation and attenuation of immune responses to fungi and other chitin-containing parasites.[8] Therefore, the study of chitin synthase is important to the understanding of fungal growth and its potential as a target for the development of antifungal drugs. In addition, identifying the species of the isolates and determining the susceptibility pattern are essential for clinical management.

To the best of our knowledge, this is the first study to investigate the species distribution and antifungal susceptibility profile of bloodstream yeast infections in Jordan.

The aim of this study was to identify yeast species prevalent in bloodstream infections (also known as candidemia) and to determine their antifungal susceptibility profiles.

MATERIALS and METHODS

This retrospective study was ethically approved by Jordan University of Science and Technology Internal Review Board (IRB) for research on human specimens.

A total of 52 yeast nonduplicate isolates that were submitted to the microbiology laboratory for blood culture between July 2013 and August 2014 were collected, each recovered from one patient with bloodstream infection. Blood cultures were routinely done from in-patients, who have high body temperature. By definition, all positive blood cultures for *Candida* species were considered infections.

Isolates were inoculated onto Sabouraud dextrose agar (SDA) (Oxoid, Hampshire, UK) plates to obtain pure cultures. Gram-stained smears were prepared from overnight cultures and were viewed under the microscope for morphology. For the rapid presumptive identification of *Candida* species, single colonies were inoculated on Brilliance™ *Candida* agar (Oxoid, Hampshire, UK) supplemented with chloramphenicol. All plates were incubated at 30°C for 24-48 hours. The color and morphology of colonies were recorded, and isolates were stored in Sabouraud dextrose broth at –80°C until tested.

Main Points

- Understanding the role of dominant *Candida* species in BSI and their antifungal susceptibility are crucial to optimize therapeutic and prophylaxis measures.
- *C. albicans* was the predominant species identified in 63.4% of the cases, and *C. tropicalis* was predominant among non-*albicans* species (12.2%). Most *Candida* species were susceptible to amphotericin B, most *C. albicans* isolates were resistant to posaconazole and voriconazole (69% each), and 61.5% were resistant to fluconazole.
- The identification of newly emerging bloodstream fungal pathogens such as *Cryptococcus neoformans*, *Rhodotorula mucilaginosa*, *Blastoschizomyces capitatus*, and *Kluyveromyces* spp. should alert the clinical laboratories and increase the awareness of physicians and to their potential in similar cases.

The Germ tube test was used to differentiate *C. albicans* from other yeasts. A well isolated colony from overnight SDA culture was suspended into 2 mL of freshly pooled human sera, incubated at 35°C for 2-4 hours, and a drop of the suspension was microscopically examined for the formation of germ tubes. *C. albicans* grew as a yeast form at 30°C and the mycelial form at 35-42°C.[9]

Speciation of the *Candida* isolates was done using the Remel RapID™ YEAST PLUS System (Thermo Fisher Scientific, Lenexa, KS, USA) following the manufacturer instructions. This system is based on enzyme technology that employs conventional and chromogenic substrates to identify yeasts and yeast-like organisms. The interpretation of reactions was done according to ERIC® electronic code compendium (http://www.remel.com/eric/) designed for Remel RapID Systems.

The antifungal susceptibility testing of selected isolates was done by disk diffusion method on Mueller–Hinton agar supplemented with 2% glucose and methylene blue dye according to Clinical Laboratory Standard Institute, 2009.[10] Isolates were tested in triplicate against seven antifungal agents, namely, amphotericin B, caspofungin, and five azoles, which are fluconazole, itraconazole, ketoconazole, posaconazole, and voriconazole (Liofilchem®, Italy). The minimum inhibitory concentration (MIC) was determined for 24 isolates (19 *C. albicans* isolates and five *C. tropicalis* isolates) that showed resistance to two or more antifungal agents by the disk diffusion. MIC was determined using the MIC Test Strips (Liofilchem, Italy) on RMPI 1640 medium supplemented with 0.2% glucose and 1.5% agar. The 0.5 McFarland inoculum was swabbed in three directions on the entire RPMI-agar plate, and the MIC Test Strips were applied. The MIC was read after 24-48 hours, where the border of the elliptical inhibition zone edge intersected the scale on the strips (at the point of approximately 80% growth inhibition). The MIC50 and MIC90 values are the lowest concentration of the antibiotic at which 50% and 90% of the tested isolates were inhibited, respectively.

Control Strains

The quality of test performance was controlled by including the reference strains *C. albicans* (ATCC 10231), *C. parapsilosis* (ATCC 22019), and *C. krusei* (ATCC 6258) in the antifungal susceptibility testing and in the MIC test.

Detection of *CHS*I Gene by PCR

This virulence gene is present in four *Candida* species (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*).[11] DNA was extracted from the isolates and controls using Gentra Puregene Yeast/Bact Kit (Qiagen, USA) according to the manufacturer's instructions, and DNA was stored at –20°C until tested.

The PCR master mixture contained 1 µL of the prepared DNA and GoTaq® Green Master Mix (Promega, USA). This premixed ready-to-use solution contains bacterial-derived *Taq* DNA polymerase, $MgCl_2$, dNTPs, and reaction buffers at optimal concentrations for efficient amplification of DNA templates; it

also contains blue and yellow dyes for monitoring the progress during electrophoresis. The sequences of primers used in PCR-targeted sequences within the *CHSI* gene of *C. albicans* were primer 1, 5′-CGCCTCTGA TGGTGATGAT-3′, and primer 2, 5′-TCCGGTATCACCTGGCTC-3′. Primers, PCR mix ,and amplification conditions are those described by Jordan.[11] *C. parapsilosis* (ATCC 22019) was used as positive control, and the negative control tube containing the mixture with no DNA template was used in every PCR run. The PCR thermal cycler (Bio-Rad, USA) was used in all amplifications.

The amplicon size for each of the four *Candida* species is *C. albicans* (122 bp), *C. parapsilosis* (311 bp), *C. tropicalis* (519 bp), and *C. glabrata* (535 bp). Five microliters of the PCR products was electrophoresed along with 100 DNA ladder (Promega, USA) in 2% agarose gel stained with ethidium bromide. The DNA bands were visualized with UV light in a gel documentation system (Bio-Rad, USA), and images were taken.

Statistical Analysis

Descriptive statistics were used to describe the isolates and the antifungal susceptibility testing of selected isolates. Categorical data are expressed as numbers and percentages.

RESULTS

A single species was identified in each sample. The Remel RapID YEAST PLUS System identified 41 isolates as *Candida* spp., except one isolate that could not be identified by this system (Table 1).

The colonies of the isolates exhibited different colors based on the chromogenic color reactions on Brilliance *Candida* agar. All *C. albicans* and *C. tropicalis* species produced green and blue colonies, respectively. *C. krusei* produced light pink and beige brown colonies, while *C. glabrata* produced white or beige brown/yellow colonies.

The distribution of the identified yeasts is shown in Table 1. Seven different *Candida* species were identified in the 41 *Candida* isolates, and *C. albicans* (63.4%) was the predominate species. Ten NCA isolates were identified, and *C. tropicalis* (12.2%) was the most common followed by *C. glabrata* (9.6%). The isolates also included *C. parapsilosis* species complex, *C. zeylanoides*, *C. apicola*, and *C. krusei*.

TABLE 1. Distribution of Yeast Genera as Identified by Remel RapID YEAST PLUS System

Identified yeasts		Total (%)
	Candida spp.*	41 (78.8)
	C. albicans	26 (63.4)
	C. tropicalis	5 (12.2)
	C. glabrata	4 (9.8)
	C. parapsilosis	2 (4.9)
	C. zeylanoides	2 (4.9)
	C. apicola	1 (2.4)
	C. krusei	1 (2.4)
Cryptococcus neoformans		3 (5.7)†
Rhodotorula mucilaginosa		5 (9.6)
Blastoschizomyces capitatus		1 (1.9)
Kluyveromyces spp.		1 (1.9)
Not identified		1 (1.9)
Total		52 (100)

*Percentage of *Candida* species calculated out of 41 isolates.
†Percentage of non-*Candida* species calculated out of 52 isolates.

TABLE 2. Diameter of Zone of Inhibition in Millimeters for One *Candida apicola* (N = 1) and Two *Candida zeylanoides* isolates

Antifungals	*C. apicola*	*C. zeylanoides*
Amphotericin B	7	7, 20
Caspofungin	7	21, 15
Fluconazole	0	33, 41
Itraconazole	0	32, 32
Ketoconazole	27	34, 48
Posaconazole	17	12, 25
Voriconazole	0	33, 40

The presumptive color identification of the isolates on the Brilliance *Candida* agar media was confirmed by the Remel RapID YEAST PLUS System. In addition, 10 isolates were identified as yeast genera other than *Candida* (Table 1).

The diameter of zone of inhibition for susceptibility was interpreted (Table 2) as described using the CLSI[10] document for caspofungin, fluconazole, and voriconazole. It varied between the antifungal agents and was as follows: *C. albicans* and *C. parapsilosis* (≥19), *C. tropicalis* (≥25), and *C. krusei* (≥16). The criteria used for amphotericin B and posaconazole were those proposed by Nguyen et al.[12] and Carrillo-Munoz et al.,[13] respectively. As for itraconazole and ketoconazole, we followed the criteria by Rosco diagnostics (www.rosco.dk/gfx/pdf/yeasts.pdf).

The diameter of inhibition zone for *C. apicola* and *C. zeylanoides* is presented in Table 2 as there were no standard interpretive criteria for these species. *Candida* species were highly susceptible to amphotericin B and caspofungin (Table 3). *C. albicans* resistance to the azole group ranged from 38 to 69%.

All five *Candida tropicalis* isolates were susceptible to amphotericin B, caspofungin, 4 (80%), were susceptible to itraconazole, and isolates showed variable resistance to the remaining azoles (60% to ketoconazole and 100% to fluconazole).

The two *C. parapsilosis* isolates were susceptible to all the azoles. All *C. glabrata* isolates were susceptible to most of the azoles; however, they showed less susceptibility to posaconazole and voriconazole (Table 3). *C. krusei* is known to have intrinsic resistance to fluconazole, and the one isolate in this study was only resistant to posaconazole.

The MIC test was performed for 24 isolates (19 *C. albicans* and five *C. tropicalis*) that were resistant to more than one antifungal agent by the disk diffusion method. After 24 hours of incubation, a symmetrical inhibition ellipse centered along the strip was formed. The MIC was read directly from the scale on each strip in μg/mL, at the point where the edge of the inhibition ellipse intersects with the MIC Test Strip. The macrocolonies in the ellipse were read at 80% inhibition, and the partial inhibition of growth giving trailing microcolonies of decreasing size end points was ignored.

The supplementation of RPMI agar with additional glucose (2% final concentration) provided optimal growth of these species

TABLE 3. Susceptibility Pattern of *Candida* Species Isolated from Bloodstream Infections

	Number of isolates of each species				
Antifungal agents	***C. albicans* (N = 26)**	***C. glabrata* (N = 4)**	***C. krusei* (N = 1)**	***C. parapsilois* (N = 2)**	***C. tropicalis* (N = 5)**
Amphotericin B (20 µg)					
S	23 (88.5)	4 (100)	1 (100)	2 (100)	5 (100)
DD/I	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
R	2 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)
Caspofungin (5 µg)					
S	23 (88.5)	4 (100)	1 (100)	1 (50)	5 (100)
DD/I	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
R	3 (11.5)	0 (0)	0 (0)	0 (0)	0 (0)
Fluconazole (100 µg)					
S	10 (38.5)	4 (100)	NA	2 (100)	0 (0)
D/I	0 (0)	0 (0)	NA	0 (0)	0 (0)
R	16 (61.5)	0 (0)	NA	0 (0)	5 (100)
Itraconazole (50 µg)					
S	9 (34.6)	4 (100)	1 (100)	2 (100)	4 (80)
DD/I	1 (3.8)	0 (0)	0 (0)	0 (0)	0 (0)
R	16 (61.5)	0 (0)	0 (0)	0 (0)	1 (20)
Ketoconazole (15 µg)					
S	5 (19.2)	3 (75)	1 (100)	2 (100)	1 (20)
DD/I	11 (42.3)	1 (25)	0 (0)	0 (0)	1 (20)
R	10 (38)	0 (0)	0 (0)	0 (0)	3 (60)
Posaconazole (5 µg)					
S	6 (23.1)	1 (25)	0 (0)	2 (100)	1 (20)
DD/I	2 (7.7)	1 (25)	0 (0)	0 (0)	0 (0)
R	18 (69.2)	2 (50)	1 (100)	0(0)	4 (80)
Voriconazole (1 µg)					
S	8 (30.8)	3 (75)	1 (100)	2 (100)	1 (20)
DD/I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
R	18 (69.2)	1 (25)	0 (0)	0 (0)	4 (80)

S, susceptible; R, resistant; DD/I, dose dependent/intermediate; NA, not applicable; percentages are shown in parentheses.

and minimized the problem of trailing endpoints due to partial inhibition of growth by azoles.[14]

The MIC breakpoints for anidulafungin, fluconazole, voriconazole, and caspofungin were carefully interpreted according to CLSI[15] (Table 4). However, the criteria used for amphotericin B and posaconazole were as previously proposed.[12,13]

There was no current break point for itraconazole, and six *C. albicans* isolates and three *C. tropicalis* did not have zone of inhibition.

The lowest MIC_{50} of the tested isolates was recorded for anidulafungin (0.012 µg/mL) followed by itraconazole with an MIC_{50} at 0.016, and amphotericin B and voriconazole had low MIC_{90} (1.5 µg/mL); however, no clinical breakpoint is available at present (Table 4).

Amplicons of the *CHSI* gene (122 bp) were observed in *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and the positive control (*C. parapsilosis* ATCC 22019), and not in the negative control (*C. krusei* ATCC 6258).

DISCUSSION

The results of this study report on the species distribution and newly emerging and rare yeast species isolated from bloodstream infections. *C. albicans* was the predominant species identified as 63.4%, and *C. tropicalis* was predominant among NCA isolates (12.2%), followed by *C. parapsilosis* species complex and *C. zeylanoides* each (4.9%).

TABLE 4. MIC_{50} and MIC_{90} Range and the Breakpoints for the Multidrug Resistant Isolates

Antifungal agents	**No of *C. albicans* isolates**	**No of *C. tropicalis* isolates**	**MIC (µg/mL)**			**Breakpoint (µg/mL)**
			Range	**MIC_{50}**	**MIC_{90}**	
Amphotericin B	18	5	0.23-1.5	1	1.5	≥1
Anidulafungin	18	5	0.002-0.12	0.012	0.047	≥1
Caspofungin	17	5	0.125-12	1	3	≥1
Fluconazole	12	3	0.012-192	1	192	≥8
Itraconazole	13	2	0.002-2	0.016	0.25	NA
Posaconazole	14	3	0.064-8	0.19	15	≥4
Voriconazole	13	3	0.008-4	0.047	1.5	≥1

MIC, minimum inhibitor concentration; NA, not applicable.

Candida species rank fourth in the United States and seventh in Europe among the causative agents of BSI in hospitals.[16,17] Although *C. albicans* remains the most common isolate obtained from invasive fungal infections and ICU patients, *C. glabrata* has emerged as the second most common cause of invasive candidiasis in the United States.[18] Despite the reported shift of species distribution, the results of our study showed that *C. albicans* is still the dominant species in bloodstream infections. The higher incidence of *C. albicans* infection can be largely explained by the presence of this yeast among the normal mucosal flora of most humans. These results are in agreement with earlier studies that recovered *C. albicans* in 47.5% of the BSI.[19] The bloodstream NCA identified in our study is in accordance with those reported earlier in candidemia cases (35-65%) in the general patient population.[20] However, another study reported greater frequency of the NCA species than that of *C. albicans* with *C. parapsilosis* as the most frequent in BSI.[21] Unlike other systemic mycoses, invasive *Candida* infections in immune compromised patients originate most frequently from endogenous reservoirs; however, exogenous infections are frequently reported in hospitalized patients.[22]

Our antifungal susceptibility testing results showed that all *Candida* species were susceptible to amphotericin B, with the exception of two *C. albicans* isolates that were resistant. The MIC_{50} and MIC_{90} for most species fall within what is considered a resistant range. The resistance of *C. albicans* isolates to the azoles group was 38-69%. The azole antifungals are the most frequent class used to treat *Candida* infections. Recent exposure to azoles and antibacterial drugs were reported as important risk factors for infection with fluconazole-resistant *Candida* spp.[23]

C. parapsilosis species complex in our study was susceptible to all tested antifungal agents (Table 3). Most of the *C. albicans* isolates were resistant to posaconazole and voriconazole (69% each), and 61.5% were resistant to fluconazole. The least resistance was observed to caspofungin (11.5%) and ketoconazole (38%). All *Candida* strains were reported earlier to be susceptible to amphotericin B and caspofungin, while 10.8% of the isolates were resistant to fluconazole.[24] Our four *C. glabrata* isolates were susceptible to several azoles, but they were less susceptible to posaconazole (Table 3). The one single *C. krusei* isolate in this study was only resistant to posaconazole.

A retrospective, case-comparative study in the United States reported that *C. albicans*, *C. tropicalis*, and *C. parapsilosis* that are generally considered to be susceptible to fluconazole accounted for 36% of isolates with a reduced susceptibility and 48% of resistant isolates.[25] One mechanism of resistance identified in *C. albicans* is the presence of point mutations in *ERG11*.[26]

Cross-resistance between the azoles among *Candida* species to multiple antifungal agents is also an important concern. It has been demonstrated for *C. glabrata*, *C. albicans*, *C. tropicalis*, and *C. parapsilosis*.[3]

Acquired resistance is generally less common than intrinsic resistance; however, data from various studies suggest that it is beginning to emerge in some countries. There are intrinsically resistant strains of *C. albicans* that can be part of a commensal growth or can be acquired from the environment or other individuals. Resistant strains of *C. albicans* can occur as a result of the normal distribution of MICs that all species exhibit, or they can develop resistance through several mechanisms.[27] There is the possibility that a strain can be induced to be resistant by exposure to the drug over long periods. In these cases, the drug itself does not cause resistance but, rather, selects for the growth of the more resistant cells in the population. In addition, a large population of yeast cells under selective drug pressure, specific random mutations that render the cell slightly more resistant, will eventually become the dominant strain in the population. Antifungal drug resistance can lead to the development of infections in high-risk patients receiving antifungal prophylaxis.

Primary resistance among certain fungi without prior exposure to the drug, eg, resistance of *C. krusei* to fluconazole and *Cryptococcus neoformans* to echinocandins, emphasizes the importance of the identification of fungal species in clinical specimens. However, secondary resistance develops among previously susceptible strains after exposure to the antifungal agent such as strains resistance of *C. albicans* and *C. neoformans* to fluconazole, which is usually dependent on altered gene expression.[28]

The *CHS1* gene was selected for PCR amplification because this virulence gene can identify four medically important *Candida* species, namely, *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*, and it was detected in the four species of our isolates. This method allows the relatively rapid identification of *Candida* species in conjunction with morphologically and physiologically based identification procedures.

It is worth mentioning the identification of rare and newly emerging bloodstream fungal pathogens in this study by Remel RapID YEAST PLUS System (Table 1). They were three *C. neoformans*, five *Rhodotorula mucilaginosa*, one *Blastoschizomyces capitatus*, and one *Kluyveromyces* spp. These rare invasive yeast pathogens were defined by the European Society of Clinical Microbiology and Infectious Diseases, Fungal Infection Study Group, and the European Confederation of Medical Mycology.[29]

C. neoformans was first reported in dialysis-associated bacteremia in a patient with renal failure, who developed fungemia during the treatment, where this yeast species grew in the catheter tip and blood culture.[30] Cryptococci generally are found in soil contaminated with pigeon feces and are transmitted to humans primarily through inhalation. *C. neoformans* is one of the most important agents of the opportunistic mycoses.[3] *Cryptococcus* invasive infections are commonly associated with immunosuppressed individuals, those with liver cirrhosis, diabetes mellitus, or other medical conditions,[31] while being extremely rare in healthy individuals.[32]

Rhodotorula species were considered as nonvirulent saprophytes and common contaminant organisms. However, they have emerged as opportunistic pathogens, particularly in immunocompromised patients. *Rhodotorula* infection was first reported in 1985 in the medical literature, and the number of human infections increased after that time,[33] most likely because of the wider use of immunosuppressant and/or the use of central venous catheters. Bloodstream infections with *Rhodotorula* species were reported in 43 cases at Duke University Medical Center, USA, between 1960 and 2000.[34]

B. capitatus (formerly known as *Trichosporon* spp. and *Geotrichum capitatum*) was identified in one bloodstream case in our study. Infection with this rare yeasty was first reported in Switzerland[35] in a leukemic patient. *Kluyveromyces* spp. was identified in one case in our study, and this uncommon yeast species (*Kluyveromyces marxianus*, anamorph *Candida kefyr*) was earlier isolated from a patient with fungemia.[36]

The limitations of our study are mainly related to its retrospective nature, and therefore, we were unable to include the initial treatment and the follow-up on whether there was a persistence of positive blood cultures after the initiation of antifungal therapy. In addition, the low number of patients has limited the statistical power of the study.

In conclusion, several newly emerging yeast pathogens are reported in this study. These results should alert the clinical laboratories to the potential of rare fungi in similar cases. Identifying the species of the isolates and determining their susceptibility pattern are essential to optimize the therapeutic decisions regarding rational antifungal therapy.

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Informed Consent: N/A

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Author Contributions: Concept - L.N., N.Q; Design - L.N., N.Q.; Supervision - L.N., N.Q.; Resource - N.Q.; Materials - L.N.; Data Collection and/or Processing - L.N., N.Q., R.I.; Analysis and/or Interpretation - L.N., N.Q., R.I.; Literature Search - L.N., N.Q., R.I.; Writing - L.N., N.Q., R.I.; Critical Reviews - L.N., N.Q.

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Original Article

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The Suitability of Silk Fibroin Based Biofilms for Cartilage Tissue Engineering

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BACKGROUND/AIMS
Silkworms and spiders produce silk fibroin (SF). SF protein has unique characteristics, which includes mechanical properties, biodegradation, biocompatibility, and the ability to support the differentiation of stem cells along the osteogenic lineage. These characteristics makes SF a favorable biomaterial for cartilage tissue engineering. The aim of this study was to design a SF biofilm and then to test the biocompatibility and cytotoxicity of the designed SF biofilm.

MATERIAL and METHODS
Characterization was executed by scanning electron microscopy and Fourier transform infrared spectrophotometer analysis spectrophotometry. Normal human articular chondrocytes were seeded on biofilm and cultured up to 2 weeks (5% CO_2, 95% air and 37°C) under the standard culture conditions. Phase contrast microscopy and cell proliferation assay (3-(4, 5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay) was applied for evaluation of cell attachment and cell growth.

RESULTS
The viability of cells was linearly correlated with optical density, and chondrocyte viability in the SF film was found to be significantly higher.

CONCLUSION
These results indicated that SF film supports cell proliferation without side effects and the SF film is a potential material as a cartilage tissue engineering matrix.

Keywords: Biofilm, silk fibroin, chondrocyte, MTT, tissue engineering

INTRODUCTION

Adult articular cartilage has a limited self-repair capacity.[1] Repair and/or regeneration of articular cartilage defects is challenging in orthopedics.[2] Autologous cell-based tissue engineering holds a promising option for the repair trauma or aging related cartilage damages.[1] Cell based therapies or tissue engineering applications for in vitro cartilage tissue development need cells that are able to undergo chondrogenic differentiation with appropriate growth factors and suitable biomaterials, which provides a favorable microenvironment of cell growth and new cartilage-specific extracellular matrix (ECM) formation.[1]

There are different types of biomaterials that can be used for cartilage repair such as naturally occurring, synthetic, biodegradable, and nonbiodegradable materials.[3,4] Silk fibroin (SF), chitosan (CHI), hyaluronan, and alginate are most frequently used polymers in cartilage tissue engineering for development of ECM in tissue regeneration applications.[5–8]

Silk proteins are produced from cocooned silk worms or spiders.[9–11] High biocompatibility capacity,[12] robust mechanical properties, biological compatibility, and blood compatibility make SF protein an important natural biomaterial in tissue engineering.[13]

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In the present study, characterization of films was carried out for cartilage tissue engineering application. Swelling kinetics, biodegradation, and antimicrobial activity of the biofilms were assessed. Then, designed SF based biofilms were used for the evaluation of cell proliferation activity of Normal Human Articular Chondrocyte (knee, NKAC-kn) cells.

MATERIAL and METHODS

Mulberry *Bombyx mori*, cocoons were collected during the harvesting time from Lapta, North Cyprus.

Preparation of SF Film

The processes involved in the synthesis of the pure SF were as follows:

- degumming process,
- dissolution process, and
- dialysis process.

In the degumming process, cocoons were cut into small pieces, and 1 g of the cocoon was weighed and put into a conical flask. Then, 100 mL of the 0.1 M sodium carbonate Na_2CO_3 solution (1%, w/v) was added into the same conical flask. Degumming process was carried out on magnetic stirrer at 1 rpm and 70°C for 3 hours. The process was repeated to ensure that sericin, the gum, was entirely removed from the silk cocoon and SF protein was dried at 25°C.

Dissolution Process

The dissolution process changes the physical state of the SF from solid to liquid and the chemical state by breaking the H-bonding of SF protein. The strong electrolyte solution with molar ratio: n_{C2H5OH}:n_{CaCl2}:n_{H2O}; 2:1:8. The required amount of degummed SF fibers were added into strong electrolyte solution and stirred at 1 rpm at 75°C. This was continued until the SF was totally dissolved.

Dialysis Process

The dialysis process was used to purify liquid SF protein. A semipermeable SnakeSkin® Dialysis Membrane (10,000 MWCO) was used for dialysis process. The pure SF was removed from the membrane using a syringe.

Preparation of the Silk Fibroin Biofilm

Five grams of the pure SF was then measured, placed into a 25 mL beaker, and eft to dry overnight at 25°C. Methanol was added to cause beta-sheet formation and later removed. The biofilm is rinsed and left to dry at room temperature.

Characterization of SF Film

Fourier transform infrared spectrophotometer analysis (in Cyprus International University, Cyprus) and scanning electron microscope analyses (Jsm-6510 model at an acceleration voltage 10 kV, at Middle East Technical University, Turkey) were carried out in order to characterize the SF biofilm produced. The SEM device produced images of the samples by focusing beam of electrons on it, and samples were coated with gold to prevent charging.[14]

Main Points

- SF film supports cell proliferation without side effects.
- The SF film is a potential material as a cartilage tissue engineering matrix.
- The SF is a potentially function as a promising articular cartilage substitute for tissue engineering applications.

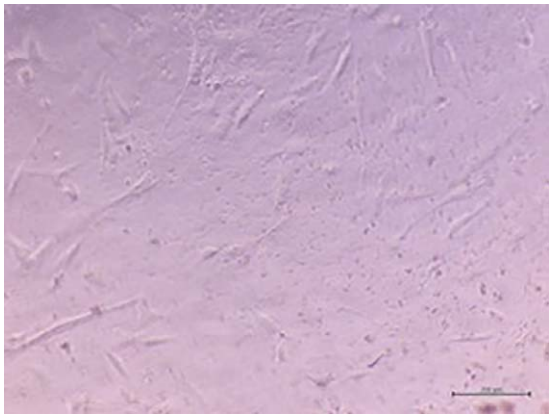

Figure 1. Cell attachment of NHAC-kn cells with phase contrast microscope; 7 days after seeding on biofilm

Antibacterial Activity

Kirby-Bauer disk diffusion method was used for the evaluation of antimicrobial activity of SF biofilm.[15] It was carried out with three gram-negative bacteria and three gram-positive bacteria. The zone of inhibition of each biofilm was measured using a scale, and photographs of the different petri plates were taken (data not supplied).

Cell Culture

NHAC-kn (Lonza, Clonetics™ Normal Human Articular Chondrocyte Cell System) culture was prepared as described previously,[14] and phase contrast microscope Olympus IX53 with camera Olympus DP22 was used for identification of cell morphology.

SF biofilms were incubated in 24-well plates in culture medium for 4 hours at 37°C before cell seeding as described previously.[14] The chondrocytes were seeded on to top of the SF biofilm at a total density of approximately 1.25×10^4 cells/ml. and growth medium replenished after 24 hours. SF biofilms were incubated for 15 days with media changes every other day. Cell attachment (30 minutes, 1, 2, and 3 hours) was evaluated by using a phase contrast microscope (Olympus IX53 with camera Olympus DP22). All images were taken at 10× and 40× magnification (Figure 1).

Analysis of Cytotoxicity

Cellular metabolic activity and relative cell viability were analyzed by methyl thiazolyl tetrazolium (MTT, Invitrogen) assay. MTT assay was applied for evaluation of cell viability after the NHAC-kn cells were cultured in a 24-well plate for 7, 9, and 15 days. The MTT assay was performed as described previously[14] (Figure 2).

RESULTS

Cell Culture and MTT Assay

Phase contrast microscope micrographs of NHAC-kn seeded biofilm were taken after 24 hours, 48 hours, 7 days, 9 days, and 15

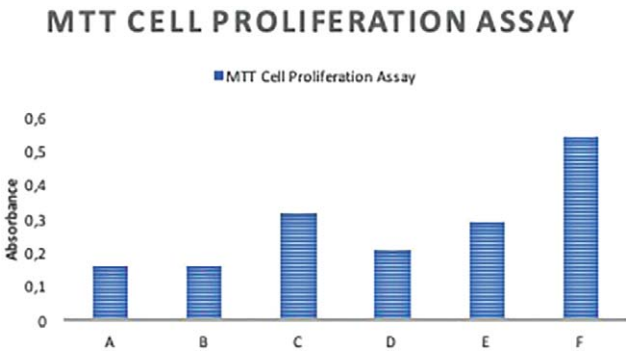

Figure 2. Cell viability in SF biofilm after 7 days, 9 days, and 15 days under the standard culture conditions. (A) Media only (0.157); (B) biofilm+media (0.157); (C) control NHAC-kn cell (0.313); (D) 7 days biofilm+cell (0.206); (E) 9 days biofilm+cell (0.289); (F) 15 days biofilm+cell (0.537).

days of culturing (data not shown). Cell growth was evaluated by measuring the MTT assay as shown in Figure 2. The viability of cells was linearly correlated with optical density; the chondrocyte viability seeded onto the SF based biofilm was significantly higher than controls. The chondrocyte viability on the SF biofilm was observed to significantly increase after 9 days.

DISCUSSION

SF is a nonsynthetic biomaterial used in tissue engineering applications. Various researchers used SF based films, fibers, meshes, membranes, scaffolds, hydrogels together with different natural materials like curcumin, hyaluronic acid (HA), poly(L-lactic-acid), CHI, cellulose, gelatin, CHI and hydroxyapatite, HA and fibrin/HA to test the cell proliferation activity, cell adhesion, and differentiation on these materials. Cartilage has a limited capacity for regeneration. Repair of cartilage injury by using specific cells, growth factors, and biomaterials is the main aim of cartilage tissue engineering. Selection of the biomaterial plays an important role within the process of cartilage engineering. Ideal scaffold should be biocompatible, biodegradable and have a three dimensional (3D) nanoporous structure.

Kim and colleagues worked with rabbit chondrocytes and used SF scaffolds and silk/curcumin blend scaffolds. They detected that curcumin with SF exhibited a high ECM production and provided an acceptable level of cell viability.[16] Nematollahi et al.[17] used rabbit chondrocytes together with silk-CHI blended scaffolds with glutaraldehyde (GA) concentrations. They concluded that tensile strength was raised with increase in freezing rate and GA concentration.[17] Zhang et al.[18] worked with bone marrow mesenchymal stem cells (BMSCs) on different materials such as collagen hydrogel (CH), collagen/sodium alginate hydrogel, collagen sponge, and silk sponge. They concluded that the induced and cocultured scaffolds exhibited high differentiation potential than control group.[18] Sawatjui et al.[19] used SF and SF-GCH scaffolds (SF/gelatine chondroitin sulfate (CS) hyaluron) with BM-MSCs and concluded that BM-MSCs proliferation, chondrogenic differentiation, and mimicking of cartilage structure and environment were identified in the SF-GCH scaffold.

Chomchalao et al.[20] used articular chondrocytes on SF, SF/C (SF/ceramic), and SF/G (SF/gelatin) scaffolds and enhanced cell attachment, proliferation of chondrocytes was observed on SF/C and SF/G scaffolds. Agrawal et al.[21] cultured hMSCs on SF:CHI (SF:CS) scaffold, and they detected cell distribution, proliferation, higher cell density, and increased expression of cartilage-specific genes. Li et al.[22] designed SF/CHI film and tried to identify rat bone marrow-derived mesenchymal stem cell proliferation activity on this material. They conclude that the SF/CHI based biofilm is a promising material for tissue engineering of bone, cartilage, adipose, and skin.[22] Wang et al.[1] used SF scaffolds and human articular chondrocytes successfully in cartilage tissue engineering. For bone tissue applications, contribution of the soluble form of ECM[23] to the SF improved wettability, mechanical property, and cyto-compatibility of the product by using a crosslinking method.[24,25] Zhou et al.[26] noted that together with SF, CS is safe and showed high biocompatibility for tissue engineering scaffolds. However, the problem is rapid degradation rate of pure CS scaffolds. Thus, SF is generally used because of its mechanical properties, long-lasting in vivo stability, and hypoimmunity.[26] They showed promoted articular cartilage defect repair with a combination of SF and CS and concluded that the silk-CS scaffold preserves better chondrocyte phenotype than silk scaffold.[26]

Lee et al.[25] developed SF (SF) and polyvinyl alcohol (PVA) hydrogels and used different methods, like salt leaching, silicone mold casting, and freeze–thawing methods, to test the auricular cartilage viability. They analyzed the swelling ratio, tensile strength, pore size, thermal properties, morphologies, and chemical properties of the hydrogels. These researchers found that the hydrogel, which is composed of 50% PVA and 50% SF (P50/S50), is the best-fabricated hydrogel for articular cartilage.[25] Shi et al.[27] used SF and gelatin scaffold to be able to repair cartilage injury via 3D printing technology. They detected high performance for cartilage repair in a knee joint and highlighted that the SF and gelatin combination scaffold was promising biomaterial for knee cartilage repair. Kim et al.[16] developed curcumin and SF scaffold for testing clinical replacement for defected cartilage. They detected cell proliferation and glycosaminoglycans (GAGs) and showed higher cell viability rate on 1 mg mL^{-1} curcumin/silk scaffold. Singh et al.[28] designed agarose/SF hydrogels of mulberry and nonmulberry SF. Higher amount of GAG and upregulation of cartilage-specific aggrecan, sox-9, and collagen type II was detected in nonmulberry SF hydrogel. They highlighted that the nonmulberry SF/agarose was an alternative biomaterial for cartilage tissue engineering.[28]

Jaipaew et al.[29] designed scaffolds from SF/HA and tested cartilage cell viability of this material by using human umbilical cord-derived mesenchymal stem cells (HUMSCs). They detected chondrogenesis related marker expression like Col2a, Agg, and Sox9 and suggested that the SF/HA scaffolds were suitable in cartilage tissue engineering and in surgery for osteoarthritis.[29] Ribeiro et al.[30] used horseradish peroxidase (HRP)-crosslinked SF scaffolds and cultured human adipose-derived stem cells. They detected under the chondrogenic culture conditions that cells showed adhesion, proliferation, and high GAGs synthesis and produced cartilage-specific extracellular matrix. They showed that the structural, mechanical, and biological performance of the proposed scaffolds could be used as 3D matrices for cartilage regeneration.[30]

Cai et al.[31] used SF coating PLA film systems for attachment and proliferation of rat osteoblasts. The usage of SF biomaterials on cartilage tissue regeneration has been used by many researchers.[1,32–36]

Tigli et al.[37] evaluated chondrogenesis by using different SF- or CS-based biomaterials and concluded that adoption of the cells was an important factor to be able to consider chondrogenic outcomes.[37] Silva et al.[38] used SF/CS composite scaffolds.

Proliferation of chondrocyte-like cells was observed in SF/CS composite scaffolds.[38,39]

SF based biomaterials, a combination of SF and other natural polymers, have been investigated for different cell types, like hMSCs, HUMSCs, and cartilage tissues. Due to its mechanical strength, elasticity, biocompatibility, bioactivity, adaptability, and biodegradability, SF protein has been widely used as a natural polymer in TE. Our results showed that, the NHAC-kn cell viability in the SF biofilm was significantly higher and indicated that SF film enhanced cell proliferation without side effects, and the SF film was a potential material for a cartilage tissue engineering matrix.

Natural polymers offer various advantages in cartilage tissue engineering applications but until now, there is no report to show ideal method for engineering of articular cartilage. SF is a naturally occurring protein, which has unique properties and that makes it a favorable matrix for the incorporation and delivery of a wide range of agents. This study highlighted that pure SF biofilm is an important material for tissue engineering applications for cartilage tissue engineering.

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Disease and Treatment Experiences of COVID-19 Patients: A Qualitative Study

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BACKGROUND/AIMS
The study aimed to explore the perceptions of hospitalized COVID-19 patients' experiences regarding the disease and treatment process.

MATERIAL and METHODS
The study was carried out as qualitative research at a hospital in Turkey between June 17, 2020 and July 7, 2020. The sample consisted of eight COVID-19 patients hospitalized in service after intensive care treatment. The data were collected through an in-depth individual interview form. Each interview was transcribed verbatim, and a thematic analysis was performed. The Standards for Reporting Qualitative Research checklist was followed.

RESULTS
Three main themes were identified for patients' perceptions of the disease experiences and treatment process. The patients experienced negative emotions after being diagnosed with COVID-19, and they spent the isolation and treatment process communicating with their beloved ones over the phone, watching television, and praying. Having an infectious disease caused them to have anxiety and sadness, and they reported satisfaction with the physicians and nurses during the treatment process.

CONCLUSION
Psychological and sociocultural factors, religious values, and health policies can be effective in patients' perception of disease and treatment. It is recommended for healthcare professionals to be aware of the psychosocial problems of the hospitalized COVID-19 patients during the disease and treatment process, monitor them for post-traumatic stress disorder, and provide the necessary support.

Keywords: COVID-19, patient, disease perception, qualitative research

INTRODUCTION

Coronaviruses have been reported to cause mild and moderate respiratory infections in the last 50 years.[1] However, recently detected coronaviruses severe acute respiratory syndrome (SARS) CoV (2002) and Middle East respiratory syndrome (MERS)-CoV (2012) have changed all current information as they cause severe acute respiratory infections and nosocomial pandemics. Novel coronavirus, now known as SARS-CoV-2 (2019), appeared in Wuhan, China, at the end of 2019, and the World Health Organization (WHO) declared the outbreak as an alarming international public health issue on January 31, 2020.[2]

Just after the first case was confirmed in Turkey on March 11, 2020, the WHO declared COVID-19 as a pandemic. According to the data by WHO on February 24, 2021, the number of people infected with SARS-CoV-2 exceeded 111 million, and more than 2.5 million people have lost their lives so far. Additionally, COVID-19 cases have been reported on all continents except Antarctica, and the number of cases and deaths continues to increase in many countries.[3] An increase in

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the number of cases has been observed since the detection of the virus in Turkey. The total numbers of COVID-19 cases and deaths were 2,665,194 and 28,285, respectively, until February 24, 2021.[4]

The ongoing COVID-19 pandemic is a serious global public health issue.[5] The literature cites that pandemics create significant psychosocial problems for healthcare workers, children, and the older adults.[6–8] The most affected group in this process is undoubtedly the patients treated with the diagnosis of COVID-19. Kong et al.[9] reported that 34.7 and 28.4% of COVID-19 patients showed anxiety and depression symptoms, respectively. Yang et al.[10] revealed varying rates of depression, anxiety, and sleep problems in COVID-19 patients treated in the isolation service. Qi et al.[11] determined post-traumatic stress disorder in 12.2%, anxiety/depression in 26.8%, and fatigue symptoms in 53.6% of 105 COVID-19 patients included in their study.

Asymptomatic or paucisymptomatic forms, respiratory failure, requiring mechanical ventilation and support in the intensive care unit, and even systemic multiorgan failure syndromes can be observed among the signs and symptoms of COVID-19.[12,13] However, the impacts of the COVID-19 outbreak on survivors are not just physical signs and symptoms. The psychological effects of the disease are immediately apparent and even permanent for a long time. Survivors of previous infectious disease outbreaks such as SARS and the MERS coronaviruses have been reported to have fear, paranoia, panic, anxiety and mental health disorders, and behavioral and attitudinal psychological responses.[14,15] Determining the patients' perception of the disease and the associated factors is crucial to reduce anxiety, adaptation, and help them to express their feelings. Knowing patients' perception of the disease and the associated factors is also essential for the governments and health authorities to effectively intervene and be prepared for the psychosocial aspects of the disease.

This study was conducted to guide psychosocial interventions during and after treatment by defining the perceptions of the disease experiences and treatment processes of the hospitalized COVID-19 patients. It is believed that the research will contribute to the literature by revealing the feelings and situations that are prominent in the patients' perception of the disease, the psychosocial needs of the patients, and their perceptions about the treatment and discharge process.

Main Points

- Three main themes were identified for patients' perceptions of the disease experiences and treatment process.
- Many participants experienced negative feelings during the treatment process, but they were only able to develop short-term coping mechanisms to cope with these emotions.
- Having a fatal and infectious disease had negative psychological impacts on the participants.
- COVID-19 patients mentioned their satisfaction with the physicians and nurses during the treatment process.
- Participants worry about being excluded and stigmatized by their social environment after discharge.

MATERIAL and METHODS

Design

This study is qualitative research in a phenomenological pattern. The Standards for Reporting Qualitative Research guideline and Colaizzi's phenomenological method were followed in the study.[16,17] Colaizzi's phenomenological method focuses on the experience and feelings of subjects and reveals shared patterns rather than individual characteristics. It is a scientific approach that ensures that the experiences collected from the participants adhere to scientific standards.

Research Group Feature and Reflexivity

The research group in the study includes an instructor (PhD), a research assistant (PhD), a physician (PhD), and an intensive care nurse (MSc). One of the researchers (M.A.) works in the Department of Mental Health and Psychiatric Nursing at the Faculty of Health Sciences. The interviewer (A.U.) possessed a Master of Science in Psychiatric Nursing and has worked as a chief nurse in the COVID-19 intensive care unit. Two of the researchers were nurses in the past (C.U.S. and M.A.). Two of the researchers are women, and two are men, and all of them have been conducting research on qualitative research. Two researchers (C.U.S. and M.A.) took courses from an expert in Qualitative Research Methods at the Department of Psychology at Oxford University and continue to take advanced courses on the related field.

Setting and Time

The data were collected at a hospital located in Rize, a town in the Black Sea Region, through semistructured in-depth interviews performed between June 17, 2020, and July 7, 2020. The sample was calculated using the purposeful sampling method.[18] The basic understanding of the criteria sampling method is to work with the studying group meeting a set of predefined criteria. Accordingly, patients hospitalized in COVID-19 service were planned to be included in the study.

The inclusion criteria for the study are as follows:

- being diagnosed with COVID-19 and receiving treatment;
- being a COVID-19 patient hospitalized in service after intensive care treatment;
- being 18 years old and above;
- not having any mental disorder diagnosis;
- being able to communicate (not being connected to the breathing apparatus, speaking Turkish); and
- participating in the study voluntarily.

In total, eight patients fulfilling the preset criteria were included in the sample ($n = 8$).

Data Collection

In qualitative research, the data are collected until the concepts for the possible answer to the research question start to repeat.[19] At that point, the researcher decides the sample number sufficiency. Based on this principle, the sample number

of the research is maintained until the researcher reaches the saturation point.[20]

The data were collected using a Semi-Structured Interview Form and the questionnaire form developed by the researchers. The questionnaire form consists of two parts and 13 questions. In the first part, nine questions are investigating sociodemographic characteristics (age, gender, marital status, place of residence, education level, employment status, perception of income level, having children, and having a chronic disease). The second part includes four questions about the COVID-19 pandemic (having a family member diagnosed with COVID-19, symptoms of COVID-19, being connected to a ventilator, and being a volunteer for plasmapheresis treatment).

During the preparation of interview questions, two experienced external academics in qualitative research were consulted. The researchers then reviewed the literature and prepared the Semi-Structured Interview Form,[21,22] including three open-ended questions as follows:

- What are your feelings/experiences about being diagnosed with COVID-19?
- How did you feel during treatment in isolation, and how did you manage this process?
- What do you think about the treatment (process and personnel)?

We informed the population about the purpose and significance of the study in advance and planned the appropriate meeting time with the participants who voluntarily accepted to take part in the study. We also explained that the interviews would be recorded using a tape recorder, their privacy and confidentiality would be protected, and obtained their consent. The questions that were not clear to the patient were explained without any guidance. The data were collected using face to face interview method in a patient room in the COVID-19 clinic. One researcher (A.U.) conducted in-person semistructured interviews, and each took between 40 and 60 minutes. The interviewer, wearing protective equipment (N95 mask, gloves, goggles/face protector, apron), made an individual interview face to face, providing a minimum distance of 1 m.

Analysis of the Data

Within 24 hours after each interview, the recordings were transcribed by a researcher (M.S.) and analyzed following the steps of Colaizzi's phenomenological analysis method. Two researchers (C.U. and M.A.) independently examined the interview materials, identified meaningful statements, created codes, and agreed on common codes. Then, the themes were developed after the data were read several times and coded.[23] The themes and codes were checked by an external expert. The steps of Collaizi's phenomenological data analysis are as follows:

1. The researcher read the accounts of the participants several times to familiarize and understand the meanings attributed to the phenomenon and the emotions.
2. Significant statements that are of direct relevance to the phenomenon are selected.
3. The significant statements are examined, and meanings are formulated.
4. The formulated meanings are clustered into subthemes, themes, and categories.
5. The emerging themes are combined with full and comprehensive life experiences.
6. The fundamental conceptual structure of the phenomenon is defined.
7. Some participants are reinterviewed, and the results obtained are verified by comparing them with their own experiences.[17]

Validity and Reliability

There is no specific method to ensure the validity and reliability of qualitative inquiry. The validity and reliability of qualitative research are ensured through credibility, dependability, confirmability, and transferability.[24] For credibility, the relationships between the themes obtained as a result of this study and the subthemes were checked for integrity. The audio recordings of the interviews with the participants were transcribed verbatim. A purposeful sampling method was used for transferability, and homogeneity was ensured. To check the reliability of the findings, the researchers analyzed the data independently. In the coding process, each researcher coded the transcripts independently, and then they agreed on common codes. For confirmability, semistructured interviews form and thematization were checked by an external expert.

Ethical Considerations

Ethical approval for the study was received from the local Ethics Committee of Recep Tayyip Erdoğan University (No: 40465587-050.01.04-127), and permission was granted by the Council for Scientific Research Studies of the Directorate General of Health Services affiliated to the Ministry of Health, Republic of Turkey (No: 2020-05-02T14-15-59). At the beginning of the interviews, the participants were first informed about the study and the provisions of the 1995 Declaration of Helsinki (as revised in Brazil, 2013), and their written and verbal consents were obtained.

RESULTS

The mean age of eight participants was 38.86 ± 7.66 years (min: 28; max: 47), four (50%) were female, five (62.5%) were married, six (75%) were living in the city, six (75%) were high school graduates, and five (62.5%) had a family member diagnosed with COVID-19 (Table 1). COVID-19 patients' experiences about the disease and treatment were collected under three main themes depending on the data analysis: "psychosocial effects," "coping styles," and "provision of health services." The themes, categories, and codes identified through interviews with patients are presented (Table 2).

Theme 1: Emotional Reflections about the Disease

Many participants (n = 5) stated that they had negative emotional responses such as fear, panic, anxiety, and anger, but few participants (n = 3) gave positive emotional responses such as hope, belief, and adaptation due to COVID-19 diagnosis. They reported that having an infectious disease caused fear, anxiety, and sadness, they were anxious about harming and infecting others, the social isolation process caused introversion, mental distress, and that they were worried about the possibility of being excluded and stigmatized by their social environment after discharge. Some of the participants

TABLE I. Distribution of Socio-demographic and COVID-19 Characteristics of Patients (n = 8)

Descriptive characteristics	n	%
Gender		
Male	4	50.0
Female	4	50.0
Marital status		
Married	5	62.5
Single	3	37.5
Place of residence		
Metropolis	1	12.5
City	6	75.30
Countryside	1	12.5
Education level		
Primary+secondary school	2	25.0
High school	6	75.0
Employment status		
Employed	4	50.0
Unemployed	4	50.0
Perception of income level		
Low	2	25.0
Moderate	4	50.0
High	2	25.0
Having children		
Yes	5	62.5
No	3	37.5
Having a chronic disease		
Yes	2	25.0
No	6	75.0
Having a family member diagnosed with COVID-19		
Yes	5	62.5
No	3	37.5
Symptoms of COVID-19*		
Weakness, fatigue	7	87.5
Dry cough	5	62.5
Fever	5	62.5
Muscle, joint pain	2	25.0
Inability to taste and smell	1	12.5
Shortness of breath	2	25.0
Headache	4	50.0
No symptom	1	12.5
Being connected to a ventilator		
Yes	4	50.0
No	4	50.0
Being volunteer for plasmapheresis treatment		
Yes	8	100
Age (year) (mean± SD)	38.86 ± 7.66	

*Multiple responses were given and percentages were taken based on n.

expressed their feelings when they were first diagnosed with COVID-19 as follows:

My dreams shattered. My blood pressure dropped a little. I was afraid a bit because of my heart disease. A disease coming from God, of course, but you are still scared. I wonder if I will die (7th participant).

I thought I would be one of those who survived this disease. I was scared, I was panicked a lot. I thought about how to overcome this situation. I believed that gradually but eventually, I would succeed (the patient moved from a lying position to a sitting position and replied the question) (4th participant).

Being infected with corona, of course... when I am talking on the phone, people are talking to me as if I were an AIDS patient. When we go out, because we are corona patients, now I am worried about how people will treat us in our social environment. I have such thoughts. I wonder how people will look at me. In other words, I know that being a corona patient is right now in our society...I don't know, people are a little prejudiced, so they can abstain from you. I haven't been out yet. I don't know what it will be like. (1st participant)

A terrible feeling. I am glad that I am fine now, but when I get out of here and go home, I am afraid of infecting it. I haven't asked the doctors a lot about this topic. They haven't said. I'm afraid I will transmit it. I hope we will all get rid of it. (6th participant)

If necessary, we have to lock ourselves in a room and protect. When we go somewhere, we need to isolate ourselves. Of course, there is a fear of infecting others. I don't want to cause this situation (replied the question by fixing the mask). (7th participant)

I believe that I will recover, but I am worried about transmitting it to my family. (8th participant)

Theme 2: Coping Styles

The participants stated that after being diagnosed with COVID-19, they spent time in isolation by doing hobby-activity, talking with their beloved ones on the phone, watching television, and praying. The participants expressed their expectations for discharge, recovery, and life goals as meeting their beloved ones and fulfilling their longing for them, spending time at home, and they had values and beliefs such as gratitude and acting modestly.

I am praying a lot...for all patients, doctors, healthcare professionals... They have been really good, nice, and sensitive. I spend time doing something on the smartphone. There's not much to do in the room. I communicate with my beloved ones (through telephone). But I feel bored. (3rd participant).

I'm doing embroidery now. I'm looking out of the window. I am reading the Quran. I usually spend time in this room. There is not much opportunity. I sometimes watch TV and go for a walk. I am talking to my relatives on the phone. (5th participant)

First I will go to my home and isolate myself for 14-15 days. I will go to my village. I'll look out of the window onto my garden. Maybe I won't go out for a month. I just want to get out alive (smiling). (2nd participant)

Theme 3: Provision of Health Services

The patients in the study stated that they were satisfied with the attitudes and behaviors of physicians and nurses during the treatment.

TABLE 2. Themes, Subthemes, and Codes in Interviews with Participants

Themes	Subthemes	Codes
Theme I: emotional reflections about the disease	Negative emotional responses	Fear
		Panic
		Anxiety
		Anger
	Positive emotional responses	Hope
		Adaptation
		Faith
	Having an infectious disease	Sadness
		Despair
	Worried about infecting others	Social isolation
		Introversion
	The concern that she will be excluded by her/his social environment	Concern
		Decreased self-confidence
		Stigmatization
Theme 2: coping styles	Nonfunctional coping styles	Crying
	Functional coping styles	Doing hobby-activity
		Talking with their beloved ones on the phone
		Praying
	Individual's expectations, life goals	Discharge and recovery
		Life goals as meeting their beloved ones
Theme 3: provision of health services	Attitude of health professionals	Satisfaction with the nurse
		Satisfaction with the physician
	Health policies	Satisfaction with the treatment and services provided

If it weren't for them, our situation would be terrible. May God be with our nurses. (7th participant)

Very much. Believe me, I am very pleased. Believe me, if I were in the service, I would not be able to stay there. There's no one there. There is always someone here. Even seeing other patients makes me happy. Here, there are passersby. There are nurses. I mean the name of intensive care is bad, but actually, that is not the case here. (3rd participant)

May Allah bless everyone. Our doctors, nurses never leave us alone. They always care for us. They give us food and clean our beds. I am very satisfied with the nurses. May Allah be pleased with all of them. May Allah bless our country. (6th participant)

DISCUSSION

The study was carried out with volunteer COVID-19 patients receiving treatment in the service after intensive care to investigate their perceptions of the disease and the treatment experiences.

Emotional Reflections about the Disease

Most of the patients expressed that they had negative emotional responses such as fear, panic, anxiety, and anger owing to the diagnosis of COVID-19. It is known that there is a relationship between the way the disease is represented in the mind of individuals and their reactions to the disease.[25] Relevant literature shows that the anxiety of the public and healthcare workers increased during epidemic diseases.[26–28] A study conducted in Hong Kong during the SARS outbreak in some countries in 2003 emphasized that psychological reactions such as high levels of stress, helplessness, and post-traumatic symptoms were common in the subjects.[29] Another study in China reports that the COVID-19 outbreak means a severe trauma for humans which has elevated their anxiety level.[22] It is thought that COVID-19 disease has a risk of infection and a fatal effect, causing feelings such as fear, anxiety, and panic in patients. Considering that patients undergoing COVID-19 treatment may experience psychological difficulties, it is recommended that physicians and nurses be sensitive, provide psychological support, and seek psychiatric consultation when necessary.

Participants in the study indicated that having an infectious disease caused fear, anxiety, and sadness, they were anxious about harming and infecting others, the social isolation process caused introversion, mental distress, and they are worried that they would be excluded and stigmatized by the social environment after discharge. In the literature, it is seen that the anxiety levels of individuals differ from country to country during epidemics. Taylor et al.[26] conducted a study to evaluate the psycho-social effects of the equine flu epidemics in Australia and reported that 34% of the participants had high-stress levels. In the study conducted by Wang et al.,[21] in the first phase of the COVID-19 pandemics in China, more than half of the respondents evaluated the levels of psychological impact and anxiety as moderate.

Being stigmatized is defined as "the patient's feeling that he/she has been rejected, isolated by society, and feeling embarrassed and insecure due to his/her illness." In a study of 3,011 people in Hong Kong, participants listed the most stigmatizing diseases as HIV/AIDS, tuberculosis, and SARS, respectively.[29] Society tends to stigmatize individuals with diseases that have infectious characteristics. We believe that there is a need to increase social awareness about the harmful effects of stigmatizing COVID-19 patients' mental health; otherwise, stigma may reduce their general functionality as a serious risk factor.

Coping Styles

Participants were spending the isolation period after being diagnosed with COVID-19 by doing hobby-activity, talking with

their beloved ones on the phone, watching television, and praying. Coping is a cognitive and behavioral strategy that the person poses consciously or unconsciously to deal with the difficulties, needs, demands, and changes stemming from the environment. Coping styles vary according to the individual's internal resources and responses, family support, and cultural structure.[30] The literature suggests that individuals with physical illnesses often benefit from the strategies such as seeking information and social support, learning new skills, actively participating in treatment, coping through confrontation, adopting a positive approach, avoiding, sharing anxiety and emotions about the disease, maintaining hope, and seeking for emotional support in line with their beliefs.[30,31] It is thought that the presence of social support resources, leisure activities, and the opportunity to worship according to their beliefs help the participants in our study to struggle with the disease.

The participants hoped for discharge and recovery, made plans such as meeting their beloved ones, fulfilling their longing for them after being discharged, spending time at home, and had values and beliefs such as gratitude, praying, acting modestly.

Psychological adaptation is a process that has biological and psychological dimensions associated with gains, losses, and struggles in the life of the patient. In the literature, the presence of psychological factors including denying, protecting hope, sense of trust, difficulty, and control are reported as positive compliance behaviors.[32] Our study is consistent with the literature in this regard.

Provision of Health Services

The patients in the study stated that they were satisfied with the treatment and care they received during the treatment and the attitude and behavior of the healthcare professionals. Health personnel's personality traits, kindness, interest, professional attitudes, ways of presenting their knowledge and skills, and especially patient–nurse relationships play a critical role in patient satisfaction.[33,34] It is reported in the literature that the most significant factor affecting patient satisfaction is communication and providing the patient with sufficient information,[35,36] because the ability of healthcare providers to communicate with patients has a pivotal role in making patients feel valued or worthless. Patient-centered and individualized care makes the patient feel valued and creates a trust relationship between people. In addition, feeling valued as an individual is in a sense related to being adequately informed. In many cases, healthcare professionals who determine the needs of individuals and provide information about these needs make patients feel valued. Various studies noted that the trust and satisfaction level of patients who are adequately informed about their condition and procedures with the healthcare personnel increased.[33,35] Due to the nature of COVID-19 disease, visitors are not accepted, and patients receive treatment and care in single and isolated rooms, so it is thought that positive communication and information provided by healthcare workers positively affects patient satisfaction.

Psychological and sociocultural factors, religious values, and health policies can be effective in patients' perception of disease and treatment. It is recommended that healthcare professionals be aware of the psychosocial problems faced by the COVID-19 patients receiving treatment in the hospital, provide appropriate environments where patients can express their feelings and thoughts, establish therapeutic communication with the patients, provide information about the disease and treatment process, and monitor them for post-traumatic stress disorder.

Strengths and Limitations

The scientific rigor of our study is that it is the first qualitative study in Turkey to examine the perception of the disease and treatment of individuals diagnosed with COVID-19. The study reflects patients' perceptions of disease and their experiences during the COVID-19 treatment. Patients' disease perceptions may change after discharge. We aim to carry out a longitudinal follow-up study to determine the changes in perceptions of the disease over time. The limitation of the study is that although the researcher who interviewed the patients was not involved in the treatment and care of the patients, the patients did not report any negative feelings and experiences regarding their treatment. They may not have mentioned their negative experiences, because they thought that their treatment process would be negatively affected.

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Informed Consent: Written informed consent was obtained from all participants who participated in this study.

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Sternoclavicular Joint Distances and Degenerative Changes in Computed Tomography

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BACKGROUND/AIMS
To evaluate sternoclavicular joint distances and age-related degenerative changes in thorax computed tomography images.

MATERIAL and METHODS
Computed tomography (CT) was performed between April 2019 and October 2019 at the Department of Radiology of State Hospital for chest pain and lung parenchymal nodule follow-up. One hundred and forty-six sternoclavicular joints of 73 patients (38 males, 35 females, median 58 years, mean 57.23 ± 13.25 years) aged 35-78 years were evaluated retrospectively. Measurements were taken for bilateral sternoclavicular joint from anterior, mid, and posterior in the axial plain and superior, mid, and inferior sections in the coronal plan. Measurements of sternoclavicular joint were compared according to gender for right and left sides. The presence of osteophyte, sclerosis, subchondral cyst, and gas showing degeneration in the sternoclavicular joint were compared according to age and sex.

RESULTS
Sternoclavicular joint distances measured in axial and coronal planes were found to be significantly lower in female gender group than in men. In the study group, there was statistically significant difference between the measurements made from the coronal plan superior section ($P = .016$). There was no statistically significant difference between mid and inferior measurements in coronal plane and anterior, mid and posterior measurements in axial plane. Sternal osteophytes were significantly more common in females than males ($P = .021$). Osteophyte located in the clavicle was significantly more frequent with increasing age.

CONCLUSION
There is no significant asymmetry in the sternoclavicular joint in asymptomatic individuals, and an increase in degenerative markers of the sternoclavicular joint is observed with the progression of age.

Keywords: Computed tomography, sternoclavicular joint, osteophyte

INTRODUCTION

Sternoclavicular joint is a diarthrodial joint located between the upper extremity and axial skeleton. Joint cartilage is composed of fibrous and hyaline cartilage intertwined with each other. Anterior and posterior sternoclavicular ligaments, costoclavicular ligament, and interclavicular ligament form the ligamentous part of the sternoclavicular joint.[1,2] Apart from traumatic conditions, rheumatological disorders, infectious disorders, calcium pyrophosphate deposit disease, and degenerative disorders also affect the joint.[3,4] Osteoarthritis does not cause joint instability.[2,5] Computed tomography (CT) is widely used for traumatic and rheumatological conditions affecting the joint.[6–8] A number of studies have investigated the normal anatomic properties and variations of the sternoclavicular joint.[9,10] This study aimed to assess degenerative changes affecting the sternoclavicular joint spaces and the joint itself in computed tomographic imaging.

MATERIAL and METHODS

Study Group

This study was approved by the regional Ethics Committee of Pamukkale University Faculty of Medicine (no: 60116787-020/18110). Sternoclavicular joint properties were retrospectively analyzed on CT images of 138 patients undergoing CT

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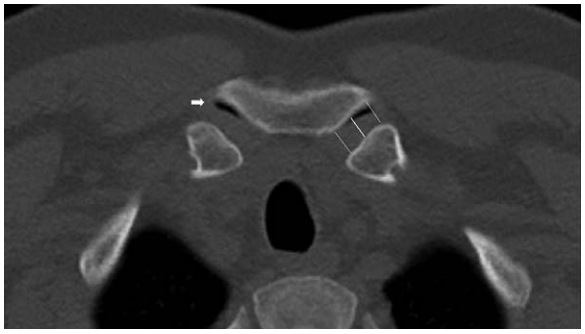

Figure 1. CT image of the sternoclavicular joint in the axial plane. Gas is present in both SCJ joints (white arrow indicates gas in right SCJ). The left SCJ measurements were made from the anterior, mid, and posterior section(lines)

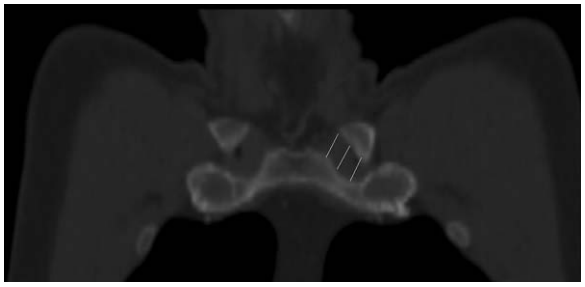

Figure 2. CT image of the sternoclavicular joint in the coronal plane. The left SCJ measurements were made from the superior, mid, and inferior section(lines)

for various reasons such as chest pain or parenchymal lung nodule monitoring at State Hospital, Department of Radiology between April 2019 and October 2019. Among those patients, the CT images of those with thoracic roto-scoliosis, history of bypass surgery or severe chest trauma, sternoclavicular anomaly, oncological disorders, or metabolic bone disease were excluded from the analysis. One hundred and forty-six sternoclavicular joints of 73 patients aged 35-78 years (38 males, 35 females, median age 58 years, mean age 57.23 ± 13.25 years) were retrospectively analyzed.

Computerized Tomography

CT was performed with a 16-detector row multi-slice helical CT device (16 MDCT CT scanner, Toshiba Alexion, Japan). All thoracic CT examinations were performed over ascenogram image, by scanning the region between the neck and upper pole of kidney, with the patients lying in supine position and their hands resting on both sides of the head. The imaging parameters were as follows: tube voltage 120 kV, tube current 70 mAs, slice thickness 2.0 mm, rotation time 750 ms, and "pitch" 0.938. All CT images were analyzed at mediastinal (*WW:350, WL:50*), parenchymal (*WW:–600, WL:1600*), and bone (*WW:2500, WL:480*) windows at the workstation.

Image Analysis

Bilateral sternoclavicular joint distances of patients enrolled between April 2019 and October 2019 were measured on axial and coronal planes on CT images. Measurements were made from the superior, mid, and inferior aspects on axial plane and from the anterior, mid, and posterior aspects on coronal plane for both joints (Figures 1 and 2). Measurements of the sternoclavicular joint were compared between the right and left sides by gender. The study population was grouped by age range. Osteophytes, sclerosis, subchondral cyst, and gas, all of which indicate sternoclavicular joint degeneration, were compared by age range and sex.

Statistical Analysis

Data analysis was performed using a Statistical Package for the Social Sciences (SPSS) version 21 (IBM SPSS Corp.; Armonk, NY, USA). Descriptive statistics included mean ± standard deviation for continuous variables and % for categoric variables. Chi-square test was used to compare categoric variables. Comparison of two sternoclavicular joint spaces on axial and coronal planes was performed with t test. $P < .05$ was considered statistically significant.

Main Points

- The sternoclavicular joint is a diarthrodial type joint composed of cartilage and ligament complex between the upper extremity and the axial skeleton.
- Sternoclavicular joint instability is often caused by rheumatological diseases and trauma.
- Sternoclavicular joint distances are observed as lower in women compared to men.
- The frequency of osteophytes in the clavicle increases with aging.
- Gas in the sternoclavicular joint is more common in 35-49 years and men.

RESULTS

Sternoclavicular joint space distances measured on coronal and axial planes from CT images are presented in Table 1. Sternoclavicular joint distances were significantly lower in women compared to men. In measurements made from the superior aspect on coronal plane, mean sternoclavicular joint distance was 11.37 ± 2.97 mm on the right side and 10.87 ± 2.89 mm on the left side. There was a statistically significant difference between measurements made from the superior aspect on coronal plane ($P = .016$). The measurements made from the mid and inferior aspects on coronal plane and the measurements made from the anterior, mid, and posterior aspects on axial plane showed no significant difference between both sides. Seventy-three patients whose CT images were analyzed were grouped by age. Accordingly, there were 24 patients aged 35-49 years, 15 patients aged 50-59 years, 19 patients aged 60-69 years, and 15 patients aged 70 years or older. Degenerative signs in the sternoclavicular joint were compared by age range and sex (Table 2) (Figure 3). Women had a significantly higher rate of osteophytes located in the sternum ($P = .021$) (Figure 4). The rate of clavicular osteophytes significantly showed a significant increase with advancing age. Presence of gas was significantly more common in patients aged 35-49 years and men compared to women (Table 3). Five patients were found to have sternal ossicle, and seven patients had clavicular notch (Figure 5).

TABLE 1. Sternoclavicular Joint Distances

Sternoclavicular joint			Man (n = 38)				Woman (n = 35)				
			Min	Max	Mean	Std. Dev.	Min	Max	Mean	Std. Dev.	*P*
Coronal	Right	Süperior	8	19	12.45	2.61	3	14	10.20	2.91	.000
		Mid	7	18	11.57	2.73	4	16	9.08	2.60	.001
		Inferior	6	14	10.26	2.11	3	16	8.71	3.73	.031
	Left	Süperior	6	17	11.94	2.67	3	14	9.71	2.69	.001
		Mid	6	16	11.05	2.69	4	13	8.77	2.35	.000
		Inferior	5	15	10.60	2.36	4	16	8.74	3.33	.007
Axial	Right	Anterior	6	15	10.21	2.24	5	15	8.88	2.54	.021
		Mid	5	15	8.76	2.24	3	12	7.45	2.18	.014
		Posterior	5	15	9.52	2.37	5	14	9.48	2.47	.943
	Left	Anterior	4	14	10.23	2.28	4	14	8.22	2.10	.000
		Mid	6	14	9.00	1.94	4	11	7.48	1.73	.001
		Posterior	6	14	9.71	1.95	5	15	9.14	2.15	.243

n: number of patients.

TABLE 2. Markers of Degeneration in the Sternoclavicular Joint

	Osteophytes		Sclerosis		Subchondral cyst	
	Clavicle	Sternum	Clavicle	Sternum	Clavicle	Sternum
Man	10	2	3	2	4	2
Woman	12	9	8	3	5	3
P	.214	.021	.104	.418	.334	.294
35-49 years	0	2	1	1	1	1
50-59 years	6	2	3	2	2	0
60-69 years	5	2	5	3	4	3
70 years and older	11	5	2	0	4	1
P	.000	.164	.219	.280	.224	.289

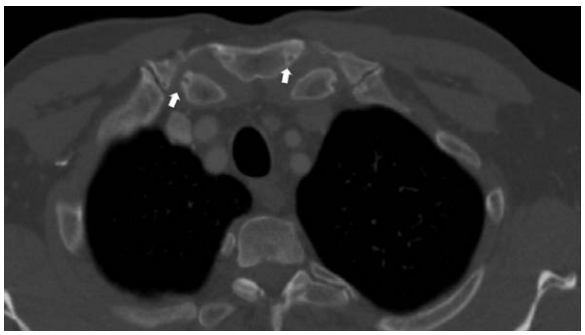

Figure 3. Sternal and clavicular subchondral cysts (white arrows)

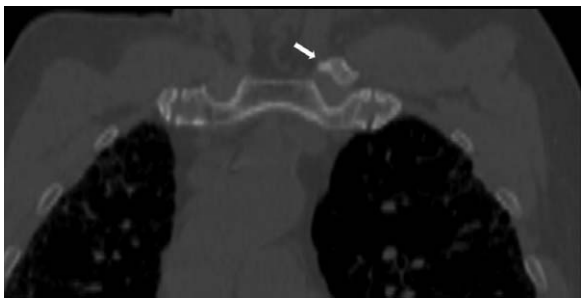

Figure 4. Left clavicular osteophyte (white arrow)

DISCUSSION

Standard radiography cannot provide adequate detail for the assessment of sternoclavicular joint. Brossmann et al.[11] provided anatomic details of joint cartilage of the sternoclavicular joint using magnetic resonance imaging (MRI). MRI has a limited use for the evaluation of the sternoclavicular joint due to respiratory and pulse artifacts. Several studies have used ultrasonography to demonstrate joint effusion and infectious conditions involving the sternoclavicular joint.[4] CT is a widely used imaging modality in routine practice to detect abnormalities of the sternoclavicular joint, which do not appear in plain radiograms. A study that examined the high-resolution CT properties of the sternoclavicular joint showed that all individuals older than 50 years had degenerative changes affecting the joint.[12] It was reported that people of advanced age had a greater rate of osteophytes, subchondral cysts, and sclerosis.[8] Our study also demonstrated an aging-related increase in the prevalence of signs of degeneration, such as osteophytes, subchondral cysts, and sclerosis.

We detected a smaller sternoclavicular joint distance on both sides in women compared to men. When the whole study group was concerned, we detected a ~0.5 mm difference

TABLE 3. Gas in the Sternoclavicular Joint

Gas in the sternoclavicular joint	Man	Woman	Age range			
			35-49 years	50-59 years	60-69 years	70 years and older
Positive	21	10	18	5	6	2
Negative	17	25	6	10	13	13
P	.033		.001			

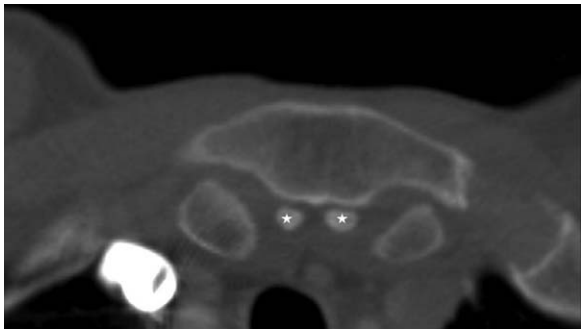

Figure 5. CT image of the sternal ossicle in the axial plane (stars)

between sternoclavicular joint distances in measurements made from the superior aspect on coronal plane. We did not detect any asymmetry between both sides in measurements made from the mid and inferior aspects on coronal plane and in measurements made from the anterior, mid, and posterior aspects on axial plane. We believe that this was caused by the patients' arms being positioned on both sides of the head. Tuscano et al.,[9] in a study examining the sternoclavicular joint in axial thoracic CT images of 104 patients, reported that there may be asymmetry of up to 5.7 mm between the sternoclavicular joints on both sides. The present study also used thoracic CT images, and we believe that asymmetry resulted from patient position. Another study retrospectively examined the sternoclavicular joint in carotid CT angiography images, and it showed symmetry in both joints.[10] We believe that it stemmed from neutral arm position. Celikyay et al.[8] reported a lesser amount of reduction in joint distance in the control group than the patient group with rheumatoid arthritis. De Maeseneer et al.[10] reported that joint distance tended to decrease with advancing age and joint distance may be minimally reduced with aging. Sternoclavicular joint asymmetry was reported to cause a tendency for osteoarthritis and joint instability.[2]

We detected osteophytes, subchondral cysts, and sclerosis on clavicular and sternal aspects of the sternoclavicular joint. The prevalence of these degenerative signs increased with advancing age. We found a significantly higher prevalence of clavicular osteophytes among patients aged 70 years or older. We identified degenerative signs more commonly on the sternal aspect than the clavicular aspect. We believe that this finding is attributable to motion of the clavicular head with arm movements. Baker et al.[12] detected sclerosis and subchondral cysts on the outer clavicular surface more commonly than the joint surface in almost 50% of the study population. However, another study reported that osteophytes and subchondral cysts were located at equal rates on clavicular and sternal tips.[10]

Baker et al.[12] reported that there was diffuse intraarticular gas content within the sternoclavicular joint. Tuscano et al.[9] detected intraarticular gas in 32% of patients, with 8% having bilateral gas content. We found that women had a greater rate of intraarticular gas content than men. On the other hand, we revealed that gas content was reduced by advancing age. We also showed that while there occurred a reduction in gas content, degenerative signs increased.

Shirazian et al.[3] reported a rate of 17% for chondrocalcinosis within the sternoclavicular joint. Another study reported a rate of 3% for the same condition.[10] We did not examine the presence of calcification within the sternoclavicular joint. We detected sternal ossicles in five patients and clavicular notch in seven patients.

Sternoclavicular joint subluxations are known to be rare and have difficulties in treatment. In patients with subluxation, we think the joint distances in this study will help with diagnosis. However, sternoclavicular joint distance and asymmetry of the joint show importance in arthroscopy in insertion surgical approach.[13]

Our study had some limitations. The major one is area small sample volume and a single-center design. Another limitation was its retrospective nature. We did not assess the soft tissue component of the sternoclavicular joint when we examined sternoclavicular joint distance and degenerative changes affecting the joint. As patients' hands were technically positioned at the level of head during the acquisition of CT images, we found a significant difference in the measurements made from the superior aspect on coronal plane. Therefore, we believe that using CT images acquired when both arms on both sides would be more useful for the evaluation of the sternoclavicular joint.

In conclusion, women have a narrower sternoclavicular joint distance than men, and sternoclavicular joint of healthy people does not show marked asymmetry. The study prevalence of degenerative signs of the sternoclavicular joint increased with advancing age. Gas content of the sternoclavicular joint is reduced with aging.

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Evaluation of Methods for Determining Working Length in Root Canal Treatment for Primary Molars: An In-Vivo Study

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BACKGROUND/AIMS
The aim of this study was to evaluate the effectiveness of tactile sensation, digital periapical radiography, and two varieties of electronic apex locators (EALs) as methods of determining working length in root canal treatment for primary molars.

MATERIAL and METHODS
In this study, 30 infected mandibular primary second molar teeth in 12 children aged 5-8 years were analyzed. The working length was determined for each tooth using the tactile sensation, digital radiography, the ProPex Pixi®, and Ipex® apex locators separately.

RESULTS
The mean root canal measurements taken using tactile sensation, Ipex, radiography, and Propex Pixi were 11.02 ± 2.05 mm, 9.47 ± 1.71 mm, 9.73 ± 1.57 mm, and 8.85 ± 1.58 mm, respectively. The radiographic method yielded results that were similar to those derived with the apex locators but differed from the measurements obtained via tactile sensation.

CONCLUSION
EALs can be used to safely determine the working length in root canal treatment for primary molars.

Keywords: Apeks locators, primary molar, root canal treatment

INTRODUCTION

Although dental caries in children has decreased with increasing parental awareness and fluoride applications, numerous children still need pulp treatment.[1] This endodontic treatment maintains the health of primary teeth until their expected exfoliation when their pulp is necrotic or infected. Successful endodontic treatment crucially depends on accurately determining the working length in primary teeth as this prevents harm to periapical tissues and tooth germs.[2] The techniques for working length determination should, therefore, generate precise and reproducible results.[3] The problem is that the accurate determination of the working length in primary teeth is difficult because of the altered anatomy of the teeth and physiological or pathological root resorption.[4,5]

The anatomic apex is the end of a root to be determined morphologically, whereas the radiographic apex is the end of a root to be determined radiographically.[6] In clinical practice, tactile sensation and conventional radiography have long served as methods of choice for determining the working length, but these approaches suffer from certain limitations. For example, the accuracy of tactile sensation changes with experience, and radiographic examination involving children is typically difficult because of poor cooperation of patients or an unsuitable sensor size for a child's small mouth.[7] These techniques may also yield inaccurate information, especially in cases with root resorption.[8] For these reasons, electronic apex locators (EALs), which are based on electrical principles instead of visual determinants, have been used more frequently to determine working length in primary teeth in recent years.

The first generation of EALs, developed in 1969, were resistance based. The second, third, fourth, and fifth generations developed in succeeding years were created on the grounds of impedance, frequency ratio, dual frequencies, and

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multiple frequencies, respectively. Fifth-generation EALs, such as Propex Pixi, measure the capacitance and resistance of a circuit separately to determine the position of the file tip in the root canal.[9]

Limited studies have been devoted to the techniques used to determine working length in root canal treatment for primary teeth.[10–12] To address this gap, the present in vivo study was conducted to compare the accuracy of tactile sensation, the digital radiographic method, and EALs in ascertaining working length. Thus, it was aimed to obtain a guiding result for physicians in determining the canal length in primary tooth canal treatments in pediatric dentistry clinical practice.

MATERIALS and METHODS

This study was conducted in the Department of Pediatric Dentistry at the Faculty of Dentistry in Eskişehir Osmangazi University, Turkey. The study protocol was approved by the university's ethics committee (E25403353-050.99-107474). Before performing any clinical procedures, informed consent was obtained from each child and parent or guardian.

Study Sample

To evaluate the accuracy of the working length measurement techniques, the sample size required for this study was calculated using G*Power (version 3.1.9.2), with consideration for a significance level of 5%, an effect size of 0.215, and a power of 90%. A sample size of 90 root canals per group was determined as enabling sufficient sensitivity to detect a difference of 0.4 mm.

Inclusion and Exclusion Criteria

Accordingly, 30 infected mandibular primary second molar teeth (90 root canals) of 12 children aged between 5 and 8 years were treated at the Department of Pediatric Dentistry.

Children with any systemic diseases as contraindications to endodontic treatment were excluded from the study. The teeth that were subjected to previous root canal manipulation and exhibiting radiographic evidence of calcification have perforated pulpal floor, excessive internal root resorption, external resorption to more than two-thirds of a root, excessive bone loss in furcation, uncontrollable bleeding, and insufficient structure for restoration were also excluded.

Pilot Study

Before initiating the clinical intervention, a pilot study involving six children was conducted. Pulpectomies of six primary molars were performed in a single appointment to standardize the procedure and to train the researcher on implementing the intervention and the researcher's measurements. For training and calibration, a standard reference researcher and an evaluator measured working length for the six primary molars using tactile sensation, the radiographic method, and two EALs (the ProPex Pixi and Ipex). The inter- and intraexaminer kappa coefficients were >0.91 and 1.00, respectively. All the root canal treatments were completed, but these measurements were not included in the main research.

Main Points

- This study showed that EALs and radiographic methods exhibit similar performance in measuring the working length of root canals in primary molar teeth.
- The use of EALs in root canal treatments for primary molars reduce the need for radiography.
- EALs can be used to safely determine the working length in root canal treatment for primary molars.

Access Cavity Preparation

The teeth were first anesthetized with Ultracaine D-S Forte and then isolated using a rubber dam. Caries was removed, and the access cavity was prepared using a round diamond bur as copious water was sprayed onto each tooth. Barbed broaches were used to extirpate pulpal tissue, and 2.5% sodium hypochlorite served as the irrigation solution. Sterile cotton pellets were used to dry the cavity.

Study Groups

To determine working length for 90 root canals, the teeth were categorized into the following measurement groups:

Group 1: Tactile sensation method

Group 2: Radiographic method (digital periapical radiography)

Group 3: Ipex EAL

Group 4: Propex Pixi EAL

Root Canal Length Determination via Tactile Sensation. In determining working length using tactile sensation (group 1), a K-file with a tip that is best adjusted to the apical area was selected and gently inserted into the canal until the operator detected the narrowest region. A silicone stop was then placed at the coronal reference, and the tooth length was measured with an endodontic ruler (0.5 mm accuracy), with consideration for the end of the root.

Root Canal Length Determination by Radiography. In group 2, working length determination was performed using digital periapical radiography. Files, which were 1 mm shorter than the tooth length (as determined using a preoperative radiograph), were inserted into the canals. Before the radiographic evaluation, children were fitted with a protective thyroid lead and a protective lead apron. Digital periapical radiographs were taken using the paralleling technique while the files were in the canals. An X-ray positioning device was used to standardize the distances between the source and the tooth, and the tooth and the radiographic film. The cusp adjacent to the canal was regarded as the occlusal reference. The difference between the tip of the file and the end of the root was calculated on the basis of image. In cases wherein the file did not pass the apex, this amount and the original length were calculated. In cases where the file passed the apex, the amount of length protruding from the apex was subtracted from the original length. In cases where the file did not pass the apex, the amount of length retruding from the apex was added from the original length. Finally, 1 mm was subtracted from the adjusted length to confirm the cemento-dentinal junction and was recorded as the radiographic working length.

Root Canal Length Determination Using EALs. Electronic working length determination was performed either with Ipex (group 3) or ProPex Pixi (group 4). A lip clip was attached to the

patient's lower lip to complete the circuit. Then, the root canals were moistened with 0.9% saline solution, and a No. 15 K-file mounted onto a holder was gently inserted into the canals until a distance of 0.5 appeared on the screen (meaning that the tip of the file was at the apical constriction). Under a reading that was stable for at least 5 seconds, the file was pulled back, and the length between the silicone stopper and the tip of the file was measured with an endodontic ruler. The endodontic treatments were completed in a single appointment.

Statistical Analysis

All the data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM SPSS Corp.; Armonk, NY, USA). Descriptive analyses were initially performed. The accuracy of the electronic and radiographic methods was ascertained on the grounds of the total values of the measurements. The data were also examined via one-way repeated-measures analysis of variance and Sidak post hoc tests. A *P*-value of .05 was considered indicative of statistical significance.

RESULTS

The working length measured via tactile sensation ranged from 9.50 to 13.50 mm with a mean value of 11.02 ± 2.05 mm. The measurements taken using Ipex ranged from 8.00 to 11.30 mm with the mean value of 9.47 ± 1.71 mm, whereas the measurements derived through Propex Pixi ranged from 7.00 to 10.30 mm, with the mean value of 8.85 ± 1.58 mm. The working length measured using digital periapical radiography ranged from 8.00 to 11.50 mm, with a mean value of 9.73 ± 1.57 mm.

The results of the radiographic method were similar to those obtained using Ipex ($P = .938$) and Propex Pixi ($P = .212$), but these results differed from those acquired via tactile sensation ($P = .023$). The measurements taken using tactile sensation statistically and significantly differed from those taken using Ipex ($P = .004$) and Propex Pixi ($P = .000$). Tactile sensation also yielded longer measurements than those derived using the other techniques.

DISCUSSION

Maintaining the unity and functioning of primary teeth until physiological exfoliation is the main purpose of pediatric dentistry. Teeth with traumatic injury or excessive carious lesions may require endodontic treatment in the primary dentition.[13,14] In the endodontic treatment of primary teeth, establishing working length accurately is vital for thorough cleaning and disinfecting root canals.[5,15,16] However, working length determination in primary teeth is challenging because of issues such as oblique physiological root resorption, underlying succedaneous tooth germs, and poor cooperation of children.[2] Different techniques are used to determine the working length of primary teeth, but no definite judgment has been provided as to an ideal approach. The most important motivation for the current research was to clarify this issue.

Various ex vivo studies have evaluated the accuracy of root canal length determination in primary teeth using different methods, such as tactile sensation, radiography, and EAL usage. However, the precise simulation of the oral environment is impossible under ex vivo conditions and, therefore, cannot be a true representative of clinical situations in which the treatment is carried out entirely in the mouth. Only a few studies have been exclusively performed in in vivo conditions for primary teeth.[17–19] To the best of our knowledge, no in vivo report has evaluated and compared the use of Propex Pixi and Ipex with different methods of determining working length (tactile sensation, radiographic method).

Physiological root resorption is not continuous. It has resting periods, which sometimes have cementum deposition on the resorbed root surface. These resorption–deposition processes cause changes in the shape, dimension, and position of the root apex.[17–19] When apical construction is destroyed by root resorption, it could be difficult to determine the working length with radiography and tactile sensation. Physiological root resorption usually starts after the age of 8 in primary molar teeth.[19] Therefore, primary molars (without root resorption) of patients aged 5-8 years were included in the present study to compare the reliability of root canal working length measurements.

Previous studies indicated that determining the working length using only tactile sensation produces incorrect results.[4,5,8] This poor quality is attributed to the physiological resorption that causes variations in canal constriction. The results of these studies agree with those derived in the present study.

Generally, radiographs have been the main tool for establishing root canal length, but this approach has some drawbacks in the establishment of canal length.[20] Radiographs are two-dimensional depictions of a three-dimensional complex, and correct canal length determination may be complicated given root resorption and the superimposition of succedaneous tooth germs over the roots of primary teeth.[15] Radiographic distortion is another drawback, along with issues in patient cooperation, especially in children, which also affects the quality of a radiograph.[21–23] Radiography also extends treatment time and, more importantly, subjects a patient to ionizing radiation. Despite these disadvantages, however, radiography remains the most frequently used method for determining the working length.

Some of the problems listed above have been eliminated with the introduction of intraoral digital radiography. The most important of these advancements is the reduction in radiation dose due to decreased exposure time.[8] Such reduction in digital radiography is approximately 60%. Other advantages include the prompt display, improvement, magnification, storage, retrieval, and transmittal of images.[24] The main drawback to intraoral digital radiography is the high cost. Furthermore, as with conventional radiography, the adjustment of a sensor inside a child's mouth continues to be a problem. Both conventional and digital radiographic methods have been reported to produce misleading results for primary teeth because of variations in apical constriction and apical outcomes being located more coronal after oblique physiological resection.[2] Nevertheless, studies have concluded that digital radiographic methods and the use of apex locators reliably and accurately determine root canal length in primary teeth.[2,8] In the present research, the radiographic method yielded results that were similar to those obtained with the apex locators.

EALs eliminated some of the inherent limitations of radiographic methods. They have gained popularity because they rely on electrical principles instead of visual determinants. They are more reliable and possess high reproducibility in locating

apical foramen despite the presence of electrolytes inside canals.[25] They also solve the radiation problems associated with radiography.[26] The first versions of EALs measure the electrical resistance between the oral mucosa and the periodontal ligament. But unfortunately, they were generally insufficient in locating true apical constriction in the presence of conductive fluids.[9] These devices cannot provide accurate measurements in the presence of vital tissue or fluid in the canal. In the recent past, multifrequency-ratio type EALs have been developed. The working mechanism of these devices is based on detecting the ratio between different electrical proportions for each impedance using different frequencies. With multifrequency-ratio type EALs, the shortcomings of the previous types were tried to be eliminated. These types of EALs, which have the ability to locate apical narrowing according to the rate of change in the impedance of signals of different wavelengths, have been the most frequently used and preferred devices.[9–18]

In this study, two different multifrequency-ratio type EALs (Propex Pixi and Ipex) were used and give similar results. The manufacturer of Propex Pixi and Ipex claim that these EALs perform highly accurate measurements given their use of multifrequency technology under any canal conditions. Although frequency dependent EALs improve the accuracy of determining apical constriction, the performance of apex locators affected by the presence of liquids such as blood, saline, local anesthetics, and endodontic irrigants remains unclear.[27]

An ex vivo study showed that compared with tactile sensation, conventional radiography, tactile sensation+conventional radiography, and digital radiography, EALs perform best in determining root canal length in primary teeth. Nonetheless, tactile sensation+conventional radiography can be an alternative when electronic resources are unavailable.[28] Another study reported that radiovisiography and Propex Pixi generate similar results in determining working length in the presence of irrigation solutions, with these methods showing no statistically significant difference in prediction rates.[29] Consistent with the literature, no statistically significant difference was found between the working lengths determined by radiographic methods and apex locators in the current study.[17–19,28]

To conclude, the results indicated no significant difference between the use of Ipex and Propex Pixi and periapical digital radiography in determining the working length in root canal treatment for primary molars. The use of EALs may be useful as a means of protecting children from exposure to recurrent ionizing radiation, over-instrumentation, overfilling, damage to permanent tooth germs, and radiation exposure. These locators may also be useful in cases wherein the radiographic determination of root lengths is encumbered by limitations.

Clinical Significance

Apex locators, which are routinely used in determining the canal length in permanent dental endodontic treatments, have a definite place in determining the length of the primary tooth for pediatric dentists in clinical use.

Ethics Committee Approval: Ethical committee approval was received from Eskişehir Osmangazi University's ethics committee (E25403353-050.99-107474).

Informed Consent: Verbal informed consent was obtained from each child and parent or guardian who participated in this study.

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Clinical Trial Registry Number: NCT04638972.

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Original Article

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Correlation of the Epworth Sleepiness Scale with Polysomnography Parameters in Obstructive Sleep Apnea Syndrome Patients

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BACKGROUND/AIMS
The aim of the present study was to find out whether Epworth Sleepiness Scale (ESS) is correlated with polysomnography (PSG) and which specific polysomnographic parameter is most closely associated with ESS scores and thus excessive daytime sleepiness.

MATERIAL and METHODS
The study included patients with an initial diagnosis of obstructive sleep apnea syndrome (OSAS). All patients completed a validated Turkish version of the ESS. Patients were divided into two groups based on their ESS scores as those with an ESS score below 10 and those scoring above 10. The differences in mean values of PSG parameters were compared between the two groups. Correlations between ESS scores and PSG parameters were investigated for all patients.

RESULTS
The study included 174 patients. The group with ESS scores above 10 was found to have significantly greater apnea-hypopnea index (AHI), arousal index, oxygen desaturation (ODI), and total sleep time spent with an oxygen saturation less than 90% in comparison to the group with ESS scores below 10. A moderate positive correlation between ESS scores and ODI and weak positive correlations between ESS scores and AHI and arousal index were found.

CONCLUSION
Given the finding that ODI had the strongest correlation with ESS, it can be concluded that as well as being closely related to the subjective symptoms of OSAS, ODI is the PSG parameter that best reflects excessive daytime sleepiness.

Keywords: Epworth Sleepiness Scale, excessive daytime sleepiness, obstructive sleep apnea, polysomnography

INTRODUCTION

Sleep is a critical component of our lives, and we spend about one-third of our life sleeping. Breathing problems during sleep and daytime sleepiness are prevalent complaints in modern societies. Sleep-related breathing disorders are the most common type of sleep disorders. Obstructive sleep apnea syndrome (OSAS) is the most frequent form of sleep related breathing disorder.[1] Obstructive sleep apnea is characterized by repetitive pharyngeal collapses during sleep. Complete or partial pharyngeal collapses result in oxygen desaturation, hypercapnia, and sleep fragmentation. Interruptions of breathing caused repeated pharyngeal collapses are associated with blood gas deterioration and arousals and disruption of sleep continuity.[2,3] Disrupted sleep continuity causes daytime sleepiness, which is a major symptom of OSAS. Several subjective and objective tests have been described to investigate daytime sleepiness of individuals. The Epworth Sleepiness Scale (ESS) is a simple, validated subjective tool, which was first developed in 1991. The ESS is a self-administered questionnaire in which respondents rate their usual chances of dozing off or falling asleep while engaged in different activities. Due to ease of use, the ESS is currently the most widely used subjective test for assessment of sleepiness.[4,5] Many studies exist in literature addressing the correlation of ESS with polysomnographic findings.

This study was presented at the 40th Turkish Otorhinolaryngology and Head and Neck Surgery Congress November 7-11, 2018, Antalya, Turkey.

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However, to our best knowledge, there are no studies that focused on the correlation of the ESS with polysomnographic parameters and a specific polysomnographic parameter that is most closely associated with ESS scores and thus excessive daytime sleepiness (EDS). This study was designed to seek answer to the following question: "Which polysomnography parameter best reflects excessive daytime sleepiness?"

MATERIALS and METHODS

Approval from the institutional ethics committee was obtained before initiation of the study from Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (Decision no: E-18-2127). Patients were informed, and their signed consents were taken. Medical records of patients presenting with complaints of snoring, witnessed apnea, and daytime sleepiness between January 2013 and June 2018 who underwent overnight polysomnography were reviewed retrospectively. Patients of both sexes with an apnea-hypopnea index (AHI) equal to or greater than 5 were included in the study. Patients with a sleep efficiency below 60% and total sleep duration less than 240 minutes, patients with suspected central sleep apnea, patients with a mental illness or neurological pathology, patients with a history of insomnia, narcolepsy, hypersomnia, and periodic limb movement disorder, and patients whose ESS questionnaires were not available were excluded.

At the time of initial examination, patients were asked to complete the validated Turkish version of the ESS.[6] The ESS is an instrument with eight questions that is used for assessment of daytime sleepiness. Respondents are asked to rate, on a 4-point scale (0-3), their usual chances of dozing off or falling asleep while engaged in eight different activities. The total ESS score is the sum of 8 item scores.

Polysomnography was conducted overnight during spontaneous sleep of patients under the supervision of a sleep technician in a single room at our hospital's sleep center. Audio monitoring and digital video recording were performed all through the night. During polysomnography, data from four-channel electroencephalogram, electromyogram (EMG-submental), EMG (right-left tibialis), two-channel electrooculogram (right-left), electrocardiography, nasal airflow, thoracic, and abdominal breathing movements, blood oxygen saturation by pulse oximetry, and body position were recorded overnight. Manual scoring was performed by an otorhinolaryngologist with special knowledge on sleep disturbance and polysomnography certificate using the criteria established by the American Academy of Sleep Medicine. Apnea was scored when there was a drop in the peak signal excursion by ≥90% of the pre-event baseline and the duration of the ≥90% drop in sensor signal was ≥10 seconds. The respiratory event to be defined as apnea as per the criteria was scored as obstructive when it was accompanied by a sustained or increasing inspiratory effort during the whole period without airflow, or as central when no inspiratory effort was present. The event was scored as mixed type apnea when an inspiratory effort was absent in the beginning but started later on. Hypopnea was scored when the peak signal excursions dropped by ≥30% of pre-event baseline using nasal pressure (diagnostic study) for ≥10 seconds in association with ≥3% arterial oxygen desaturation or an arousal.[7]

Main Points

- The Epworth Sleepiness Scale is a useful tool for assessing excessive daytime sleepiness.
- Oxygen desaturation index is the most strongly correlated polysomnography parameter with Epworth sleepiness scale.
- High oxygen desaturation index values may have the potential to predict increased daytime sleepiness.

Patients were divided into two groups based on their ESS scores as those with an ESS score below 10 and those scoring above 10. The differences in mean values of polysomnography parameters were compared between the two groups including AHI, sleep latency, sleep efficiency, the percentages of non-rapid eye movement (REM) stage 1 sleep (NREM1%), non-REM stage 2 sleep (NREM2%), non-REM stage 3 sleep (NREM3%) and REM sleep (REM%), arousal index, oxygen desaturation index (ODI), total sleep time spent with an oxygen saturation less than 90% (TST < 90%), mean oxygen saturation, minimum oxygen saturation. Correlations between ESS scores and age, body mass index and polysomnography parameters were investigated for all patients.

Statistical Analysis

Kolmogorov–Smirnov test was used to analyze the distribution of the data. Mean values were provided with their standard deviations. The significance of difference between means was tested with the T-test when data were normally distributed and with the Mann–Whitney U test when data were non-normally distributed. Spearman correlation analysis was used to test correlations between ESS and polysomnography (PSG) parameters. A *P* value less than .05 was considered significant. Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analyses.

RESULTS

A total of 174 patients (132 males and 42 females) were included in the study. The mean age of the patients was 47.8 years. Polysomnographic parameters for the two groups with ESS scores below or above 10 were as follows: AHI, 29.95 ± 23.56 and 48.14 ± 30.16; sleep latency, 17.94 ± 12.73 and 18.25 ± 13.02; sleep efficiency %, 83.37 ± 9.52 and 82.43 ± 10.21; NREM1%, 8.26 ± 5.61 and 11.34 ± 9.18; NREM2%, 51.6 ± 10.9 and 53.94 ± 13.95; NREM3%, 23.81 ± 10.06 and 19.83 ± 11.81; REM%, 15.21 ± 6.03 and 14.15 ± 6.21; arousal index, 15.8 ± 16 and 28.25 ± 22.54; ODI, 22.75 ± 23.41 and 48.96 ± 34.67; TST <90%, 7.56 ± 14.42 and 18.48 ± 24.33; mean oxygen saturation, 93.41 ± 2.54 and 91.62 ± 5.25; minimum oxygen saturation, 78.66 ± 12.08 and 72.59 ± 17.35, respectively (*P* values < .001, .909, .660, .062, .257, .043, .443, <.001, <.001, .007, .049, and .048, respectively). Statistically significant differences were found between the two groups in the mean values of AHI, NREM3%, arousal index, ODI, TST < 90%, mean oxygen saturation, and minimum oxygen saturation (Table 1).

ESS scores were not correlated with age or body mass index. ESS scores were positively correlated with AHI, arousal index, ODI, and TST < 90% and negatively correlated with NREM3%, mean oxygen saturation, and minimum oxygen saturation (*P* and r values: <.001 and 0.265, .001, and 0.256, <.001 and 0.416, .002, and 0.228, .036 and –0.159, .049 and –0.144, .006 and –0.208, respectively). A moderate positive correlation between ESS scores and ODI, weak correlations between ESS scores and AHI, arousal index, TST < 90% and minimum oxygen saturation

TABLE 1. Mean Values of Polysomnography Parameters for Groups Stratified by ESS Scores

	Group with ESS scores below 10 (n = 112)	Group with ESS scores above 10 (n = 62)	*P* value
AHI	29.95 ± 23.56	48.14 ± 30.16	<.001
Sleep latency (minutes)	17.94 ± 12.73	18.25 ± 13.02	.909
Sleep efficiency (%)	83.37 ± 9.52	82.43 ± 10.21	.660
NREM1%	8.26 ± 5.61	11.34 ± 9.18	.062
NREM2%	51.6 ± 10.9	53.94 ± 13.95	.257
NREM3%	23.81 ± 10.06	19.83 ± 11.81	.043
REM%	15.21 ± 6.03	14.15 ± 6.21	.443
Arousal index	15.8 ± 16	28.25 ± 22.54	<.001
ODI	22.75 ± 23.41	48.96 ± 34.67	<.001
TST < 90%	7.56 ± 14.42	18.48 ± 24.33	.007
Mean oxygen saturation	93.41 ± 2.54	91.62 ± 5.25	.049
Minimum oxygen saturation	78.66 ± 12.08	72.59 ± 17.35	.048

All values are provided as means with standard deviations.
ESS, Epworth Sleepiness Scale; AHI, apnea-hypopnea index; NREM1%, non-REM stage 1 sleep percentage; NREM2%, non-REM stage 2 sleep percentage; NREM3%, non-REM stage 3 sleep percentage; REM%, REM sleep percentage; ODI, oxygen desaturation index; TST < 90%, total sleep time spent with an oxygen saturation less than 90%.

TABLE 2. Correlations of ESS Scores with Age, Body Mass Index, and Polysomnographic Parameters

	P value	r
Age	.468	
Body mass index	.193	
AHI	<.001	0.265
Sleep latency (minutes)	.263	
Sleep efficiency (%)	.203	
NREM1%	.387	
NREM2%	.436	
NREM3%	.036	−0.159
REM%	.313	
Arousal index	.001	0.256
ODI	<.001	0.416
TST < 90%	.002	0.228
Mean oxygen saturation	.049	−0.144
Minimum oxygen saturation	.006	−0.208

ESS, Epworth Sleepiness Scale; AHI, apnea-hypopnea index; NREM1%, non-REM stage 1 sleep percentage; NREM2%, non-REM stage 2 sleep percentage; NREM3%, non-REM stage 3 sleep percentage; REM%, REM sleep percentage; ODI, oxygen desaturation index; TST < 90%, total sleep time spent with an oxygen saturation less than 90%.

and very weak correlations between ESS scores and NREM3% and mean oxygen saturation were found (Table 2) (Figure 1).

DISCUSSION

In this study, AHI, arousal index, ODI, and TST < 90% were significantly greater in the group with ESS scores above 10 compared to the group with ESS scores below 10. NREM3%, mean oxygen saturation, and minimum oxygen saturation were significantly lower in the group with ESS scores above 10 compared to the group with ESS scores below 10. ESS scores were positively correlated with AHI, arousal index, ODI, and TST < 90% and negatively correlated with NREM3%, mean oxygen saturation, and minimum oxygen saturation. A moderate correlation between ESS scores and ODI, weak correlations between ESS scores and AHI, arousal index, TST < 90% and minimum oxygen saturation and very weak correlations between ESS scores and NREM3%, mean oxygen saturation were found.

Discordant results have been reported in literature on the correlation of the ESS with polysomnographic parameters, with some studies showing positive correlations whereas others reporting none.[1,2,8] In their study, Ozcan et al.[2] investigated the consistency of ESS results with polysomnographic findings and reported no statistically significant associations between patient ESS scores and AHI, TST < 90% and arousal index values. A separate study found slightly greater AHI and arousal index in patients with EDS than those without EDS.[8] In our study, the group of patients with EDS showed highly significantly greater mean values of AHI, ODI, and arousal index in comparison to the group without EDS.

A cut-off of 10 points is recommended for ESS scoring.[9] Patients with an ESS score above this cut-off may be considered to have EDS. In a study in which patients were considered to have EDS if the ESS was >10, sleep latency was found to be shorter in the group with EDS.[8] In the same study, patients with EDS were found to have less NREM sleep in stages 1 and 2 and greater sleep efficiency and longer NREM sleep in stage 3 than those without EDS. In light of these data, the authors concluded that EDS was not associated poor sleep quality.[8] We found no significant differences between the groups with an ESS score below or above 10 with respect to sleep latency, sleep efficiency, and NREM sleep in stages 1 and 2. The group with an ESS score below 10 had longer NREM sleep in stage 3. Thus, EDS was not considered to be directly associated with sleep quality.

While the exact mechanisms of sleepiness have not been fully elucidated, EDS attributed to nocturnal hypoxemia, sleep fragmentation, or both in OSAS patients.[10–15] Roure et al.[8] stated that nocturnal hypoxemia might be a contributing factor to the development of EDS. Additionally, they found higher arousal index values in patients with EDS, which led them to suggest that sleep fragmentation may also have a role in EDS. Temirbekov et al.[1] examined the correlation of ESS scores with AHI and ODI values and found a stronger correlation of ESS with ODI than that with AHI and also suggested that the subjective symptoms of OSAS were closely related to ODI. When we examined the correlation of ESS scores with PSG parameters, ODI was most strongly correlated with ESS scores.

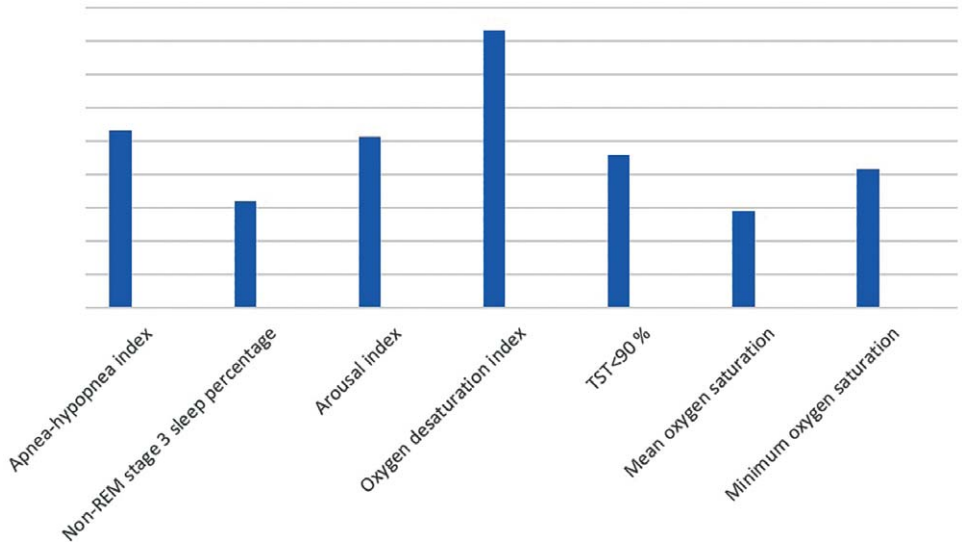

Figure 1. Correlation levels between polysomnography parameters and Epworth Sleepiness Scale scores according to correlation coefficient (r)

Positive correlations were also found between the parameters of nocturnal hypoxemia other than ODI (TST < 90%, meanoxygen saturation, minimum oxygen saturation) and ESS scores. Considering the correlations of ESS scores with polysomnographic parameters that indicate sleep fragmentation and sleep quality, we did not find any correlations between ESS scores and sleep latency, sleep efficiency, NREM1%, NREM2%, and REM% but observed a very weak negative correlation of ESS scores with NREM3% and a weak positive correlation of ESS scores with arousal index. Based on these results, it may be suggested that nocturnal hypoxemia plays a more prominent role in the development of EDS in comparison to sleep fragmentation.

As a conclusion, while the ESS is a subjective tool that does not take into account cognitive function, work conditions, and sociocultural status of individuals, it has the potential to indicate the severity of OSAS and disrupted nocturnal oxygenation. Given the finding that ODI had the strongest correlation with ESS, it can be concluded that as well as being closely related to the subjective symptoms of OSAS, ODI is the PSG parameter that best reflects EDS. Higher ODI values may predict EDS in OSAS patients.

Ethics Committee Approval: Ethical committee approval was received from Ankara Numune Training and Research Hospital Clinical Research Ethics Committee (Decision no: E-18-2127).

Informed Consent: Written or verbal informed consent was obtained from all participants who participated in this study.

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Author Contributions: Concept - Ş.A., S.E.K., Z.O., F.C.E.; Design - Ş.A., S.E.K., F.C.E.; Supervision - Ş.A., M.Ç., M.F.K.; Resource - Ş.A., S.E.K., Z.O.; Materials - Ş.A., S.E.K., Z.O.; Data Collection and/or Processing - M.Ç., M.F.K.; Analysis and/or Interpretation - S.E.K., MÇ., M.F.K.; Literature Search - Z.O., M.F.K., F.C.E.; Writing - Ş.A., M.Ç. S.E.K.; Critical Reviews - Ş.A., F.C.E., Z.O.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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Antimicrobial Effects of Gutta-Percha Points Containing Root Canal Medications against Some Anaerobic Bacterial Species and *Enterococcus faecalis*

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BACKGROUND/AIMS
The purpose of this study was to examine the ability of gutta-percha points to reduce bacterial counts.

MATERIAL and METHODS
ISO #40-sized Calcium Hydroxide Plus Points or Activ Points were used for all experiments. Three pieces of each type of gutta-percha points were placed in sterile 0.5-mL eppendorf tubes. After incubating the plates in an anaerobic chamber at 37°C for 2 days, colony forming units' (CFUs) numbers per milliliter of suspension were calculated, and CFU numbers relating to each time interval were statistically analyzed by using Kruskal–Wallis 1-way analysis of variance and multiple comparison tests.

RESULTS
The results showed that Calcium Hydroxide Plus Points or Activ Points have similar effects to kill the tested microorganisms except *Enterococcus faecalis* and *Peptostreptococcus micros*.

CONCLUSION
Calcium Hydroxide Plus Points or Activ Points have similar effects to kill the tested microorganisms and did not kill 100% of *E. faecalis* and *P. micros*.

Keywords: Active point, anaerobic bacteria, antimicrobial activity, calcium hydroxide plus point, *E. faecalis*

INTRODUCTION

Endodontic treatment plays an important role in eliminating bacteria and their substrates by instrumentation, irrigation, usage of intracanal medicaments, and antibacterial obturation materials. If root canal treatment is not performed, the infection can reach the area surrounding the dental root apex and cause an inflammatory response.[1] Several cultural and molecular studies have revealed that more than 500 various bacterial species or phylotypes have been determined in infected root canals.[2] Obligatory anaerobic bacteria predominate the root canals, including *Porphyromonas*, *Prevotella*, *Fusobacterium*, *Peptostreptococcus*, and *Eubacterium*. Some gram-positive facultative anaerobes, such as *Actinomyces*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Gemella*, and *Staphylococcus*, have also been frequently isolated from acute abscesses of endodontic origin.[3]

To achieve local healing and stop the pathologic process, the bacteria need to be eliminated as far as possible from all parts of the root canal system.[4] Tests have been conducted on many substances, which have been utilized for intracanal medication. Calcium hydroxide represents the most frequently utilized intracanal medication. Calcium hydroxide exerts an antimicrobial effect, and it is also able to neutralize bacterial endotoxins.[5–7] Chlorhexidine is an alternative substance for root canal medication that is capable of eliminating gram-negative and gram-positive bacteria,[9,10] but it can not

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TABLE I. Contents of the Gutta-Percha Points Used in this Study

Gutta-percha points	Contents
Calcium Hydroxide Plus Points	52% calcium hydroxide, 42% gutta-percha, sodium chloride, surfactant, coloring agents
Activ Points	5% chlorhexidine diacetate, gutta-percha, ZnO, $BaSO_4$, coloring agents

neutralize bacterial endotoxins.[8] For the selection of an appropriate intracanal medication, the antimicrobial properties of local endodontic disinfectants should be known.

The present research aimed to examine the ability of calcium hydroxide and chlorhexidine-containing gutta-percha points to reduce the bacterial counts of some obligatory anaerobe bacterial species and *Enterococcus faecalis*.

MATERIALS and METHODS

A method presented by Podbielski et al.[11] was used in this study. Gutta-percha points, which were ISO #40-sized, were used. ROEKO Calcium Hydroxide PLUS Points (ROEKO, Langenau, Germany) and ROEKO chlorhexidine diacetate-impregnated active points (ROEKO, Langenau, Germany) were used to test the efficiency of bacterial reduction in all tests. Table I contains information on the points' contents.

Microorganisms

Peptostreptococcus micros (NCTC 11808), *Eubacterium lentum* (NCTC 11813), *Prevotella intermedia* (NCTC 13070), *Prevotella melaninogenica* (NCTC 12963), *Porphyromonas endodontalis* (NCTC 13058), and *E. faecalis* (ATCC 29212) were utilized in the present research. All bacterial strains were cultured in thioglycolate broth (Merck, Darmstadt, Germany) added with 0.5 mg/L of vitamin K (Sigma, St. Louis, USA) and 5 mg/L of hemin (Sigma, St. Louis, USA), and Schaedler agar (BBL, Cockeysville, USA) and *E. faecalis* on 5% sheep blood agar (Lab M, IDG, Lancashire, UK).

Procedure

The bacterial count per milliliter was detected by using a Nano Photometer Pearl spectrophotometer (Implen GmbH, Munich, Germany) to measure the optical densities of cultures at a 600 nm wavelength (OD_{600}) for each strain. Individual standard curves were formed as a result of plotting different bacterial concentrations, demonstrated by viable plate counting of colony forming units (CFUs) against the optical density of the suspension at a 600 nm wavelength. The obtained standard curves were utilized for the spectrophotometric detection of bacterial concentrations utilized in the tests.

Main Points

- There were statistically significant differences between the control group and the experimental groups at 14 days.
- Calcium Hydroxide Plus Points and Activ Points presented antimicrobial activity against all of the strains.
- Calcium Hydroxide Plus Points or Activ Points have similar effects to kill the tested microorganisms and both material did not kill 100% E. faecalis and P. micros.

For quantitative assays, bacteria suspensions at OD_{600} levels previously determined to contain 2×10^7 CFU/mL were prepared. All the test microorganisms were sedimented as a result of centrifuging at 2000 g for 10 minutes using a UniCen 15DR (Herolab GmbH, Wlesloch, Germany) and were then resuspended in 3.1 mL of a mixture containing equivalent volumes of a newly prepared human serum and 0.9% NaCl solution. Serum collection was performed from four different donors. Inactivation of the serum was carried out at a temperature of 56°C for a period of 30 minutes in a water bath, GFL model 1083 (LabortechnikmbH, Burgwedel, Germany), and it was then reduced by incubation in an anaerobic chamber for 4 hours before use. Three pieces of every type of gutta-percha points were put in sterile 0.5-mL Eppendorf tubes (Greiner bio-one GMBh, Frickenhausen, Germany). The pieces were acquired as a result of cutting the points into three parts by utilizing sterilized surgical blades and tweezers. Afterward, aliquots (100 µL) of the bacteria that had been suspended in the diluted serum were put into the Eppendorf tubes. The tubes that contained only bacteria suspensions were used as growth controls. Different tubes for every experimental day (1, 2, 3, 4, 7, and 14 days) were arranged for every condition. The final active compound concentrations were found to be 1 µg/100 µL chlorhexidine diacetate for the active points and 6.29 µg/100 µL calcium hydroxide for Calcium Hydroxide PLUS Points.[12]

For *E. faecalis*, the tubes were kept in an incubator at a temperature of 37°C, and for anaerobic bacteria, the tubes were kept in an anaerobic chamber, Bactron model 1-2 (Sheldon Manufacturing Inc., USA). The removal of tubes was performed at days 1, 2, 3, 4, 7, and 14. At the above-mentioned time, the tubes were vortexed for a period of 15 seconds using the Vortex Mixer MX-S (DragonLab, Beijing, China). The tubes were then opened, and serial dilution of 10-µL aliquots of the bacterial suspensions was performed in a sterile solution of 0.9% NaCl. Aliquots (10 µL) of the dilution steps were streaked onto a solid medium (Schaedler agar or blood agar). Following the incubation of the plates in an anaerobic chamber at a temperature of 37°C for a period of 2 days, CFU numbers per milliliter of the suspension were computed. Every assay was performed again on six independent cases.

Statistical Analysis

Statistical analysis was carried out by the Kruskal–Wallis one-way analysis of variance (ANOVA) and multiple comparison tests. The Kruskal–Wallis one-way ANOVA test was conducted for the purpose of evaluating the alteration in CFU numbers with regard to every time interval; when the *P*-value was statistically significant ($P < .01$), multiple comparison tests were used to evaluate which groups differed from which others. The Bonferroni correction was used for multiple comparisons.

RESULTS

The inhibition of bacterial growth was assessed as a result of employing viable plate counting of cultures that had been grown when the gutta-percha points that contained calcium hydroxide or chlorhexidine diacetate were present. Figures 1–6 demonstrate the susceptibilities of the studied microorganisms to the gutta-percha points that contained the two root canal medicaments.

Calcium Hydroxide PLUS Points were able to kill *P. endodontalis* and *E. lentum* within 1 day and *P. intermedia* and *P.*

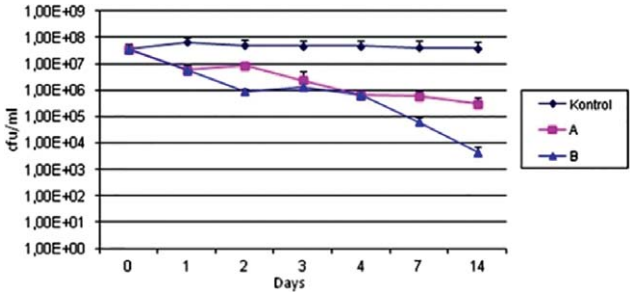

Figure 1. Survival of *Enterococcus faecalis* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

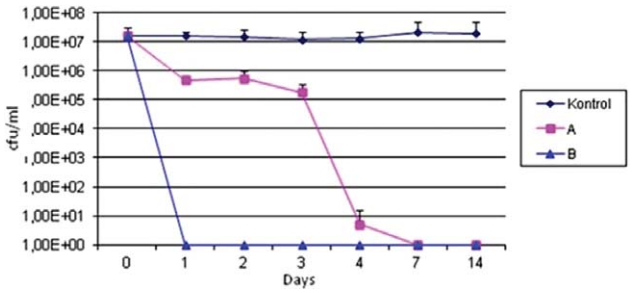

Figure 2. Survival of *Eubacterium lentum* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

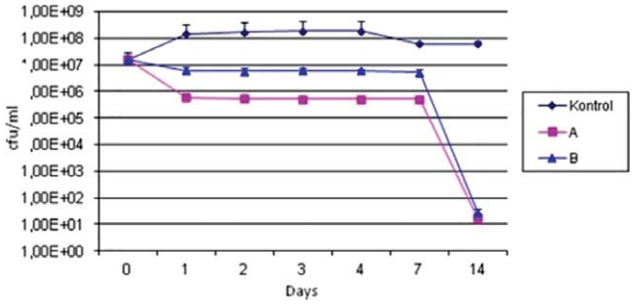

Figure 3. Survival of *Peptosreptococcus micros* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

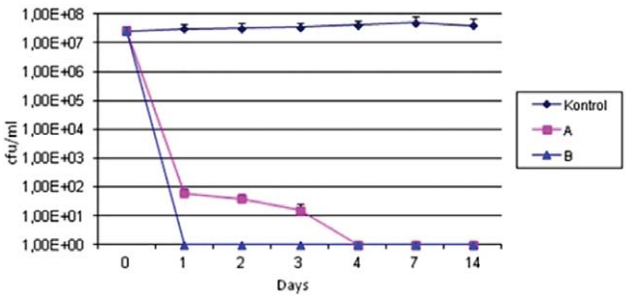

Figure 4. Survival of *Porhyromonas endodontalis* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

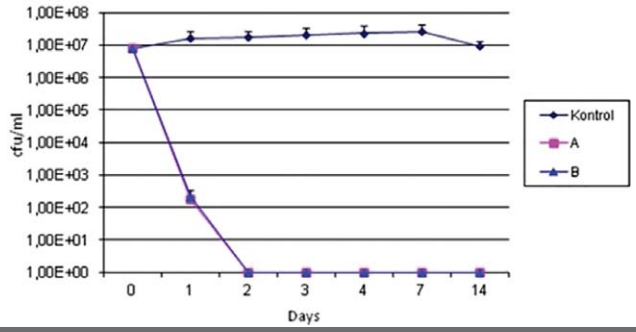

Figure 5. Survival of *Prevotella melaninogenica* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications

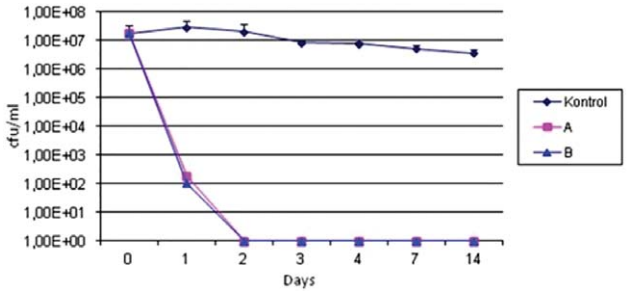

Figure 6. Survival of *Prevotella intermedia* in quantitative in vitro assays to measure the antimicrobial activity of gutta-percha points containing root canal medications. (A) Activ Point (chlorhexidine diacetate); (B) Calcium Hydroxide Plus Points

melaninogenica within 2 days. The active points were able to kill *P. intermedia* and *P. melaninogenica* within 2 days, *P. endodontalis* within 4 days, and *E. lentum* within 7 days. However, neither Calcium Hydroxide PLUS Points nor the active points exhibited adequate antimicrobial activity to kill *E. faecalis* or *P. micros* in a period of 14 days. The results showed that Calcium Hydroxide PLUS Points and the active points have comparable killing effects on the studied microorganisms apart from *E. faecalis* and *P. micros*.

When the antibacterial activity of Calcium Hydroxide PLUS Points and the active points against *E. faecalis* was examined, a statistically significant difference was found between Calcium Hydroxide PLUS Points and the active points at only 2 days ($P = .012$). Statistically significant differences were determined between the experimental groups and the control group for all days ($P < .01$), with the exception of the active points and the control group at 2 days ($P > .05$). For *P. micros*, statistically significant differences were found among the groups at days 1, 2, 3, 4, and 7 ($P < .001$). No statistically significant difference was determined between the two experimental groups at 14 days ($P > .05$). However, statistically significant differences were identified between the control group and the experimental groups at 14 days ($P = .015$). Statistically significant differences were found between the experimental groups and the control group for *E. lentum* for all days ($P < .001$), whereas statistically significant differences were determined at only 1, 2, and 3 days between the two experimental groups ($P < .001$). For *P. endodontalis*, statistically significant differences were identified between Calcium

Hydroxide PLUS Points and the active points at 1, 2, and 3 days ($P \leq .01$). The differences were found to be statistically insignificant at 4, 7, and 14 days for the experimental groups ($P > .05$), but the control group and the experimental groups differed statistically significantly at 1, 2, 3, 4, 7, and 14 days ($P < .001$).

For *P. melaninogenica* and *P. intermedia*, no statistically significant differences were found between Calcium Hydroxide PLUS Points and the active points for the whole experimental period ($P > .05$). The control group and the experimental groups differed statistically and significantly at 1, 2, 3, 4, 7, and 14 days ($P < .001$).

DISCUSSION

The primary goal of successful root canal treatment is eliminating microorganisms from root canals and preventing the following reinfection. Despite the elimination of most bacteria by the biomechanical preparation of the root canal space, a number of microorganisms may survive and increase rapidly in the anatomical complexities of root canals. The microbiota in infected root canals has polymicrobial properties and anaerobic gram-positive cocci dominate it.[13] The bacterial species that were used in our study are most frequently isolated from root canal infections, and *E. faecalis* represents the main pathogen that is associated with root-filled teeth with persistent lesions, since it exhibits resistance especially to numerous conventional antimicrobial agents utilized in clinical practice.[11,14]

Growth conditions that are similar to the common in situ conditions as much as possible represent a significant parameter in assays that investigate antimicrobial effects. Human serum was utilized as the most appropriate test medium in the current research, because a liquid that seeps into the instrumented root canal will represent interstitial fluid and a filtrate of blood components.

Intracanal medicaments are routinely used for root canal disinfection as part of controlling sepsis in root canal treatment.[14,15] The antimicrobial efficiency of the gutta-percha points that contained different medications on different bacterial species was determined in the present research.

Calcium hydroxide has emerged as a popular endodontic intracanal medicament. Calcium hydroxide is available in several formulations due to the number of vehicles that can be used along with it. The most common formulation is calcium hydroxide powder that is mixed with distilled water. A problem associated with it is its incomplete removal of calcium hydroxide remnants from the canal walls.

Calcium hydroxide points are designed for releasing calcium hydroxide from the gutta-percha matrix.[16] Calcium hydroxide ionization and the release of hydroxyl ions promoting the increased pH of the medium and its maintenance determine the antimicrobial mechanism of calcium hydroxide-containing gutta-percha points. Moreover, hydroxyl ions influence some action on bacterial cells as a result of destructing unsaturated fatty acids or phospholipids of the bacterial cytoplasmic membrane.[17]

In vitro studies have shown that gutta-percha points that contain calcium hydroxide may not be effective for calcium hydroxide release.[18,19] It is possible to explain this by the lower hydroxyl-releasing potential of medicated points compared to that of calcium hydroxide paste. Furthermore, it is possible that gutta-percha containing calcium hydroxide will not work as a good physical and chemical barrier as calcium hydroxide paste. Nevertheless, Stojanovic et al.[20] found out that intracanal medication with gutta-percha containing calcium hydroxide efficiently eliminated *E. faecalis* from infected root canals. In our study, Calcium Hydroxide PLUS Points significantly reduced bacteria compared to the control group for all experimental periods.

Chlorhexidine gluconate represents a broad-spectrum antibacterial agent, which functions by adsorbing onto the microorganism's cell wall and leading to the leakage of intracellular components. The formulation of the active points allows large quantities of chlorhexidine to be released from the points' surface.[16,21] Barthel et al.[22] and Lenet et al.[23] found out that gutta-percha points that contain chlorhexidine have a lower antimicrobial impact in comparison with chlorhexidine in other vehicles. In this research, the active points significantly reduced bacteria compared to the control group for all experimental periods.

Some controversies exist about calcium hydroxide effectiveness against *E. faecalis*. A number of theories have been suggested for explaining the survival of *E. faecalis* following treatment using calcium hydroxide.[24,25] It has been demonstrated that *E. faecalis* is capable of maintaining pH homeostasis in a passive and active way by a proton pump. Furthermore, it is capable of surviving in a severe environment, including a high pH value of 11.5, and it can penetrate into the dentin tubules and escape from the medicament's effective concentration. Moreover, the buffering impacts of dentin may ensure that a sufficiently high pH is not obtained in the dentin tubules.[20] Gutta-percha points that contain calcium hydroxide or chlorhexidine have also been found to have no effect against *E. faecalis* in a number of in vitro studies.[12,26,27] Barthel et al.[22] showed the inadequate antimicrobial activity of gutta-percha points that contain calcium hydroxide or chlorhexidine under in situ conditions. In our study, neither Calcium Hydroxide PLUS Points nor chlorhexidine diacetate-impregnated active points exhibited sufficient antimicrobial activity against *E. faecalis* and *P. micros*, even after 14 days. Moreover, Calcium Hydroxide PLUS Points were more effective against *E. faecalis* than the active points at 2 days period.

The effectiveness of chlorhexidine as a potential option to calcium hydroxide as an intracanal medicament when *E. faecalis* is suspected has been shown.[18] Rathke et al.[28] demonstrated that gutta-percha points containing chlorhexidine were significantly more effective against *F. nucleatum* and *P. micros* than gutta-percha points that contained calcium hydroxide. Bozza et al.[29] conducted in vitro research for the purpose of evaluating the antimicrobial activity of gutta-percha that contained calcium hydroxide, gutta-percha that contained chlorhexidine, and conventional gutta-percha points, and concluded that gutta-percha that contained chlorhexidine proved to be effective against most tested species. Tanomaru et al.[17] found out that sufficient antimicrobial activity against *E. faecalis* was not exhibited by gutta-percha containing chlorhexidine inside infected dentine tubules. Identical findings were obtained in the research carried out by Jhamb et al.,[21] who evaluated gutta-percha that contained chlorhexidine, which showed higher antimicrobial activity against *P. aeruginosa, S. aureus*, and *E. coli* than gutta-percha containing calcium hydroxide.

Although some studies have reported gutta-percha containing chlorhexidine as a more efficient intracanal medicament in comparison with calcium hydroxide, Stojanovic et al.,[20]

Podbielski et al.,[11] and Oztan et al.[12] showed that gutta-percha containing chlorhexidine exhibited a similar antibacterial effect against *E. faecalis* in comparison with gutta-percha that contained calcium hydroxide.[11,12,20]

In this research, Calcium Hydroxide PLUS Points exhibited significantly higher efficiency against *P. endodontalis* and *E. lentum* than the active points for 1, 2, and 3 days; *P. micros* for 1, 2, 3, 4, and 7 days. For *P. melaninogenica* and *P. intermedia*, no statistically significant differences were determined between Calcium Hydroxide PLUS Points and the active points during 14 days.

It was assumed that the rapid release of the active ingredients occurred from the gutta-percha matrix. The short-term release in question can be among the reasons why, in this research, the highest antibacterial effect of both gutta-percha containing chlorhexidine and calcium hydroxide was achieved at the beginning, and a decrease occurred in it during the period of 3 days.[28] A considerably higher pH was obtained only in the immediate proximity of the gutta-percha containing calcium hydroxide following 1 day, and it was not possible to detect it following 3 days.[30] The factors that have made the comparison more difficult are associated with the fact that all the studies presented above were carried out in vitro and utilized various methods in order to detect pathogens.[20]

Under the limitations of the in vitro setting, the findings of the present study suggested that Calcium Hydroxide PLUS Points and the active points had an antimicrobial effect against all of the strains utilized in the current research. Calcium Hydroxide PLUS Points and the active points had similar effects to kill the tested microorganisms. However, neither material eradicated *E. faecalis* and *P. micros*.

Ethics Committee Approval: N/A

Informed Consent: N/A

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Original Article

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Evaluation of the Apoptotic Effects of the Humic Acid Treatment on Chronic Myeloid Leukemia Cell Line K562

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BACKGROUND/AIMS
Humic acid (HA) formed by the decomposition of organic matter is a high-molecular-weight polymer. Humic-derived substances are potential drugs for human health, and their role in the prevention and treatment of diseases, like diabetes, antiviral, UV-B protective effect, cancer, and cardiovascular disease, is emphasized in various studies. It has antiulcerogenic, antitumor. Yang et al. demonstrated that HA exerts an antiproliferative effect and growth inhibition on HL-60 cells through the induction of apoptosis. A study showed that the antiulcer effect of Shilajit (HAs extracted from Shilajit) samples may be due to antimicrobial, anti-inflammatory, antianxiety, antioxidant, healing, and regenerative effects. The present study aimed to investigate the effects of HA on cultures of the chronic myeloid leukemia cell line in vitro.

MATERIAL and METHODS
We determined the apoptotic effect of HA in K562 cells by using various molecular methods. For cell viability, HA was tested in vitro against K562 cell lines using the MTT colorimetric process. Bax, bcl-2, casp-3, and casp-9 expressions were quantified by Western blot. Apoptosis analysis has been made with the *Annexin-V/PI* method.

RESULTS
The IC_{50} values of HA were determined 100 μg mL^{-1} for the K562 cell line. HA has been shown to inhibit cell proliferation. As the bcl-2/bax ratio decreased and increasing the levels of caspase-3 and caspase-9, the cells underwent apoptosis.

CONCLUSION
As a result, HA showed the anticancer effect on leukemia cells. In the future, HA can be used as a chemopreventive agent in leukemia treatments.

Keywords: Anticancer treatment, caspase-3, caspase-9, bcl-2, bax

INTRODUCTION

Humic acid (HA) formed by the decomposition of organic matter is a high-molecular-weight polymer.[1,2] HA, which has cation exchange capacity, makes the soil fertile by increasing the mineral forming capacity of the plants. In the literature, studies are reporting that polyphenol, quinone, and polycarboxylic groups in the structure of humic substances impart antiulcerogenic, antitumor, and anti-inflammatory effects to the substance.[3–5] Humic substances are used in traditional Chinese medicine because of their many pharmacological properties.[2,6] The presence of studies emphasizing the role of humic substances in the prevention and treatment of wound healing, antiviral, cardiovascular diseases, osteoarthritis, cancer, and inflammation can be regarded as evidence emphasizing that humic substances are potential drugs for human health.[7–10] HA, therefore, has the potential for medical use in cancer treatment.

Chronic myeloid leukemia (CML), a myeloproliferative disease identified by the increased and unregulated increase of myeloid cells in the bone marrow and accumulation of these cells in the blood. Although white cells work close to normal in CML patients, their numbers are higher than normal. As this numerical height continues to increase, leukemia

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begins to develop in parallel with this increase. Although there is a lack of data on the global incidence of CML, which seems to have no relationship with race or ethnicity, which accounts for 15% of all leukemias worldwide, it is evident that more than 10,000 patients will be affected by CML each year, and this will cause significant global health burden.[11,12] In addition to chemotherapy, some natural antitumor drugs, such as paclitaxel and camptothecin, are used in the treatment of CML, which is a difficult malignant hematological disease to treat.[13] Due to the side effects and drug resistance of the drugs used, there is a continuing need to develop effective natural antitumor drugs for the treatment of CML.

Recent research has shown that a wide variety of chemotherapeutic agents provide the initiation of apoptosis. Bcl-2 (inhibitor) and bax (activator) proteins from the family of the bcl-2 associated X members of proteins have a crucial apoptosis function. The levels of protein inhibitors and activators play a key role in the apoptotic cycle. The improvement in the inhibitor–activator–protein ratio is informative regarding apoptosis regulation.[14] Most anticancer drugs used by apoptosis to destroy some forms of tumor cells suggest that apoptosis often plays a function in the death of cancer cells caused by drugs.

Our objective in this research was to examine HA's anticancer activity in the K562 cell line and to identify possible cell death pathways that are impaired in vitro. Here, we demonstrate that HA keeps from proliferation and cause apoptosis in the human CML cell line K562 at approximately 100 μM concentrations.

MATERIAL and METHODS

Purification of HA

HA (Sigma-Aldrich Co., Darmstadt, Germany) was acidified with a pH < 2.0 HCl solution after dissolution in pH > 10 NaOH solution. After half an hour of centrifugation at 3,000 × *g* as described by Schnitzer's,[15] the procedure was repeated three times to obtain the purest form of HA dissolved in NaOH solution. Prior to the preparation of stock solutions at 5, 10, 20, 50, and 100 HA μg mL^{-1}, the powdered HA was dissolved with PBS (pH = 7.4).

Cell Culture

The K562 cells (ATCC: CCL-243) were cultured in RPMI 1640 medium (Biochrom, FG1215, Berlin, Germany), supplemented with 10% inactivated fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin (supplied from Capricorn Scientific, FBS-11B, Ebsdorfergrund, Germany; EMD Millipore, K0282, Darmstadt, Germany; Biochrom, A2213; respectively). The cell culture experiments were performed at 37°C, physiological pH, and in a 5% CO_2 humidified atmosphere. Our study was conducted according to the Helsinki Declaration.

Main Points

- Humic acid (HA) has anticancer activity on chronic myeloid leukemia cell line.
- HA helped decrease the bcl-2/bax ratio in K562 cell lines.
- HA helped increase the level of casp-9 in K562 cells.

Cell Viability Assay

Following 24 hours of incubation, proliferation and viability measurement of K562 cells against HA at a concentration of 5-100 μg mL^{-1} was evaluated by the cell proliferation kit I (MTT) (Roche 11465007001). 96-well culture dishes were seeded with K562 cells containing about 5 × 10^3 cells well and cultured in RPMI medium that contains 5, 10, 20, 50, and 100 μg mL^{-1} concentrations of active ingredient for 24 hours. Then, 1 mg mL^{-1} MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was used to incubation for 4 hours, at 37°C in 5% CO_2 humidified incubator. Experiments were studied in three replicates. Finally, the formazan absorbance was immediately measured at 540 nm using UV-spectrophotometer (Synergy HI, BioTek Instruments, Inc., Vermont, USA).

Apoptosis Assay

Before the cells were assayed Tali image-based cytometer, cells were stained with Annexin V. Annexin-V/PI cells were realized as apoptotic cells (Propidium Iodide, Molecular Probes A10788, and Tali® Apoptosis Kit-Annexin V Alexa Fluor® 488, respectively). Apoptosis analysis that determined percentages of the life, death, and apoptotic cells was performed as described by Mega-Tiber et al.[16] and using the Tali Image-Based Cytometer.[16]

Mitochondrial Membrane Potential Assay

Moreover, apoptosis was also investigated by examining probable changes in the mitochondrial membrane. This assay deterioration in the initial stage of apoptosis was assessed by the change in the permeability of the membrane. To determine probable changes in the mitochondrial membrane using the JC-1 (ab113850) probe, cells were washed with PBS, and JC solution was added, finally analyzed using a fluorescent microplate reader filter. The ratio of green fluorescence from low $\Delta\psi M$ to red fluorescence apoptotic cells from healthy cells with high $\Delta\psi M$ was calculated using multimode microplate readers with correct filters.

Western Blot Assay

Cells (5.0 × 10^5 cells mL^{-1}) were composed with 1 × radioimmune-precipitation assay buffer containing protease inhibitor cocktail (Cat no: 11873580001; Sigma-Aldrich). According to established in the literature, the cells were prepared, loaded on the gel, and transferred onto nitrocellulose-membranes.[16,17] The membranes were blocked with bovine serum albumin, incubated primary antibodies [caspase-3 (casp-3, 1:200), casp-9 (1:200), bcl-2 (1:200), bax (1:100), and β-actin (1:200); Santa Cruz, CA, USA] for 1 hour and incubated with secondary antibody for 12 hours. Beta-actin was used as a housekeeping standard. Finally, the membranes were analyzed using a free software program (Bio-Rad Molecular Analyst, reached from www.totallab.com). Molecular weights were for β-actin (was used for standardization in all membranes), casp-3, casp-9, bcl-2, and bax; 43, 20, 46, 26, and 23 kDa, respectively.

Data Analysis

For statistical analysis, GraphPad software (Prism 3.0; GraphPad Software, San Diego, CA, USA) was used. All data were expressed means ± standard error. The groups of data were analyzed by one-way analysis of variance followed by *Bonferroni* multiple comparison post hoc tests. *P* values less than .05 were considered as a statistically significant difference.

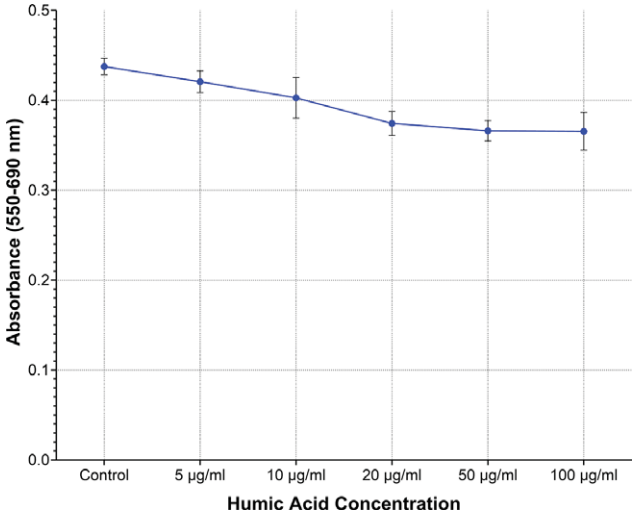

Figure 1. The evaluation of the antiproliferative effects of humic acid using the MTT assay at 24 hours in K562 cells (n = 3, triplicate)

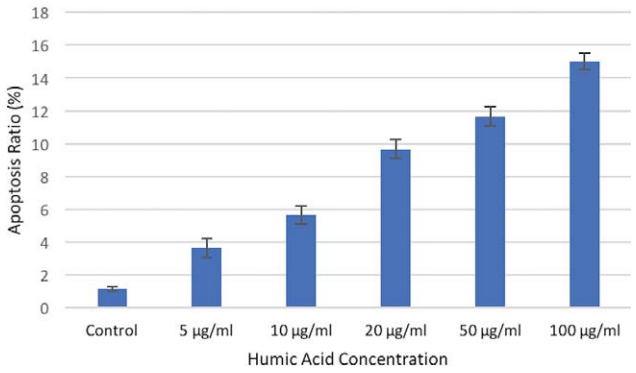

Figure 2. Apoptotic cells were evaluated using the Tali apoptosis kit in K562 cell lines (n = 3)

RESULTS

The Cytotoxic Effect of HA on K562 Cells

Cell proliferation and decreased viability are inhibited by 24-hour treatment of K562 cells with HA. MTT assay was used to determine the cytotoxic effect. Also, cell viabilities were handled after trypan blue staining using a TALI image-based cytometer. After 24 hours of incubation, the treatment of K562 cells with HA was determined by the MTT method, in which the viability of the cells was dose-dependent and the cell proliferation was suppressed (Figure 1). Cell proliferation was inhibited by treatment of K562 cells with HA for 24 hours, in particular, it was found that cell proliferation 25% decreased in 100 µg mL^{-1} of HA (Figure 2).

The Evaluation of Apoptosis and Membrane Potential Assay

When quantifying annexin V/PI staining, the number of apoptotic cells at 100 µg mL^{-1} a dosage of HA was found to be 10% of the total cell population. It was reached to 15% at the max concentration of HA (100 µg mL^{-1}). When analyzed with JC-1 assay, the obtained apoptotic cell values are the same with a 16% decrease in cell proliferation (Figure 3).

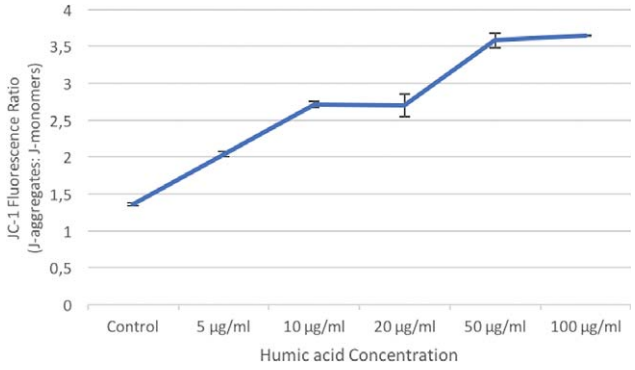

Figure 3. Mitochondrial membrane potential was evaluated using JC-1 dye (n = 3)

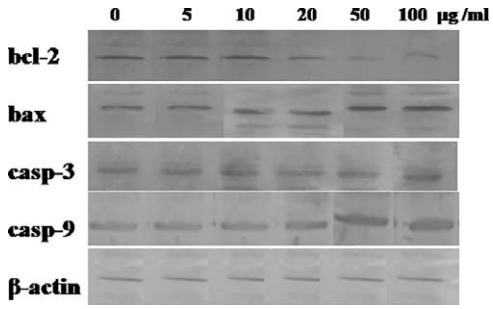

Figure 4. Representative images of western blotting experiments used to determine bcl-2 (≅26 kDa), bax (≅ 23 kDa), casp-3 (≅ 20 kDa), casp-9 (≅ 46 kDa), and ***β***-actin (≅ 43 kDa) expressions' levels (n = 3, duplicate)

Immunoblotting Assay

The bax/bcl-2 ratio is a clear calculation of the permanent apoptosis. Representative images of western blotting experiments used to determine bcl-2/bax ratio and casp-3 and casp-9 expression levels are shown in Figure 4. As the amount of HA added increased, there was a minor upregulation in the bax/bcl-2 ratio, but this was only evident at 50 and 100 µg mL^{-1} ($P <$ .05, and $P <$ .001 for bcl-2; $P <$ .01, and $P <$ .001 for bax, Table 1). At high concentration (50 and 100 µg mL^{-1}) of HA, casp-9 level increased significantly ($P <$ .001 for both concentration), whereas at low concentrations such as 10 µg mL^{-1} and 20 µg mL^{-1}, casp-9 level decreased (Table 1). Regardless of the low concentration of HA, it was determined that casp-3 was increased only in the highest concentration of HA (100 µg mL^{-1}) ($P <$ .05, Table 1).

DISCUSSION

Although there is information in the literature on the pharmacological properties of HA and the inhibition of the growth of certain types of cancer cells, such as A549 and human acute promyelocytic leukemia cell line (HL-60), its effect on the growth of different tumor cells, such as K562, and the underlying mechanisms are not fully elucidated. We examined the effect of HA against the K562 cell line and showed that HA inhibited the increase of K562 cells at concentrations greater than 20 µg mL^{-1}. After 24 hours of treatment, 100 µg mL^{-1} a dosage of HA to K562 cells was 21 µg mL^{-1}. This result shows

TABLE I. The Expression Levels of bcl-2, bax, casp-3, and casp-9 after HA Treatment in K562 Cells. For Each Group n = 3 and the Analyses were Performed as Duplicate

Relative density of	Control	5 μg mL^{-1}	10 μg mL^{-1}	20 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}
Bcl-2	0.66 ± 0.02	0.64 ± 0.04	0.61 ± 0.04	0.52 ± 0.09	0.48 ± 0.04*	0.41 ± 0.04**
Bax	0.42 ± 0.03	0.43 ± 0.01	0.48 ± 0.03	0.55 ± 0.04	0.77 ± 0.12***	0.81 ± 0.02***
Bax/bcl2	0.64	0.67	0.79	1.06	1.60	1.98
Casp-3	0.22 ± 0.05	0.23 ± 0.02	0.26 ± 0.03	0.29 ± 0.02	0.34 ± 0.03	0.38 ± 0.01*
Casp-9	0.28 ± 0.01	0.29 ± 0.02	0.33 ± 0.06	0.35 ± 0.03	0.48 ± 0.07***	0.56 ± 0.03***

*$P < .05$,
**$P < .01$, and
***$P < .001$, represents comparison with control group.
Casp-3 and casp-9: caspase-3 and caspase-9; HA: humic acid.

that HA significantly inhibits the proliferation of K562 cells in a dose-dependent manner. Moreover, our study showed that 100 μg mL^{-1} HA induces apoptosis in K562 cells by increasing the expressions of proapoptotic proteins at 24 hours. Apoptosis research is important both in explaining cancer development and in developing anticancer therapies. The data obtained from this study suggest that the inhibitory effect of HA on the K562 cell line may be due to the induction of apoptosis.

The molecular structure of HA with the current study was measured apoptosis-related proprotein and antiapoptotic expression levels in HA-treated K562 cells. We determined the expression levels of bax/bcl-2 ratio after HA treatment in K562 cells. Bax/bcl-2 ratios were found 0.64 (control), 0.67 (5 μg mL^{-1}), 0.79 (10 μg mL^{-1}), 1.06 (20 μg mL^{-1}), 1.60 (50 μg mL^{-1}), 1.98 (100 μg mL^{-1}), respectively (Table I). BCL-2 gene family proteins play a role in the regulation of apoptosis.[18] BCL-2 proteins can make cancer cells resistant to some chemotherapeutic agents. Therefore, BCL-2 related antiapoptotic proteins are important targets in the development of new anticancer agents.[19] BAX is proapoptotic protein. BAX protein enables the release of apoptogenic factors such as cytochrome *c*, activation of the caspase cascade.[20]

Bax protein's role in apoptosis is the opposite, while bcl-2 protein levels play a role in apoptosis suppression. Our objective in evaluating the bcl-2/bax ratio and casp-3 and casp-9 levels was to assess whether the apoptotic effect of HA was inducing or inhibiting in various concentrations on the K562 cell line. In our results, it was determined that at the 50 and 100 μg mL^{-1} concentrations, the bcl-2 expression level decreased and the increase in the bax expression at the same high concentrations caused a increase in the bax/bcl-2 ratio. Moreover, the results of our study showed that both casp-3 and casp-9 expressions increased in the treatment of 100 μg mL^{-1} HA. Raisova et al.[21] evaluated the change in bcl-2 and bax ratio and emphasized that the decrease in bcl-2/bax ratio is important for apoptosis. There are a large number of studies showing that a wide diversity of chemotherapeutic agents cause apoptosis, an active cell death pattern.[2] Although there are many in vitro or in vivo studies of HA related to different cancer cells in the literature, its relation with cancer remains unclear. HL-60 cell line treatment using diverse chemotherapeutics is reported to be accompanied by increased cytosolic translocation of cytochrome-*c* and activation of casp-3. Incubation of HL-60 cells with HA significantly increased bax protein levels. However, the level of bcl-2 is not changed. This result suggests that reducing the level of bcl-2/bax may cause apoptosis. HA (100 μg mL^{-1})) induction into HL-60 cells resulted in an increase in casp-3 activity from 4 hours to 24 hours.[2] Similar to the results in the literature, our results suggest that HA has an apoptotic effect on K562 cell lines. Additionally, we investigated the activity of caspase-9; HA concentrations of 50 and 100 μg mL^{-1} important increases were analyzed.

The in vitro study results of A549 (adenocarcinoma *human* alveolar basal epithelial *cells*) and SiHA cells (human cervical cancer cells) are showed that low-dose HA increases the progression of the lung cancer cells.[22,23] Another high-dose HA research was documented to induce apoptosis via death receptor activation, mitochondrial and endoplasmic reticulum stress signaling cascades.[24] Also, HA has been shown to produce reactive oxygen species and leads to the depletion of glutathione.[25]

In the literature, HA has been reported to induce oxidative damage, growth retardation, and cell death in human primary fibroblasts. The administration of a single dose of HA 100 mg kg^{-1} bw by gastric intubations to mice has been reported to result in abnormalities in the structure of induction in intestinal cells. It is reported that HA induces apoptosis in HL-60 and post-initiated mouse epidermal cells.[26] Similarly, our study results showed that a high concentration of HA contributed to apoptosis of the K562 cells via decreased bcl-2/bax ratio and increased casp-3 and casp-9 levels.

These findings showed that HA effectively inhibited the proliferation of the K562 cell line and indicated that apoptosis could be induced by a decrease of the bcl-2/bax ratio and upregulation of casp-3 and casp-9. The results of this study indicate that in the prevention and treatment of CML, HA may be a potential for future drug development.

Including the use of humic substances as homeopathic or nutritional supplements, the use as an anticancer agent can be beneficial in the treatment of patients with leukemia.

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Informed Consent: N/A

Peer-review: Externally peer-reviewed.

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and/or Interpretation - A.A., P.M.T., T.B.O., M.K.; Literature Search - A.A.,P.M.T.,T.B.O.; Writing - A.A., P.M.T., T.B.O., M.K.; Critical Reviews - A.A., P.M.T.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Original Article

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Role Conflict and Role Ambiguity in Pediatric General Duty and Intensive Care Unit Nurses

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BACKGROUND/AIMS
The aim of this study is to investigate the role conflict and role ambiguity in pediatric nurses who work outside and in the pediatric intensive care units and whether the situation varies according to some characteristics of the nurses.

MATERIAL and METHODS
The descriptive study was carried out in intensive care units with featured units, other featured units (emergency service, operating room, and burn unit), and inpatient clinics in a pediatric hospital between October 1, 2019 and February 1, 2020. All nurses were included in the sample using the total count sampling method (N = 210). The data were collected using a Nurse Personal Information Form and the Role Conflict and Role Ambiguity Scale.

RESULTS
The mean role conflict score of the nurses who worked in the hospital was 35.74 ± 9.34 and above the moderate level, while the mean role ambiguity score was 39.90 ± 5.71 and they experienced no role ambiguity. The mean role conflict score of the nurses in the clinic (37.38 ± 9.46) was significantly higher than those in the intensive care unit (33.80 ± 9.15) ($F = 3.42$, $P = .03$). The role conflict was higher in nurses who spent more years in the profession, in the hospital, and have not received orientation training in the hospital, whereas the role ambiguity was higher in nurses who have not received orientation training in the hospital ($P < .05$).

CONCLUSION
The working environments in health institutions create a favorable ground for role conflict and role ambiguity.

Keywords: Pediatric nursing, critical care nursing, intensive care unit, role conflict, role ambiguity

INTRODUCTION

Nursing in our country has experienced a swift transformation in recent years and has turned into an autonomous profession with the developments regarding its qualifications and competence.[1] Nursing is legally defined as a profession that requires an undergraduate degree,[2] and its duties, authorities, and responsibilities are explained according to the unit worked in.[3] The development of medical science and the changes in health needs have also affected the roles in nursing.[1] Different demands are made on the nurses in different working environments. When the tasks in the acute and long-term care institutions are not clearly defined, the working environment can be stressful.[4] Intensive care unit (ICU) is a complex and challenging environment. When physicians hold the authority, ICU nurses may not work with clear roles and responsibilities.[5] The development of new roles in ICU nursing is a response to the advances in health technology. Many nurses face problems of role conflict, role ambiguity, excessive workload, ambiguity of role boundaries, and autonomy.[6]

Health institutions are environments that are open to conflict, since they bring together different employees. One of the most commonly defined sources of conflict is role conflict and role ambiguity.[7] Status-related barriers among healthcare

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workers are one of the communication barriers.[8] Hierarchy, interpersonal differences of power, role conflict, and role ambiguity cause erroneous communication between nurse and physician.[9] According to Weiss et al.,[4] role conflict, lack of a clear job description, and inability to adapt to the job are the causes of stress in the work environment.

Role conflict is incompatible role expectations and may occur among and within roles.[10,11] Conflicting instructions and demands of physicians, nursing managers, and head nurses cause role conflicts.[10] The literature holds studies with different results showing that nurses do not have any role conflicts[12] or have conflicts at various levels, ranging from middle to high.[13–22] Intensive care nurses have been reported to have a moderate level of living[5] and live longer than other nurses.[10] Role ambiguity is a lack of information about the expected role behavior. There is an inadequate understanding of what the job is about because of the unclear job definition.[10,23] The literature holds studies with different results, showing that nurses do not have any role conflicts[12] or have conflicts at various levels, ranging from middle to high.[13–22] It has been shown that the level in intensive care nurses is middle[5] and higher than other nurses.[10]

Role conflict and role ambiguity have been shown to increase work-related tension,[12] burnout,[10,16,20,22] unethical behavior,[23] staff turnover,[13] and intention to leave work,[18] and decrease self-efficacy,[10] job performance,[19] job satisfaction,[14,17,19] dedication to work,[15] and organizational commitment.[16] In the study of Roch et al.,[24] it was determined that it affected care practices. In qualitative studies, it was stated that it was among the causes of in-team conflict perceived by the nurses,[11] resulting in the transfer of care practices to the auxiliary personnel.[24]

Collaboration, coordination, and compliance and clarity in role expectations are considered necessary for the fulfillment of care practices by nurses.[24] Pediatric ICU is a unit where complex treatment plans of critical patients are coordinated with more than one member. Perfection in a pediatric ICU is achieved through a combination of many factors and is dependent on a healthy work environment.[25] Given that the role conflict and role ambiguity results in many unfavorable situations for nurses, and that this is reflected in the quality of care; the importance of the studies for determining the level of the conflict and ambiguity becomes apparent. In national and international literature, no study investigating the role conflict and role ambiguity in pediatric nurses has been found. We believe that this study may open the way for future studies for the improvement of the working environment by the hospital and healthcare services managers.

Main Points

- We observed that pediatric nurses had a moderate level of role conflict and no role ambiguity.
- We demonstrated that nurses working in the clinic experienced more role conflicts than nurses working in the ICU.
- We found that nurses working in the intensive care and in the hospital, nurses who spent more years in the hospital and in the profession, and nurses who have not received orientation training from the hospital experienced more role conflicts.
- We also found that pediatric nurses who have not received orientation training in the hospital experienced more role ambiguity.

Objective

The aim of this study is to investigate the role conflict and role ambiguity in pediatric nurses who work outside and in the pediatric ICU and whether the situation varies according to some characteristics of the nurses.

MATERIALS and METHODS

Type of the Study

This study was conducted in accordance with the descriptive research type.[26]

Place and Time of the Study

This study was conducted between October 1, 2019 and February 1, 2020 in in-patient clinics, ICUs with featured units (neonatal, pediatric, pediatric surgery, and cardiovascular surgery ICU), and other featured units (emergency, operating room, and burn unit) of a pediatric hospital.

The Target Population and the Sample of the Research

The target population of the study was composed of nurses working in the hospital (N = 353). The sample consisted of nurses who worked in the hospital and met the sampling criteria. The sampling criteria were as follows: to be working in a featured unit or inpatient clinic, being a bedside nurse, and having worked in the unit for at least 1 month. Total count sampling method was used. The whole target population of the study was included in the sample of the study[26] (N = 295), and this study was completed with 210 nurses.

Data Collection Tools

Nurse Personal Information Form. It was developed based on the literature by researchers to determine the sociodemographic and professional characteristics of nurses.[5,10,12,14,27] It consists of 17 questions in total.

Role Conflict and Role Ambiguity Scale. The original "Role Conflict and Role Ambiguity Scale" was developed by Rizzo et al.[28] Later, it was finalized by Schuler et al.[29] It was first adapted to Turkish by Kaygin[30] and later by Yildirim,[31] who provided its face validity. The scale measures the role conflict and role ambiguity that the individuals experience. It consists of 14 items, eight items measure the role conflict and six items measure the role ambiguity.[31] The last eight items in the scale are about role conflict, and the first six items are about role uncertainty.[29] The scale has been prepared according to the seven-point Likert scale (1 = absolutely wrong and 7 = absolutely correct). Total points for role conflict and role ambiguity are recorded separately. The minimum total point is 8 and maximum is 56 for role conflict, while the minimum total point is 6 and maximum is 42 for role ambiguity. The high score for role conflict indicates that the conflict is experienced more, whereas the lower score for role ambiguity indicates that the ambiguity is experienced more. The test–retest reliability coefficient of the adapted scale was 0.81 for role conflict and 0.72 for role ambiguity, and the internal consistency reliability coefficient was determined as 0.82 for role conflict and 0.63 for role ambiguity.[31] In our study, it was found 0.83 for role conflict and 0.78 for role ambiguity.

Collection of the Data

Nurses were provided to fill in the data collection tools by providing the necessary information through individual face-to-face interviews by the researchers. In order to contact all nurses, the units were visited at different working hours. Filling the tools took 5-10 minutes for each nurse.

Evaluation of the Data

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM SPSS Corp.; Armonk, NY, USA). Descriptive statistics (number, percentage, mean, standard deviation, and median) were used to evaluate the data. The reliability of the scale was tested by reliability analysis. In the scale, role conflict was found in accordance with normal distribution, whereas role ambiguity was not. In comparison of the data, for the scale that showed normal distribution, the independent samples t-test was used in analyzing two groups and one-way analysis of variance for more than two groups, and for the non-normally distributed scale, the Mann–Whitney U test was performed analyzing two groups, and the Kruskal–Wallis analysis was performed for more than two groups. The Bonferroni test was used to find the group that created a difference in the analysis of more than two groups. The level of statistical significance was accepted as $P < .05$.[26]

Ethical Aspect of the Research

Approval for this study was obtained from the University of Health Sciences Dr. Behcet Uz Child Disease and Pediatric Surgery Training and Research Hospital Clinical Research Ethics Committee (approval date: September 26, 2019; approval number: 2019/14-11), hospital management, and Mrs. Yildirim, who adapted the scale. Nurses' consent was obtained using an informed consent form.

RESULTS

The mean age of the nurses in the hospital was 34.87 ± 8.30, 63.8% of them had an undergraduate degree, and the average years in the profession were 12.59 ± 8.37. It was found that the weekly working time in the hospital was 52.74 ± 9.14 hours, and those who worked most for 57-72 hours were the ICU nurses, with a rate of 42.1%. The average number of nurses during day shift was 2.17 ± 0.38 for ICU and 5.83 ± 1.62 for clinic, while during night shift, it was 2.62 ± 0.80 for ICU and 9.49 ± 2.37 for clinic. The rate of nurses receiving a certificate was the highest among the ICU nurses (30.7%). Most of the nurses in the hospital had received general (85.2%) and unit-specific (79.5%) training in duties, authorities, and responsibilities, and had received orientation training in the hospital (89%) and the unit (91.9%) (Table 1).

The mean score for role conflict of the nurses in the hospital was 35.74 ± 9.34, and the mean score of role ambiguity was 39.90 ± 5.71. According to the unit worked in, the mean score for role conflict was 37.38 ± 9.46 in the clinic, 36.63 ± 8.93 in other featured unit, and 33.80 ± 9.15 in the ICU, and the mean score for role ambiguity was 30.05 ± 5.96 in other featured unit, 30.48 ± 5.36 in the clinic, and 31.68 ± 5.90 in the ICU (Table 2). The mean score for role conflict of nurses in the clinic was significantly higher than those in the ICU ($F = 3.42$, $P = .03$) (Table 3).

In the analysis of the mean score for role conflict, it was found that the nurses in the hospital with a period of more than 10 years spent in the profession (37.06 ± 8.82) had significantly higher scores than those who spent less than 5 years (32.21 ± 10.66) ($F = 3, 87$, $P = .02$). Similarly, for the nurses in the ICU, those who had 5-10 years (35.02 ± 8.22) and more than 10 years in the profession (35.38 ± 8.26) had significantly higher scores compared with those with less than 5 years (27.94 ± 10.81) ($F = 4.70$, $P = .01$). For the nurses in other featured units, those who spent more than 10 years in the profession (40.75 ± 6.91) also had significantly higher scores than those who spent less than 5 years (34.11 ± 11.77) ($F = 3.33$, $P = .04$). It was observed that the nurses in other featured units who worked for 40 hours weekly (44.50 ± 6.89) had higher scores compared with those who worked for 41-56 hours weekly (33.94 ± 9.21) ($F = 3.58$, $P = .03$). The mean score for role conflict was significantly higher in the clinic nurses who received certificates (45.66 ± 6.74) when compared with those who did not (36.74 ± 9.37) ($t = 2.28$, $P = .02$). The distribution of the mean role conflict scores varied according to orientation, in such a way that in hospital nurses, those who did not receive a certificate (39.43 ± 10.75) had significantly higher scores when compared with those who did (35.29 ± 9.08) ($t = -2.01$, $P = .04$). Similarly in ICU nurses, those who did not receive a certificate (41.00 ± 7.41) had significantly higher scores than those who did (33.18 ± 9.06) ($t = -2.21$, $P = .02$) (Table 4).

In evaluation of the median scores for role ambiguity, it was seen that there was a statistically significant difference only the nurses in other special units had a significant difference in terms of working time spent in the profession, the hospital, and the unit. Those who had more than 10 years of work experience in the profession (26.00) had highly significantly lower scores than those who spent less than 5 years (34.00) and 5-10 years (36.00) ($KW = 9.65$, $P = .008$); those who had more than 10 years of work experience in the hospital (26.00) had significantly lower scores than those who had less than 5 years (34.00) ($KW = 8.64$, $P = .01$); and those who had more than 10 years of work experience in the unit (26.00) had highly significantly lower scores than those who had less than 5 years (34.00) ($KW = 10.30$, $P = .006$). The median score for role ambiguity was significantly lower in clinic nurses who received a certificate (27.00) when compared with those who did not receive a certificate (31.00) ($U = 121.50$, $P = .05$). The median score for role ambiguity in nurses in other featured units who received unit-specific training in duties, authorities, and responsibilities (25.50) was significantly lower than those who did not (29.50) ($U = 43.00$, $P = .03$). The median score for role ambiguity in hospital nurses who did not receive orientation (30.00) was significantly lower when compared with those who did (32.00) ($U = 1500.50$, $P = .01$) (Table 5).

DISCUSSION

The ability of pediatric nurses to provide quality care to the child and the family depends on their ability to perform their roles.[32] The working environment is dynamic and complex since it requires the performance more than one role at a time.[11] Factors such as job-oriented work, inadequate employment, and lack of information about the role negatively affect the nurses in performing their roles.[32]

In this study, it was seen that the role conflict in nurses in the hospital was above the moderate level. Similar results to our study have been achieved in other national studies.[14,19,22] On the other hand, some studies suggest that the conflict was not

TABLE I. Mean and Percentage Values of the Sociodemographic and Professional Characteristics of Nurses

	Hospital (N = 210)		ICU (n = 88)		Other featured unit (n = 38)		Clinic (n = 84)	
Characteristic	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
Age	34.87	8.30	33.44	7.70	32.89	8.39	37.26	8.39
Number of patients during day shift	5.28	4.38	2.17	0.38	10.84	6.81	5.83	1.62
Number of patients during night shift	7.05	5.45	2.62	0.80	12.86	7.43	9.49	2.37
Characteristic	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Gender								
Female	189	90.0	76	86.4	31	81.6	82	97.6
Male	21	10.0	12	13.6	7	18.4	2	2.4
Level of education								
High school	12	5.7	4	4.5	2	5.3	6	7.1
Associate	48	22.9	18	20.5	7	18.4	23	27.4
Undergraduate	134	63.8	59	67.0	29	76.3	46	54.8
Graduate	16	7.6	7	8.0	0	.0	9	10.7
Years in profession								
<5 years	38	18.1	17	19.3	9	23.7	12	14.3
5-10 years	67	31.9	35	39.8	13	34.2	19	22.6
>10 years	105	50.0	36	40.9	16	42.1	53	63.1
Years at hospital								
<5 years	83	39.5	37	42.0	21	55.2	25	29.8
5-10 years	62	29.5	31	35.3	8	21.1	23	27.4
>10 years	65	31.0	20	22.7	9	23.7	36	42.8
Period of service in the unit								
<5 years	129	61.4	47	53.4	23	60.5	59	70.2
5-10 years	61	29.0	29	33.0	9	23.7	23	27.4
>10 years	20	9.6	12	13.6	6	15.8	2	2.4
Type of service								
Day and night shifts	179	85.2	77	87.5	34	89.5	68	80.0
Day shift only	22	10.5	4	4.5	3	7.9	15	17.8
Night shift only	9	4.3	7	8.0	1	2.6	1	1.2
Weekly working time								
40 hours	46	21.9	20	22.7	6	15.8	20	23.8
41-56 hours	99	47.1	31	35.2	18	47.4	50	59.5
57-72 hours	65	31.0	37	42.1	14	36.8	14	16.7
Received a certificate?								
Yes	37	17.6	27	30.7	4	10.5	6	7.1
No	173	82.4	61	69.3	34	89.5	78	92.9
Received training in general duties, authorities, and responsibilities?								
Yes	179	85.2	75	85.2	32	84.2	72	85.7
No	31	14.8	13	14.8	6	15.8	12	14.3
Received training in unit-specific duties, authorities, and responsibilities?								
Yes	167	79.5	70	79.5	32	84.2	65	77.4
No	43	20.5	18	20.5	6	15.8	19	22.6
Received orientation at the hospital?								
Yes	187	89.0	81	92.0	36	94.7	70	83.3
No	23	11.0	7	8.0	2	5.3	14	16.7
Received orientation at the unit worked in?								
Yes	193	91.9	82	93.2	37	97.4	74	88.1
No	17	8.1	6	6.8	1	2.6	10	11.9

ICU, intensive care unit.

TABLE 2. Mean and Median Scores of the Nurses' Role Conflict and Role Ambiguity

	Role conflict				Role ambiguity			
Unit worked in	**M**	**SD**	**Mdn**	**Min-max***	**M**	**SD**	**Mdn**	**Min-max***
Hospital	35.74	9.34	37.00	8.00-55.00	39.90	5.71	32.00	10.00-42.00
ICU	33.80	9.15	35.00	8.00-54.00	31.68	5.90	33.00	10.00-42.00
Other featured unit	36.63	8.93	37.00	20.00-53.00	30.05	5.96	29.00	16.00-42.00
Clinic	37.38	9.46	39.00	14.00-55.00	30.48	5.36	30.50	17.00-42.00

ICU, intensive care unit.
*Min-max: minimum-maximum.

TABLE 3. Comparisons of the Mean Scores for Role Conflict and Median Scores for Role Ambiguity of Nurses According to the Unit They Work in

	Role conflict				
Unit worked in	**N**	**M**	**SD**	**F**	***P***
ICU[a]	88	33.80	9.15	3.42	.03*
Other featured unit[b]	38	36.63	8.93		c > a
Clinic[c]	84	37.38	9.46		
	Role ambiguity				
Unit worked in	**n**	**Mdn**	**Min-max†**	**KW**	***P***
ICU	88	33.00	10.00-42.00	4.10	.12
Other featured unit	38	29.00	16.00-42.00		
Clinic	84	30.50	17.00-42.00		

ICU, intensive care unit.
*$P < .05$.
†Min-max: minimum-maximum.

TABLE 4. Comparison of the Mean Scores for Role Conflict According to the Sociodemographic and Professional Characteristics of Nurses

	Hospital (N = 210)				**ICU (n = 88)**				**Other featured unit (n = 38)**				**Clinic (n = 84)**			
Characteristic	**M**	**SD**	**F**	***P***	**M**	**SD**	**F**	***P***	**M**	**SD**	**F**	***P***	**M**	**SD**	**F**	***P***
Level of education																
High school	31.08	10.80	1.21	.30	28.00	9.12	0.97	.40	40.00	11.31	0.19	.82	30.16	11.87	2.31	.08
Associate	36.12	8.19			35.72	6.64			37.42	5.68			36.04	9.98		
Undergraduate	35.82	9.47			33.91	9.52			36.20	9.63			38.02	9.00		
Graduate	37.50	10.19			31.28	11.45			36.63	8.93			42.33	6.04		
Years in profession																
<5 years[a]	32.21	10.66	3.87	.02*	27.94	10.81	4.70	.01*	34.11	9.77	3.33	.04*	36.83	9.40	0.18	.83
5-10 years[b]	35.68	8.96		c > a	35.02	8.22		b > a	33.30	9.12		c > b	38.52	9.89		
>10 years[c]	37.06	8.82			35.38	8.26		c > a	40.75	6.91			37.09	9.47		
Years at hospital																
<5 years[a]	33.65	10.18	3.65	.02*	30.91	9.78	3.34	.04*	35.04	9.37	1.78	.18	36.52	10.75	0.20	.81
5-10 years[b]	36.72	7.82		c > a	35.90	8.03		b > a	35.37	9.10			38.30	7.13		
>10 years[c]	37.49	9.18			35.90	8.50		c > a	41.44	6.52			37.38	9.99		
Period of service in the unit																
<5 years	35.19	9.72	0.89	.41	32.78	9.75	0.73	.48	35.78	9.29	1.97	.15	36.88	9.63	1.37	.26
5-10 years	36.16	8.60			35.41	8.21			34.55	9.05			37.73	9.04		
>10 years	38.05	9.02			33.91	9.04			43.00	4.38			48.00	0.00		
Weekly working time																
40 hours[a]	36.95	10.36	0.49	.61	33.00	10.84	0.38	.68	44.50	6.89	3.58	.03*	38.65	9.32	1.28	.28
41-56 hours[b]	35.34	8.96			34.96	8.81			33.94	9.21		a > b	36.08	9.07		
57-72 hours[c]	35.50	9.24			33.72	8.60			36.71	7.70			40.21	10.80		
Characteristic	**M**	**SD**	**t**	***P***	**M**	**SD**	**t**	***P***	**M**	**SD**	**t**	***P***	**M**	**SD**	**t**	***P***
Received a certificate?																
Yes	37.18	9.21	1.03	.30	34.74	8.58	0.63	.53	41.00	9.34	1.03	.30	45.66	6.74	2.28	.02*
No	35.43	9.37			33.39	9.43			36.11	8.88			36.74	9.37		
Received training in general duties, authorities, and responsibilities?																
Yes	35.73	9.15	−0.03	.97	33.89	8.76	0.21	.83	36.21	8.70	−0.65	.51	37.44	9.50	0.15	.88
No	35.80	10.55			33.30	11.54			38.83	10.66			37.00	9.63		
Received training in unit-specific duties, authorities, and responsibilities?																
Yes	35.80	9.22	0.18	.85	34.08	9.19	0.56	.57	35.96	8.71	−1.05	.29	37.58	9.28	0.35	.71
No	35.51	9.92			32.72	9.17			40.16	10.10			36.68	10.27		
Received orientation at the hospital?																
Yes	35.29	9.08	−2.01	.04*	33.18	9.06	−2.21	.02*	35.97	8.67	−2.00	.06	37.38	8.91	0.01	.99
No	39.43	10.75			41.00	7.41			48.50	4.94			37.35	12.24		
Received orientation at the unit worked in?																
Yes	35.76	9.29	0.07	.94	33.73	9.36	−0.28	.77	36.81	8.98	0.74	.45	37.48	9.04	0.27	.78
No	35.58	10.27			34.83	6.11			30.00	–			36.00	12.72		

ICU, intensive care unit.
*$P < .05$.

TABLE 5. Comparison of the Median Scores for Role Ambiguity According to the Sociodemographic and Professional Characteristics of Nurses

	Hospital (N = 210)				ICU (n = 88)				Other featured unit (n = 38)				Clinic (n = 84)			
Characteristic	**Mdn**	**Min-max***	**KW**	***P***	**Mdn**	**Min-max***	**KW**	***P***	**Mdn**	**Min-max***	**KW**	***P***	**Mdn**	**Min-max***	**KW**	***P***
Level of education																
High school	34.00	21.00-36.00	1.95	.58	33.50	32.00-36.00	0.98	.80	30.50	26.00-35.00	2.54	.28	34.50	21.00-36.00	1.32	.72
Associate	31.50	10.00-41.00			32.50	10.00-41.00			26.00	21.00-34.00			31.00	22.00-39.00		
Undergraduate	32.00	16.00-42.00			34.00	16.00-42.00			29.00	16.00-42.00			30.50	17.00-42.00		
Graduate	29.50	16.00-37.00			32.00	16.00-35.00			–	–			27.00	23.00-37.00		
Years in profession																
<5 years[a]	33.00	22.00-39.00	1.21	.54	27.00	38.00-34.00	2.15	.34	34.00	24.00-39.00	9.65	.008†	30.00	22.00-35.00	2.65	.26
5-10 years[b]	32.00	10.00-42.00			10.00	42.00-32.00			36.00	25.00-42.00		a < c	28.00	17.00-40.00		
>10 years[c]	31.00	16.00-42.00			16.00	41.00-34.00			26.00	16.00-34.00		b < c	32.00	20.00-42.00		
Years at hospital																
<5 years[a]	33.00	16.00-42.00	1.31	.51	33.00	16.00-42.00	1.11	.57	34.00	16.00-39.00	8.64	.01†	30.00	17.00-40.00	0.69	.70
5-10 years[b]	31.50	10.00-42.00			32.00	10.00-42.00			28.00	25.00-42.00		a < c	32.00	24.00-42.00		
>10 years[c]	31.00	20.00-40.00			34.00	21.00-40.00			26.00	21.00-33.00			31.00	20.00-40.00		
Period of service in the unit																
<5 years[a]	32.00	16.00-42.00	0.28	.86	33.00	16.00-42.00	1.83	.39	34.00	16.00-42.00	10.30	.006†	30.00	17.00-42.00	0.03	.98
5-10 years[b]	32.00	10.00-42.00			34.00	10.00-42.00			28.00	22.00-36.00		a < c	31.00	23.00-37.00		
>10 years[c]	30.50	20.00-40.00			35.00	22.00-40.00			26.00	21.00-26.00			29.50	20.00-39.00		
Weekly working time																
40 hours	32.00	16.00-42.00	1.32	.51	33.50	16.00-42.00	3.25	.19	26.00	21.00-38.00	2.09	.35	32.00	23.00-42.00	3.09	.21
41-56 hours	32.00	10.00-42.00			34.00	10.00-41.00			31.00	16.00-42.00			30.00	21.00-40.00		
57-72 hours	32.00	17.00-42.00			33.00	20.00-42.00			29.00	22.00-38.00			31.00	17.00-37.00		
Characteristic	**Mdn**	**Min-max***	**U**	***P***	**Mdn**	**Min-max***	**U**	***P***	**Mdn**	**Min-max***	**U**	***P***	**Mdn**	**Min-max***	**U**	***P***
Received a certificate?																
Yes	32.00	16.00-42.00	2881.50	.34	34.00	16.00-42.00	818.00	.96	24.00	16.00-30.00	27.50	.06	27.00	22.00-32.00	121.50	.05†
No	32.00	10.00-42.00			33.00	10.00-42.00			29.00	21.00-42.00			31.00	17.00-42.00		
Received training in general duties, authorities and responsibilities?																
Yes	32.00	10.00-42.00	2490.50	.36	33.00	10.00-42.00	436.50	.54	29.00	21.00-42.00	68.50	.27	31.50	17.00-42.00	313.50	.12
No	30.00	16.00-39.00			34.00	25.00-39.00			26.50	16.00-36.00			28.50	22.00-35.00		
Received training in unit-specific duties, authorities, and responsibilities?																
Yes	32.00	16.00-42.00	3016.50	.10	33.50	16.00-42.00	601.00	.76	29.50	21.00-42.00	43.00	.03†	31.00	19.00-42.00	527.00	.33
No	31.00	10.00-39.00			33.00	10.00-39.00			25.50	16.00-33.00			30.00	17.00-36.00		
Received orientation at the hospital?																
Yes	32.00	10.00-42.00	1500.50	.01†	34.00	10.00-42.00	193.50	.16	29.00	21.00-42.00	15.00	.20	31.50	17.00-42.00	372.00	.15
No	30.00	16.00-35.00			32.00	24.00-34.00			22.00	16.00-28.00			30.00	20.00-35.00		
Received orientation at the unit worked in?																
Yes	32.00	16.00-42.00	1440.50	.40	33.50	16.00-42.00	140.00	.07	29.00	16.00-42.00	7.50	.42	30.00	17.00-42.00	357.50	.86
No	33.00	10.00-36.00			28.00	10.00-34.00			36.00	36.00-36.00			32.50	20.00-35.00		

ICU, intensive care unit.
*Min-max: minimum-maximum.
†$P < .05$.

experienced,[12] was experienced below the moderate level (15.20), or was experienced at the moderate level.[13] The results from international studies are also compatible with those of this study.[16,17] In two studies, it is seen that the conflict is experienced at a moderate level.[18,21] It is common to experience a role conflict in a profession that plays many roles simultaneously, such as nursing. Within the healthcare team, each member may consider his/her own demand to be more important. Responding to the family's demands while performing the roles of pediatric nurses may have an impact on their role conflict.

It can be stated that the nurses in the hospital have not experienced any role ambiguity. In a study conducted in our country, similar results were reported,[12] while in other studies, the ambiguity was reported to be below the moderate level,[20] at the moderate level,[13] or above the moderate level.[14,15,19,22] In other countries, findings supporting this study have been reported.[17,21] However, some other studies showed that it was experienced at a moderate level.[16,18] This difference between the results of the pediatric nurses and adult nurses may be due to the nature of the pediatric patient. Working in child clinics, the team carries out many initiatives, within or outside its role, taking the best interests of the child into account. Role ambiguity may also result from differences in the institutional structure. It can be said that job descriptions in the hospital are very clear. Considering that most of the nurses in the hospital hold at least an undergraduate degree (71.4%), it can be stated that nurses with higher education levels are more aware of their roles.

It is seen that the nurses in the clinic experienced more role conflicts than those in the ICU. In another study, it was found that the nurses in surgical clinics lived longer than those in the ICU, operating room, and emergency room.[27] In contrast, other studies reported that those in the ICU experienced more conflict[10] at a moderate level.[5] Pediatric intensive care nurse is the person who coordinates the team for the care of critically ill and highly vulnerable children.[25] In this study, it can be said that a team work was adopted in ICU, expectations from the intensive care nurse were compatible, and that the nurse did not have to choose between the roles despite the stressful

environment. The fact that the lowest percentage of holding an undergraduate degree was among the clinic nurses (54.8%) can be considered as a cause. A stable, permanent, and competent nursing workforce in the ICU improves patient outcomes.[25] The fact that the ICU nurses had the highest percentage in having a certificate and the clinic nurses had the lowest may have caused this result. The average number of patients in the ICU during day shift (2.17) and night shift (2.62) is considered to be standard, which indicates that their workloads are normal. On the other hand, it can be asserted that the difference in the number of patients between day and night shifts in the clinic caused this result.

We found that the role ambiguity score of the nurses did not change according to the unit they worked in. The findings of other studies are not in line with ours; the scores were higher[10] and above the moderate level.[5] The result of this study can be explained by the institutional arrangements and pediatric nurses' full comprehension of their duties, authorities, and responsibilities depending on their professional characteristics. Accountability, responsibility, and autonomy are required to improve patient outcomes in the intensive care environment.[5] We also observed that the nurses working in the ICU experienced the least role ambiguity. Relocation of the interrelated personnel in the institution is among the causes of role ambiguity.[14] Half of the nurses in the ICU have been working here for at least 5 years.

Role conflict and role ambiguity results of the nurses were different based on some of their characteristics. We found that this difference was not based on the nurses' educational background. The same result was obtained for role conflict[27] in one study and for role ambiguity in another.[15] In another study, those with an undergraduate degree experienced more role conflict than high school graduate.[14] While it is expected that the nurses with a higher level of educational would experience it less, the actual result can be explained by the average period of 12.59 years spent in the profession. In addition, it seems that this may be caused by the fact that the majority of the nurses have received training in duties, authorities, and responsibilities and orientation.

The process of gaining professional competence has been defined by Benner in five stages, namely, the "Novice to Expert" model: novice, advanced beginner, competent, proficient, and expert. The expert stage requires graduate and certified training with at least 5 years of experience and includes intuitive decision making based on knowledge and experience that does not require rules.[33] The role conflict in the nurses in hospitals, ICUs, and other featured units increases as the working time increases. In two studies, the exact opposite results of this study were obtained,[12,27] while in another study, there was no difference.[14] Role ambiguity in nurses in other featured units increases as the time spent in the profession increases. In other studies, however, it did not change.[12,14] This unexpected finding may be attributed to the fact that the graduate education (7.6%) and the certified education (17.6%) required by the expert stage are not sufficient. It is stated that nurses spend 30-40% of their time on jobs other than care, which are their main roles.[10] In comparison to the clinic, the weekly working time was higher and working in the night shifts was more in the hospital, ICU, and other featured units, which shows that the workload was also higher. From another point of view, this result can be explained by the decision-making feature of the expert stage that is not limited by the rules. Also, the concept of autonomy can be mentioned. Professional autonomy is to make its own decisions about own practices, and it emphasizes independent nursing roles.[1] It can be said that pediatric nurses possess professional autonomy.

Clinic nurses who own a certificate experience more role conflicts and role ambiguities than those who do not. This unexpected result can be explained by the fact that, in this group, the percentage of high school graduates (7.1%) and the percentage of those who worked in the unit for a period of less than 5 years (70.2%) was the highest, whereas the percentages of those who received orientation training from the hospital and from the unit worked were the lowest.

Role ambiguity was higher in nurses in other featured units who were not trained in unit-specific duties, authorities, and responsibilities. This result emphasizes the importance of clearly defining the responsibilities and limits of the nurse in featured units such as emergency, operating room, and burn unit, which are special branches of nursing that have its own special structure and functioning.

Newly started nurses have difficulty in integrating their professional roles with the institution's expectations regarding their roles.[34] At this point, orientation training helps these nurses acquire the necessary knowledge and skills.[35] Role conflict in the hospital and ICU nurses, and role ambiguity in the hospital nurses are more commonly observed in those who have not received orientation training from the hospital. It can be thought that an orientation training given on the hospital basis clarifies nursing roles.

Conclusion and Suggestions

We demonstrated that nurses in the hospital experienced role conflict above the moderate level; however, they experienced no role ambiguity. We found that nurses in the clinic experienced more role conflicts than those in the ICU. Hospital, ICU, or other featured unit nurses who spent more years in the profession; hospital and ICU nurses who spent more years in the hospital; nurses in other featured units who had a low total of weekly working time; clinic nurses who received a certificate; and hospital and ICU nurses who have not received orientation training from the hospital experienced more role conflicts. Other featured unit nurses who spent more years in the profession, the hospital, and the unit; clinic nurses who received a certificate; other featured unit nurses who have not received unit-specific training in duties, authorities, and responsibilities; and hospital nurses who have not received orientation training from the hospital experienced more role ambiguity.

The working environments in health institutions create a favorable ground for role conflict and role ambiguity. Developing strategies suitable for the institution in order to reduce role conflict and role ambiguity by executive nurses is recommended. For this purpose, a system where nurses can communicate their problems can be used to determine the root causes. Encouraging graduate and certificate education can yield results. This issue can be addressed in in-service training and orientation training programs organized at the institution or unit level for the newcomers. During the adaptation period, counselor nurses can be used. It is important to address this issue during nursing education before graduation in order to train nurses who are capable of responding to incompatible role expectations and

are aware of what their job is about. Conducting studies comparing pediatric and adult nurses, and revealing the nurses' perceptions and experiences about their roles with qualitative studies are recommended.

Limitations of the Research

This study had some limitations. The first one concerns the measuring tool. Scale scorings were performed differently in different studies. In this study, the study about the adapted scale was taken as the basis. It is important to take this situation into account when interpreting the findings and planning researches in the future.

The generalizability of the findings is limited due to the fact that our study was a single-center study. This study was conducted in a training and research hospital of a public university. Therefore, the results may not be generalized to all hospitals. Since the sample consisted of pediatric nurses, the results may not represent all nurses.

Ethics Committee Approval: Ethics committee approval was received for this study from the University of Health Sciences Dr. Behcet Uz Child Disease and Pediatric Surgery Training and Research Hospital Clinical Research Ethics Committee (approval date: September 26, 2019; approval number: 2019/14-11).

Informed Consent: Written informed consent was obtained from nurses who participated in this study.

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Original Article

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Systemic Inflammatory Markers as a Prognostic Factor in Parotid Gland Tumors

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BACKGROUND/AIMS
In this study, it was aimed to evaluate systemic inflammatory markers as prognostic factors in patients with parotid gland tumors.

MATERIAL and METHODS
Between 2014and2019, retrospective analysis of 181 patients who applied to Otorhinolaryngology Clinic and operated due to parotid gland tumor was performed. Additionally, 63 patients without tumor were included in the study as a control group. According to the post-operative histopathological diagnosis, it was divided into two groups as patients with benign and malignant tumors. The patients' age, gender, pre-operative blood tests, neutrophil-lymphocyte ratio(NLR), platelet-lymphocyte ratio(PLR), MPV-platelet ratio(MPVPR), MPV-lymphocyte ratio(MPVLR) and Systemic inflammatory index(SII) parameters calculated.

RESULTS
RBC, hemoglobin, hematocrit and lymphocyte levels were statistically significantly lower in patients with malign tumor compared to other groups. In addition, compared to benign tumor group, NLR, PLR and SII levels significantly increased and MPVLR level significantly decreased in patients with malign tumor. Consequently, according to ROC analysis for malign tumor, RDW, lymphocyte, NLR, PLR, MPVLR and SII were significant prognostic factors ($P = .036$, $P = .002$, $P = .033$, $P = .004$, $P = .001$, $P = .049$, respectively).

CONCLUSION
RDW, lymphocyte, NLR, PLR, MPVLR and SII parameters, which are fast, cheap and easy to use, can be used in the differential diagnosis of benign and malignant masses in patients with parotid gland tumors.

Keywords: Parotid gland tumors, red cell distribution width, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, systemic inflammatory index

INTRODUCTION

Salivary gland tumors (SGTs) constitute about 5–10% of all head and neck tumors, and approximately 80–85% of these tumors originate from the parotid gland.[1,2] Treatment of parotid tumors is primarily surgery. However, the planning of surgery varies depending on the histopathology and local spread of the tumor. Therefore, it is vital to distinguish whether the lesions are benign or malignant in the preoperative period. Fine needle aspiration biopsy (FNAB) is the most commonly used diagnostic method today. FNAB has over 95% sensitivity in the separation of tumor and nontumor pathologies, while 90% in the benign/malignant distinction.[1] Parotid tumors contain a large group of tumors with similar clinical and histopathological features. In these tumors, cystic degeneration is also frequently encountered. It is very difficult to make a definitive diagnosis in aspirates containing cystic rash.[1] An experienced cytopathologist is required for a definitive diagnosis.

In recent years, the number of studies evaluating the relationship between cancer and immune system has increased considerably. Epithelial inflammation is an important and diffuse pathological event that occurs alone or as a result of neoplasia.[3] First, inflammatory cells acts as a defense mechanism to prevent the formation of tumors task; however, preventing apoptosis may facilitate tumor proliferation and metastasis secondary to angiogenesis and DNA damage as a result of chronic inflammation.[4,5] Many researchers have reported that systemic inflammatory markers are important

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independent predictive markers in the diagnosis and prognosis of head and neck cancers, based on the idea that inflammation contributes to cancer onset and progression.[6–12] We aimed to evaluate the importance of preoperative systemic inflammatory markers (red cell distribution width [RDW], platelet, mean platelet volume [MPV], neutrophil–lymphocyte ratio [NLR], platelet–lymphocyte ratio [PLR], MPV–platelet ratio [MPVPR], MPV–lymphocyte ratio [MPVLR], and systemic inflammatory index [SII]) as a prognostic factor in the differential diagnosis of benign and malignant parotid masses in our clinic.

MATERIALS and METHODS

In our study, between 2014 and 2019, retrospective analysis of 181 patients who applied to University Research Hospital Otorhinolaryngology Clinic and operated on for parotid tumor was performed. In addition, as a control group, 63 patients without cancer were included in the study. Patients with active infection, hematological diseases, second primary cancer, and chronic inflammatory diseases, such as systemic lupus erythematosus and missing data, were excluded from the study. The study was approved by Gaziantep University Clinical Research Ethics Committee (2020/134). Written informed consent was obtained from the patients who participated in this study.

The patients' ages, genders, and preoperative blood tests (one day before surgery), and pathology results (tumor type, benign or malign) were obtained from the patients' files. In addition, NLR, PLR, MPVPR, MPVLR, the systematic inflammatory index (SII) values were calculated and recorded. The following parameters were calculated from the hemogram results performed at the preoperative stage.

The NLR was calculated by dividing the neutrophil count by the lymphocyte count. PLR was found by dividing platelet count by lymphocyte count. MPVPR was found by dividing the number of MPV by the number of platelets. MPVLR was found by dividing the number of MPV by lymphocyte count. The SII was found by multiplying neutrophil count and PLR value.

The tumor classification of the patients was made according to the TNM classification determined by The American Joint Committee on Cancer, which was modified in 2017. The stage of the tumor and lymph node involvement were evaluated based on histopathological evaluation. Analyses of all samples were performed on Sysmex XN-9100TM (Kobe, Japan) hematological auto-analyzer devices.

Main Points

- Compared to benign tumor group, NLR, PLR, and SII levels significantly increased and MPVLR level significantly decreased in patients with malign tumor.
- According to the results of ROC analysis in patients with malign tumor, sensitivity 60.7% and specificity 54.2% for RDW ($P = .036$), sensitivity 41.0% and specificity 40.8% for lymphocyte ($P = .002$), sensitivity 59.0% and specificity 56.7% for NLR ($P = .033$), sensitivity 59.0% and specificity 56.7% for PLR ($P = .004$), sensitivity 37.7% and specificity 35.0% for MPVLR ($P = .001$) were found.
- The risk factors found to be significantly related to differentiation in the regression analysis involved lymphocyte, NLR, PLR, and MPVLR, while the effect of age for differentiating malign tumor from benign tumor in multiple regression analysis was not statistically significant.

Statistical Analysis

All analyzes were done using Statistical Package for the Social Sciences (SPSS) version 25 (IBM SPSS Corp.; Armonk, NY, USA). *P* values less than .05 were considered significant. The normality control of the data was done with the Shapiro–Wilk test. Student's t test was used for comparing the means of normally distributed parameters, and Mann–Whitney U test was used for the comparison of parameters that did not conform to normal distribution. In multiple comparisons, Kruskal–Wallis variance analysis was used. Descriptive statistics were expressed with odds ratio and 95% confidence intervals. ROC analysis was used to determine the cut-off point, the area under the curve (AUC), the sensitivity (sensitivity), and the specificity (specificity) of the data. Multivariate logistic regression analysis performed to distinguish benign from malignant tumor in terms of factors age, MPV, platelet, RDW, lymphocyte, NLR, PLR, MPVPR, MPVLR, and SII.

RESULTS

Comparison of laboratory and socio-demographic findings between the groups of patient and control is shown in Table 1. The mean age of the patients was 49.59 ± 15.7. In the control group, the mean age was 42.76 ± 13.1. There was a statistically significant difference between the groups in terms of age ($P = .002$). Compared to the control group, WBC, MCV, MCH, RDW, and neutrophil levels were statistically significantly higher in the patient group ($P = .027$, $P = .007$, $P = .004$, $P = .032$, $P = .033$, respectively). Hemoglobin and hematocrit levels were statistically significantly lower in the patient group compared to the control group ($P = .020$, $P = .003$, respectively). There was not any statistical significance between the groups in terms of other parameters (Table 1).

Comparison of laboratory results according to tumor status is shown in Table 2. There was a statistically significant difference between groups in terms of WBC, RBC, hemoglobin, hematocrit, MCV, MCH, RDW, platelet, and lymphocyte levels ($P = .040$, $P < .001$, $P = .002$, $P < .001$, $P = .028$, $P = .017$, $P = .012$, and $P = .004$, respectively). WBC, MCV, MCH, and lymphocyte levels were statistically significantly higher in patients with benign tumor compared to control. RBC, hemoglobin, hematocrit, and lymphocyte levels were statistically significantly lower in patients with malign tumor compared to other groups. However, there was statistical significance between the groups in terms of NLR, PLR, MPVLR, and SII parameters ($P = .022$, $P = .010$, $P = .003$, and $P = .049$, respectively). In addition, compared to benign tumor group, NLR, PLR, and SII levels significantly increased and MPVLR level significantly decreased in patients with malign tumor (Table 2).

ROC analysis results in patients with malign tumor are shown in Table 3. According to the results of ROC analysis in patients with malign tumor, sensitivity 47.5% and specificity 48.3% for MPV ($P = .448$). sensitivity 47.5% and specificity 50.8% for platelet ($P = .638$), sensitivity 60.7% and specificity 54.2% for RDW ($P = .036$), sensitivity 41.0% and specificity 40.8% for lymphocyte ($P = .002$), sensitivity 59.0% and specificity 56.7% for NLR ($P = .033$), sensitivity 59.0% and specificity 56.7% for PLR ($P = .004$), sensitivity 54.1% and specificity 50.0% for MPVPR ($P =$

TABLE 1. Comparison of Laboratory and Sociodemographic Findings

	Patient (n = 181)	Control (n = 63)	*P* value
Age (years)	49.59 ± 15.7	42.76 ± 13.1	**.002***
Gender (n, %)			.658
Male	107 (59.1%)	35 (55.6%)	
Female	74 (40.9%)	28 (44.4%)	
WBC (mm^{-3})	8.20 (3.7-13.7)	7.64 (3.9-13.8)	**.027****
RBC (μL)	5.02 ± 0.5	5.19 ± 0.4	**.016***
Hemoglobin (g dL^{-1})	14.61 ± 1.8	14.67 ± 1.4	**.020***
Hematocrit (%)	43.65 ± 4.9	44.24 ± 3.7	**.003***
MCV (fL)	87.40 (61.2-98.3)	85.20 (69.6-94.9)	**.007****
MCH (pg)	29.20 (18.6-33.2)	28.60 (21.9-31.3)	**.004****
MCHC (g dL^{-1})	33.44 ± 1.2	33.14 ± 1.3	.561*
RDW (%)	13.30 (11.5-22.7)	12.90 (11.8-17.2)	**.032***
Platelet (mm^{-3})	270.71 ± 58.3	268.79 ± 59.4	.501*
MPV (fL)	10.20 (6.8-12.3)	10.40 (9.1-13.8)	**.049****
Neutrophil (mm^{-3})	4.89 (2.0-11.1)	4.17 (1.4-8.9)	**.033****
Lymphocyte (mm^{-3})	2.47 (0.7-5.7)	2.34 (1.3-4.7)	.274**
NLR	1.92 (0.81-14.75)	1.89 (0.67-5.00)	.503**
PLR	105.94 (38.1-374.1)	112.06 (39.3-217.7)	.417**
MPVPR	0.038 (0.01-0.10)	0.037 (0.02-0.08)	.647**
MPVLR	4.08 (1.2-15.5)	4.48 (2.2-9.9)	.150**
SII	498.62 (125.9-2714.0)	490.82 (163.9-1395.7)	.423**

WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red cell distribution width; MPV, mean platelet volume; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; MPVPR, MPV–platelet ratio; MPVLR, MPV–lymphocyte ratio; SII, systemic inflammatory index.
*Student t test.
**Mann–Whitney U test.

TABLE 2. Comparison of Laboratory Results According to Tumor Status

	Control (n = 63)	Benign (n = 120)	Malign (n = 61)	*P* value
WBC (mm^{-3})	7.64 (3.9–13.8)	8.46 (3.7–13.7)*	7.98 (3.9–13.5)	**.040**
RBC (μL)	5.19 ± 0.4	5.14 ± 0.5	4.82 ± 0.5†	**<.001**
Hemoglobin (g dL^{-1})	14.67 ± 1.4	14.92 ± 1.7	13.99 ± 1.7†	**.002**
Hematocrit (%)	44.2 ± 3.7	44.6 ± 4.5	41.6 ± 5.0†	**<.001**
MCV (fL)	85.20 (69.6–94.9)	87.50 (65.7–97.5)*	86.90 (61.2–98.3)*	**.028**
MCH (pg)	28.60 (21.9–31.3)	29.25 (20.4–33.2)*	29.00 (18.6–32.9)*	**.017**
MCHC (g dL^{-1})	33.14 ± 1.3	33.38 ± 1.3	33.55 ± 1.1	.216
RDW (%)	12.90 (11.8–17.2)	13.20 (11.5–19.8)	13.50 (11.9–22.7)*	**.012**
Platelet (mm^{-3})	268.79 ± 59.4	271.44 ± 51.7	269.28 ± 69.9	.949
MPV (fL)	10.40 (9.1–13.8)	10.20 (7.7–12.3)	10.10 (6.8–12.3)	.305
Neutrophil (mm^{-3})	4.17 (1.4–8.9)	4.95 (2.1–10.9)	4.83 (2.0–11.1)	.103
Lymphocyte (mm^{-3})	2.34 (1.3–4.7)	2.62 (0.7–5.7)*	2.31 (0.7–5.5)†	**.004**
NLR	1.89 (0.6–5.0)	1.82 (0.8–8.6)	2.00 (0.9–14.7)†	**.022**
PLR	112.06 (39.3–217.7)	101.71 (46.8–374.1)	120.58 (38.1–318.6)‡	**.010**
MPVPR	0.037 (0.02–0.08)	0.038 (0.02–0.06)	0.038 (0.01–0.10)	.876
MPVLR	4.48 (2.2–9.9)	4.33 (1.2–15.6)	3.72 (1.6–9.5)*	**.003**
SII	490.82 (163.9–1395.7)	481.96 (222.6–2192.1)	511.12 (125.9–2714.0)‡	**.049**

WBC, white blood cell; RBC, red blood cell; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red cell distribution width; MPV, mean platelet volume; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; MPVPR, MPV–platelet ratio; MPVLR, MPV–lymphocyte ratio; SII, systemic inflammatory index.
*Statistically significant compared to control.
†Statistically significant compared to other groups.
‡Statistically significant compared to benign.

.888), sensitivity 37.7% and specificity 35.0% for MPVLR (P = .001), sensitivity 55.7% and specificity 56.7% for SII were found. The cut-off point for these values were <10.15, >263.5, >13.25, <2.40, >1.92, >106.9, >0.037, <3.91, and >505.8, respectively. As a result, according to ROC analysis for malign tumor, MPV, platelet, and MPVPR were not found to be a prognostic factor, although the RDW, lymphocyte, NLR, PLR, MPVLR, and SII were significant prognostic factors (Table 3 and Figure 1).

Multivariate logistic regression analysis performed to distinguish benign from malignant tumor is shown in Table 4. The risk factors found to be significantly related to differentiation in the

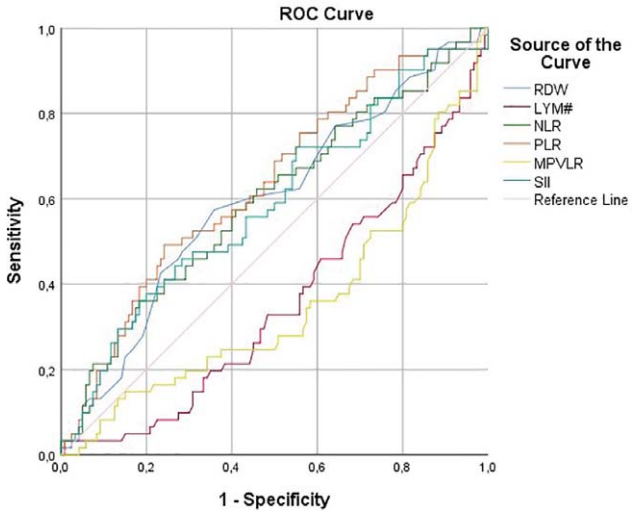

Figure 1. ROC analysis of RDW, lymphocyte, NLR, PLR, MPVLR, and SII (RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; MPVLR, mean platelet volume–lymphocyte ratio; SII, systemic inflammatory index)

regression analysis involved lymphocyte, NLR, PLR, and MPVLR while the effect of age for differentiating malign tumor from benign tumor in multivariate logistic regression analysis was not statistically significant (Table 4).

DISCUSSION

In our study, WBC, hemoglobin, RBC, hematocrit, MPV, RDW, MCV, MCH, and neutrophil levels were statistically significant in parotid tumor patients compared to healthy individuals. However, compared to benign tumor group, NLR, PLR, and SII levels significantly increased and MPVLR level significantly decreased in patients with malign tumor. As a result, according to ROC analysis for malign tumor, MPV, platelet, and MPVPR were not found to be a prognostic factor, even though the RDW, lymphocyte, NLR, PLR, MPVLR, and SII were significant prognostic factors. In addition, the risk factors found to be significantly related to differentiating malign tumor from benign tumor in the regression analysis included lymphocyte, NLR, PLR, and MPVLR.

MPV was an early marker of activated platelets. Greater platelet reactivity has more than smaller ones.[13] The relationship between MPV and cancer has been researched in different cancer types.[14,15] It has been shown that thyroid papillary carcinomas have higher MPV levels than benign goiter patients and the control group, and MPV levels decrease after surgical treatment.[15] In a study by Fu et al.[16] in 216 laryngeal cancer patients, they showed that MPV was significantly lower in laryngeal cancer patients compared with control. It is also stated that MPV is one of the independent risk factors for distinguishing malignant laryngeal tumor from benign laryngeal tumor.[16] Eryilmaz et al.[17] reported that MPV levels of the patients were significantly high in their study on 96 head and neck cancer patients. In contrast to, MPV levels were statistically significantly lower in the parotid tumor group compared to the control group in our study.

RDW is a parameter that shows the degree of heterogeneity of erythrocyte volume. Its level increases in diseases, such as venous thromboembolism, diabetes, cardiovascular disease, and cancer.[18] It is stated that RDW is a prognostic factor in various types of cancer, such as lung, breast, esophagus, brain, kidney, stomach, colon, pancreas, prostate, multiple myeloma, and lymphoma.[19,20] Kara et al.[8] showed that the RDW prognostic effects in 103 larynx carcinoma patients. In our study, RDW levels were statistically significantly higher in the patient with malign tumor group compared to control group. As a result, according to ROC analysis for malign tumor, it was determined that RDW is one of the prognostic factors.

Lymphocytes have a potent antitumor response of cellular immune and are important immune system components.[21] Compared to neutrophils, lymphocytes supply host defense against tumors; hence, lymphocyte increase is related to a better prognosis.[22] On the contrary, lymphopenia shows impaired cellular immunity against tumors and is related to poor prognosis.[23] In our study, lymphocyte levels were statistically significantly lower in patients with malign tumor compared to control and benign tumor group. In addition, as a result according to ROC analysis for malign tumor, it was determined that lymphocyte is one of the important prognostic factors.

In the study of Damar et al.[2] on 182 patients covering all SGTs, they reported that high NLR and low lymphocyte count can be used to distinguish malignant tumors from benign tumors. The PLR was also presented as prognostic value in various different tumor types (tongue cancer, oral cancer, etc.).[24,25] In the study of Ekici and Kuran[26] on 134 patients, they stated that NLR and PLR can be used in the differential diagnosis of benign and malignant masses in parotid gland tumors patients. In the study of Kuzucu et al.[10] in 145 patients with parotid tumors, they stated that preoperative PLR and NLR values are higher malignant parotid tumors patients than benign parotid tumor patients. In our study, there was statistical significance between the groups in terms of PLR and NLR parameters. In addition, compared to benign tumor group, NLR and PLR levels significantly increased in patients with malign tumor. However, according to the results of ROC analysis in patients with malign tumor, the RDW, PLR, and NLR were significant prognostic factors.

Deveci and Sürmeli[7] reported that high SII values were associated with increased perineural/lymphovascular invasion and extranodal involvement. Ekici and Kuran[26] did not find a statistically significant difference in their study on 134 patients with parotid gland tumors, although the SII value was higher in the malignant tumor group. In our study, compared to benign tumor group, SII levels significantly increased in patients with malign tumor. However, as a result according to ROC analysis for malign tumor, it was determined that SII is one of the important prognostic factors.

Recently, the MPVLR was shown as one of the complete blood count parameters. It was first shown by Kuzucu et al.[10] in patients with myocardial infarction and diabetes as a potential prognostic marker in 2016. In the study of Sut et al.[27] in 78 patients with breast cancer, compared to control, they reported that MPVLR, NLR, and PLR levels were significantly higher in patients with breast cancer. In addition, a significant association was found between NLR, PLR but not MPVLR and low dietary polyphenol intake in breast cancer patients.[27] In our study, NLR, PLR, and SII levels significantly increased and MPVLR

TABLE 3. ROC Analysis Results in Patients with Malign Tumor

	Cut-off	Sensitivity	Specificity	AUC (95% CI)	*P* value
MPV (fL)	<10.15	47.5%	48.3%	0.465 (0.371–0.560)	.448
Platelet (mm^{-3})	>263.5	47.5%	50.8%	0.479 (0.381–0.576)	.638
RDW (%)	>13.25	60.7%	54.2%	0.596 (0.507–0.684)	**.036**
Lymphocyte (mm^{-3})	<2.40	41.0%	40.8%	0.358 (0.274–0.442)	**.002**
NLR	>1.92	59.0%	56.7%	0.597 (0.508–0.686)	**.033**
PLR	>106.9	59.0%	56.7%	0.631 (0.545–0.717)	**.004**
MPVPR	>0.037	54.1%	50.0%	0.494 (0.394–0.593)	.888
MPVLR	<3.91	37.7%	35.0%	0.351 (0.263–0.439)	**.001**
SII	>505.8	55.7%	56.7%	0.589 (0.499–0.678)	**.051**

AUC, area under the curve; MPV, mean platelet volume; RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; MPVPR, MPV–platelet ratio; MPVLR, MPV–lymphocyte ratio; SII, systemic inflammatory index.

TABLE 4. Multivariate Logistic Regression Analysis of Factors Used for Differentiating Malign Tumor from Benign Tumor

	β	OR (95% CI)	*P* value
Age (year)	0.018	1.018 (0.997–1.039)	.091
MPV (fL)	–0.267	0.766 (0.534–1.097)	.145
Platelet (mm^{-3})	–0.002	0.998 (0.992–1.004)	.589
RDW (%)	0.049	1.050 (0.776–1.422)	.176
Lymphocyte (mm^{-3})	–0.566	0.568 (0.369–0.873)	**.010**
NLR	1.239	1.239 (1.007–1.525)	**.043**
PLR	0.007	1.007 (1.001–1.013)	**.028**
MPVPR	0.001	0.011 (0.008–0.014)	.398
MPVLR	–0.279	0.757 (0.597–0.959)	**.021**
SII	0.001	1.001 (1.000–1.002)	.079

MPV, mean platelet volume; RDW, red cell distribution width; NLR, neutrophil–lymphocyte ratio; PLR, platelet–lymphocyte ratio; MPVPR, MPV–platelet ratio; MPVLR: MPV–lymphocyte ratio; SII, systemic inflammatory index.

level significantly decreased in patients with malign tumor. As a result according to ROC analysis for malign tumor, RDW, lymphocyte, NLR, PLR, MPVLR, and SII were significant prognostic factors. However, as a result of multivariate logistic regression analysis, the risk factors found to be significantly related to differentiation in the regression analysis, including lymphocyte, NLR, PLR, and MPVLR used for differentiating malign tumor from benign tumor. According to the literature knowledge, this is the first research to examine the relation between MPVLR and in patients with parotid cancer. We consider that reduced MPVLR may reflect an impaired interaction between tumor cells, blood platelets, and lymphocyte dependent immune response.

Studies in the literature report that most of the systemic inflammatory markers change with age (e.g., RDW decreases, MCV, NLR increase) in a healthy population.[28–32] In our study, although there was a significant difference between the patient group and the healthy group in terms of age, the effect of age for differentiating malign tumor from benign tumor in multivariate logistic regression analysis was not statistically significant.

As limitations in our study, our study is a single center and retrospective study. The small sample size and age differences were other limitations. Moreover, multicenter and prospective studies could be planned to backup these preliminary results. Compared to other studies in the literature,[2,8,17,26] the strengths of our study, our sample was larger and our results were supported by a logistic regression analysis.

In conclusion, as far as we know, this study is the first study to evaluate different numbers and rates of leukocytes (especially MPVLR) together before surgery in patients with benign and malign parotid gland tumors. These parameters which are cheap and easy can be used as a potential prognosis factor in patients with parotid tumors. Our results propound that the combination of RDW, lymphocyte, NLR, PLR, and MPVLR could be utilized as a potential inflammatory marker in patients to differentiate benign from malignant parotid gland tumors. We think that these values should be defined in laboratory devices and added to blood results in order to facilitate the clinician's work. However, new prospective researches with a larger group of patients are needed to determine the reference intervals of these values and to use them as prognostic factors.

Ethics Committee Approval: Ethical committee approval was received from the Gaziantep University Clinical Research Ethics Committee (2020/134).

Informed Consent: Informed consent was obtained from all participants who participated in this study.

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Interpretation - O.T., M.K.; Literature Search - O.T., B.G., M.K.; Writing - O.T., B.G., M.K.; Critical Reviews - O.T., M.K.

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Case Report

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An Uncommon Cause of Hypernatremia in Very Low Birth Weight Premature Infants: Idiopathic Central Diabetes Insipidus

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Central diabetes insipidus (CDI) is a water homeostasis disorder characterized by an inability to concentrate urine because of insufficient production of antidiuretic hormone. Dehydration with hypernatremia can occur during the neonatal period in preterm neonates in association with insensible water loss, high urine output, and reduced sodium excretion. A high index of suspicion is required to diagnose CDI in preterm neonates. We report two cases, who presented persistent hypernatremia with polyuria despite increased fluid supply and low sodium intake. CDI diagnosis was confirmed by the therapeutic test with oral vasopressin analog. Investigations were all normal; CDI was considered idiopathic. Persistent hypernatremia despite increased fluid intake with polyuria, hyposthenuria, low urine output, and high plasma osmolality is the key point for the diagnosis.

Keywords: Central diabetes insipidus, premature infants

INTRODUCTION

Diabetes insipidus (DI) is a water homeostasis disorder characterized by an inability to concentrate urine because of insufficient production of antidiuretic hormone (ADH) (central diabetes insipidus, CDI) or due to impaired kidney response to ADH (nephrogenic diabetes insipidus).[1,2] CDI in a neonate is usually associated with congenital abnormalities of the central nervous system, hypoxic-ischemic encephalopathy, meningitis, encephalitis, or severe intraventricular hemorrhage (IVH) in preterm neonates.[3] During neonatal period, dehydration with hypernatremia can occur due to insensible water loss, high urine output, and reduced sodium excretion in preterm neonates. Therefore, a high index of suspicion is required to diagnose CDI in very low birth weight infants.[2–4]

To alert for CDI diagnosis and early treatment in the neonatal period, two cases of very low birth infants with idiopathic CDI, who were successfully controlled through lyophilized sublingual desmopressin, were reported.

CASE PRESENTATION

Case 1

A male preterm neonate with a gestational age of 31 weeks and a birth weight of 1215 g was born by cesarean section (C/S) with Apgar scores of 4 and 7 at 1st and 5th minutes, respectively. He was on nasal intermittent positive-pressure ventilation and did not require surfactant therapy. Antibiotic therapy was begun because of high CRP level. On the 5th day of life due to increase in CRP, oxygen requirement ($FiO_2 > 40\%$), and respiratory acidosis, he was intubated, surfactant was given, and antibiotic treatment was changed. He was extubated the next day followed by noninvasive ventilation for 2 weeks. On the 13th day of life, although the daily Na intake was 1.2 meq kg^{-1} d^{-1} and the daily fluid supply was 180 mL kg^{-1} d^{-1}, the serum Na level and urine output were 152 mmol L^{-1} and 8 mL kg^{-1} h^{-1}, respectively. The urinary and plasma osmolalities were 98 and 302 mOsm kg^{-1}, respectively. The density of urine was around 1005. The ADH level was 2.30 pmol L^{-1}. Thus, DI was diagnosed and desmopressin treatment was begun (2 × 1.5 mcg per oral). The urine output decreased to 4-5 mL kg^{-1} h^{-1}, and the plasma Na level decreased to 140 mmol L^{-1}. Transfontanelle sonography did not demonstrate IVH. The evaluation of the pituitary axes revealed normal thyroid, adrenal, and gonadal functions. Serological tests for syphilis, toxoplasmosis, cytomegalovirüs, herpes simplex, and rubella (TORCHS) ruled out

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these congenital infections. No abnormalities were observed in serum urea nitrogen, creatinine, potassium, calcium, and bicarbonate levels. Magnetic resonance imaging (MRI) of the brain was planned to evaluate pituitary gland and the other structures of the brain, but parents did not approve it because of sedation. The etiology remains unknown. He was discharged on postnatal on 52nd day of life, at 38/4 corrected age, weighing 2285 g, with a prescription of 2 × 1.5 mcg of oral desmopressin per day.

Case 2

A male preterm neonate with a gestational age of 31 weeks and a birth weight of 1015 g was born by C/S with Apgar scores of 4 and 7 at 1st and 5th minutes, respectively. He was intubated in the delivery room, surfactant was given for the diagnosis of respiratory distress syndrome, and then extubated followed by noninvasive ventilation for 10 days. On the 12th day of life, respiratory distress reappeared and oxygen requirement increased (FiO_2 40%). He was thought to develop pneumonia and re-intubated. He was extubated on the next day followed by noninvasive ventilation for 2 weeks. On the 24th day of life, we noticed that the urine output (7.78 mL kg^{-1} h^{-1}) and plasma Na level were high, while he was receiving Na supply at a maintenance dose of 3 meq kg^{-1} d^{-1}. The urine osmolality was low (153 mOsm kg^{-1}), serum osmolality (292 mOsm kg^{-1}), and plasma Na level (146 mmol L^{-1}) were high. DI was thought and lyophilized sublingual desmopressin (2 × 3 mcg) was begun. After treatment, the serum Na level was between 139 and 142 mmol L^{-1}, and the urine output decreased to 4-5 mL kg^{-1} d^{-1}. Due to desmopressin response, CDI diagnose was made. Transfontanelle sonography did not demonstrate IVH. The evaluation of pituitary axes revealed normal thyroid, adrenal, and gonadal functions. Serological tests for TORCHS were normal. No abnormalities were observed in serum urea nitrogen, creatinine, potassium, calcium, and bicarbonate levels. Desmopressin was used for 23 days. Before discharge, we stopped desmopressin treatment. After 2 days without desmopressin treatment, the urine density was 1015, and plasma and urine osmolalities were 281 and 123 mOsm kg^{-1}, respectively; the plasma Na and plasma ADH levels were 142 mmol L^{-1} and 3.41 pmol L^{-1}. Therefore, the patient was discharged without treatment. Ten days after discharge, the serum Na level and urine density were 141 mmol L^{-1} and 1010, respectively. CDI disappeared, but the etiology remained unknown.

DISCUSSION

In very-low-birth weight infants, fluid–electrolyte homeostasis in the first week of life is generally characterized by low urine output in the first few days and polyuria thereafter. This causes physiological weight loss and an increase in the serum N concentration, which is aggravated by transepidermal water loss. Generally, this diuretic phase gets over by the end of the first week of life, and hypernatremia is rare thereafter.[5] Persistent hypernatremia despite increased fluid intake with polyuria, hyposthenuria, low urinary osmolality, and high plasma osmolality should alert the clinician for the diagnosis of DI.[6] In present cases, despite the increase in the total fluid intake, patients' serum Na and urine outputs remained elevated. The detection of low urine osmolality with high serum osmolality and good response to desmopressin confirmed the CDI diagnosis. Until now, underlying causes of CDI reported are intraventricular hemorrhage, meningitis, septo-optic dysplasia, Listeria monocytogenes sepsis, congenital cytomegalovirus infection, midline intracranial defects, and following surgical resection of a suprasellar mass.[7–9] Idiopathic CDI accounts for 12-24% of the cases,[8–10] but idiopathic CDI prevalence in premature infants is higher.[10] Diagnostic work-up, including the level of hypothalamic-pituitary axis hormones, infectious causes such as serological tests for TORCHS of present cases, were all normal. Transfontanelle sonography revealed no intraventricular hemorrhage.

ADH analog–desmopressin, which is available in three different forms, namely, oral, parenteral, and intranasal preparations, is used to reduce urine output and to decrease serum Na levels to normal range.[11] Many studies recommended oral desmopressin lyophilisate because of its efficiency, ease of use, and better tolerance.[10,12,13] Regardless of the route, treatment requires careful adjustment of dose as neonates rely on liquid diet and desmopressin may cause fluid overload with wide fluctuations in serum Na levels. Thus, beginning with low dosage twice a day and then increasing according to serum Na levels and urine output should be better.[11,12] Both the cases were treated with sublingual lyophilized desmopressin twice a day. Similarly, Ozaydın et al.[14] and Atasay et al.[15] used sublingual desmopressin lyophilisate at a dosage of 2.5 µg kg^{-1} d^{-1} to manage CDI in very low birth weight premature infants.

Karthikeyan et al.[10] reported the first case series of CDI. The most common cause was septo-optic dysplasia but of note idiopathic isolated CDI was diagnosed in three of every premature infants. None of the preterm infants had significant intracranial hemorrhage. The median serum Na and serum and urine osmolalities at diagnosis were 156 mmol L^{-1} (range: 145-175), 320 (range: 300-345) and 112 mOsm kg^{-1} (range: 66-322), respectively, as reported cases.

In conclusion, polyuria despite increased fluid intake, hyposthenuria, low urinary, and high plasma osmolality and persistent hypernatremia are the key points for the diagnosis.

Main Point

- Central diapedes insipidus is rare and diagnosis is difficult in preterm infants.
- Persistent hypernatremia, dehydration and polyuria beyond first week of life should alert neonatologists.
- Oral desmopressin should be started as soon as the diagnosis is suspected.
- This treatment should be begun with low dosage and be closely monitored.

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CYPRUS JOURNAL OF MEDICAL SCIENCES

Case Report

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A Rare Complication of Central Venous Catheter: Innominant Vein Perforation and Endovascular Treatment

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The incidence of central venous catheterization is increasing worldwide. It is important to consult an experienced physician after a failed procedure or in the case of complications. The use of ultrasound during the procedure will increase its success. Left internal jugular vein catheterization is at high risk for vascular injury due to its anatomical position. A 65-year-old female patient with chronic renal failure was referred to our interventional radiology unit with suspected innominant vein perforation. We aimed to present our successful endovascular treatment method in the patient.

Keywords: Central venous catheter, venous perforation, coiling

INTRODUCTION

Central venous catheters are used for different reasons such as hemodialysis, fluid infusion, apheresis, and central venous pressure measurements. Secondary to the advances in medical technologies, prolongation of life expectancy leads to the increase of hypertension and diabetic diseases, and the prevalence of chronic renal failure also causes an increase. Hemodialysis (HD) is the most common renal replacement therapy in patients with chronic renal failure. Due to the low risk of thrombosis and infection, 50% of patients are treated with mature AV fistula hemodialysis. Temporary catheters are preferred in patients requiring emergency HD treatment and in patients whose catheter is expected to remain for less than 3-4 weeks. Permanent catheters are used in patients with heart failure, peripheral arterial disease, short life expectancy, and severe uremia, which cannot be expected for the maturation of the fistula. The use of a permanent catheter in chronic HD patients is reported as 32%.[1] Internal jugular vein and subclavian vein are the first options for central venous catheterization, and femoral vein with a higher risk of mechanical complications is preferred if these veins cannot be accessed. The risk of mechanical complications in central venous catheterization varies between 5 and 19%, where infectious complications occur in 5-26% and thromboembolic complications occur in 2-26%.[2]

CASE PRESENTATION

A 65-year-old female patient who had been followed-up with a diagnosis of chronic renal failure for 15 years underwent left internal jugular vein catheterization for hemodialysis at the outer center. After the procedure because of the absence of blood from the hemodialysis catheter, PA-chest direct radiography was performed. In the radiograph, it is observed that the catheter tip extends to the mediastinum. The patient was evaluated together by our hospital's cardiovascular surgeons and interventional radiologists. It was decided that mediastinal access might be required during the operation and, considering the possible risks, endovascular treatment would be appropriate. The patient's coagulation parameters were within normal limits. The patient was admitted to the angiography unit. A 6F sheath was inserted into the axillary vein, and then a 10 cc 50% dilated contrast medium was taken from the sheath. We shoved that the hemodialysis catheter was perforated in the innominate vein at the junction of the left internal jugular vein and subclavian vein.

The 5F vertebral catheter passed through the lumen of the hemodialysis catheter, and when the tip extended to the mediastinum, the hemodialysis catheter was slowly removed from the vertebral catheter (Figure 1). Then, the coiling was

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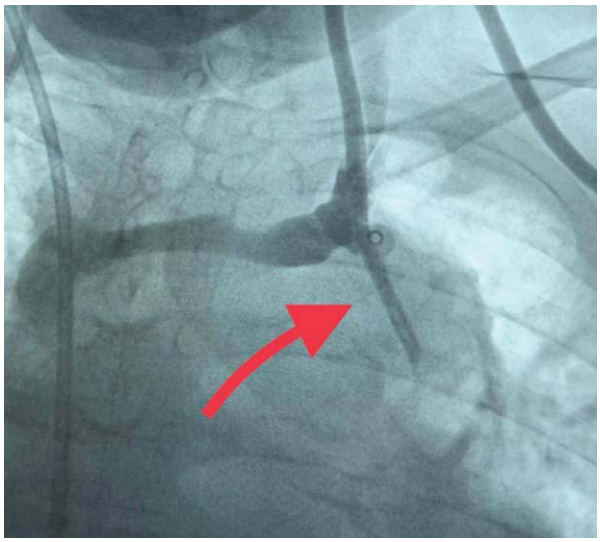

Figure 1. 5F vertebral catheter (red curved arrow) extending from the innominant vein to the mediastinum is seen with the contrast material given from the 6F sheath in the axillary vein

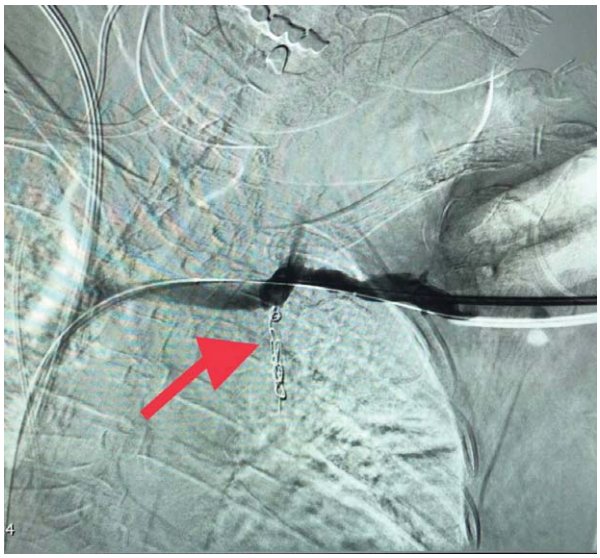

Figure 2. Coil materials (red arrow) extending to the posterior wall of the innominant vein are observed

quickly performed from the distal of the 5F vertebral catheter to the vessel lumen. Coiling was terminated when the distal end of the 5F vertebral catheter leaned against the inferior wall of the innominate vein. Afterward, 10 cc 50% dilated iodinated contrast agent injection was performed from the 6F sheath in the axillary vein. Angiographic evaluation showed that no contrast agent extravasation and the procedure were terminated by withdrawing the 6F sheath and the 5F vertebral catheter (Figures 2 and 3). Then, a hemodialysis catheter was placed with femoral venous access.

Main Points

- Experience and multidisciplinary approach are important in interventional procedures.
- The risk of complications is lower in procedures performed with imaging.
- Smart decisions can be life-saving in case of complications.

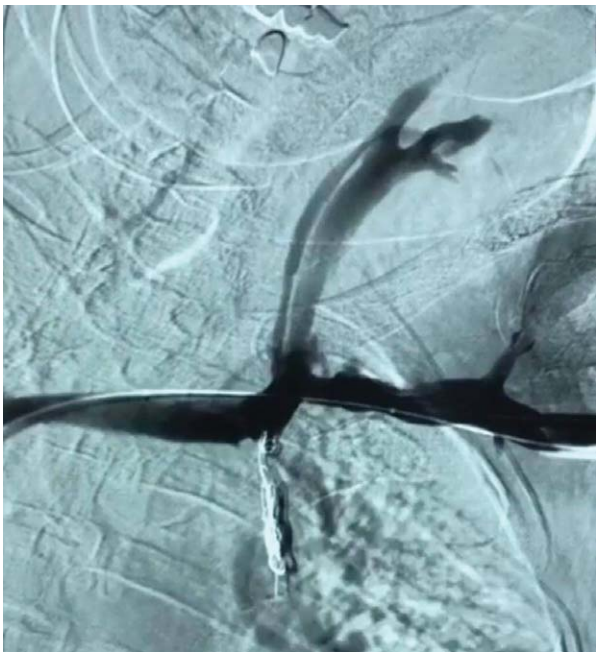

Figure 3. After coiling, the contrast agent given from the 6F sheath in the axillary vein showed no extravasation

DISCUSSION

Early complications of central venous catheterization are catheter malposition, catheter fracture, arterial puncture, air embolism, large vessel perforation, pneumothorax-hemothorax-chylothorax, and cardiac arrhythmia, and late complications include central vein thrombosis, development of infection leading to sepsis, and arteriovenous fistula and pseudoaneurysm at the site of intervention. fibrin sheath formation and catheter break (pinch-off syndrome). The risk of complications varies depending on the experience of the person performing the procedure, the weight of the patient, history of radiotherapy or surgical procedures at the site of intervention, and the number of interventions. The ultrasound-guided procedure significantly reduces the risk of complications.[3–5] Left subclavian and internal jugular vein catheterization are high risk procedures of venous perforation because the innominate vein forms a vertical angle to the SVC. Vascular perforation due to catheter can be seen between days 1 and 60 after catheter insertion, whereas 50% occurs on day 2.[6] Catheter malposition should be suspected in the absence of blood or any other thing such as air, chylous fluid, and nonpulsatile blood.[7] Previous case reports have reported that vascular injury/perforation due to catheter malposition is corrected by surgical intervention.[8,9] In the case report of Singh, it was reported that the patient with innominant vein perforation underwent successful stent graft application with femoral vein access.[10] In our case, this method was not suitable for our patient because the stent application would prevent the internal jugular vein flow. Central venous

catheterization should be performed under the control of an experienced physician. Catheter location must be checked by chest X-ray after the procedure. In order to reduce the possibility of complications, it is useful to perform the procedure with ultrasonography.

In conclusion, central venous catheters will continue to be applied with the increasing frequency. Imaging-guided procedures reduce the risk of complications. We believe that this smart method will guide physicians interested in central venous catheterization.

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Letter to the Editor

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Overweight and Obesity among School Children

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Dear Editor,

Despite the significant ethnic differences, the prevalence of overweight and obesity in primary school students is a serious public health problem in Cyprus, Turkey, and Brazil.[1-4] Excess of body weight has been a global burden associated with metabolic and cardiovascular disorders with a significant reflex in both the quality and expectancy of life in adulthood.[1-4] Health policies must control and reduce the "overweight and obesity pandemic" across the world. Comparisons among data of some studies developed in school children of Cyprian and Brazilian populations between 2004 and 2019 seem useful to know about the modified parameters.

Lazarou et al.[2] evaluated 1140 children aged between 9 and 13 years with a mean age of 10.7 years, in which 177 boys and 231 girls were obese [p: .004], whereas 187 boys and 259 girls had overweight [p: .177]. The authors found a correlation of obesity with screen viewing time, mainly among girls, and emphasized interventions to avoid sedentary behaviors in this young Cyprian population.[2] More recently, Arikan et al.[1] studied the body mass index of 10,781 students from 16 primary schools of Konya city center in Turkey. They were aged between 6 and 15 years, in which 52% were boys. Also, they found that 5.8% of overall prevalence of obesity were among boys and 5.3% among girls, while 7.4% of overall prevalence of overweight occurred in individuals of both genders.[1] The authors suggested new studies to define the best strategies for weight control in childhood. Xavier et al.[4] evaluated 229 Brazilian students aged between 5 and 15 years with a mean age of 8.0 years, in which 51.5% were girls, and found that the prevalence of overweight and obesity was 11.8% and 13.5%, respectively. There was no significant difference in the data between the gender and age groups of students. Weight gain showed a nonstatistical association with television viewing time for more than two hours daily; however, there was no relation between physical inactivity or screen viewing time and obesity. The authors commented on the role of parents and healthcare workers in preventive tasks.[4] Pereira et al.[3] investigated weight gain and high blood pressure (HBP) in 888 Brazilian students aged between 6 and 10 years with a mean age of 7.7 years, in which 51.7% were girls, and found that the prevalence of overweight and obesity were 17.7% and 16.2%, respectively. Obesity was associated with HBP, even after adjusting gender, ie, 43.6% in the 6-7 years old group and 62.9% in the 8-9 years-old group. There was no significant difference in the variables between the gender and age groups of students.[4]

The commented manuscripts highlighted the requirement of preventive programs including weight excess and arterial hypertension among school children.

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