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European Journal of Therapeutics (Eur J Ther) is the double-blind peer-reviewed, open access, international publication organ of the Gaziantep University School of Medicine. The journal is a quarterly publication, published on March, June, September, and December. The journal publishes content in English.

European Journal of Therapeutics aims to contribute to the international literature by publishing original clinical and experimental research articles, short communication, review articles, technical notes, and letters to the editor in the fields of medical sciences. The journal's target audience includes researchers, physicians and healthcare professionals who are interested or working in in all medical disciplines.

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Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html).

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Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards (Altman DG, Gore SM, Gardner MJ, Pocock SJ. Statistical guidelines for contributors to medical journals. Br Med J 1983: 7; 1489–93). Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

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Review Article	5000	250	50	6	10 or total of 20 images
Short Communication	1500	200	20	5	1 or total of 5 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

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Contents

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Original Article

Should Women Between the Ages of 25 and 30 Get Tested for HPV?

Reyhan Gündüz[1], Elif Ağaçayak[1], Özlem Polat Bozbay[1], Ulaş Alabalık[2], Mehmet Sait İçen[1], Talip Gül[1]

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ABSTRACT

Objectives: The aim of this study is to discuss whether performing the human papillomavirus test on women aged 25-30 in Turkey has any effect on preventing cervical cancer. It is aimed to reevaluate the screening program.

Methods: A total of 400 patients between the ages of 25-30 who had the Papanicolaou smear and the human papillomavirus test were included in our study. Pap smear and the human papillomavirus test were performed again on the patients with a positive human papillomavirus test for high-risk types in accordance with the screening program. Demographical and clinical characteristics of the patients were recorded. The incidence of human papillomavirus test positivity with a high risk among patients aged 25-30, regression, and persistence ratios were calculated.

Results: The incidence of human papillomavirus test positivity with a high risk among patients aged 25 to 30 was found to be 7%. Human papillomavirus persistence ratio was 17.6% and the regression ratio was 82.4%. Among patients with a positive high-risk human papillomavirus test between the ages of 25 and 30, human papillomavirus 16 was found in 47.1% of the patients. For one of our patients with a persistent human papillomavirus 16 positivity, conization was performed after the cervical biopsy.

Conclusion: We believe that human papillomavirus, which plays an important role in the etiology of cervical cancer, should be screened from the age of 25. This way, we can catch and treat precursor lesions of cervical cancer at earlier ages and lower the incidence and mortality of cervical cancer.

Keywords: Cervical cancer, human papillomavirus test, Papanicolaou smear test, screening program, young women

INTRODUCTION

Cervical cancer is the fourth most common cancer among women worldwide.[1] Most of the cases are seen in developing countries.[2] The low incidence and mortality of cervical cancer in developed countries depend on screening programs and human papillomavirus (HPV) vaccination programs.

Almost all cases of cervical cancer are caused by HPV infections. High-risk types of HPV can be counted as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Human papillomavirus 16 is seen in almost 50% of the patients and HPV 18 is seen in 20%.[3] In addition, 19% of cervical cancer is suspected to be caused by HPV types 31, 33, 45, 52, and 58.[4]

It takes 10-20 years for HPV infections to progress into invasive carcinoma. This duration helps us catch the disease during the premalignant stage when treatment is most effective. Premalignant cervical lesions are asymptomatic and they can only be detected with appropriate screening tests. The aim of the screening of cervical cancer is to find the high-grade lesions in asymptomatic women, to treat them, and to prevent them from progressing into invasive carcinoma.[5]

Since 2014, the Papanicolaou (Pap) smear test and the HPV test are used for the screening of cervical cancer in our country. The cytological evaluation of the cervical Pap smear and simultaneous HPV DNA study of the same sample is defined as the "co-test." Today, the co-test is the most widely accepted screening method for women over the age of 30. American Society of Colposcopy and Cervical Pathology and the American College of Obstetricians and Gynecologists recommend that screening be done for women between the ages of 30 and 65 with co-testing once every 5 years.[6-8] Because HPV infections are mostly transient in patients under the age of 30, co-testing is not recommended for this age group as it has low persistence and it might increase the false-positive rate of the test. For that reason, screening with only cytology once every 3 years is recommended for women aged 21-30.[9]

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The Cobas HPV test is the only Food and Drug Administration (FDA) approved test for primary HPV screening in women over the age of 25. It has perfect reproducibility with solid and automatically end-to-end encrypted procedures.[10]

There is no clinical study comparing mortality rates between various screening strategies. However, a microsimulation model for US Preventive Services Task Force found out that screening strategies including the HPV test correlated less with cervical cancer mortality when compared to screening strategies with the Pap smear test.[11]

The aim of our study is to evaluate the persistence and regression ratios of high-risk HPV in women aged 25-30 in our country. We also aim to discuss the effect of performing HPV test for women aged 25-30 in Turkey on preventing cervical cancer, to ensure further evaluation of the screening program, hence contributing to the literature.

METHODS

A total of 400 patients aged 25-30 who were incidentally tested for HPV after presenting to Dicle University Obstetrics and Gynecology Clinic for the Pap smear test and patients who were referred from other hospitals to receive the HPV test between 2015 and 2018 were included in our retrospective study. For our study, approval no. 277 was received from Dicle University Ethics Committee on December 5, 2019.

Human papillomavirus test results were evaluated one by one and the high-risk positive ones were determined (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, and HPV-68). Patients with hysterectomy, cervical cancer, radiotherapy or chemotherapy histories, and immunosuppressive diseases were left out. From the patients included in our study, the ones with past abnormal cytology results in their Pap smear and/or cervical lesions were left out. Healthy women were included in the study. Human papillomavirus negative and high-risk positive ones were determined, and then a control HPV test and a Pap smear were performed in accordance with the screening program in patients that were high-risk positive. Women that had the control HPV test were over the age of 30 in accordance with the criteria of our study, and at least 2 years had passed since their first HPV test.[12] According to the control HPV and the Pap smear results, the diagnostic algorithm was employed, cervical biopsy through colposcopy and conization were performed when necessary and the results were noted down. Pap smear and HPV test results of patients between 25 and 30 and patients over the age of 30 were noted down and evaluated. As to the patients aged 25 to 30, regression and persistence ratios of the Pap smear and HPV test results and high-risk HPV positivity incidence were calculated. Persistent HPV infection was defined as 2 consecutive positive HPV tests at least 12 months apart.[12] Demographical characteristics of the patients, the time between the HPV tests (in years), age at first coitus, marital status, smoking status, previous HPV vaccination, history of cervical cancer within the family, and the methods of contraception that they used were acquired either from the hospital's information management system or through phone calls with the patients.

Main Points

- Cervical cancer is the fourth most common cancer among women worldwide.
- Almost all cases of cervical cancer are caused by human papillomavirus infections.
- Cervical cancer can be prevented with regular and effective screening programs.
- Cervical cancer screening programs should be revised at certain intervals for each country.

Our hospital has been performing HPV tests since 2015. The Cobas test is the chosen test for HPV. A Pap smear test is performed as liquid-based cytology. Sample taken from the cervix with a brush is placed inside of the SurePath vial and both the Pap smear and the HPV test are done as a co-test using the same sample. The sample is taken by a gynecology specialist working at our clinic. During the testing, it was made sure that the patient had no history of coitus within the last 48 hours and that the patients were not in their menstruation period. As to the Cobas HPV testing, a sample preparation module was employed to prepare the master mix and to aliquot it, and then conduct the sample addition.[13] For this purpose, 25 µL of sample was added to 25 mL of master mix in a 96-well polymerase chain reaction plate.[13] The plate was sealed by manual means and taken to the z480 real-time amplification and detection module of the Cobas 4800 system in line with the manufacturer's protocol, making use of spectrally unique fluorescent dyes to mark TaqMan probes for HPV16, HPV18, and 12 other high-risk human papillomavirus (HR HPV) genotypes.[13] The assay aims at the 14 high-risk types included in the Cobas assay and also HPV6, 11, 26, 40, 42, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39(82 subtype), and 89 (CP6108).[13] The results of the Pap smear were reported in accordance with the 2001 Bethesda System.[14] The results were reported as follows: High-grade squamous intraepithelial lesion (HGSIL), atypical squamous cells cannot rule out HGSIL (ASCH), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LGSIL), atypical squamous cells of undetermined significance (ASCUS), and negative for intraepithelial lesion or malignancy. As a routine at our clinic, patients that are HPV 16 or 18 positive, or the ones with an ASCUS and higher smear result with HR HPV positivity are given information about cervical biopsy through colposcopy. Consent is taken from the patients that accept the procedure and then the procedure is performed.[11] A second co-test after 12 months is recommended for patients with a normal Pap smear with an HR HPV positivity.[11] For patients with an HGSIL biopsy result from the colposcopy, conization is recommended.

Statistics were evaluated by means of the Statistical Package for Social Sciences (SPSS) 21 (SPSS, Inc., Chicago, Ill, USA) package program. Percentage, frequency, arithmetic mean and standard deviation were used for the analysis of the data.

RESULTS

A total of 400 patients aged 25-30 who fit the inclusion criteria and who received both Pap smear and HPV test between 2015 and 2018 were included in the study. The incidence of high-risk HPV positivity was calculated as 7% (28) for these patients. Additionally, 17 of the high-risk HPV-positive patients wanted to receive control HPV and Pap smear tests. The remaining 11 patients either did not want to get retested or could not be reached (Figure 1).

The mean age of HPV high-risk positive patients was found to be 31.7 ± 0.7, age at first coitus was 20.7 ± 3.5 and the interval between the 2 HPV tests was 4.7 ± 2.1 years (Table 1). Two of the patients were single (11.8%), 2 of them had an HPV vaccine after testing positive for the first time (11.8%), 5 of them were smokers (29.4%), and 8 of them used condoms as their protection method (47.2%) (Tables 1 and 2).

Among high-risk HPV-positive patients aged 25-30 that we were able to reach, 8 patients had HPV 16 (47.1%), 8 patients had HR HPV (47.1%), and 1 patient was positive for both HPV 16 and HR (5.8%). The results of the Pap smear came as ASCUS or higher lesions for 13 patients (76.5%) (Figure 2).

Among patients over the age of 30, 14 patients (82.4%) were HPV negative, 2 patients were HR HPV positive (11.8%) and 1 patient was HPV 16 positive (5.8%). Pap smear results were reported as

Figure 1. Flow chart of the study.

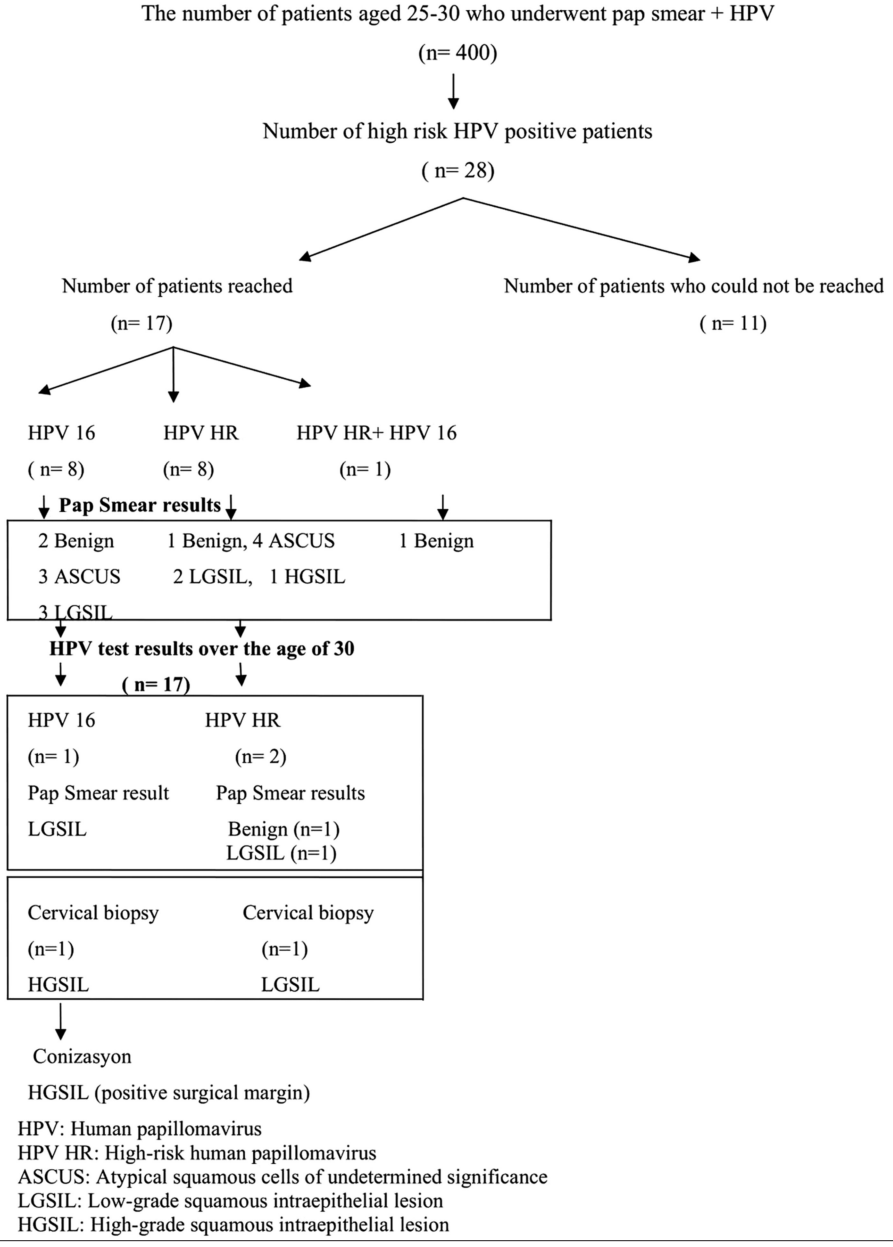


Table 1. Evaluation of Demographic and Clinical Data

	Mean ± SD	(Min–Max)
Age	31.7 ± 0.7	(31–33)
Gravidity	1.9 ± 1.7	(0–5)
Parity	1.7 ± 1.7	(0–5)
Number of living children	1.6 ± 1.6	(0–4)
Abortion	0.2 ± 0.7	(0–3)
The time between the HPV tests (in years)	4.7 ± 2.1	(2–5)
Age at first coitus	20.7 ± 3.5	(16–28)

Data are presented as mean ± SD (min–max).
HPV, human papillomavirus; SD, standard deviation.

Table 2. Evaluation of Clinical Data

		n	Percentage
Marital status	Married	15	88.2
	Single	2	11.8
Smoking status	Yes	5	29.4
	No	12	70.6
HPV vaccination	Yes	2	11.8
	No	15	88.2
History of cervical cancer within the family	Yes	0	0
	No	17	100
The methods of contraception	No	7	41.2
	Condom	8	47.2
	Intrauterine device	1	5.8
	Depoprogesterone	1	5.8

Data are presented as percentage.
HPV, human papillomavirus.

ASCUS or higher for 3 patients (17.6%). Two of these (11.8%) were ASCUS patients between the ages of 25 and 30, and one of these (5.8%) was a patient with a past Pap smear result that was reported as benign (Figure 3). None of the patients that were included in our study tested positive for HPV 18.

When high-risk HPV positivity results of the patients aged 25-30 and of the patients over the age of 30 were evaluated (Figure 4), the persistence ratio of HPV was calculated as 17.6% (3) and the regression ratio was found to be 82.4% (14). As to the Pap smear results (ASCUS and higher lesions), the regression ratio was 84.6% (11) and the persistence ratio was calculated as 15.4% (2).

Of 17 patients aged 25-30, 8 had cervical biopsy through colposcopy. Either LGSIL or HGSIL was found in 75% of them. Control HPV tests and Pap smears taken from these patients were reported as normal cervical epithelium. Three patients over the age of 30 (17.6%) had indications for cervical biopsy through colposcopy, and they accordingly received the procedure. One of these patients' results was reported as normal cervical epithelium, another had LGSIL, and the third patient had HGSIL. The patient that had HGSIL was given information and she went through the conization procedure. The result was reported as HGSIL and a positive surgical margin. This case was a single patient with persistent HPV 16.

DISCUSSION

It is widely accepted that almost all cervical cancer is caused by HPV. In our country, screening with HPV is not recommended for women under the age of 30 within the screening program

Figure 2. Human papillomavirus test and pap smear results between the ages of 25-30.

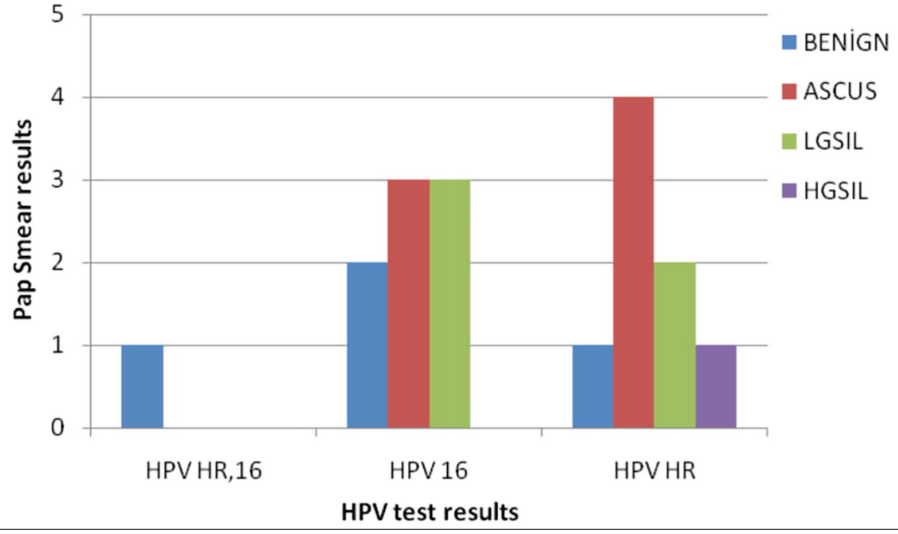


Figure 3. Human papillomavirus test and pap smear results over the age of 30.

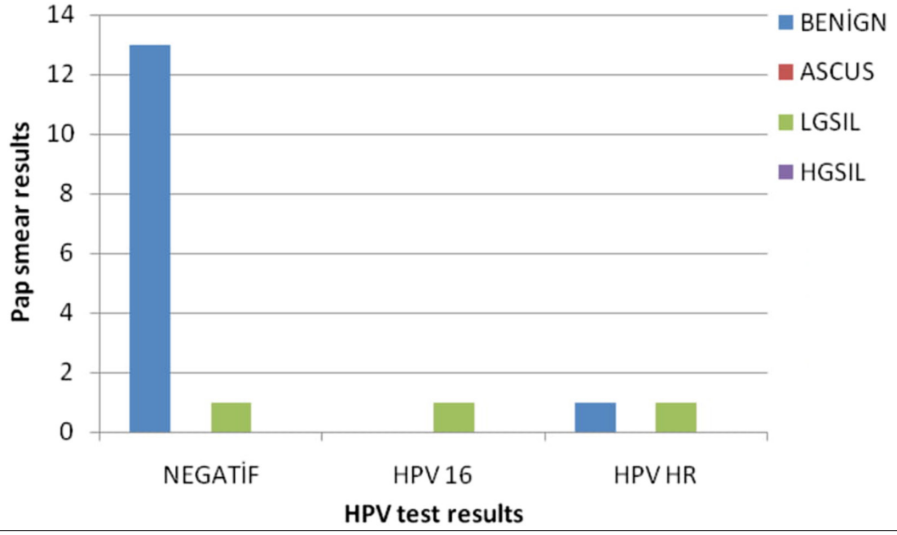
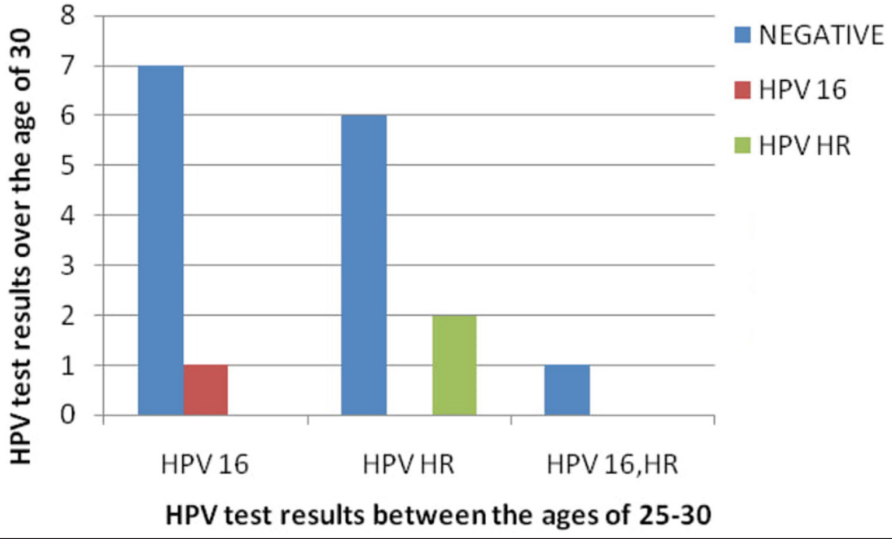


Figure 4. Human papillomavirus test results for 25-30 years old and over 30 years old.



which had its last update in 2014.[6-8] In our study, we evaluated the HPV test results of women aged 25-30 and also of women over the age of 30 with at least 2 years interval from their last test together with the Pap smear results to analyze the HPV incidence, regression, and persistence ratios. It was our wish to blaze a trail for further studies for the HPV screening to be performed from the age of 25 in our country to reduce the mortality and the incidence of cervical cancer.

In a study conducted by Veldhuijzen et al. the incidence of high-risk HPV-positive patients between the ages of 24 and 28 was found to be 8.6%. It was stated that as patients got older, the incidence and prevalence decreased. High-risk HPV incidence between the ages of 24 and 60 was reported as 3.13%. The most common types were found as HPV 16 (1%), HPV 31 (0.54%), HPV 51 (0.46%), HPV 18 (0.41%), and HPV 56 (0.29%).[15] In their study, Castle et al performed HPV and Pap smear tests for patients over the age of 25. They found 10% HPV 16/18 positivity and 6% abnormal cytology. Performing the HPV test as a co-test or performing it as a primary testing method was found to be reliable and cost-effective in cervical cancer screening.[16] In our study, we calculated the high-risk HPV positivity incidence in women aged 25-30 as 7%, which is comparable with the literature. Moreover, 76.5% of these HPV-positive patients had ASCUS or higher lesions as their Pap smear results. Conde-Ferráez et al had 76.1% of women under the age of 32 and 43.7% of women under the age of 25 in their study. Even though HPV infections are common and mostly transient around these ages, some studies have shown that after the infection, the incidence of high-risk lesions is higher after a very short while and cervical cancer mortality

in young women also increases; thus, they have emphasized the importance of screening at younger ages.[17] In our study, 1 of 17 patients (5.8%) was found to have a high-risk lesion with a positive surgical margin after conization. This case was a patient who did not want to get the cervical biopsy between the ages of 25 and 30 and had persistent HPV 16 after the age of 30, and the conization was performed when she was over the age of 30, 2 years after the first HPV test. The lesion might not have progressed this much had we performed the cervical biopsy between the ages of 25 and 30. For that reason, we believe that extensive research is necessary within this field. Sasieni et al also reported in their study that the increasing cervical cancer rate in the United Kingdom in 2009 did not correlate with the fact that patients aged 20-24 were not being tested under the screening program; however, HPV did play a part.[18] That is the reason why we believe that it is important to perform screening tests on younger patients and that it should start even from the age of 25 as recommended by the FDA, also as stated in these studies.

In a study performed by You et al.[19] the HPV test was performed on patients between the ages of 21 and 65 who had abnormal Pap smear results, and the age groups with the highest risk were found to be women between the ages of 26 and 30, and between the ages of 51 and 55. Fındık et al stated that the incidence of HPV between the ages of 30 and 65 in Turkey was 3.16%, and it was most common among women aged 30-40 with 39%. Furthermore, 18.3% of HPV-positive cases had cytological abnormalities and it was most commonly seen in women aged 30-40 with 43%. As a result, it was stated that HPV-positive women were found to have more cervical intraepithelial lesions.[20] Because HPV is not routinely tested in Turkey before the age of 30, we believe that this is the reason why HPV is most commonly seen in women aged 30-40 and that this information might change if the screening starts to include women under 30. Unlike the aforementioned studies, in our study, women that were high-risk HPV positive at 25-30 years of age were tested for HPV and Pap smear when they were over 30. We calculated the persistence ratio of HPV as 17.6% and the regression ratio as 82.4%. For the Pap smear results (as ASCUS and higher lesions) the regression ratio was 84.6% and the persistence ratio was 15.4%. We deeply believe that these ratios should be taken into consideration and further studies should be conducted to support our findings.

Testing for HPV under the age of 30 is not included within the screening program due to the fact that it might cause unnecessary colposcopies and overtreatment. When we review the literature, we see that Felix et al compared primary HPV with Pap smear in women aged 25 to 30 and co-testing with primary HPV in women aged 30-70 with regards to clinical and economic advantages. It was stated that primary HPV testing in women aged 25-30 had a minimal effect on diagnosing cancer and reducing mortality, and it was not cost-effective when compared with cytology. They found that co-testing 3 years apart between the ages of 30 and 70 was more cost-effective and prevented cancer even more compared to primary HPV testing.[21] In a study conducted by Bhatla et al. it was mentioned that most countries started to perform primary HPV tests in cervical cancer screening for women over the age of 25. However, in their study, they found that more colposcopies were performed after primary HPV tests compared to the patients that either had the co-test or just cytology.[22] We suggest that in our country, the co-test currently being performed for women over the age of 30 should be performed for women from the age of 25. Even though this might increase the costs of colposcopies, we still stand by this suggestion and believe that it will be indirectly more cost-effective because it is known that HPV is more sensitive when it comes to preventing cervical cancer or detecting precancerous lesions.

In our study, age at first coitus being 20.7, 29.4% of patients being smokers, 41.2% having completely unprotected sexual intercourse, and just 2 of the patients getting the HPV vaccine after testing positive for HPV show us that risk factors for cervical cancer are present in our country. That is the reason why we believe that in addition to further improving the screening program, women in their reproductive ages should be educated about HPV and vaccination to prevent cervical cancer.

One of the limitations of our study is that we were not able to reach all of the patients that tested high-risk positive for HPV, because 11 of them were hesitant to get the control test. In addition, we were not able to determine the positive and negative predictive values of HPV because it was not ethical to perform colposcopy on every patient included in the study. Besides, as we only included the patients that applied to our hospital, the number of positive patients was limited. Another limitation of our study is that because we acquired the patient information either from the patient files or by phone calls for some questions, we had to depend on the answers given by the patients. The strength of our study is that all of our patients were otherwise healthy, and because the HPV test was done in a random manner, we were able to include patients with different socio-cultural backgrounds. Also during our literature review, we did not come across any past study that evaluated HPV test results of women between the ages of 25 and 30 in Turkey, which can also be seen as another strength of our study.

CONCLUSION

Papanicolaou smear test has been the chosen method for screening since the 1960s and the last update to the screening program in Turkey was done in 2014. However, cervical cancer still creates a big health risk within our society. For that reason, the screening program should be revised at certain intervals in accordance with the risk factors present in the society. We believe that screening for HPV, which has an important role in cervical cancer etiology, could be reconsidered for potential coverage of women from the age of 25. That way, precursor lesions of cervical cancer can be caught at earlier ages and the incidence and the mortality of cervical cancer can be reduced.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Dicle University (Date: December 5, 2019, Decision no: 277).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – R.G., E.A., O.P.B., U.A., M.S.I., T.G.; Design – R.G., E.A., M.S.I.; Supervision – R.G., E.A., U.A., M.S.I.; Funding – R.G., O.P.B.; Materials – R.G., U.A.; Data Collection and/or Processing – R.G., O.P.B., U.A.; Analysis and/or Interpretation – R.G., E.A., U.A., M.S.I., T.G.; Literature Review – R.G., T.G.; Writing – R.G., M.S.I.; Critical Review – R.G., E.A., O.P.B., U.A., M.S.I., T.G.

Declaration of Interests: The authors declare that they have no competing interest.

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Original Article

Evaluation of Liver Masses and Accompanying Findings by Diffusion–Weighted Magnetic Resonance Imaging

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ABSTRACT

Objective: The aim of this study was to investigate the contribution of diffusion-weighted magnetic resonance imaging to differential diagnosis in the characterization of liver masses.

Methods: Diffusion-weighted sequences were added to conventional sequences in cases in which a mass was detected during upper abdominal magnetic resonance imaging performed for any reason. Diffusion-weighted images were obtained by applying diffusion-sensitive gradients at the b0, b600, and b1000 values with a single shot echo-planar spin echo sequence in the axial plane using the 1.5T magnetic resonance imaging device, and apparent diffusion coefficient maps were automatically constructed from these images by the magnetic resonance imaging device. The mean apparent diffusion coefficient values were calculated for 56 masses and 45 liver parenchyma in 45 cases with histopathological diagnoses.

Results: Of the 56 masses, 28 were benign and 28 were malignant. The benign masses consisted of 11 hemangiomas, 8 hydatid cysts, 3 simple cysts, 4 abscesses, and 2 focal nodular hyperplasia. The malignant masses comprised 13 hepatocellular carcinomas, 12 metastases, 2 cholangio cellular carcinomas, and 1 carcinosarcoma. The mean apparent diffusion coefficient value of the benign masses was calculated to be 2.67×10^{-3} s/mm^2 and that of the apparent diffusion coefficient value was 1.21×10^{-3} s/mm^2, indicating a statistically significant difference between the 2 groups. Apparent diffusion coefficient combined with diffusion-weighted magnetic resonance imaging had 100% sensitivity and 69% specificity in the differentiation of benign and malignant masses

Conclusion: Diffusion-weighted magnetic resonance imaging is a technique that provides results in a short time without using any contrast agent and contributes to the differential diagnosis of liver masses and should be added to conventional sequences.

Keywords:: Hepatic masses, diffusion-weighted imaging, magnetic resonance imaging, cyst hydatid, hepatic hemangioma

INTRODUCTION

The liver is an organ in which benign and malignant lesions are frequently located.[1] The characterization of focal mass lesions in the liver can be performed with high accuracy (97%) using T1-weighted, T2-weighted, and dynamic contrast-enhanced examinations and fat-suppressed sequences in routine liver examination by magnetic resonance imaging (MRI).[2]

Diffusion refers to the random motion of molecules with their kinetic energy, which is also called Brownian motion.[3] Diffusion-weighted imaging (DWI) is one of the functional MRI techniques sensitive to the Brownian motion of molecules. Image contrast depends on the microscopic movements of water molecules. Images are obtained without contrast in a short exposure time. The disadvantage of this technique is that it is sensitive to magnetic field inhomogeneity, images of low geometric resolution, and low signal/noise ratio. Diffusion-weighted imaging shows significant sensitivity to current and motion.[4]

In DWI, images are T2-weighted, and apparent diffusion coefficient (ADC) maps are constructed in which only diffusion effect is seen to eliminate the T2 effect.[3] An ADC map comprises synthetic images created by processing the data obtained at the pixel base to prevent T2 shine-through. The resulting images are independent of the direction of diffusion and the T2 effect. In restricted diffusion, low ADC values, that is low signal, are observed, while high ADC values are observed in increased diffusion due to high signal. As the gradient intensity (b value) used in diffusion measurement increases, phase distribution and signal loss in mobile protons also increase.[4]

The main objective of this study was to determine the DWI findings of various liver masses and investigate their contribution to diagnosis in terms of benign and malignant differentiation by calculating the characteristic features and ADC values that may be useful in the differential diagnosis.

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METHODS

After the decision of the ethics committee of Erciyes University Faculty of Medicine (09/130, Kayseri) was taken, DWI was added to patients with liver mass detected in abdominal MRI for any reason between March 2009 and January 2010 in our hospital. Between the specified dates, liver masses were detected in 128 cases in the upper abdominal MRI. Twelve patients who could not hold their breath for any reason, 32 with masses smaller than 1 cm, and 39 without histopathological diagnoses were excluded from the evaluation. Fifty-six masses of the remaining 45 cases (26 men and 19 women) with histopathological diagnoses were examined. The age of the patients varied between 26 and 80 years, and the mean age was 59.3 ± 15.6 years.

Routine upper abdominal MRI was performed on the patients using a 1.5 Tesla MRI device (Philips Gyroscan Intera, Best, the Netherlands) with 4 phased-array coils. Before injecting the contrast agent, DWI were obtained in the single-shot echo-planar sequence in the axial plane at different b values (b0, b600, and b1000 s/mm^2) [TR (Time to Repetation), 3656 ms; TE (Time to Echo), 89 ms (b1000); TR, 2673; TE, 60 (b600); matrix, 128 × 256; FOV (Field-of-view), 35-40 cm; section thickness, 7 mm; cross-section, 1 mm). Oil pressure pulses were used to prevent serious chemical-shift artifacts. The ADC maps of isotropic images were created automatically by the device.

Apparent diffusion coefficient values were calculated by placing the regions of interest (ROI) on the lesions to cover 2/3 of the area. In large lesions, measurements were taken by placing ROI at the locations corresponding to the contrasted areas of the lesions. The histopathological diagnoses of the patients were compared with the mean ADC values measured. In addition, 1 cm^2 ROI was placed in the liver parenchyma to calculate the ADC value of this tissue.

Statistical Package for the Social Sciences 15.0 for Windows program (SPSS Inc, Chicago, Ill, USA) was used for statistical evaluation. Quantitative data were defined as mean ± standard deviation. The difference between the 2 groups was analyzed using the Student's *t* test. Countable data (qualitative) were defined as a percentage. Diagnosis the sensitivity and specificity of the criteria were calculated. Kappa match between 2 tests was analyzed using statistics and the difference between them was determined by using the Mc Nemar test. The significance level was taken as .05.

Main Points

- Many benign and malignant masses may be seen in the liver.
- Diffusion-weighted images are a magnetic resonance imaging (MRI) sequence that was obtained without contrast in a short exposure time.
- Benign and malignant differentiation of liver masses can be done by diffusion-weighted magnetic resonance imaging sequence.

RESULTS

Fifty-six masses of 45 cases with histopathological diagnoses were evaluated. Of these masses, 28 were benign and 28 were malignant. The benign masses consisted of 11 hemangiomas, 8 hydatid cysts, 3 simple cysts, 4 abscesses, and 2 focal nodular hyperplasia (FNH). The malignant masses comprised 13 hepatocellular carcinomas (HCCs), 12 metastases, 2 cholangiocellular carcinomas, and 1 carcinosarcoma. The size of the benign masses ranged from 2 to 13 cm with a mean value of 7.36 ± 2.70 cm, and the size of the malignant masses varied between 1.5 and 20 cm, with a mean value of 7.55 ± 4.42 cm (Table 1).

In the quantitative evaluation of the lesions, the mean ADC measurements were obtained from the ADC maps of the b600 and b1000 images. The ADC values of 28 benign masses varied between 4.29 and 1.28 × 10^{-3} s/mm^2, with the mean value of 2.93 ± 0.29 × 10^{-3} s/mm^2, and the highest ADC in the benign group was observed in simple cysts with a mean value of 4.29 × 10^{-3}s/mm^2 and the lowest ADC in abscesses with a mean ADC value was measured as 1.28 × 10^{-3} s/mm^2. Among the 28 malignant masses, the ADC values ranged from 1.42 to 1.17 × 10^{-3}s/mm^2, with a mean value of 1.24 ± 0.24 × 10^{-3}s/mm^2. The mean ADC values of the liver parenchyma and cirrhotic livers were 1.54 ± 0.2 × 10^{-3} s/mm^2 and 1.43 ± 0.4 × 10^{-3}s/mm^2, respectively. The difference between these 2 groups (normal liver parenchyma and cirrhotic liver) was statistically significant (Table 2). There was a statistically significant difference between the mean ADC values of the benign and malignant masses (Table 3).

The hydatid cysts (n = 8) showed loss of signal as the b value increased in DWI, but they were only slightly more hyperintense at the b1000 value compared to the simple cysts (Figure 1). For the hemangiomas (n = 11), hydatid cysts (n = 8), and simple cysts (n = 3), the ADC appearance presented with a higher value compared to the liver, while the malignant lesions except for hypovascular metastases (n = 17) and the benign lesions of abscesses (n = 4) and FNH (n = 2) had lower values than the liver in ADC maps

Table 1. Number and Size of Lesions According to the Lesion Type

	Number of Lesions	Lesion Size (cm)
Hemangioma	11	2–11
Hydatid cyst	8	5–13
Simple cyst	3	3–6
Abscess	4	5–9
FNH	3	3–4.5
HCC	13	2.5–15
Metastasis	12	1.5–20
CCC	2	9–10
Carcinosarcoma	1	20

FNH, focal nodular hyperplasia; HCC, hepatocellular carcinomas; CCC, cholangiocellular carcinomas.

Table 2. Mean ADC Values at b600 and b1000 According to the Lesion Type

Lesion Type	ADC 600 ($\times 10^{-3}$ s/mm^2)	ADC 1000 ($\times 10^{-3}$ s/mm^2)	*P*
Hemangioma (n = 11)	2.99 ± 0.17	2.75 ± 0.31	.43
Hydatid cyst (n = 8)	4.27 ± 0.17	3.71 ± 0.51	.07
Simple cyst (n = 3)	4.29 ± 0.18	4.09 ± 0.41	.40
Abscess (n = 4)	1.28 ± 0.59	1.21 ± 0.69	.52
FNH (n = 2)	1.32 ± 0.06	1.25 ± 0.02	.33
HCC (n = 13)	1.28 ± 0.10	1.12 ± 0.11	.50
Metastasis (n = 12)	1.17 ± 0.10	1.05 ± 0.91	.54
CCC (n = 2)	1.42 ± 0.11	1.30 ± 0.48	.38
Carcinosarcoma (n = 1)	1.27	1.11	
Normal liver (n = 38)	1.54 ± 0.2	1.41 ± 0.3	.44
Cirrhotic liver (n = 7)	1.43 ± 0.4	1.37 ± 0.5	.32

FNH, focal nodular hyperplasia; HCC, hepatocellular carcinomas; CCC, cholangiocellular carciomas ADC, apparent diffusion coefficient.

(Figure 2). The hypovascular metastases (n = 11) had peripheral hyperintensity and central hypointensity on DWI and low peripheral and high central values in ADC maps (diffusion restriction in the peripheral area) (Figure 3). The hypervascular metastasis (n = 1), on the other hand, was observed to have homogeneous hyperintensity on DWI and a low value in ADC maps (Figure 4). The periphery of the abscesses (n = 4), especially the capsule, was iso-hypointense in DWI and had a high value in ADC maps. A significant diffusion restriction was observed in the central of the abscesses, which were observed to have hyperintensity on DWI and a low value (diffusion restriction in the central area) in ADC maps (Figure 5). Focal nodular hyperplasia (n = 3) showed hyperintensity in DWI and a low value (diffusion restriction) on ADC maps, which could be confused with malignant masses (Figure 6).

DISCUSSION

Diffusion describes the random motion of water molecules, which is also called Brownian motion.[3] The amount of diffusion is determined by the diffusion coefficient. The measurement of the diffusion coefficient is affected by many factors in biological tissues, including capillary perfusion, temperature, magnetic sensitivity in tissue, and movement. Therefore, ADC maps are used instead of the diffusion coefficient.[3] Diffusion-weighted imaging is important due to its rapid examination time (20-30 seconds) and no requirement of contrast material.[5] The disadvantage of this technique is that SGN is low, and therefore problems occur in the evaluation of lesions smaller than 1 cm.[6]

Table 3. Mean ADC Values of the Benign and Malignant Masses

Masses (n = 56)	ADC 600 ($\times 10^{-3}$ s/mm^2)	ADC 1000 ($\times 10^{-3}$ s/mm^2)	*P*
Benign (n = 28)	2.93 ± 0.29	2.68 ± 0.26	>.05
Malign (n = 28)	1.24 ± 0.24	1.10 ± 0.72	>.05
P	<.05	<.05	

ADC, apparent diffusion coefficient.

The most suitable b values for tissue characterization in the liver have been reported as b0 and b500-600 s/mm^2.[7] When we evaluated the image quality in our study, we observed that the images obtained at b600 were higher quality and contained fewer artifacts than those obtained at b1000 values.

Diffusion-weighted images have been found to be highly sensitive and specific in the characterization of focal liver masses and diffuse liver diseases.[6,8-10] In the literature, the mean ADC value of benign liver masses ranges from 2.45 to 1.94 s/mm^2, while that of malignant varies between 10.8 and 1.04 s/mm^2. This difference between the ADC values of the malignant and benign masses has been attributed to the former containing more cells than the latter.[6,10] However, abscesses, FNH, adenomas (benign lesions with high cell density), and cystic necrotic tumors (malignant lesions with low cell density) are exceptions to this rule.[7,11] In the current study, the mean ADC values of the benign and malignant masses were found to be $2.93 \pm 0.29 \times 10^{-3}$ s/mm^2 and $1.24 \pm 0.24 \times 10^{-3}$ s/mm^2, respectively, and the difference between the ADC values of these 2 groups was statistically significant, consistent with the literature. The numerical differences between ADC values in different studies are due to the changes in these values according to the b parameter, device and imaging protocol used, gradient changes, and the shooting technique.[12]

In cases where fatty liver, fibrosis, and accumulation of collagen deposit in the liver, a decrease is observed in the ADC value of the liver parenchyma.[13] In the literature, the mean ADC value of normal liver parenchyma ranges between 0.69×10^{-3} s/mm^2 [10] and 1.83×10^{-3} s/mm^2,[6] while the ADC value of cirrhotic liver parenchyma has been measured as 0.60×10^{-3} s/mm^2 and 1.37×10^{-3} s/mm^2, respectively, in the same studies. In the current study, the mean ADC value of normal liver parenchyma was determined as $1.54 \pm 0.2 \times 10^{-3}$ s/mm^2 and that of cirrhotic liver parenchyma was $1.43 \pm 0.4 \times 10^{-3}$ s/mm^2, indicating a statistically significant difference, which is in agreement with the literature.

In the literature, the mean ADC values of hemangiomas have been reported to vary between 2.95 and 1.92×10^{-3} s/mm2, which is higher compared to malignant lesions and liver parenchyma and lower compared to cysts.[6,10,14] In our study, we determined the mean ADC value of the hemangiomas as $2.99 \pm 0.17 \times 10^{-3}$ s/mm^2.

In the literature, the mean ADC values of simple cysts range from 3.63 to 2.91×10^{-3} s/mm^2, which is reported to be higher than those of liver masses.[6,10,14] In the current study, we calculated the ADC value of the simple cysts as $4.29 \pm 0.18 \times 10^{-3}$ s/mm^2.

In a study conducted by İnan et al.,[15] hydatid cysts and simple cysts were examined at b500 and b1000 on DWI, and it was reported that both groups were hyperintense compared to the liver parenchyma at b500 (T2 shine-through effect), while at b1000, hydatid cysts were minimally hyperintense and simple cysts became isointense compared to the liver parenchyma.

Figure 1. A 49-year-old patient with a hydatid cyst. On diffusion-weighted images obtained at b600, the hydatid cyst localized in segment 8 is observed as hyperintense, which is similar to simple cysts, while at b1000, it shows moderate hyperintensity to the liver unlike simple cysts.

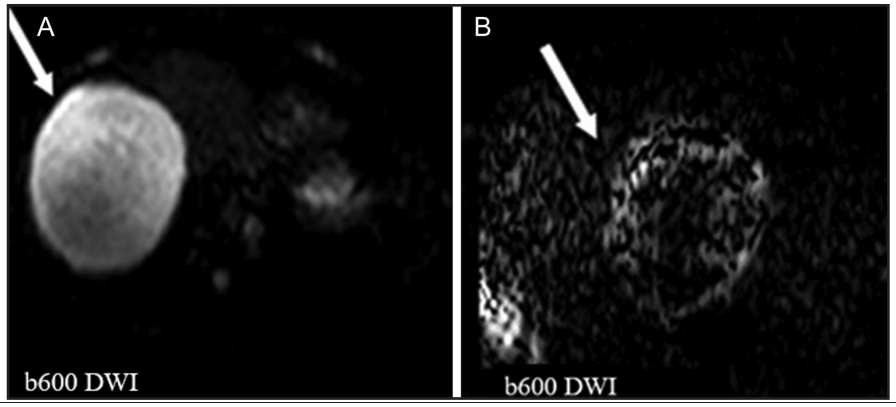


Figure 2. ADC appearance of a hydatid cyst (A), hemangioma (B), and hypervascular metastatic lesion (C). While the hydatid cyst and hemangioma are observed to have high values, the metastatic lesion has a low value. ADC, apparent diffusion coefficient.

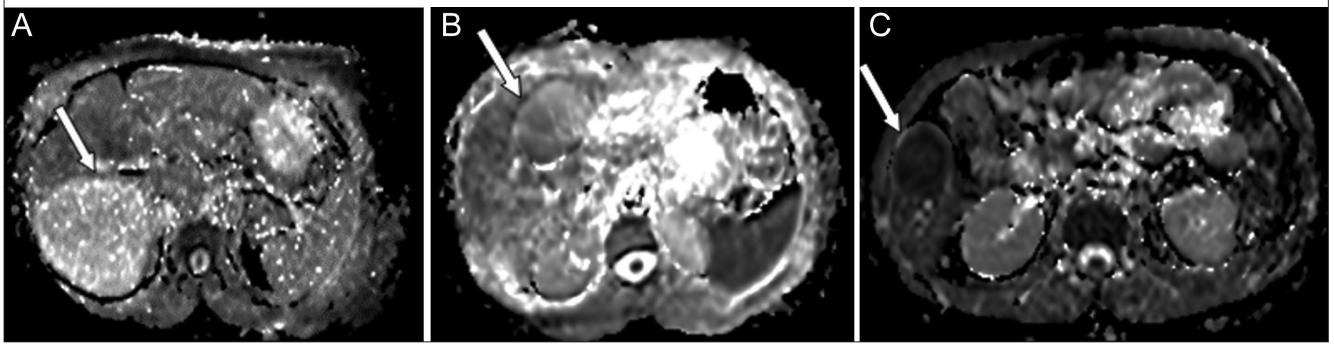
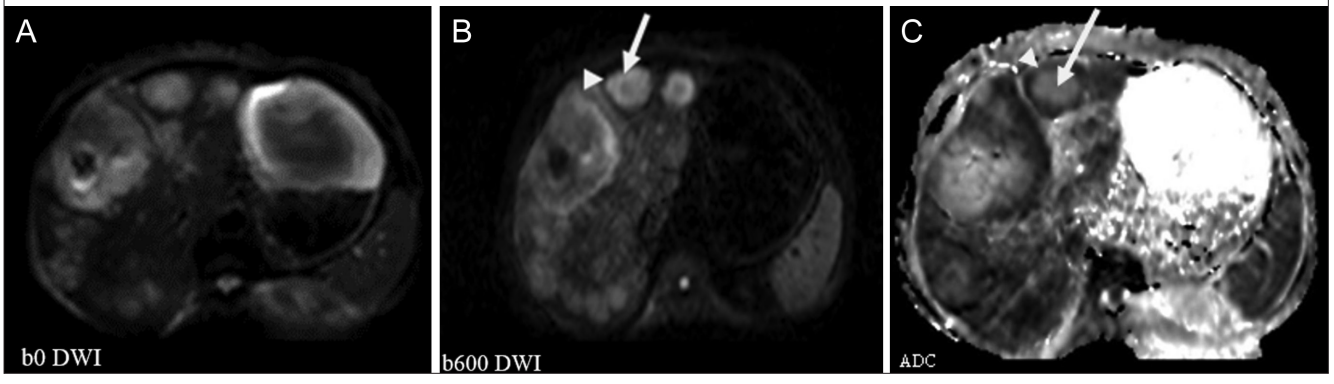


Figure 3. A 68-year-old patient with a gastric adenocarcinoma. There are numerous metastatic lesions in the liver. On DWI, the center of the lesion is hypointense (arrow) and the periphery is hyperintense (arrowhead). In the ADC map, the center has a high value (arrow) and the periphery has a low value (arrowhead). ADC, apparent diffusion coefficient; DWI, diffusion-weighted imaging.



Therefore, the authors reported that the b1000 values provided significant results. In the same study, the mean ADC value was 3.5×10^{-3} s/mm^2 for simple cysts and 2.9×10^{-3} s/mm^2 for hydatid cysts, with a statistically significant difference between the 2 groups. The authors attributed this finding to hydatid cysts being more denser than simple cysts due to their scolex, protein, glucose, lipid, and polysaccharide content. In our study, in cases with 7 hydatid cysts and 3 simple cysts, the hydatid cysts were observed to be iso-hyperintense, while the simple cysts were isointense compared to the liver at b1000. The mean

Figure 4. A 57-year-old patient with a pancreatic neuroendocrine tumor. Liver metastasis is observed. Hypervascular metastases show homogeneous hyperintensity in diffusion-weighted images and a low value in the ADC map. ADC, apparent diffusion coefficient.

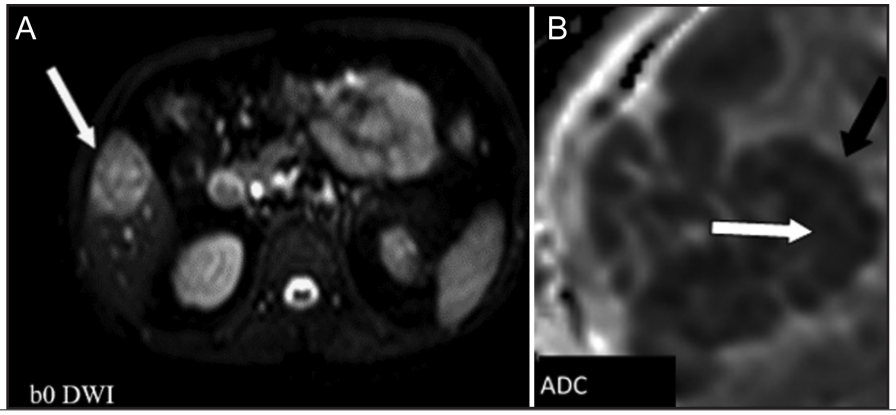


Figure 5. The abscess capsule is hypointense and the center is hyperintense on DWI. In the ADC map, the abscess capsule has a high value and the center has a low value. ADC, apparent diffusion coefficient; DWI, diffusion-weighted imaging.

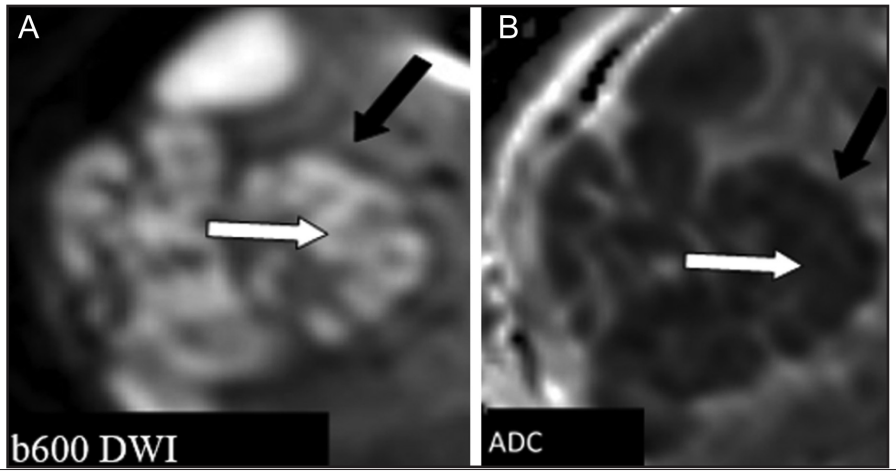
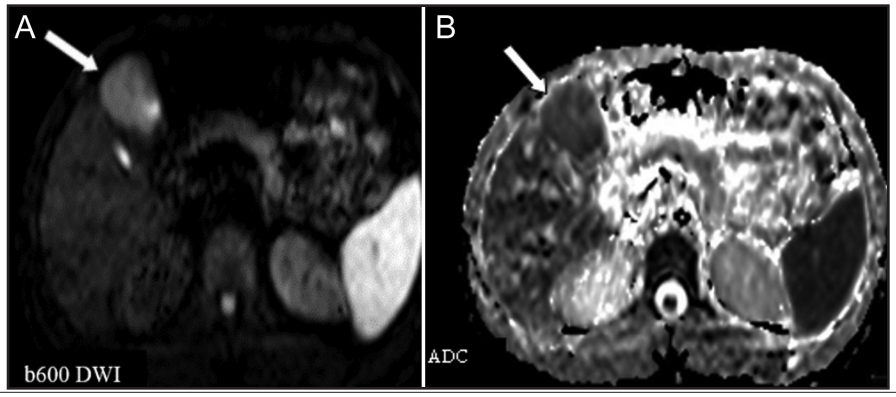


Figure 6. A 31-year-old female patient with a liver mass detected during a routine examination and diagnosed as FNH as a result of biopsy. The lesion is hyperintense on DWI and has a low value in the ADC map. ADC, apparent diffusion coefficient; DWI, diffusion-weighted imaging; FNH, focal nodular hyperplasia.



ADC values were calculated as $4.27 \pm 0.17 \times 10^{-3}$ s/mm^2 and $4.29 \pm 0.18 \times 10^{-3}$ s/mm^2 for the hydatid and simple cysts, respectively, but a statistical analysis could not be undertaken due to the insufficient number of cases.

In a previous study, the ADC values of FNH were reported to vary between 1.75 and 1.49 × 10−3 s/mm^2.[6] This can be explained by the high cell density of these lesions.[14] In our study, we determined the mean ADC value of FNH as $1.32 \pm 0.06 \times 10^{-3}$ s/mm^2. For abscesses, the mean ADC value was previously measured as 0.65×10^{-3} s/mm^2. This value being similar to malignant lesions is considered to be due to inflammatory cells, bacteria, necrotic tissue, and protein-containing pus within the abscess cavity.[16] In the current study, the mean ADC value of the abscess cavity was found to be $1.28 \pm 0.59 \times 10^{-3}$ s/mm^2.

In the literature, the mean ADC values of metastases have been reported to range from 0.94 to 1.51 × 10^{-3} s/mm^2,[6,10,14] while the range for the mean ADC values of HCCs is 0.97 to 1.33 ×10^{-3} s/mm^2.[6,11,14] This has been attributed to the cell density of malignant lesions.[15] In our study, the mean ADC value was observed to be $1.17 \pm 0.10 \times 10^{-3}$ s/mm^2 for the metastases and $1.28 \pm 0.10 \times 10^{-3}$ s/mm^2 for the HCCs.

When only ADC values are examined, DWI can significantly differentiate between benign and malignant lesions, while the ADC values of FNH and abscesses are similar to malignant lesions.[16] Necrotic areas in the center of hepatic metastases cause an increase in ADC values, which is comparable to the ADC value of benign lesions.[5,6,9] In brief, DWI determines cellularity, and therefore FNH, adenomas, and abscesses, which have higher cell density among benign lesions, show diffusion restriction, while cystic necrotic malignant lesions have high ADC values.[17] In the current study, the ADC values of FNH and abscesses were similar to those of malignant lesions.

In all our hemangioma cases, we observed that as the b value on DWI increased, the hemangiomas showed loss of signal, but this was not so pronounced as in cysts, and at higher b values, hemangiomas were still observed as mildly hyperintense compared to the liver. The ADC values of the hemangiomas were also higher compared to the liver parenchyma. These findings led us to consider that a diagnosis of hemangioma can be made by DWI without the need for a contrast-enhanced examination. This assumption should be verified by further studies with larger series.

Cysts show loss of signal at increased b values on DWI due to the free diffusion of water molecules.[17] In our study, as the b values increased in DWIs, the signal intensity of the cysts decreased, and they became isointense to the liver at b1000. This appearance was not observed in the remaining liver lesions. Therefore, we consider that DWI can differentiate between cystic and solid masses.

In a study conducted by Chan et al.[16] the central and peripheral areas of abscesses and necrotic tumors were evaluated separately. The centers of all abscesses showed diffusion restriction, while the centers of necrotic tumors showed free diffusion. In our study, when the 4 abscess cases were evaluated in the same manner, although all showed low ADC values suggesting malignant masses, the marked peripheral hyperintensity in DWI differentiated the lesions from malignant masses based on iso-hypointensity.

Similar to malignant lesions, FNH also has low ADC values while they are hyperintense on DWI. Therefore, it is not possible to distinguish FNH from malignant masses using DWI.[17] In both of our FNH cases, hyperintensity was observed on DWI, suggesting malignant masses, but the ADC values were low, and thus a benign-malignant differentiation could not be made.

In our study, when we evaluated our cases based on ADC values alone, abscesses were successfully identified when the qualitative evaluation was included in the analysis, increasing the sensitivity value to 100% and specificity to 89%. Therefore, such evaluations should be made both qualitatively and quantitatively.

Limitations

In our study, neither parallel nor respiratory triggered imaging, which are methods to increase image quality, was used. Therefore, the rate of SGO rate was very low. Since only cases with histopathological results were included in the study, the number of our cases was also low. Considering the mass subgroups, we did not have any cases with an adenoma or cystic metastasis.

CONCLUSION

Diffusion-weighted imaging is effective in differentiating benign–malignant liver masses. The evaluation of liver masses with both DWI and ADC values is important for accurate mass characterization, especially for abscesses. Apparent diffusion coefficient values can be used to differentiate cirrhotic and non-cirrhotic livers. Solid cysts, hemangiomas, simple cysts, and hydatid cysts can be differentiated by adding b1000 DWI to the examination. This can facilitate the diagnosis of hemangiomas without using contrast material. Focal nodular hyperplasia imitates malignant lesions in terms of DWI characteristics and ADC value.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Erciyes University Faculty of Medicine. (Date: March 20, 2009, Decision no: 09/130).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – U.G.O.G, O.I.K.; Design – U.G.O.G, O.I.K.; Supervision – O.I.K.; Resources – U.G.O.G.; Materials – U.G.O.G.; Data Collection – U.G.O.G and/or Processing – U.G.O.G.; Analysis and/or Interpretation – U.G.O.G., Literature Search – U.G.O.G.; Writing Manuscript – U.G.O.G.; Critical Review – O.I.K.

Declaration of Interests: The authors declare that they have no competing interest.

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Original Article

Morphometric and Morphological Evaluation of the Atlas: Anatomic Study and Clinical Implications

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ABSTRACT

Objective: Atlas is located at a critical point close to the vital centers of the medulla oblongata, which can be compressed by the dislocation of the atlantoaxial complex or instability of the atlantooccipital joint. This study aimed to determine in detail the morphometric and morphological characteristics of the atlas to guide the reduction of the risk of complications and increase the success rate in various surgical approaches for the craniovertebral junction.

Methods: In this study, 17 atlas vertebrae whose measurement parameters were pronounced and unknown gender, age, and ethnic characteristics were examined.

Results: Totally 16 parameters, 11 of which were bilateral and 5 were unilateral, were examined on the atlas. Also, no accessory foramen transversarium was found in these atlas vertebrae. Of the 23 foramina transversaria that were prominent and not broken, 7 were found to be round-shaped (30.43%), and 16 were oval-shaped (69.57%).

Conclusion: It is deducted that the results obtained in this study will help to have information about the morphometry and morphology of atlas vertebrae. Although information such as age, gender, and ethnic origin is not known about the bones evaluated, it is the advantage of this study that a large number of parameters are evaluated and compared with previous publications. Nevertheless, it seems that there is a need for studies in which much more cases are assessed, and information such as age, gender, and ethnic origin is known.

Keywords: Atlas, craniovertebral junction, dry bone, morphometry, morphology

INTRODUCTION

Craniovertebral junction (CVJ) surgery is one of the essential parts of spinal surgery.[1] The CVJ is an anatomical transition zone between the skull and the cervical spine. It contains the caudal part of the occipital bone, atlas and axis vertebrae, ligaments, many cranial nerves, blood vessels, and lymphatics.[2,3] Atlas vertebrae within the CVJ have anatomical properties that differ from other cervical vertebrae.[4] In CVJ surgery, it is necessary to have knowledge about the anatomy of this region, particularly the atlas vertebrae.[1] Cacciola et al[5] stated that the anatomy of the vertebral artery at the level of atlas and axis vertebrae is significantly different from the relatively straightforward course of the C3 to C6 vertebrae. Due to these anatomical differences and the location of the vertebral artery groove in a vital place, surgical procedures in this region are very difficult.[1] However, the number, size, and shape of the foramen transversarium can affect the morphology of the vertebral artery. Besides, conditions such as vertebrobasilar insufficiency, headache, migraine, and fainting attacks may occur as a result of pressure on the vertebral artery due to these variations.[6]

Atlas has a joint relationship with the occipital bone and axis.[1] Also, the atlas is located at a critical point close to the vital centers of the medulla oblongata, which can be compressed by the dislocation of the atlantoaxial complex or instability of the atlantooccipital joint.[7] Besides, the placement of the pedicle screw can damage essential structures such as the spinal cord, nerve roots, cranial nerves, and vertebral arteries.[4] Moreover, as different methods developed for the treatment of pathologies of this region, the bone structure's anatomy should be better known.[8] Cacciola et al[5] stated that understanding the atlas is crucially essential for any surgery in the CVJ.

This study aimed to determine in detail the morphometric and morphological characteristics of the atlas to guide the reduction of the risk of complications and increase the success rate in various surgical approaches for the CVJ.

METHODS

In this study, 17 atlas vertebrae whose measurement parameters were pronounced and unknown gender, age, and ethnic

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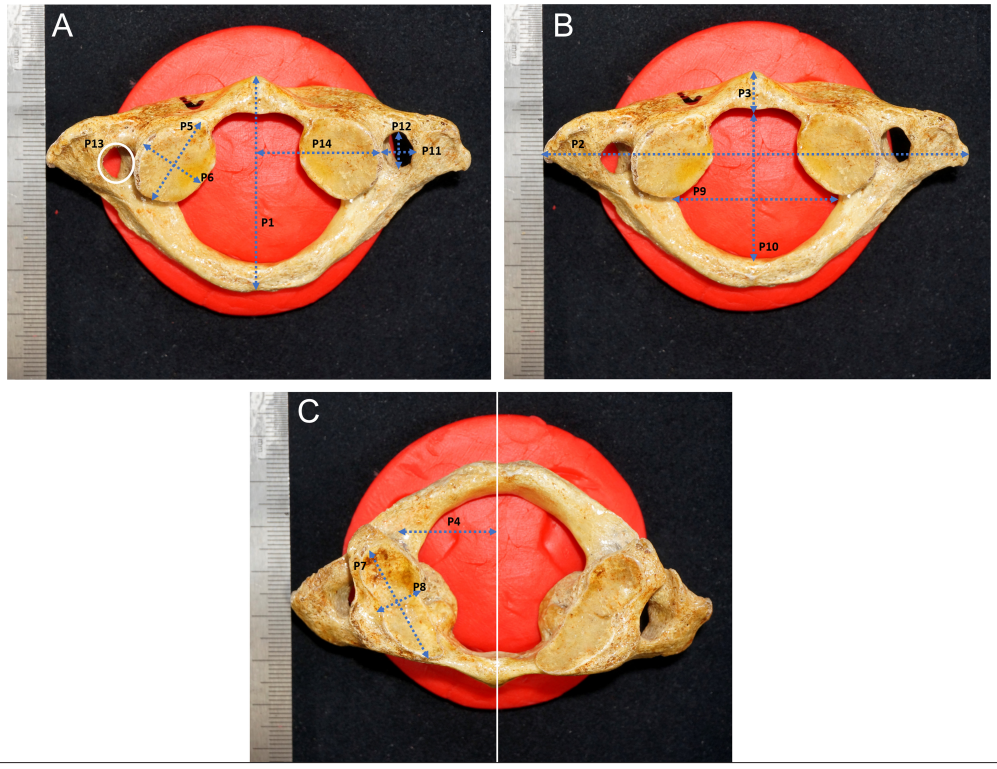
Figure 1. A-C. Inferior (A, B) and superior (C) views of the atlas.



characteristics in the Department of Anatomy of Gaziantep University Faculty of Medicine were examined. Playdough was used to keep the bones in position. Photos of the bones were taken from the top, front, and lateral sides with the Sony Nex 6 camera, Canon 50 mm, and 35 mm macro lenses. A ruler was placed next to the bones to ensure standardization and calibration while measuring on the photograph. To achieve better image quality, a mechanism that distributes the light evenly was used. The following 16 parameters, 11 of which (P4, P5, P6, P7, P8, P11, P12, P13, P14, P15, and P16) were bilateral and 5 (P1, P2, P3, P9, and P10) were unilateral, were examined on the atlas (Figure 1 A-C). The first 14 of them were carried out on the photograph of the bones using ImageJ 1.50 software. Also, the shape of the foramen transversarium (P15) and the presence of the accessory foramen transversarium (P16) were morphologically evaluated.

Main Points

- Atlas is located at a critical point close to the vital centers of the medulla oblongata, which can be compressed by the dislocation of the atlantoaxial complex or instability of the atlantooccipital joint.
- It is deducted that the results obtained in this study will help to have information about the morphometry and morphology of atlas vertebrae.
- Although information such as age, gender, and ethnic origin is not known about the bones evaluated, it is the advantage of this study that a large number of parameters are evaluated and compared with previous publications.
- Nevertheless, it seems that there is a need for studies in which much more cases are assessed, and information such as age, gender, and ethnic origin is known.

P1: Length of the atlas

P2: Width of the atlas

P3: Anteroposterior thickness of the anterior arch of atlas

P4: Distance between midline and groove for vertebral artery

P5: Anteroposterior diameter of inferior articular facet

P6: Transverse diameter of inferior articular facet

P7: Anteroposterior diameter of superior articular facet

P8: Transverse diameter of superior articular facet

P9: Transverse diameter of vertebral foramen

P10: Sagittal diameter of vertebral foramen

P11: Transverse diameter of foramen transversarium

P12: Anteroposterior diameter of foramen transversarium

P13: Area of foramen transversarium

P14: The distance between the most medial point of the foramen transversarium and the midline

P15: Shape of the foramen transversarium

P16: Presence of the accessory foramen transversarium

Statistical Analysis

Descriptive statistics were evaluated for the morphometric measurements, and their statistical distribution was analyzed. Shapiro–Wilk test was used for normality check. Mann–Whitney *U* test was applied to non-parametric data. Statistical Package for the Social Sciences 22.0 package program was used for all analysis (IBM Corporation; Armonk, NY, USA). Values with $P < .05$ were considered statistically significant.

RESULTS

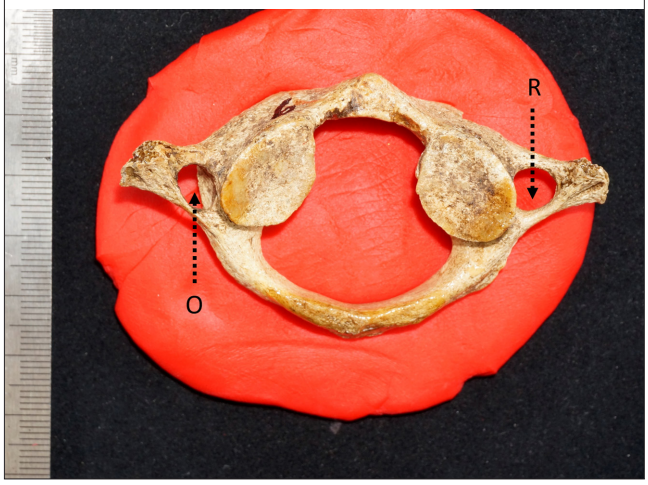
The values of 14 parameters that were examined on the atlas vertebrae are shown in Table 1. Nine of these parameters (P4, P5, P6, P7, P8, P11, P12, P13 and P14) were examined bilaterally, and no statistically significant difference was found between the sides ($P = .865$, $P = .962$, $P = .339$, $P = .394$, $P = .394$, $P = .091$, $P = .566$, $P = .976$, and $P = .838$, respectively). Also, no accessory foramen transversarium was found in these atlas vertebrae (P16). Of the 23 foramina transversaria that were prominent and not broken, 7 were found to be round-shaped (30.43%), and 16 were oval-shaped (69.57%) (Figure 2).

Table 1. Results of Measured Parameters in the Atlas Vertebrae

Parameters	N	Mean ± SD	Range
P1	17	44.10 ± 3.50	39.40–53.70
P2	12	76.32 ± 8.09	64.10–90.20
P3	17	11.60 ± 1.82	9.20–15.20
P4 (R)	16	17.58 ± 2.29	14.20–24.00
P4 (L)	16	17.59 ± 2.14	14.50–23.80
P5 (R)	17	22.71 ± 3.15	16.70–29.00
P5 (L)	17	22.10 ± 3.41	17.70–32.10
P6 (R)	17	8.52 ± 1.61	5.10–11.00
P6 (L)	17	9.15 ± 1.88	5.40–13.00
P7 (R)	17	18.25 ± 2.05	13.40–22.00
P7 (L)	17	17.60 ± 2.06	13.80–20.50
P8 (R)	17	13.29 ± 1.42	9.90–15.60
P8 (L)	17	12.82 ± 1.70	9.60–15.90
P9	17	29.61 ± 3.21	24.40–36.70
P10	17	31.27 ± 2.76	27.60–38.60
P11 (R)	12	5.90 ± 0.97	4.70–7.80
P11 (L)	11	6.48 ± 0.69	5.30–7.40
P12 (R)	12	7.56 ± 0.93	6.30–9.30
P12 (L)	11	7.29 ± 0.62	6.30–8.20
P13 (R)	12	37.43 ± 7.85	23.70–51.70
P13 (L)	11	37.31 ± 6.27	27.60–45.70
P14 (R)	16	24.47 ± 2.14	20.50–28.80
P14 (L)	16	24.29 ± 1.81	21.70–28.10

R, right; L, left.
The unit for all parameters is mm except P13, and the unit for P13 is mm^2.

Figure 2. Shape of the foramen transversarium. O, oval-shaped; R, round-shaped.



DISCUSSION

The bones that make up the CVJ are the occipital bone, atlas, and axis. This region is the most complex area of the spine.[3,9] The atlas vertebra supports the skull, providing a unique positioning of the atlantoaxial complex.[7] Miller et al[10] stated that many techniques could be used during the stabilization of cervical spine injuries such as anterior plating, posterior wirings or Harrington/Luque rods with wires, posterior lateral mass plating, and posterior pedicle screw fixation. On the other hand, new surgical techniques and instrumentation for the treatment of unstable cervical spine continue to evolve. Therefore, detailed knowledge of the anatomy of the bones in the CVJ and surrounding structures becomes even more important.[4,8]

In addition, the relationship between the vertebral artery and the groove for the vertebral artery of the atlas vertebrae has an essential role in the operative approaches to be applied to this region.[4] Screw fixation can be used in atlas instabilities.[4] Although pedicle screw fixation provides the strongest stability for cervical reconstruction, there is a risk of neurovascular injury during this procedure. Moreover, various complications such as the vertebral artery, nerve root, and spinal cord injuries and infections may also occur due to this procedure.[11,12] More dramatically, even very serious problems such as cerebral infarction or death can occur due to injury to the bilateral vertebral arteries.[13] When all these complications are considered, it is extremely important to know the morphology, morphometry, and variation of the all-anatomic structures on the atlas.

Sanchis-Gimeno et al[14] stated that the cervical variants should be known before any surgery is performed. In addition, anatomical

Table 2. Literature Comparison of Parameters Related to the Atlas Vertebrae

Study	P1	P2	P3	P4		P5		P6		P7		P8		P9	P10
				R	L	R	L	R	L	R	L	R	L		
Christensen et al[2]	45.67 ± 3.61	75.61 ± 5.94	6.02 ± 1.02												
Gosavi and Vatsalaswamy[7]		69.37 ± 6.47	10.33 ± 1.67			21.24 ± 2.39	21.02 ± 2.52	10.36 ± 1.72	10.47 ± 1.61	16.57 ± 1.91	16.50 ± 1.67	14.01 ± 1.93	14.42 ± 1.67	26.89 ± 1.93	25.66 ± 2.59
Jasveen et al[16]						21.52 ± 2.36	21.51 ± 2.07	11.21 ± 1.47	11.32 ± 1.53	17.54 ± 1.50	17.70 ± 1.60	14.99 ± 1.65	14.94 ± 1.51		
Naderi et al[1a]	43.22 ± 2.45	74.74 ± 5.81	6.66 ± 0.85	15.03 ± 1.22		19.94 ± 2.42									30.24 ± 2.30
Naderi et al[1b]	43.35 ± 2.37	74.97 ± 5.53	6.23 ± 1.06	14.61 ± 1.17		19.94 ± 2.00									30.68 ± 2.45
Rocha et al[19]	46.60 ± 3.20	78.90 ± 6.40		15.0 ± 1.6	14.8 ± 1.6	23.9 ± 2.5	23.6 ± 2.5			18.8 ± 1.7	18.7 ± 1.6	16.6 ± 2.0	16.4 ± 2.0		32.6 ± 1.8
Şengül and Kadıoğlu[4]	46.20 ± 6.00	74.60 ± 9.70		16.2 ± 2.5	15.8 ± 2.4	19.9 ± 3.4	18.6 ± 3.2	9.6 ± 1.9	9.8 ± 1.5	17.1 ± 2.6	17.5 ± 2.4	14.6 ± 2.5		28.7 ± 1.8	31.4 ± 3.5
Tun[22]			7.00 ± 1.20												
Present study	44.10 ± 3.50	76.32 ± 8.09	11.60 ± 1.82	17.58 ± 2.29	17.59 ± 2.14	22.71 ± 3.15	22.10 ± 3.41	8.52 ± 1.61	9.15 ± 1.88	18.25 ± 2.05	17.60 ± 2.06	13.29 ± 1.42	12.82 ± 1.70	29.61 ± 3.21	31.27 ± 2.76

[a]Direct morphometric measurement; [b]Computed tomography measurement.
The unit for all parameters is mm.
R, right; L, left.

Table 3. Literature Comparison of Parameters Related to the Foramen Transversarium of Atlas Vertebrae

Study	P11 (mm)		P12 (mm)		P13 (mm²)		P14 (mm)	
	R	L	R	L	R	L	R	L
Rocha et al[19]	6.6 ± 0.9	6.5 ± 0.9	7.3 ± 1.1	7.2 ± 1.1			24.1 ± 1.8	23.8 ± 1.8
Lalit et al[18]	5.17 ± 1.09	5.40 ± 1.11	6.72 ± 1.05	6.90 ± 0.99				
Taitz et al[21]	5.52 ± 0.93	5.76 ± 0.98	7.26 ± 0.87	7.23 ± 0.98				
Karau Bundi et al[17]			5.11	5.16	36.30	37.20		
Sethi et al[20]			5.3	5.1	30.46	30.82		
Present study	5.90 ± 0.97	6.48 ± 0.69	7.56 ± 0.93	7.29 ± 0.62	37.43 ± 7.85	37.31 ± 6.27	24.47 ± 2.14	34.29 ± 1.81

R, right; L, left.

variations of the atlas should be investigated further before the procedure in patients undergoing spine surgery.[14] On the other hand, Kaur et al[15] stated that since there are so many variations in this region, it is challenging to operate according to predetermined size standards. Moreover, it has been reported that this region's morphological and morphometric characteristics may also differ between different ethnic groups and races.[7] Therefore, the anatomy of the bony structures in the CVJ, such as the atlas vertebrae, should be well known in order to reduce complications and increase success during the procedures to be applied to this region.

Morphometry of the Atlas Vertebra

There are many studies in the literature examining the morphometry of the atlas vertebrae.[1,4,7,16-22] In this study, the parameters evaluated in previous studies were compiled and compared (Tables 2-3). Although most of these studies evaluated dry bone, both dry bone and computed tomography images were used in the study by Naderi.[1] It is known that the most critical disadvantage of dry bone studies is the lack of information about age, gender, and ethnicity.[23] Therefore, it is deducted that these studies on dry bones will help to have an idea about this region rather than determining standard reference values.

In atlantoaxial dislocation, spinal epidural abscess, and odontoid process fractures, the spine can be reached by a transoral approach.[24] During this procedure, the anterior arch of the atlas can be resected to reach the odontoid process. In this case, it is important to know the anteroposterior dimension of the anterior arch of the atlas vertebra (*P3*).[22] Distance between midline and groove for vertebral artery (P4) is vital for the close neighborhood of the vertebral artery and the area where the surgery will be performed. This distance should be known especially in order to perform laminectomy safely.[22] According to Steel's rule of thirds, the sagittal diameter of the vertebral foramen of the atlas (P10) is divided into 3 equal parts: one-third cord, one-third odontoid, and one-third space (safe zone).[25] For this reason, knowing the sagittal diameter of the vertebral foramen of the atlas (P10) can give an idea about the safe zone in surgical procedures. The number, size, and shape of the foramen transversarium may affect the morphology of the vertebral artery, causing vertebrobasilar insufficiency. Depending on the morphology and morphometry of the foramen transversarium, vertebral artery compression may occur. This situation may cause clinical symptoms such as chronic headaches, migraines, and fainting attacks.[6] Moreover, Taitz et al[21] stated that foramen transversarium and vertebral vessels are interrelated, and it can be assumed that variations of the vertebral vessels may manifest as variations of the foramen transversarium. Therefore, it is essential to know the transverse (P11) and anteroposterior (P12) diameters, area (P13), and shape (P15) of the foramen transversarium.

Limitations

The most important limitation of this study is that information about bones such as age, gender, and ethnic origin is not known.

CONCLUSION

It is deducted that the results obtained in this study will help to have information about the morphometry and morphology of atlas vertebrae. Although information such as age, gender, and ethnic origin is not known about the bones evaluated, it is the advantage of this study that a large number of parameters are evaluated and compared with previous publications. Nevertheless, it seems that there is a need for studies in which much more cases are assessed, and information such as age, gender, and ethnic origin is known.

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Declaration of Interests: The authors declare that they do not have any conflict of interest.

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Original Article

Manual Versus Automated Volume Reduction of Cord Blood

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ABSTRACT

Objectives: All cord blood banks all over the world follow a common procedure, concentrating progenitor cells by volume reduction, with the main purpose of optimizing the use of storage space. The main objective of this study was to compare CD34 and total nucleated cell recovery rates and red blood cell depletion efficiencies following cord blood processing using automated Sepax or manual CellEffic cord blood processing systems.

Methods: Nine cord blood units with high volumes were divided into 2 equal fractions and processed with CellEffic cord blood and Sepax. Total nucleated cell, mononuclear cells, $CD34^+$, red blood cell and total nucleated cell viability, and clonogenic assays were performed, and recovery rates were calculated on pre- and post-process cord blood units and after freeze/thaw process. In the comparison group, post-thaw differential cell counting was also performed.

Results: Our results showed that post-process total nucleated cell viability with CellEffic cord blood was slightly higher than Sepax, whereas Sepax post-process total nucleated cell/ mononuclear cell values were superior to CellEffic cord blood. Post-thaw red blood cell depletion was better for CellEffic cord blood. Post-thaw Sepax colony-forming unit counts were higher than CellEffic cord blood. In addition, $CD45^+CD71^+$ cells were lower, whereas $CD45^+CD34^+CD38^-$ cells were higher for the CellEffic cord blood system.

Conclusion: Despite the fact that there is a need for well-trained personnel for processing cord blood units with CellEffic cord blood, it may be an attractive alternative to Sepax system for cord blood processing, particularly for cord blood units with low volumes, at banks with low budget where the cord blood turnover rates are relatively low.

Keywords: Hematopoietic Stem cells, cord blood, blood banking

INTRODUCTION

Cord blood (CB) is a significant graft source for hematopoietic stem cell (HSC) transplantation for patients for whom a suitable human leukocyte antigen (HLA)-matched donor is missing.[1] Since 1988, more than 40 000 umbilical CB have been transplanted, both in children and adults.[2,3] Relapse as well as graft versus host disease risk after cord blood transplantation (CBT) is considerably low.[4,5] High quality of a CB unit (CBU) is strongly correlated with shorter engraftment period and rapid immune reconstitution, thus higher survival rates.[1,6] The quality of CBU is highly dependent on the laboratory procedures; mainly processing, cryopreservation, and storage conditions.[7-9] Currently, umbilical CBUs are processed via red blood cell (RBC) depletion and volume reduction. Basically, 2 approaches, automated and manual (centrifugation) processing, are being used worldwide.[10] Three major automated systems are in use for the depletion of excess plasma and RBC from CB, most commonly used are Sepax (Biosafe S.A. Eysins/Nyon, Switzerland), AutoXpress Platform (Cryo-Cell International Inc., Florida, USA, for mononuclear cells was also mentioned in the text as MNC.), and PrepaCyte-CB (Cryo-Cell International, Inc., USA) systems.[11,12] Both automated systems are proven to be efficient, yielding high total nucleated cell (TNC) and $CD34^+$ HSC recovery rates, retaining viabilities. The major advantage of these CB processing systems is the need for a fully closed operating environment, which minimizes the risk of contamination but increases cost. On the other hand, centrifugation may be harmful to quality and quantity of CB HSCs.[13-16] The need for potentially toxic chemical usage, such as hydroxyethyl starch (HES), is another drawback of closed systems.

A novel filtration system was described recently by KANEKA Corporation (2-3-18, Nakanoshima, Kita-ku, Osaka 530-8288, Japan). This filtration system uses a non-chemical-coated/non-woven polyester fabric filter, which traps $CD34^+$ cells through

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affinity without the need for centrifugation and allows manual processing of CB.[16] The main objective of this study was to compare CD34 and TNC recovery rates and RBC depletion efficiencies following CB processing using automated Sepax (Biosafe, S.A. Eysins/Nyon) or manual CellEffic CB technology (KANEKA Corporation, Japan).

METHODS

Collection of CB

Nine CBUs collected in utero from consented maternal donors with volumes >100 mL were included in this study. Cord blood plasma and RBC depletion were performed either with both Sepax/CellEffic CB systems (n = 9). Cord blood units with collection volumes higher than 100 mL were fractioned into 2 bags in equal volumes to be processed with both systems. All CBUs and maternal donors were negative for infectious disease markers, and CBUs were processed within 48 hours after collection. Apart from one CBU which was collected from vaginal delivery, CBUs were obtained from Caesarean section (C/S) and all 9 CBUs were split into 2 equivalent fractions. Thus, 18 units were processed by both systems.

CB Processing with Both Systems – Sepax and CellEffic CB

Nine CBUs were split into 2 bags in equal volumes. Cord blood units were processed as described by Sato et al.[17] Samples from each bag were taken for complete blood count and flow cytometric analyses, in order to evaluate TNC, RBC, mononuclear cell (MNC), neutrophil and CD34 cell counts, and cellular viabilities. Following cell count assessments for each split bag, half of the initial CB was processed with automated Sepax and the other half with CellEffic CB.

Cryopreservation, Thawing, and Recovery Assessment

Five microliters of 5% dimethyl sulfoxide (DMSO) was infused (at a 25 mL/h speed) into all buffy coat (v = 20 mL) products derived either from CellEffic CB or Sepax (n = 18). Cord blood unitswere then transferred into a controlled-rate freezer and relocated into cryogenic tank at liquid nitrogen vapor phase. Reference samples (either segment or vial) from all CB products were thawed after they had been cryopreserved for 45 days for post-thaw analysis.

A single segment attached to the CB product was used to determine HSC subpopulations in split CBUs processed with Sepax or CellEffic CB. Thawed segments were diluted in Roswell Park Memorial Institute (RPMI) (StemCell Technologies [Vancouver, Canada]) with 10% fetal bovine serum (Thermo Fisher Scientific [Waltham, Massachusetts, USA]) with a dilution factor of 1/9.

Main Points

- Sepax post-thaw total nucleated cell/mononuclear cell recovery rates as well as colony-forming unit counts were higher than CellEffic cord blood (CB). Nonetheless, CellEffic CB was by far superior in terms of red blood cell depletion.
- The main drawback of CellEffic CB seems to be the labor-intensive and longer hands-on time nature with the requirement of qualified technicians.
- CellEffic CB can be an alternative system for processing CB at a much lower cost in private as well as public CBB or for immediate use for CBBs with lower turnover rates.
- To recommend CellEffic CB for routine CB banking requires more experience from CBBs.

Before and after processing and thawing, TNC, $CD34^+$ cells, MNC, RBC, and neutrophil recoveries were calculated for each CBU, and the mean values were evaluated between the 2 groups at all stages. Viability assessment was also performed on all post-process and post-thaw CBUs. Differences among pre-process, post-process, and post-thaw mean values as well as the mean recovery rates calculated from each CBU were compared for the CBUs processed with both systems.

Cell Counting, Immunophenotyping, and Viability

Pre-process, post-process, and post-thaw TNC values were calculated using white blood cell (WBC) counts (Siemens Healthcare Diagnostic Inc [Wien, Austria]). Red blood cell, TNC, MNC, and neutrophil cell counts were assessed accordingly.

Likewise, $CD34^+$ cell counts, as well as viability assessment, were carried out using Beckman Coulter Navios Cell Sorting Device. 7-Aminoactinomycin D (7-AAD)-based viability detection and $CD34^+$ cell counting were performed via Stem Cell Enumeration Kit using FITC-labeled CD45 and PE-labeled CD34 monoclonal antibodies (Beckman Coulter Stem Kit, California, USA). The analyses were performed using International Society of Hematotherapy and Graft Engineering (ISHAGE) single test platform.

Hematopoietic stem cell subpopulations were assessed, with Sepax (n = 9) or with CellEffic CB (n = 9) on a single segment attached to the CBUs. Flow cytometry analyses were carried out using the Beckman Coulter FC500 device. Cell surface markers and staining dyes used were given as follows: CD45 (ECD/FITC), CD34 (PC7), CD38 (FITC), CD3 (PC5), CD19 (ECD), CD33 (PE), CD71 (PE), and 7-AAD (P5).

CFU-GM Assay

Colony-forming unit-granulocytes and macrophages (GM) assay was performed using a commercially available methylcellulose medium [MethoCult H4445 Enriched without erythropoietin (EPO), StemCell Technologies, Canada]. Colony-forming unit-GM analyses were performed for all post-process and post-thaw CBUs. Colony-forming unit colonies were counted according to the manufacturer's recommendations with the same method used in the study by Gencer et al.[18]

Microbial Testing of the Processed CBUs

The RBC fraction was used for aerobic and anaerobic microbial testing (BACTEC Pediatrics Aerobic Plus/F Culture Vials (442194)/ BACTEC Plus Anaerobic Plus/F Culture Vials (442193). One to two microliters of RBC were used to inoculate aerobic bottles, whereas 8-10 mL of RBC fraction was seeded into anaerobic bottles, as recommended by the manufacturer. BACTEC bottles were loaded onto BD BACTEC 9240 Instrument and growth has continuously been tracked for 6 days.

Statistical Analysis

Statistical analyses for the differences between 2 processing methods were performed on Statistical Package for the Social

Table 1. Pre Process, Post Process, and Post–Thaw Data of Sepax and Celleffic Cb Systems

	Sepax			CellEffic CB		
	Pre Process (Mean ± SD)	Post Process (Mean ± SD)	Post Thaw (Mean ± SD)	Pre Process (Mean ± SD)	Post Process (Mean ± SD)	Post Thaw (Mean ± SD)
TNC ($\times10^7$/unit)	72.66 ± 27.29	58.16 ± 23.77	34.40 ± 19.76	72.87 ± 27.1	45.92 ± 14.15	29.91 ± 10.49
Neutrophil ($\times10^7$/unit)	35.95 ± 14.69	29.16 ± 12.15	18.2 ± 10.76	35.67 ±15.44	23.19 ± 7.63	15.31 ± 7.98
$CD34^+$ ($\times10^6$/unit)	2.68 ± 1.49	2.19 ± 1.49	1.15 ± 0.81	2.57 ± 1.48	2.05 ± 1.27	1.08 ± 0.63
MNC($\times10^7$/unit)	25.32 ± 15.21	23.78 ± 14.47	11.26 ± 12.14	25.48 ± 15.01	19.42 ± 9.17	13.81 ± 6.98
RBC ($\times10^{12}$/unit)	228.85 ± 52.93	94.92 ± 12.36	60.11 ± 24.93	227.79 ± 50.75	49.65 ± 7.44	39.42 ± 11.97
CFU Counts ($\times10^6$/unit)	NA	2.4 ± 1.73	1.91 ± 1.86	NA	2.73 ± 1.33	1.33 ± 0.77
Viability (%)	94.78 ± 4.94	92 ± 7.68	64.11 ± 8.27	95 ± 4.12	95.11 ± 4.01	68.01± 11.73

SD, standart deviation; CB, cord blood;TNC, total nucleated cells; MNC, mononuclear cells; RBC, red blood cells; CFU, colony forming unit.

Sciences for Windows version 20; (IBM Corporation, Armonk, NY, USA). Kolmogorov–Smirnov test and Shapiro–Wilk tests were taken into account for the assessment of the normality of the data. Paired samples *t*-test or Wilcoxon signed-ranks test was conducted to compare 2 processing methods for normally and non-normally distributed data, respectively. Results were interpreted as significant when a $P < .05$ was achieved.

RESULTS

Comparison of Processing Efficiencies and Recovery Rates of Sepax and CellEffic CB Systems

Nine splits (18 CBUs) were processed with both systems (median: 66.11; min-max: 54-94 mL). None of the pre-process parameters investigated yielded statistically significant differences between 2 groups [Sepax vs CellEffic CB]. Table 1 indicates pre-process, post-process, and post-thaw results in terms of TNC, neutrophil, $CD34^+$ cells, as well as MNC, RBC, and CFU mean counts with standard deviations (SD). Viabilities are also shown in Figure 1.

A total of 18 vials linked to associated units were thawed under similar conditions from the comparison group. Pre-process, post-process, and post-thaw data for this group are summarized in Table 1. The only major difference was that CellEffic CB system had better RBC depletion rates (Table 1).

Recovery rates between pre-process and post-thaw steps were generally similar between the systems except for RBC depletion, which was superior for CellEffic CB as expected ($P = .005$). There was a trend for better CD34 recovery with CellEffic CB (73%) compared to Sepax (59.9%) ($P = .183$) (Figure 2). Total nucleated cell and MNC recoveries were similar for the 2 groups compared.

When post-process and post-thaw results were investigated within the same 2 groups, MNC recovery and RBC depletion rates differed significantly in favor of Sepax ($P = .018$ and $P = .066$, respectively). Recovery rates of post-process/post-thaw TNC viability for CellEffic CB group were slightly higher than after Sepax but did not reach any statistical significance ($P = .161$). CellEffic CB was surpassing Sepax in terms of all parameters in relation to post-process/post-thaw recovery rates (Figure 3).

CFU GM Analysis

A total of 35 CFU-GM analysis were performed for Sepax versus CellEffic CB group; however, 31 out of 35 were accomplished. Four individual units (2 CellEffic CB and 2 Sepax) did not show visible colony growth. When 2 systems were compared, although the number of post-process CFU-GM assays was not enough for statistical evaluation of the differences between the groups, in terms of post-thaw CFU-GM counts, Sepax was statistically

Figure 1. Process recovery rates of Sepax versus CellEffic CB.

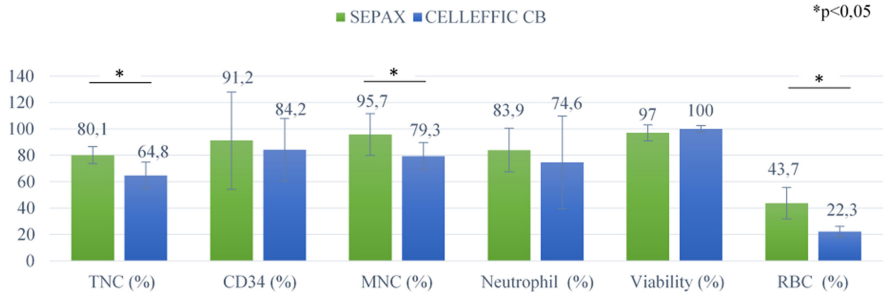


Figure 2. Pre process and post thaw recovery rates of Sepax versus CellEffic CB.

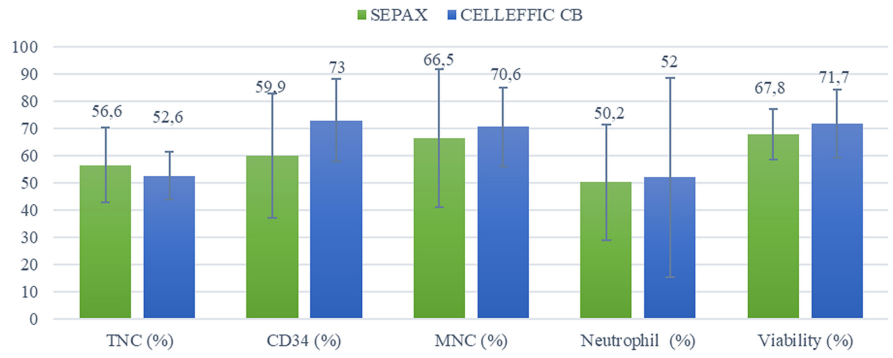


Figure 3. Post process and post thaw recovery rates of Sepax versus CellEffic CB.

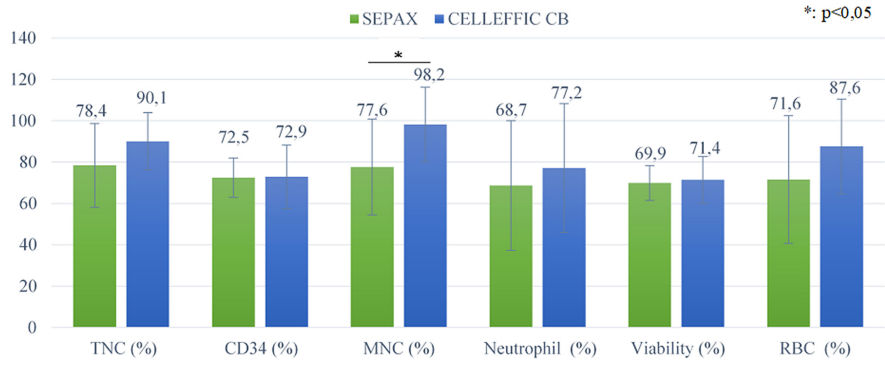
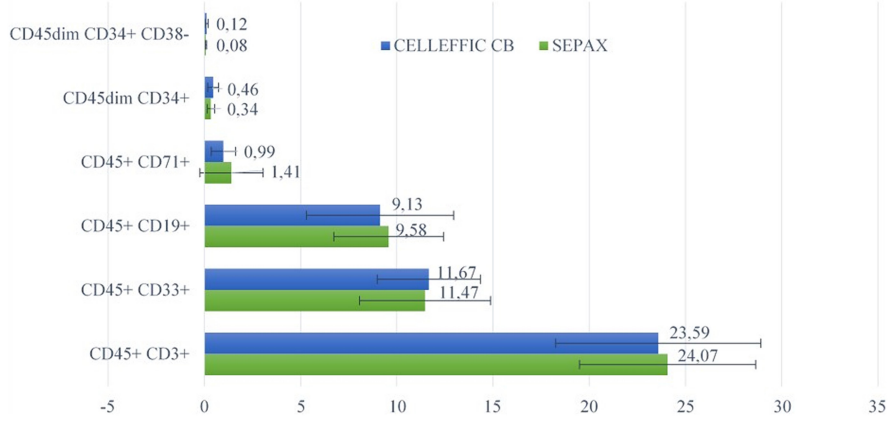


Figure 4. Post thaw cell populations (gated through viable CD45⁺ cells).



superior over the CellEffic CB group ($P = .048$), (Table 1). Colony-forming unit testing could not be performed on one of the units from CellEffic CB group.

CB HSC Subpopulation

Any likely impact of the observed processing systems on post-thaw CB HSC subpopulations was evaluated for all CBUs.

After the segments attached to the units had been thawed, mean percentages were calculated through the subpopulations gated from viable CD45⁺ population, and the results are summarized in Figure 4 which revealed no impact of the technology used.

CellEffic CB was advantageous in terms of RBC depletion ($P = .007$) and post-thaw TNC viability ($P = .017$), on the other

Table 2. Summary of CellEffic CB Versus Sepax Comparison [(+): Superior for Associated Item]

Item	Sepax	*P*	CellEffic CB
RBC depletion	(–)	.007	**(+)**
TNC recovery	**(+)**	<.001	(–)
MNC recovery	**(+)**	.016	(–)
Post process TNC viability	(–)	.017	**(+)**
Post thaw CFU counts	**(+)**	.048	(–)
Ease of use	**(+)**	–	(–)

CB, cord blood;RBC, red blood cells;TNC, total nucleated cells; MNC, mononuclear cells; CFU, colony forming unit.

hand, in terms of TNC and MNC recoveries, Sepax was superior to CellEffic CB ($P < .001$ and $P = .016$). Additionally, Sepax had a superiority over CellEffic CB ($P = .048$) for post-thaw CFU counts. While CellEffic CB was efficient for small volumes, Sepax was favorable with its user-friendly automated nature (Table 2).

Microbial Contamination

Totally 21 post-process microbial sterility testing was performed for CBUS using BACTEC system, and growth was observed on both aerobic and anaerobic bottles which were checked out daily for 6 days. None of the units showed any microbial growth regardless of the processing system used.

DISCUSSION

Processing and storage of high-quality CB are the primary goals of CB banking.[4,19,20] High post-thaw TNC/MNC, CD34 recovery rates, as well as viability, are the most important parameters to be maintained for a successful transplant.[21-23] A common procedure, volume reduction of CBU, is performed at all CB banks over the world. All FACT-NetCord accredited CB banks (including ours) have clearly defined acceptance criteria. A collection volume of ≥70 mL, a total TNC number being $\geq 100 \times 10^7$, and a previability of ≥90% are established. Provided that the CD34+ cell count is $\geq 1.5 \times 10^6$/unit, then CBU is accepted for processing. Additionally, all microbiological testing should come negative.

Sepax is the mainstream closed automated system used worldwide which has been proven to be the most efficient method with highest TNC recovery yield.[10] Nonetheless, Sepax system has a main disadvantage of utilizing very expensive disposable kits that cannot be afforded by all banks. Having perks like considerable RBC depletion rates, low cost and *in house* optimization chances, manual systems operating in open settings are prone to contamination of the product. Last but not the least, they are generally labor-intensive and time-consuming. In general, automated systems are preferred over manual methods because of better standardization and reproducibility, as well as less operator dependency.[12,24]

CellEffic CB system, which was evaluated in this present study, is a manual semi-closed system claiming to cause less stress and thus less harm to cells. This enables the system to be a promising candidate for quality products to be transferred to clinical programs.

CellEffic CB was announced for the first time in this aforementioned paper.[17] Although similar in nature, they did use nonmatching separate CBUs for the comparison of 2 systems in contrast to ours. We involved exactly the same CBUs with high volumes equally divided into 2 fragments and evaluated data in the same unit. This is a striking difference which makes our interpretations more robust. To our knowledge, this is the first study that 2 processing systems were compared on equivalent split CBUs. The other main difference between theirs and ours is that we did not use HES.[17] Hydroxyethyl starch usage might have had a slight but negligible impact on the outcomes.

Recovery rate assessment was shown to be the best reflector of cell contents of a CB product thus allows comparison of CB processing systems. In this study, our post-process TNC recovery result was higher in favor of Sepax ($P < .001$). In contrast to ours, the paper from Sato et al.[17] in which CellEffic CB was announced for the first time and compared to Sepax, have reported no statistical significance in terms of TNC recovery rate (76.6% post process for Sepax and 73.14% for CellEffic CB Saline).[17] In the study from Basford et al.[10] in which 5 CB processing systems were compared, highest TNC recovery was found to be with Sepax similar to our findings .[10] Our results indicated significant difference in favor of post Sepax MNC recovery with 95.7% versus 79.3% ($P = .016$). In contrast to our findings, Sato et al[17] found no statistical significance for post-process MNC recovery. When CD34 post-process recovery rate was investigated, there was no statistical significance between 2 systems. Sato et al[17] denoted a considerable difference between CD34 recovery rates in favor of CellEffic CB; however, none of the post CD34 recovery results reached statistical significance; main reason for this difference might be HES usage along with Sepax system, which seems to be the one and only difference between their study and ours. When compared, post-thaw TNC viability was found to be similar with both systems (64.11% and 68.01% for Sepax and CellEffic CB). Sato et al.[17] opposite to us, have found statistical significance for post-thaw TNC viability rates (85.24% for CellEffic CB and 64.8% for Sepax).[17] Although CellEffic CB viability was slightly higher at their hands, post-thaw Sepax viability was in concordance with ours.

Plasma removal/RBC depletion is crucial to obtain pure MNC cells which may otherwise interfere with HSC population of the product. Additionally, depletion of RBC will lead to smaller volumes allowing more products to be banked.[25,26] Post-process RBC count was found to be higher in Sepax, similar to the study of Sato et al.[17] Consentient to our results, Basford et al[10] have found higher RBC count Sepax. When we analyzed RBC removal rates, CellEffic CB depleted more RBC than Sepax did ($P < .001$). Our results were similar to the findings from Sato et al.[17] although their results did not reach statistical significance.[17] Basford et al[10] reported better RBC depletion rates using manual CB processing methods over Sepax, ours and the results from Sato et al[17] showed better RBC depletion rates in favor of CellEffic CB.

As indicated in all studies, we mentioned above, although Sepax is better for TNC and MNC recovery, it is less efficient in terms of RBC depletion post process. When we analyze post-thaw RBC depletion rates, RBC count was revealed higher in Sepax, Both Sato et al[17] and Basford et al[10] have observed similar results, with Sepax being the most disadvantageous system in terms of excess RBCs in both processed and thawed CBUs.

Colony-forming unit-GM is essential for the assessment of functional the clonogenic and proliferative potential of HSC in vitro, a major FACT-NetCord quality standard at the same time.[18,27,28] The CFU-GM results in our study with both systems were generally in concordance. We found post-process CFU counts higher in CellEffic CB. Sato et al.[17] have found similar results like us for post process. Colony-forming unit-GM counts after CellEffic CB showed higher colonies compared to Sepax similar to ours in 2 different papers.[16,17] When we looked at CFU counts post-thaw, Sepax group was superior to CellEffic CB ($P = .048$). Consistent with our results, Sepax was superior to all other manual systems tested in the study of Basford et al.[10] In contrast to our result, Sato et al[17] and Shima et al[16] have observed higher post-thaw CFU counts for CellEffic CB. There was no significant difference between post-process CFU-GM counts for units processed with Sepax and CellEffic. Either way, the small number of observations in this analysis prevents any firm conclusion based on statistical results.

Sustaining the essential cellular content of the CB product ensures successful transplantation.[29] In light of this information, we wanted to evaluate any likely impact of the investigated processing systems on post-thaw CB HSC subpopulations after cryopreservation. Early HSCs, namely $CD45^{dim}$ $CD34^{+}$ $CD38^{-}$ cells, were 0.08% and 0.12% of viable $CD45^{+}$ cells in Sepax and CellEffic CB, respectively. Although minimal, this difference may highlight the lack of centrifugation for a higher yield of viable HSC was slightly higher. Whereas, erythroid progenitors were found to be lower in CellEffic CB as expected, since RBC depletion rates were also higher in this group.

A CB processing system which avoids centrifuge stress on cells with better RBC depletion and TNC/MNC recovery rates will highly likely to be effectively used in the field. Moreover, RBC depletion pre-cryopreservation is crucial since removing RBCs was shown to improve post-thaw CD34+ cell viability.[30] Post-thaw CD34+ cell viability is one of the most important parameters for a successful transplant outcome. As a result, the occurrence of viscosity and clumping in the product may be another disadvantage for CB transplants.[17]

To our knowledge, this is the first study that 2 processing systems were compared on equivalent split CBUs. CellEffic CB was advantageous in terms of RBC depletion ($P = .007$) and post-thaw TNC viability ($P = .017$), on the other hand, in terms of TNC and MNC recoveries Sepax was superior to CellEffic CB ($P < .001$ and $P = .016$). Additionally, Sepax had a superiority over CellEffic CB ($P = .048$) for post-thaw CFU counts. While CellEffic CB was efficient for small volumes, Sepax was favorable with its user-friendly automated nature (Table 2).

The major drawback of our study is the sample size. Due to the valuable nature of CB, only discarded ineligible units were included in this study. Moreover, of the discarded units, only the ones with sufficient volumes were selected; those suitable for a split. Low volume is among the most common non-conformities leading to disposal, thus only restricted number of CB was available for the comparison of split units.

CONCLUSION

A CB processing system avoiding centrifuge stress on cells with better RBC depletion and TNC/MNC recovery rates on top will highly likely to be effectively used in the field. CellEffic CB was surpassing Sepax in terms of all parameters in relation to post-process recovery rates. CellEffic CB seems to be particularly useful for CBUs with lower volumes and high $CD34^{+}$ cell counts, which are generally subject to be not applicable to automated systems. The main 2 differences in favor of Sepax were post-thaw TNC/MNC recovery rates as well as CFU counts. Nonetheless, CellEffic CB was by far superior in terms of RBC depletion. The main drawback of CellEffic CB seems to be the labor-intensive and longer hands-on time nature with the requirement of qualified technician. CellEffic CB can be an alternative system for processing CB at a much lower cost in private as well as public CBB or for immediate use for CBBs with lower turnover rates. Thus, to recommend CellEffic CB for routine cord blood banking requires more experience from CBBs.

Ethics Committee Approval: Ethical committee approval was received from the Ankara University School of Medicine Ethics Committee. (Date: December 22, 2014, Decision no: 21-882-14).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – P.Y.M.; Design – M.B.; Supervision – E.B.G.O., H.Y.A.; Funding – P.Y.M., E.B.G.O., H.Y.A., M.B.; Materials – P.Y.M., E.B.G.O., H.Y.A., M.B.; Collection and Processing – H.Y.A.; Analysis and/or Interpretation – E.B.G.O.; Literature Review – P.Y.M., E.B.G.O.; Writing – P.Y.M.; Critical Review – P.Y.M., E.B.G.O., H.Y.A., M.B.

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Declaration of Interests: The authors declare that they have no competing interest.

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Original Article

Ferritin Levels in Serum and Saliva of Oral Cancer and Oral Potentially Malignant Disorders

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ABSTRACT

Objective: Oral cancer remains a substantial health burden worldwide despite creditable developments in its prevention, detection, and treatment. The early detection of oral cancer offers high chances of survival and improves response to therapy making overall healthcare affordable. The aim and objective of this study were to compare and correlate serum and saliva ferritin levels in healthy subjects, oral potentially malignant disorders, and subjects with oral cancer and to assess the role of saliva as a valuable diagnostic tool.

Methods: Totally 30 participants each in 3 groups comprising healthy subjects, oral potentially malignant disorders, and oral cancer constituted the sample size. Enzyme-linked immunosorbent assay method was employed for serum and saliva ferritin levels.

Results: The respective mean serum ferritin and saliva ferritin levels were increased significantly in subjects with oral cancer (296.62 ± 82.54 ng/mL and 80.44 ± 12.94 ng/mL, respectively) and decreased significantly in oral potentially malignant disorders (69.83 ± 17.39 ng/mL and 17.49 ± 5.40 ng/mL, respectively) with a highly significant P <.001 when compared to that of healthy subjects, (116.15 ± 21.19 ng/mL and 38.47 ± 8.08 ng/mL), P <.001. All the 3 groups had a significant positive correlation between serum and saliva ferritin levels; healthy controls (r = 0.622), oral potentially malignant disorders (r = 0.878), and oral cancer (r = 0.668).

Conclusion: The encouraging results of the present study demonstrate the potential involvement of ferritin in the pathogenesis of oral potentially malignant disorders and oral cancer. Further, the study favors saliva, as a reliable and non-invasive diagnostic tool providing a cost-effective approach for screening large populations.

Keywords: Ferritin, oral cancer, oral potentially malignant disorders, saliva, serum

INTRODUCTION

Oral cancer along with oropharyngeal cancers constitutes the 6th most common malignancy around the globe.[1] Worldwide more than 400,000 oral cancer cases are diagnosed every year, mostly in the countries such as India, Sri Lanka, Pakistan, Bangladesh, and Indonesia.[1,2] The overall 5-year survival rate of oral cancer has stayed low at 40%, but if diagnosed early the survival rates can improve up to 80%.[3] About half of all oral cancer cases are not diagnosed until in their later stages, due lack of symptoms in the early stages, and patients seeking medical help only in case of clear symptoms such as pain, growth in the mouth or surrounding areas or when lymphatic spread has taken place.[4] Oral cancer can occasionally be preceded by lesions of oral precancer which predominantly includes oral submucous fibrosis (OSMF) and leukoplakia.[5] The prevention and timely recognition of such OPMDs not only favors a decreased rate of oral cancer but also improves the chances of survival in subjects developing oral cancer[6] There is a need to develop simple, non-invasive diagnostic markers for early diagnosis, which would also aid in monitoring the progress of disease during the treatment. Recent studies have shown additional functions characterized by ferritin other than being a major iron-storage protein and these include suggestions linking ferritin to various pathways associated with cancer, such as suppressor evasion, cell proliferation, growth angiogenesis, cell death inhibition, immunomodification, immortalization, invasion, and metastasis.[7] Although several studies have assessed serum ferritin in OPMDs and oral cancer, the current study is the only till date evaluating ferritin levels of

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saliva in the aforementioned conditions. So the primary aim of this study was to assess the role of saliva as a diagnostic medium by estimating saliva ferritin levels in OPMDs and oral cancer.

METHODS

This case–control study was carried out on 90 participants reporting to the Department of Oral Medicine and Radiology of our institution, and included 3 groups (A, B, C) with 30 in each of them. Written and informed consent was obtained from all the cases before inclusion in the study. Group A comprised 30 healthy participants, group B had 30 OPMDs, and group C had 30 subjects with oral cancer. Buccal mucosa constituted the most affected anatomical site with 11 cases, followed by the tongue with 7 cases (Table 1). The OPMDs mainly consisted of 18 OSMF, 8 oral leukoplakia, and 4 cases of oral lichen planus.

Data Collection

Ethical approval was obtained from the institutional ethical committee, AB Shetty Memorial Institute of Dental Sciences (Date: October 30, 2015, Decision no: ABSM/EC 64/2015). World Medical Association (WMA)Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects was followed for sample collection.

Sample Collection

After obtaining institutional ethical clearance, informed consent from each subject participating in the current study was taken. The detailed case history of each subject was recorded, and the oral cavity was examined thoroughly.

Saliva Collection

Subjects were instructed not to eat, drink, or smoke for 1 hour before saliva sample collection. Each subject was seated with their head tilted forwardly. The subjects were instructed not to swallow any saliva and were abstained from speaking during sample collection time. "Spit Technique" was employed to collect unstimulated saliva from each subject participating in the study. The subjects were given a graduated container with a funnel and instructed to spit into it for 8-10 minutes. The collected sample consisted of secretions from major and minor salivary glands and gingival crevicular fluid and represented whole mouth fluid. The collected saliva sample was centrifuged (2500 rpm for 10 minutes) and the supernatant thus collected was stored at −20°C before analysis.

Main Points

- The functions of ferritin are manifold than just being an iron-storage protein.
- Although ferritin was elevated in malignancies, the level of ferritin in oral potentially malignant disorders (OPMDs) was downregulated. The more number of oral submucous fibrosis patients in OPMDs sample in this study could be the reason for this downregulation. Further large-scale studies are warranted to corroborate this finding.
- The current study verifies that saliva can be used as a safe and non-invasive diagnostic medium.

Table 1. Distribution of Oral Cancer Cases According to Their Site

Various Sites of Oral Cavity (Oral Cancer)	Number of Patients (n = 30)
Buccal mucosa	11
Tongue	7
Mandibular alveolus	3
Buccal vestibule	3
Maxillary alveolus	2
Retromolar region	1
Lip	1
Floor of the mouth	1
Buccal mucosa with skin	1

Blood Collection

The patients were seated in a comfortable position, and a syringe was used to draw 2 mL of venous blood from the antecubital vein. The blood collected was transferred into plain tubes and centrifuged for 10 minutes at 2500 rpm. Serum extracted from blood was stored in glass vials at −20°C and was later subjected to analysis.

Ferritin Enzyme-Linked Immunosorbent Assay

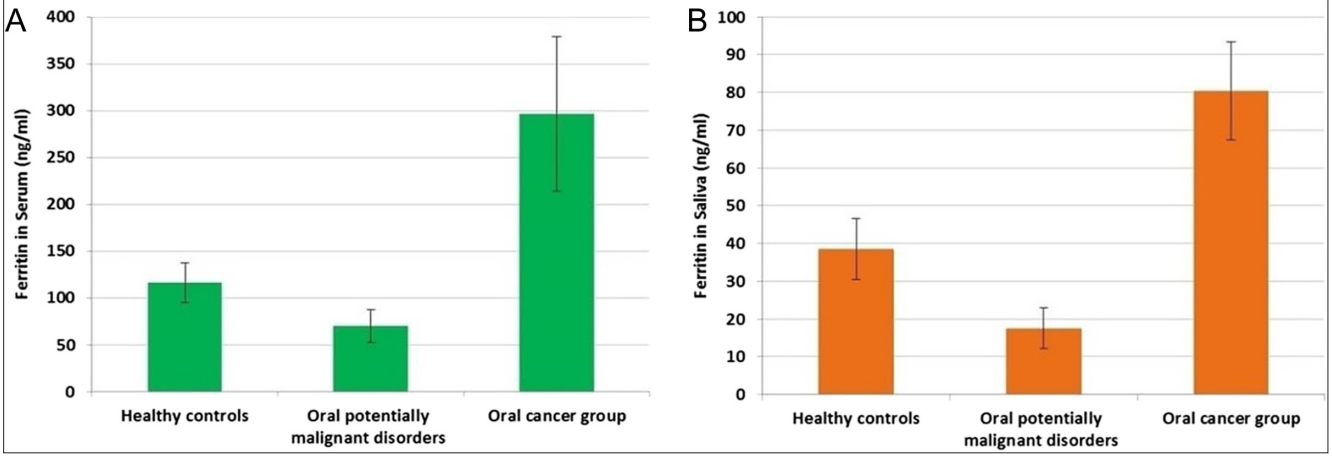
Principle of the Test

Based on a streptavidin–biotin principle, the ferritin ELISA kit involves a solid-phase sandwich assay technique. The designated wells are coated with streptavidin, and the standards, samples, and biotinylated anti-ferritin antibody reagent are added to the wells.The endogenous ferritin in the saliva and serum sample binds to the biotinylated anti-ferritin antibody at its antigenic site. The high-affinity streptavidin–biotin interaction concurrently immobilizes biotinylated antibody onto the wells. The buffer wash is used to wash off the unbound protein and excess biotin-conjugated antibody. A sandwich complex is formed, the analyte of interest lies between the 2 highly specific antibodies, and is labeled with horseradish peroxidase and biotin upon the addition of the peroxidase-conjugated anti-ferritin antibody reagent. The buffer wash is then used to wash off unbound protein and excess enzyme-conjugated antibody reagent. The intensity of the color developed upon the addition of the substrate is directly proportional to the concentration of ferritin in the samples. The color intensity relation to the concentration of ferritin is interpreted by drawing a standard curve.

Method of Analysis

Statistical Package for the Social Sciences 21.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for the statistical analysis. Mean and standard deviation of the quantitative values in control and study groups were estimated. Chi-square test was used for analyzing the distribution of gender between control and study groups. One-way analysis of variance was used for the analysis of serum and salivary ferritin levels in all the groups. Tukey multiple comparison tests were used for the comparison of study groups to the control group and comparison between the study groups. Pearson correlation was used to measure the correlation between serum and saliva ferritin levels between all the 3 groups.

Figure 1. A, B. Mean serum ferritin levels in normal healthy controls, subjects with OPMDS, and subjects with oral cancer (A) and mean saliva ferritin levels in normal healthy controls, subjects with OPMDs, and subjects with oral cancer (B). OPMDS, oral potentially malignant disorders.



RESULTS

The analysis of demographic data among the 3 groups is given as follows: the age range in all the 3 groups was chosen as 20-70 years. The normal healthy controls (Group A) had mean age of 53.53 ± 10.092 years with 33.3% (10/30) females and 66.7% (20/30) males; oral potentially malignant disorders (OPMDs, group B) with a mean age of 56.87 ± 10.954 years included 46.7% (14/30) females and 53.3% (16/30) males; oral cancer (group C) had a mean age of 60.53 ± 7.300 years and comprised of 30% (9/30) of females and 70% (21/30) of males.

Mean Serum ferritin levels

Healthy controls (group A) had a mean serum ferritin level of 116.15 ± 21.19 ng/mL and group B (OPMDs) and group C (oral cancer) had mean serum ferritin levels of 69.83 ± 17.39 ng/mL and 296.62 ± 82.54 ng/mL, respectively (Figure 1A).

Mean Saliva Ferritin levels

Group A had mean saliva ferritin levels of 38.47 ± 8.08 ng/mL and group B and group C had mean saliva ferritin levels of 17.49 ± 5.40 ng/mL and 80.44 ± 12.94 ng/mL, respectively (Figure 1B).

Analysis of Statistical Significance

Serum Ferritin Levels

The mean serum ferritin levels of healthy controls (group A) was higher than group B (OPMDs) with a highly significant *P*-value of .002. Oral cancer (group C) showed higher mean serum ferritin than both group A and group B with a highly significant *P*-value of <.001 each (Table 2).

Saliva Ferritin Levels

Similarly, the mean of saliva ferritin levels was higher in group A than in group B, and the mean was higher in group C than in both group A and group B, all having a statistically significant *P*-value of <.001. (Table 3).

Pearson correlation: Significant positive correlation in ferritin levels between serum and saliva was seen in all the 3 groups;healthy controls: ($r = 0.622$) (Figure 2), OPMDs: ($r = 0.878$) (Figure 3), and oral cancer: ($r = 0.668$) (Figure 4).

DISCUSSION

The growth of a tumor can be monitored by evaluating tumor markers, and such evaluation can prove vital in diagnosis, staging, and future prognosis. The clinical use of a tumor marker becomes vital only when it enables its continuous measurement during a patient's clinical course after being positive for the same. The advancement or remission of malignancies can be predicted by mounting or falling values of tumor markers. The early intervention employed in high-risk OPMDs after predicting the progression of their phenotype can prevent the development of oral cancer. A considerable understanding of the various cellular processes and molecular mechanisms fundamental to

Table 2. Comparison of the Mean Difference between the Serum Ferritin of the Study Groups and the Healthy Controls, and between the Study Groups

				95% CI	
(I) Group	(J)	Mean Difference (I–J)	P	Lower Bound	Upper Bound
Healthy controls	OPMDs	46.32*	.002	15.40	77.23
	Oral cancer	−180.48*	<.001	−211.39	−149.56
OPMDs	Oral cancer	−226.80*	<.001	−257.71	−195.88

*The mean difference is significant at the .05 level.

Table 3. Comparison of the Mean Difference between Saliva Ferritin of the Study Groups and the Healthy Controls and between the Study Groups

				95% CI	
(I) Group	**(J)**	**Mean Difference (I–J)**	P	**Lower Bound**	**Upper Bound**
Healthy controls	OPMDs	20.98*	<.001	15.23	26.73
	Oral cancer	−41.97*	<.001	−47.72	−36.22
OPMDs	Oral cancer	−62.95*	<.001	−68.70	−57.20

*The mean difference is significant at the 0.05 level.

Figure 2. Correlation of serum and saliva ferritin levels in group A (healthy controls).

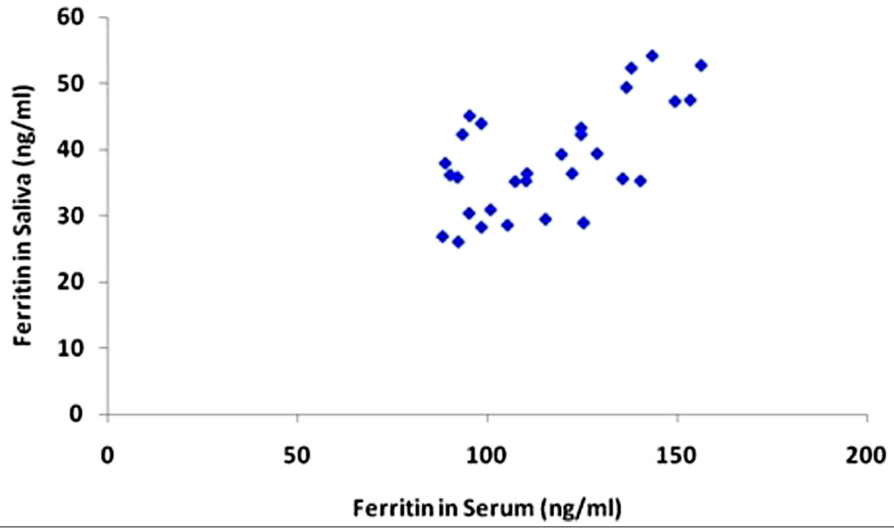
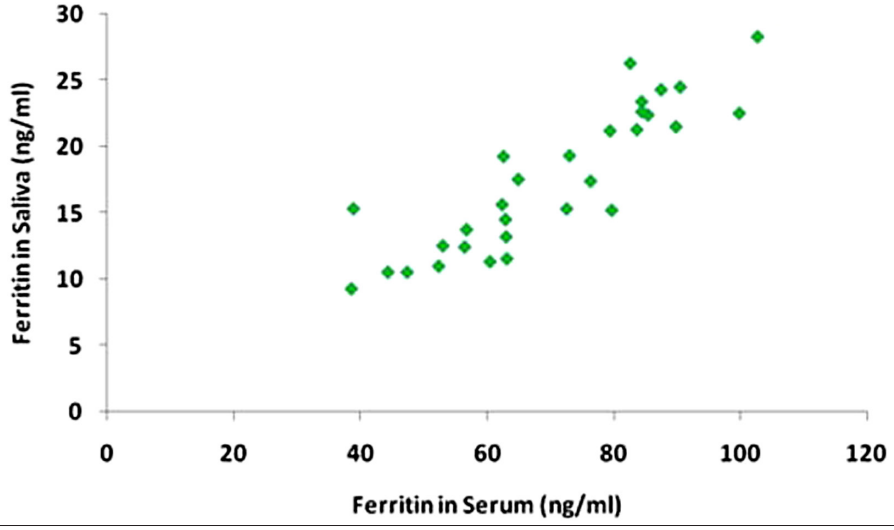
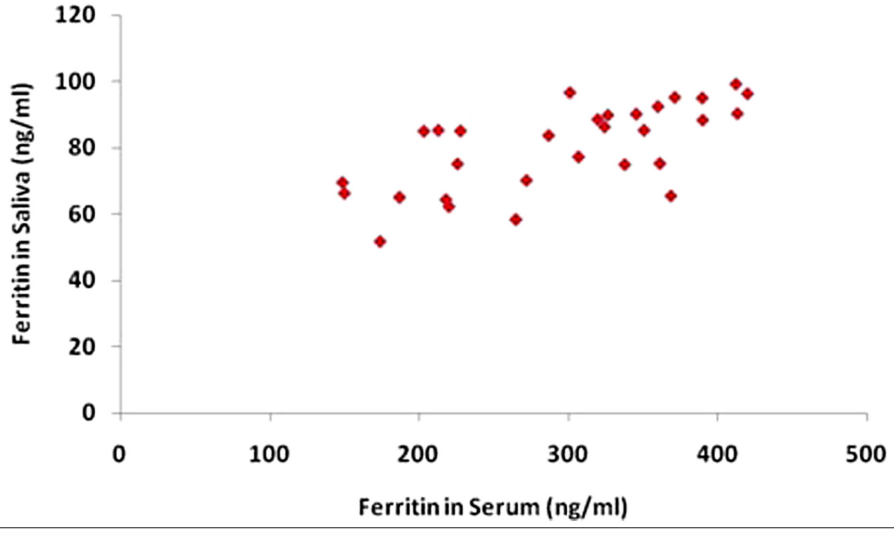


Figure 3. Correlation of serum and saliva ferritin levels in group B (oral potentially malignant disorders).



the initiation of cancer is required for the establishment of tumor markers. A special focus is given to the fact that various cellular functions can get disrupted by only a minor change in only a few regulatory proteins or genes. The tumor markers may be released as constituents in various body fluids such as serum, urine, saliva, and cerebrospinal fluid (CSF) or as substances in cells of tissues. The evaluation of tumor markers, until recently, was usually carried out in fluids other than saliva such as urine, blood, and CSF, but technological advances in the field of diagnosis have accredited saliva with certain advantages over other diagnostic media.

Figure 4. Correlation of serum and saliva ferritin levels in group C (oral cancer).



Saliva has been used for the assessment of either individual or a group of protein markers together to assist in the early recognition of oral cancer and in employing a suitable therapy. The current study was carried out with the objective of assessing the serum and saliva ferritin levels in OPMDs and subjects with oral cancer and to assess the role of saliva as a diagnostic tool.

Serum Ferritin in Oral Cancer

The statistically significant increase in serum ferritin levels in oral cancer in this study is concomitant with a cross-sectional study by Baharvand et al.[8] who evaluated serum ferritin in 60 oral cancer cases and 66 age and sex-matched controls. The elevated levels of ferritin may occur in response to chronic diseases, inflammation, and infection and may be increased despite inadequacy or deficiency of iron. Evidence gathered suggests an established role of iron in carcinogenesis.[9] Ferritin helps in maintaining the performance of important biochemical reactions and balances the process of oxidative stress.[10] Free radicals may be created as a result of increased serum ferritin, causing carcinogenesis effects. The study done by Khanna et al.[11] in conformity with our study, showed an increase in serum ferritin in oral cancer cases and confirmed much higher levels of serum ferritin in advanced stages as compared to early stages, thereby concluding that the levels of serum ferritin can be used to differentiate between early and late stages of oral squamous cell carcinoma (OSCC). Maxim et al.[12] also found increased levels of serum ferritin in head and neck cancer cases in accordance with our study and found lowered serum ferritin in cured subjects, who had no sign of clinical disease for 5 years than in those of untreated cases. Our study showed higher levels of serum ferritin in oral cancer as compared to controls, which was in accordance with studies done by Inal et al.[13] Richie et al.[14] Bhatavdekar et al.[15] Yuan et al.[16] and Vinzenz et al.[17] Elevated ferritin levels in cancer patients have been attributed to iron metabolism, hematopoiesis, and some nonspecific tissue damage. The direct secretion of ferritin from tumor cells has been postulated as the cause of the elevation of ferritin in oral cancer.

Serum Ferritin in OPMDs

Only few studies have evaluated the serum concentration of ferritin in OPMDs. Richie et al.[14] reported significantly lower serum ferritin values in oral pre-malignancies than in normal healthy subjects: the reduced ferritin level is indicative of iron deficiency, whereas raised ferritin levels can occur even in iron-deficiency states. So it can be inferred that elevated serum ferritin levels in oral cancer and low serum ferritin levels in oral precancerous lesions of oral cavity have different underlying causes. Thakur et al.[18] found a significant decline of serum ferritin in OSMF patients as compared to normal healthy controls and further showed a progressive decrease in serum ferritin levels as the histopathological grade increased. The reduced serum ferritin in OSMF can be related to the corollary of increased utilization of iron in OSMF for collagen synthesis. Thus, as the iron stores get depleted, serum ferritin level decreases and is downregulated. In the current study, serum ferritin levels in OPMDs were significantly reduced in OPMDs, and as the subjects were from a similar socioeconomic background, the decreased levels of serum ferritin could be because of the disease process itself rather than being a cause. Thus, it can be stated that the lack of proper intake of micronutrients in the diet leads to anemia initially, which worsens later due to the progression of the OPMDs. As low ferritin is one of the diagnostic criteria for iron deficiency, the increased iron utilization in case of OPMDs (as in OSMF) could be the reason for decreased levels of ferritin.

Saliva Ferritin Levels in Oral Cancer and OPMDs

The existing literature showed no earlier study employing saliva ferritin levels in oral cancer and OPMDs. In the present study, the mean saliva ferritin level in subjects with oral cancer showed a significant elevation (similar to that of serum) when compared with the mean saliva ferritin levels of normal healthy subjects. Although it is not precisely known how tumor markers manifest in saliva, they may be either derived from serum or can be produced locally. When derived from serum, ferritin in saliva can

appear as a constituent of normal saliva composition, active transport, passive diffusion, an outflow of crevicular fluid, and ultrafiltration through tight junctions. When produced locally, it can be a result of cell necrosis, apoptosis, active release, or trauma. Thus, the increase in saliva ferritin levels in oral cancer subjects could be due to direct leakage from the malignant tumors in addition to its derivation from serum, as ferritin has been considered to be a product of damaged cells.

The saliva ferritin levels in OPMDs were significantly lower than saliva ferritin levels of healthy controls. The decreased saliva ferritin levels in OPMDs could be due to increased utilization of iron, as comparable results were seen in serum ferritin levels of OPMDs. The altered epithelial turnover rate, decreased intake of micronutrients owing to the difficulty in mastication, and increased utilization of iron as seen in subjects with OSMF to produce collagen all lead to iron depletion in OPMDs. These factors, in turn, could lead to the reduction of saliva ferritin levels in OPMDs.

Limitations of the Study

The analysis of ferritin was not interpreted according to the stages in the oral cancer study group. The sample size in each group was fairly small.

CONCLUSION

The current study showed a significant increase in serum and saliva ferritin levels in oral cancer and a significant decrease in serum and saliva ferritin levels in subjects with OPMDs thereby indicating that ferritin can be used as an adjunctive diagnostic biomarker in both oral cancer and OPMDs. Furthermore, the positive significant correlation between serum and saliva ferritin levels signifies that saliva can be utilized as a reliable, non-invasive tool for diagnosing and monitoring of OPMDs and oral cancer. Saliva, which is a readily available sample containing a substantial number of proteins and peptides, is used as a biomarker for diagnosing various oral and systemic diseases. It is one of the most reliable tools for diagnosing oral squamous cell carcinomas because of its direct contact with oral cancer and OPMDs. Thus, saliva can be used in a non-invasive fashion for the diagnosis of OPMDs and oral cancer subjects, which has the potential to dramatically reduce anxiety and discomfort associated with blood sampling procedures and increases the willingness of patients to undergo frequent health inspections.

Ethics Committee Approval: This study was conducted at NITTE (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences, Mangalore- Karna taka- India from January 2016 to December 2017 and was approved by the institutional ethics committee (Date: June 30, 2017, Decision no: ABSM/EC64/2015).

Informed Consent: Informed consent was taken from all the volunteers, after having been informed of the study details and provided with clarifications.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.A.B., S.G.B.; Design – S.A.B., S.G.B., R.L.C.; Supervision – S.G.B., R.L.C.; Materials – S.A.B., D.S.P., S.B., U.L.D.; Data Collection and/or Processing – S.A.B., U.L.D.; Analysis and/or Interpretation – S.A.B., R.L.C., S.B., U.L.D.; Literature Review – S.A.B., D.S.P., S.B.; Writing – S.A.B., D.S.P.; Critical Review – S.G.B., R.L.C.

Declaration of Interests: There are no conflicts of interest.

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Original Article

Prevalence of Tooth Number Anomalies and Their Distribution by Genders

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ABSTRACT

Objective: This study aims to examine the dental anomalies (tooth number, size, and structural anomalies) in a group of pediatric dental population and the difference between the genders in terms of the prevalence of these anomalies.

Methods: In this retrospective study, digital orthopantomograms belonging to 5000 patients aged 5-14 years, taken at the Faculty of Dentistry of Erciyes University were evaluated. Tooth number anomalies (hypodontia, hyperdontia, anodontia, and mesiodens) and dental pathology (odontoma and cyst-tumor) were evaluated in orthopantomograms. The frequencies of dental anomalies were compared, and their distribution by gender was examined using descriptive tests and chi-square tests.

Result: Of 5000 patients, 169 (3.38%) had 1 dental anomaly. Of the patients, 137 (81%) had hypodontia, 27 (15.9%) hyperdontia, 6 (3.5%) mesiodens, 2 (1.1%) odontoma, and (1.7%) cysts-tumor-like structures. The prevalence of hypodontia was found to be higher in girls than in boys ($P < .05$). There was a difference between the genders in terms of the prevalence of hypodontia ($P < .05$). No difference was found between the genders in terms of other dental anomalies ($P > .05$).

Conclusion: Whether common or rare, dentists should be careful about the presence of dental anomalies while examining their patients. Detecting these anomalies and performing interventions in the required period is important to prevent complications that may occur in the future. In this way, psychological, aesthetic, phonational, and physical problems that may occur in the future lives of children and adolescents can be prevented.

Keywords: hyperdontia, hypodontia, macrodontia, Tooth number anomalies, tooth size anomalies, tooth structural anomalies

INTRODUCTION

Dental anomalies are changes in terms of morphology, position, size, and number of teeth.[1] Dental anomalies are divided into 2 sub-groups as developmental and acquired anomalies. Developmental dental anomalies (DDAs) occur during tooth developmental stages, which cover the morphodifferentiatio n and histodifferentiation periods. Acquired dental anomalies (ADAs) are caused by the changes that occur after the normal tooth developmental stages are completed.[2] Developmental dental anomalies constitute an important category of dental problems.[3] These anomalies can be observed alone (non-syndromic) or may develop as a part of a syndrome.[4] A DDA may be asymptomatic, or it may manifest itself with malocclusion, aesthetic and functional problems, and a tendency to other oral diseases.[3] These anomalies may cause deterioration in dental arches and affect dental eruption,[5] making clinical management important.[3]

Developmental dental anomalies affect tooth size (microdontia and macrodontia), tooth shape (dens invaginatus, talon tubercle, dens evaginatus, germination, fusion, root dilution, taurodontism, and concretion), tooth number (hyperdontia, hypodontia, and oligodontia), and tooth structures in dental tissues (amelogenesis imperfecta, dentinogenesis imperfecta, and dentin dysplasia).[6] These are usually detected during routine dental examinations.[3] While panoramic radiographs determine the status of dental anomalies and pathologies, they allow the diagnosis and treatment planning of various jaw and facial diseases.[7] The fact that dental anomalies mostly occur in childhood and the inexperience of dentists in diagnosing them causes incurable dental problems in pediatric patients.[8] While the early diagnosis may provide optimal patient management and treatment planning, delay in treatment complicates future treatment and causes psychological problems.[7,8]

Although dental anomalies are common in many populations,[8] their prevalence in different population groups provides important information for phylogenic and genetic studies.[3] Knowing the prevalence of dental anomalies in populations is important for dentists to be more careful about that anomaly during routine examinations as well as to prevent possible complications and wrong-site tooth extractions.

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This retrospective study aims to evaluate the prevalence of dental anomalies in a group of pediatric population living in the Anatolia (Cappadocia) region.

METHODS

Ethics committee approval for the study was obtained from Erciyes University's non-clinical research ethics committee (October 9, 2019, Decision no: 2019/687).

Study Design

In this retrospective study conducted between January 2018 and December 2019, it was calculated that at least 4994 orthopantomograms (OPTs) should be evaluated according to power analysis ($\alpha = 0.05$, $\beta = 0.80$). Considering the losses that may occur, OPTs of 5115 patients who presented to the Department of Pediatric Dentistry were evaluated.

Inclusion and Exclusion Criteria

Patients between the ages of 5 and 14, without systemic disease, and with clear orthopantomograms taken for diagnosis and treatment were included in the study. Images from patients with systemic disorders such as syndromes, or cleft lip and/ or palate, previous jaw surgery, extracted teeth, prosthodontic, or orthodontic treatment were excluded from the study. The radiographs of the patients who had more than 1 radiograph (by examining 2 radiographs together) were evaluated. One hundred fifteen of the 5115 OPTs examined in this context were excluded from the study. Wisdom tooth deficiencies were not considered as hypodontia. Assessment of the OPTs was performed directly on the same monitoring independently by 2 calibrated examiners (B.D. and C.D.) (Cohen's kappa = 80%). In case of disagreement, the discussion was made to reach a consensus. Radiographs of a total of 5000 patients were included in the study.

Statistical Analyses

The data were entered into the Statistical Package for the Social Sciences version 20.0 (IBM SPSS Corp.; Armonk, NY, USA) according to the gender and age of the patients, tooth number anomalies (hypodontia, hyperdontia, oligodontia, and anodontia), and odontoma and cyst and tumor-like dental pathologies. Descriptive analysis was made using frequency analysis, and the chi-square test was used for comparison between the genders. The statistical significance was *P* value <.05.

Main Points

- This study was researched to determine dental anomalies in a group of pediatric population in the Anatolia (Cappadocia) region.
- The study aimed to investigate the difference between the genders in terms of the prevalence of dental anomalies.
- It is aimed to increase the attention of dentists in terms of the prevalence of dental anomalies during a routine examination.

RESULTS

Dental anomalies were observed in 169 (3.38%) of 5000 patients. 50% of the patients were female. The average age of the patients was 9 ± 21.

The frequency and percentages of the probability of anomaly occurrence in all the examined patients are given in Table 1.

The distribution of the number of dental anomalies and tooth numbers by gender is given in Figure 1.

Of the 27 (15.9%) patients with hyperdontia, 40% were female. While 1 extra tooth was observed in 24 patients (14.2%) (10 girls and 14 boys), 2 extra teeth were observed in 3 (1.8%). Hyperdontia was mostly observed in the right (7 girls and 12 boys) and left (5 girls and 12 boys) quadrants of the upper jaw, while mesiodens, which was specific in the upper midline, was detected in 6 (3.5%) patients (1 girl and 5 boys).

Hypodontia was observed in 137 (75 girls and 62 boys) patients (81.0%); 73 (43.1%) of the patients (41 girls and 32 boys) had 1 tooth missing, 50 (29.5%) had 2 teeth missing, (Figure 2) 4 (2.3%) (2 girls and 2 boys) had 3 teeth missing, 9 (5.32%) (4 girls, 5 boys) had 4 teeth missing, 2 (1.1%) (1 girl and 1 boy) had 5 teeth missing, and 2 (1.1%) (1 girl and 1 boy) had 11 teeth missing (oligodontia). Missing teeth were mostly observed in the left lower quadrant (69 teeth, 40.8%) (38 girls and 31 boys), followed by the right lower quadrant (67 teeth, 39.6%) (33 girls and 34 boys), the right upper quadrant (48 teeth, 28.4%) (25 girls and 23 boys), and the left upper quadrant (46 teeth, 27.2%) (28 girls and 18 boys). Hypodontia was seen most frequently in girls, and a statistically significant difference was found between the genders in terms of hypodontia ($P = .026$).

There was no significant difference between the genders in terms of the prevalence of supernumerary teeth, mesiodens ($P > .05$).

Table 1. The Frequency and Percentages of Anomaly Occurrence in all the Examined Patients

	Gender			
Dental Anomalies	**Girls**	**Boys**	**Total, n (%)**	***P***
Hypodontia	75	62	137 (81)	**.02***
Hyperdontia	11	16	27 (15.9)	.29
Mesiodens	1	5	6 (3.5)	.09
Odontoma	1	1	2 (1.1)	.10
Cyst/tumor	2	1	3 (1.7)	.56
Total	90	85	175 (100)	

*P < .05.

Figure 1. The distribution of the number of dental anomalies and tooth numbers by gender.

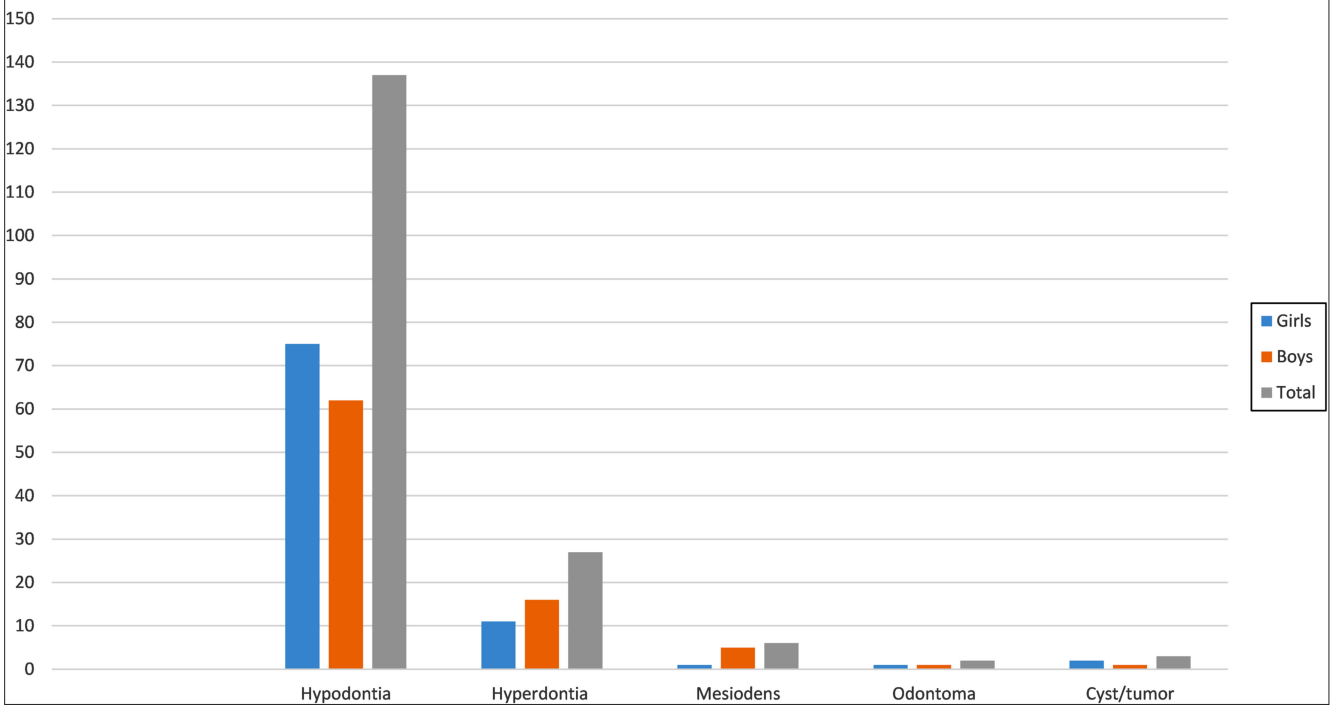
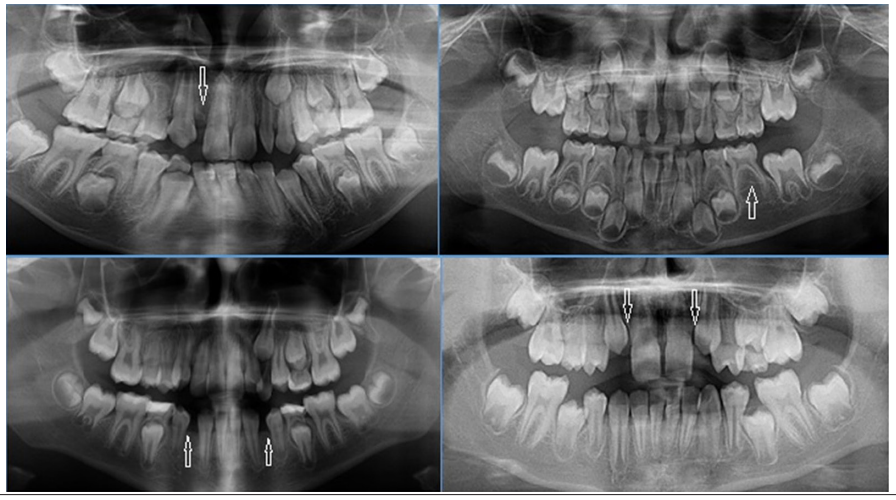


Figure 2. The panoramic radiograph of the patient with 1 and 2 hypodontia.



DISCUSSION

Early diagnosis of DDAs is important because they cause aesthetic and functional problems in the future. Raising awareness in dentists about anomalies will facilitate diagnosis and treatment. In this study, the prevalence of dental anomalies (tooth number anomalies, tooth size anomalies, and structural anomalies) in children and adolescents and their distribution by gender were investigated.

Cunha et al[9] examined 523 panoramic radiographs belonging to patients between the ages of 4 and 12 and found dental anomalies in 82 patients (15.68%). Another study conducted in Australia evaluated 1050 panoramic radiographs, and the prevalence of dental anomalies was reported as 5.14%.[1] Another study in Italy determined the prevalence of dental anomalies as 20.9%.[10] A study examined tooth shape, number, structure, and size anomalies in an Indian population and reported the anomaly prevalence as 34.2%.[11] In the study conducted in a Turkish population, 1200 panoramic radiographs of patients aged between 6 and 40 were evaluated, and the prevalence of dental anomalies was found to be 39.2%. As seen in our study, dental anomalies were detected in 169 (3.38%) of 5000 patients aged 5-14 years.

Not including shape and position anomalies in the study may be the reason for detecting the prevalence of dental anomalies lower than that reported in other studies.

Carvalho et al[12] found the prevalence of hypodontia to be 0.4% and the prevalence of hyperdontia to be 0.8% in their study on 750 Belgian children. This is similar to the findings of Brook et al[13] who found the prevalence of hypodontia as 0.3% and the prevalence of hyperdontia as 0.8% in their study in England. In the study by Ravn et al[14] conducted in Denmark, it was reported that 0.5% of 4564 patients had hypodontia and 0.6% had hyperdontia. In light of these studies, it can be stated that hypodontia is more prevalent and hyperdontia is less prevalent in the Turkish population than in Belgian, Dane, and British populations. Furthermore, Cunha et al[9] stated that hypodontia was the most common dental anomaly. In the study by Gomes et al[15] 1049 Brazilian patients (6.3%) were reported to have hypodontia. Another study from Italy reported that the most common anomaly was determined as the displacement of canine teeth, followed by hypodontia with 7.1%.[10] In our study, hypodontia was encountered most frequently (2.74%) in the studied population. Hypodontia constituted 81% (n=137) of all DDAs in this study. The findings reported by the said studies as well as our results support the assumption that the frequency of dental anomalies varies among populations.

In the study by Chen et al[16] 2611 children (1442 boys and 1169 girls) between the ages of 2 and 6 were examined and as a result, primary mandibular incisors were most commonly found missing. Another study by Bekiroğlu et al[7] showed that the most frequently missing tooth was the lower premolar tooth. In the literature, there are studies showing the lower premolars as the most frequently missing tooth[1,10] and the maxillary lateral teeth as the second most frequently missing ones.[1,17] This is supported by our finding that lower premolars were the most frequently missing teeth.

Some studies reported no difference between the genders in terms of the prevalence of anomalies. However, contrary to these findings, 2 studies[18,19] determined a higher prevalence of hypodontia in women than in men. This is also supported by our findings that show a significantly higher frequency of hypodontia in girls than in boys. This finding emphasizes the need for dentists to be more careful about early intervention, especially in girls who are more sensitive about their appearance since hypodontia may lead to aesthetic problems in the future.

In a study examining 152 children between the ages of 5 and 15, it was stated that supernumerary teeth were seen more in boys than in girls, which is in parallel with our study.[20] This result supports the finding reported by most studies that supernumerary teeth are more prevalent in men.[21,22] However, there was no statistically significant difference between the genders in terms of supernumerary teeth and mesiodens. Cunha et al[9] found a total of 7 supernumerary tooth cases in 134 anomaly cases. In their study, it was stated that there was no statistically significant difference between the genders. In our study, supernumerary teeth were observed in 27 patients, with a higher prevalence in boys (11 girls and 16 boys). However, a statistically significant relationship was not found between the presence of a supernumerary tooth and gender.

Salcido-Garcia et al[23] reported that supernumerary teeth were present in 3.2% of 2241 patients, 48.6% of whom had mesiodens. Araz et al[24] found supernumerary teeth in 4.33% of the children, and the most common supernumerary tooth was mesiodens (64.4%). In our study, 0.7% of the patients had supernumerary teeth, 22% of which were mesiodens.

A study conducted in Brazil in 2013[9] reported that dental anomalies (supernumerary teeth, endodontics, ankylosis) were more prevalent in women than in men. This can probably be attributed to the sample and race differences as well as local environmental impacts.

The fact that size, shape, and position anomalies are not included in our study prevents the generalization of our findings to all DDAs. Also, the study covers a cross-sectional region in the country. We believe that our study is important in terms of showing the prevalence of tooth number, tooth size, and structural anomalies and revealing the difference between the genders. Further studies are needed to evaluate the DDAs in different populations across the country.

CONCLUSION

It can be inferred from our findings that, apart from hypodontia, there was no significant difference between male and female patients in terms of the distribution of dental anomalies. Hypodontia may result in functional, phonational, and potential orthodontic and esthetic problems in the future. The study emphasizes the necessity for early diagnosis and management of anomalies to prevent the occurrence of psychological problems, especially in girls who care more about their appearance. In addition, the most prevalent dental anomaly was hypodontia among the patients included in our study. For this reason, dentists should be very careful about anomalies during a routine examination since preventing complications is possible with early diagnosis and treatment.

Ethics Committee Approval: This study was reviewed and approved by Erciyes University Non-Invasive Clinical Research ethics committee (Date: October 9, 2019, Decision no: 2019/687).

Informed Consent: There was no informed consent required as this was a retrospective study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – E.K.; Design – E.K, B.D, C.D., H.G.; Supervision – E.K.; Resources – E.K., B.D., C.D.; Materials – E.K., B.D., C.D., H.G.; Data Collection and/or Processing – B.D., C.D.; Analysis and/or Interpretation – E.K., B.D.; Literature Search – E.K., B.D.; Writing Manuscript – E.K., B.D.; Critical Review – E.K., B.D., C.D., H.G.

Declaration of Interests: The authors declare that they have no competing interest.

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Original Article

Exploring the Role of HPV 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx

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ABSTRACT

Objective: Human papillomavirus infections may have a role in the development of oral cavity and oropharynx carcinomas. Human papillomavirus-positive oral cavity and oropharyngeal carcinomas differ from human papillomavirus-negative in that to occur in younger, are more frequent in men, and are strongly associated with sexual behavior. These observations lead to the treatment options and outcomes in human papillomavirus-related tumors, and the questions of targeted treatment that can be performed in the coming years have come to age.

Methods: This prospective study was conducted at Gaziantep University, medical faculty, otorhinolaryngology department. Patients with squamous cell carcinomas of non-lip oral cavity and oropharyngeal admitted to our department were included in the study. Samples from the cases were immunohistochemically stained. Sections were examined by light microscopy.

Results: The 55 cases P16 (76.4%) expressions were detected to be positive, and 17 (23.6%) cases were negative. There was no statistically significant correlation between prognostic parameters and p16 expressions. However, a significant difference was detected between human papillomavirus-positive and negative groups in regard to survival in oropharyngeal carcinoma.

Conclusion: Disease management can consider human papillomavirus-positive oral cavity and oropharyngeal carcinomas as a separate group. human papillomavirus-positive oral cavity and oropharyngeal carcinomas respond better to chemotherapy and radiotherapy than human papilloma virus-negative cancers. The presence/absence of human papillomavirus 16 might be considered a prognostic marker, but its reliability has not yet been confirmed. In future clinical studies, cancer centers should classify head–neck patients with respect to human papillomavirus status. However, we must always emphasize that the best treatment for cancer in which the main pathogenic agent is known is protection.

Keywords: Oral cavity, oropharynx, neoplasms, human papillomavirus p16, immunohistochemistry

INTRODUCTION

Head and neck carcinoma is the sixth most common cancer in the whole world.[1] Head–neck cancers are more common in males and occur in fifth and sixth decades.[2] Ninety percent of cancer that appeared in head–neck region are squamous cell carcinomas (SCC). Oral cavity and oropharynx cancers are the most common cancers all over the world and the second most common cancer in our country.

The relationship between smoking and alcohol and the cancers of the oral cavity and oropharynx has been known for a long time. The opinions on some factors such as diet and oral hygiene predispose to the disease have been expressed.[3,4] Animal studies have been performed to light on the relationship between head–neck cancer and hereditary, which has begun to focus on human papillomavirus (HPV) infections in addition to other factors in recent years.[3-5] It has been understood that DNA viruses can create tumors in mammals, and Shope[6] has shown keratinous lesions to be formed in rabbits following papillomavirus infections in 1993, and some of them have also transformed into epithelial neoplasms.

Human papillomavirus is a DNA group virus in the family of Papovaviridea in which 200 different types have been identified. Molecular studies indicate that specific mechanisms play a role in HPV-induced carcinogenesis, and it has been thought to have a relationship between HPV infection and head and neck cancers.[5,7]

Various studies have shown that some specific HPV types are associated with many premalignant and malignant lesions of the cervix uteri, vulva, penis, conjunctiva, and upper respirator

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y–digestive system.[8] These patients' carcinoembryonic antigen levels increased, and such cellular immunosuppression may predispose to cancer. Methods used in virus detection are election microscope, immunohistochemical staining, hybridization techniques (Southern blot, dot blot, and in situ hybridization), and "polymerase chain reaction" (PCR). This causal association between HPV and SCCs suggests that the presence of the virus may be a high-risk indicator between HPV and SCCs. Brandwein et al[9] reported that the presence of HPV DNA in laryngeal tumors was associated with prognosis.

The present study aimed to investigate HPV p16 presence in the oral cavity and oropharynx carcinomas using histochemical methods. The expected benefits of this study are to demonstrate the relationship of HPV p16 with clinicopathologic parameters in oral cavity and oropharyngeal carcinomas, to determine the behavior model of oral cavity and oropharynx cancers in advance, and to provide the most appropriate methods for treatment.

METHODS

This study aimed to indicate the effects of the relationships of HPV 16 with oral cavity and oropharynx cancers on age, stage, relapse, metastasis, and 3-year survival. The patients were examined retrospectively. This study was approved by Gaziantep University Clinical Research Ethics Committee (Date: April 6, 2015, 2015/114).

The patients with oral cavity and oropharynx SCCs and admitted to polyclinic of Department of Otorhinolaryngology of Gaziantep University Faculty of Medicine in 2002-2015 were included in this study. The patients with lip carcinoma, histopathology other than SCC, previously treated and with additional malignancies were excluded from the study.

After receiving the detailed history of the patient who meets the above characteristics, a head and neck examination was performed and a histopathologic diagnosis was made by biopsy. Following histopathological diagnosis, at least one of the treatment methods of excision, neck dissection with excision, or chemotherapy/radiotherapy was applied to the patients. Patients with squamous epithelial cell carcinoma of language, hard palate, buccal mucosa, retromolar triangle, soft palate, tonsil, and tongue were included in our study. The case data are collected as follows:

Main Points

- Oral cavity and oropharynx squamous cell cancers should be examined for human papillomavirus (HPV) 16 positivity.
- Immunohistochemical examination is a suitable method for the diagnosis of HPV 16.
- HPV 16 positivity can be evaluated as a prognostic factor in oral cavity and oropharynx squamous cell cancers.
- Prophylactic vaccination studies should be carried out to prevent cancers that are known to be the main pathogenic agent such as HPV 16.

- General information about the demographic, medical, and current illnesses of the cases was taken from personal information form and pathology records that were routinely filled at the center where the study was conducted.
- The success of the surgeon after surgery and metastasis and relapse developments was followed by the file records and pathology records of the cases.

Immunohistochemically Staining

The study consisted of 72 cases who operated due to non-lip oral cavity and oropharynx squamous cell cancer at Otorhinolaryngology Gaziantep University Faculty of Medicine and whose specimens were sent to the Laboratory of Pathology in 2002-2015.

Paraffin blocks were sectioned with a "Leica RM 2145" model microtome to a thickness of 4 μm and followed pre-staining protocols. Subsequently, p16 antibody was immunohistochemically administrated using CINtec Histology kit containing E6H4 clone antibody against P16INK4a. Nuclear and cytoplasmic staining is the basis. Staining of over 70% was considered positive. Figure 1a and b shows the positive p16 light microscope image of SCC (×100) and Figure 2a and b shows the image of a p16 negative patient with the same disease.

Statistical Analysis

Statistical Package for the Social Sciences 22.0 (IBM Corporation, Armonk, NY, USA) program was used to analyze the variables. Normal distribution suitability of univariate variables was assessed by Lilliefors corrected Kolmogorov–Smirnov test and variance homogeneity was assessed by the Levene test. Independent–Samples *t* test was used together with Bootstrap results for comparing 2 independent groups. When comparing categorical variables, Pearson chi- and Fisher's exact tests were tested with Monte Carlo Simulation technique. The odds ratio was used to determine the most important risk factor among categorical significant risk factors. The Kaplan–Meier (product-limit method)-LogRank (Mantel-Cox) analysis was used to examine the effect of factors on mortality and lifespan. Quantitative variables were tabulated to be ±std. (standard deviation) and range (maximum–minimum), and categorical variables were shown as n (%). Variables were examined at 95% confidence level and $P < .05$ was considered significant.

RESULTS

This study included 72 patients diagnosed with non-lip oral cavity and oropharyngeal SCC at the Department of Otorhinolaryngology Gaziantep University Faculty of Medicine. Totally 26 patients (36.1%) were female, and 46 patients (63.9%) were male. The age distribution ranged from 16 to 88 (mean, 53.39). We examined the patients after dividing them first into 2 groups as oral cavity and oropharyngeal carcinomas and then grouped as positive and negative according to HPV p16 staining. The disease was located in the oropharynx of 20 patients (27.7%) and in the oral cavity of 52 patients (72.3%). Distribution of the disease in cases according to localization is shown in Figure 3.

Figure 1. A, B. Histopathological view of squamous cell carcinoma (H–E, ×100) (a) and positive p 16 image of same patient (×100) (b). H–E, hematoxylin and eosin.

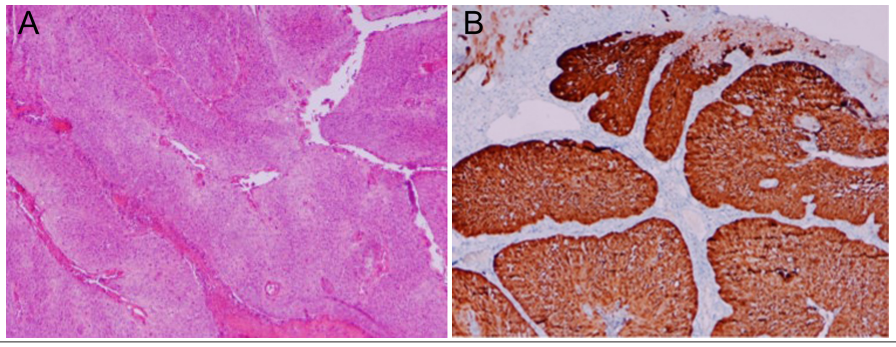


Figure 2. A, B. Histopathological view of squamous cell carcinoma (H–E, ×100) (a) and negative p 16 image of same patient (×100) (b). H–E, hematoxylin and eosin.

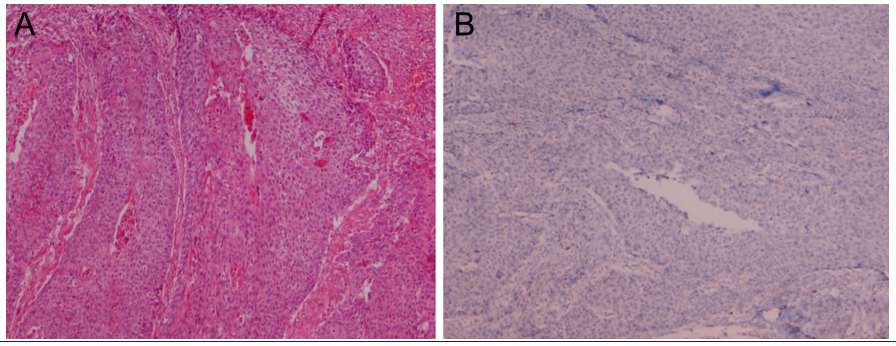
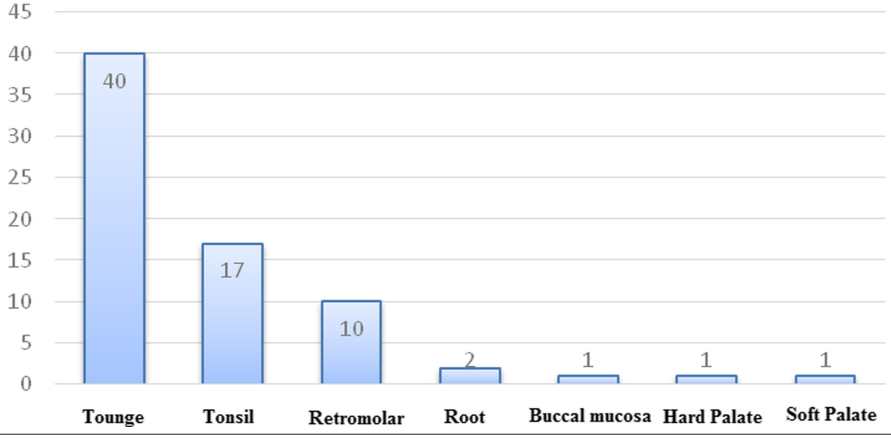


Figure 3. Distribution of cases based on localization.

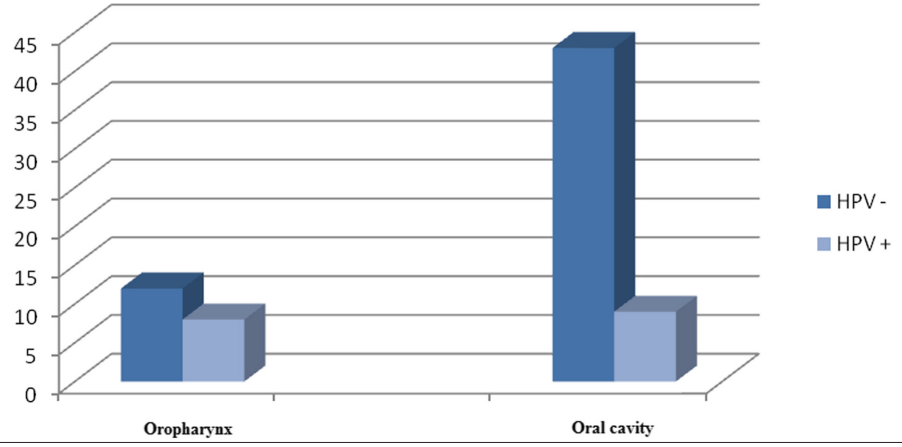


Totally 17 (85%) cases with oropharyngeal cancers were male and 3 (15%) were female and 29 (55.8%) of the patients with oral cavity cancer were male and 23 (44.2%) were female. Oral cavity and oropharynx incidence were found statistically higher in males than females ($P = .028$)

Human papillomavirus p16 was found to be positive in 17 of 72 (23.6%) patients included in the study. Nine of 52 cases (17.3%) with oral cavity cancer were found to have HPV p16 positivity and 8 of 20 cases (40%) with oropharynx cancer were found to have HPV p16 positivity. A statistical difference was not recorded between oral cavity and oropharynx cancer in terms of HPV positivity ($P = .063$; Figure 4).

A total of 63 (87.5%) patients were operated on, 9 (12.5%) patients underwent chemotherapy and radiotherapy after

Figure 4. Distribution of oral cavity and oropharynx cancers based on HPV p16 staining. HPV, human papillomavirus.



biopsy and histopathological diagnosis of SCC, and 62 surgical excisions and neck dissections were simultaneously performed in 62 of the patients who were operated, and only 1 patient was surgically excised.

When 63 patients were evaluated in terms of stage, 15 patients (23.8%) were stage 1, 14 patients (22.2%) were stage 2, 13 patients (20.6%) were stage 3, and 21 patients (33.3%) were stage 4 (Figure 5).

While there was not a significant difference in terms of stage between HPV negative and positive groups in oropharynx cancers ($P = .424$), there was a significant difference in terms of stage between HPV positive and negative groups in oral cavity cancers ($P = .017$). Human papillomavirus-positive group in oral cavitary cancers was seen at an earlier stage (Table 1).

We performed follow-up visits for our operated patients with physical examination and imaging methods in our clinic, of which we observed metastasis to the neck lymph nodes in 26 of (41.2%) 63 patients operated and relapse in 20 (31.7%). A significant difference between HPV-positive and -negative groups in oral cavity and oropharynx cancers in terms of metastasis to neck lymph nodes was not recorded. (oral cavity $P = .240$, oropharynx $P = 1$) (Table 2).

However, 3-year survival rate of HPV-positive group was statistically higher than HPV-negative group ($P = .032$).

When the difference between sex and 3-year survival rate is evaluated, although no significant difference was found between male and female groups in terms of 3-year survival in oral cavity cancers ($P = .381$), a significant difference was found between male and female groups in terms of 3-year survival in oropharynx cancers ($P = .001$). Three-year survival in oropharynx cancers was found to be significantly worse in women.

When 3-year survival is evaluated between the patients operated on, and the patients who underwent chemotherapy/radiotherapy in oral cavity and oropharynx, a significant

Figure 5. Distribution of oral cavity and oropharynx cancer in terms of stage.

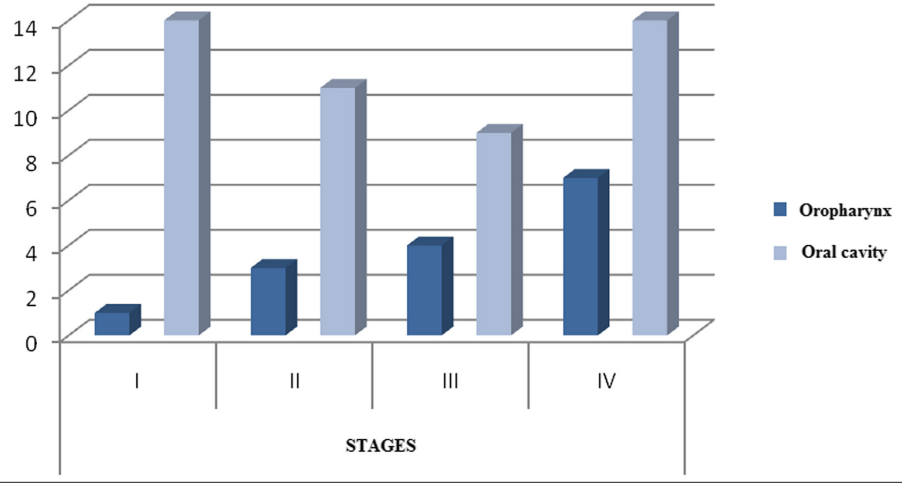


Table 1. Comparison of the Relationship Between HPV and Stages

		STAGES					
		I	II	III	IV		
	HPV	n (%)	n (%)	n (%)	n (%)	Total	*P*
Oropharynx	–	1 (14.3)	2 (28.6)	2 (28.6)	2 (28.6)	7 (100)	.424
	+	0 (0.0)	1 (12.5)	2 (25.0)	5 (62.5)	8 (100)	
Oral cavity	–	12 (30.0)	6 (15.0)	8 (20.0)	14 (35.0)	40 (100)	.017
	+	2 (25.0)	5 (62.5)	1 (12.5)	0 (0.0)	8 (100)	
Total	–	13 (27.7)	8 (17.0)	10 (21.3)	16 (34.0)	47 (100)	.330
	+	2 (12.5)	6 (37.5)	3 (18.8)	5 (31.3)	16 (100)	

Pearson chi–square test (Monte Carlo).
HPV, human papillomavirus.

Table 2. Comparison of the Relationship Between HPV and Metastasis

		Localization					
		Oropharynx		Oral Cavity		Total	
		HPV		HPV		HPV	
		–	+	–	+	–	+
Metastasis	None	3 (42.9)	2 (25.0)	25 (62.5)	7 (87.5)	28 (59.6)	9 (56.3)
	Positive	4 (57.1)	6 (75.0)	15 (37.5)	1 (12.5)	19 (40.4)	7 (43.8)
P		1		0.240		1	
Relapse	None	5 (71.4)	6 (75.0)	27 (67.5)	5 (62.5)	32 (68.1)	11 (68.8)
	Positive	2 (28.6)	2 (25.0)	13 (32.5)	3 (37.5)	15 (31.9)	5 (31.3)
P		1		1		1	

Fisher's exact test (Exact).
HPV, human papillomavirus.

difference was recorded between operated patients and those who underwent chemotherapy/radiotherapy in both oral cavity and oropharynx cancer groups (oral cavity cancers $P = .001$, oropharynx cancers $P = .016$). Three-year survival rate of operated patients was found statistically better in oral and oropharyngeal cancers.

When the relationship between the presence of neck lymph node metastasis and 3-year survival is evaluated, no significant difference in the 3-year survival rate between patients with or without metastases in oropharyngeal carcinomas was observed ($P = .611$); however, a significant difference was found between patients with or without metastasis in oral cavity cancer ($P = .049$), and non-metastatic group's 3-year survival rate was found statistically higher than metastatic group.

When the relation between relapse and 3-year survival is evaluated, no significant difference was found between the groups with or without relapse in oral cavity cancers ($P = .115$), however, a significant difference between the groups with or without relapse was found in oropharynx cancers ($P = .046$), in which non-relapsing group was higher.

DISCUSSION

The incidence of head and neck region cancers was found to be less than 5% of all cancers in developed countries. This percentage reaches up to 17 in developing countries. Oral cavity cancers constitute 25%-35% of head and neck cancers and occur 3 times more in men than in women between the age of 50 and 60.[10] These cancers are one of the major health problems with increasing frequency in many parts of the world. Despite recent advances in treatment and new protocols using alternative treatment modalities, the prognosis of patients is still poor. When lesion and treatments-caused functional and cosmetic deformities are combined with low survival rate (5-year survival rate T1-T2: 51%, T3-T4: 18%,[11] the importance of oral and oropharynx cancers is increasing even more. Although there are improvements in CT, RT, and surgical treatment techniques, the survival rates of patients have increased very little in recent years, which makes it necessary to investigate the treatment methods causing

the least mortality and morbidity. The most important factor for effective treatment is early diagnosis, which allows aesthetic, functional, and oncological successful outcomes.[11]

In general, it is thought that the most reliable parameters in treatment planning and prognostic determination can be determined by tumor node metastasis (TNM) classification. Lymph node metastasis is the most important parameter accepted.

However, even if all these features are taken into account and the same treatment modalities are administrated to the patients, there can be significant differences in terms of treatment response, relapse, tumor behavior, and overall prognosis among the patients. These differences lead to the conclusion that there are other factors affecting the outcome of oral cavity and oropharynx cancer treatment, and recently, some researchers thought viral factors might be the reason for differences.

Many studies have shown that smoking and alcohol use are major, common risk factors for head and neck SCC (HNSCC). However, for the last 10-15 years, HPV infection has been recognized as a major etiologic risk factor for a type of HNSCC,[12,13] which is mostly oropharyngeal SCC (OPSCC). For the first time, Gillison et al[14] reported that HPV infection plays a role in OPSCC etiology. Many case studies have been conducted to evaluate the prevalence of HPV infection in oropharyngeal cancers using molecular techniques such as PCR or in situ hybridization in 2000.[15,16] In fact, it has been very clear for the last 5 years that HPV plays a pathogenic role in head and neck cancers. These findings provide new opportunities for advanced therapy and primary prevention for HNSCC.[17]

It has been known for almost a century that HPV is in a relation to upper respiratory tract pathologies. However, the viral oncogenic effects have been better reported in the literature in the last 3 decades.[18-20] Human papilloma virus has been found to be associated with oropharyngeal cancers, especially tonsil cancers. The life span of HPV-positive cases and the therapeutic response were thought to be better than HPV-negative cases.[21,22]

Human papillomavirus is a DNA virus with more than 200 types defined in the PapovaViridea family.

Human papillomavirus prevents apoptosis in human genital keratinocytes and oral and tonsillar epithelial cells. Tissue culture derived from immortalized cell line results in a transformed phenotype. This data indicate that HPV plays initiator role in the transformation of malignant.

Immunohistochemical staining, hybridization techniques (Southern blot, dot blot, and in situ hybridization), and PCR techniques are used to detect viruses. But, which one of these techniques is safety is still being discussed.[23,24]

This causal relationship between HPV and SCCs suggests that the presence of the virus may be a high risk of developing cancer. The high-risk subtypes of HPV are HPV 16, 18, 31, 33, 39, 45, 52, 58, and 69 and play role in cervical and other anogenital cancers. Human papillomavirus 6 and 11 are "low risk" types and are rarely seen in malign lesions. They mainly occur in non-malignant lesions.

In some studies, the reasons for HPV infections in head and neck regions are reported as oral–genital contact, multiple sex partners, infection from mother to baby during childbirth, and hygenic behavior differences.[25]

D'Souza et al[26] reported in a case–control study that the high number of vaginal sex partners (>26) and 6 or more oral sex partners are high-risk factor for OPSCC. In women with HPV-induced anogenital cancer, the risk of HPV-induced OPSCC risk is also increased. Also, male partners of these patients had HPV contamination in oropharyngeal cavities have been seen, which has been supported by the studies of Frisch[27] and Hemminki.[28]

There are many great studies in literature that investigated HPV prevalence in head and neck cancers, which has been detected at 34.5%. However, a wide range of about 7%-59% has been found, depending on the localization of the selected tumor group, the method used, or the patient characteristics. In our study, 17 of all cases (23.6%) have been detected to be positive by immunohistochemical staining method. In our study, although HPV is positive in 40% of oropharynx cancers, it is positive in 17.3% of oral cavity cancers. This ratio is statistically significant, but HPV positivity was found to be high (40 %) in oropharynx cancers, and we have concluded that small number of cases lead this ratio to be statistically insignificant results.

Miller et al[29] found HPV to be in the ratios of 10% in normal oral mucosa, 22.2% in leukoplakia, 26.2% in intraepithelial neoplasia, 29.9% in Verrucous carcinoma, and 46.5% oral SCC.

SahebJamee et al[30] investigated the presence of HPV in the saliva of cases with oral SCC and control group using PCR method. Human papillomavirus was found to be positive in 40.9% of SCC cases and 25% of control group. Human papillomavirus 16 was found to be in 27.3% of the cases and 20% of the control group. In this study, the difference between HPV rates in the patient group and the control group was not statistically significant.

Marur et al[4] found that HPV-positive head and neck tumors were more common in males. In the same study, HPV-positive head and neck SCCs were found to be more sensitive to chemotherapy and radiotherapy. They also noted that HPV p16 has an effect on survival but was not sufficient by itself. In our study, HPV-positive tumors were more common in male, especially in oropharynx cancers, and the difference between males and females was statistically significant.

Ang et al[31] showed that HPV-positive patients were generally younger, diagnosed at 54 years of age, and had fewer cigarette and alcohol exposures. In our study, no statistically significant relationship between HPV and age has been recorded.

Many studies have shown that HPV-positive tumors are generally being presented as early T stage (T1, T2)[32] and high N stage

(generally cystic and multilevel)[33] and have generally different histologic features (moderate/weak tumor differentiation and non-keratinization or basaloid pathology).[32,33] In our study, 82.3% of HPV-positive cancers were seen in the early T-phase (T1, T2) and 17.7% in the late T-phase (T3). In terms of neck lymph node metastasis, 68.75% of HPV-positive cancers were seen in early N (N0, N1) and 31.25% in late N (N2, N3). Also, in operated patient group, the distribution of cases is as follows: 15 cases (23. 8%) are in stage 1, 14 cases are in stage 2 (22.2%), 13 cases are in stage 3 (20.6%), and 21 cases are in stage 4 (33.3%). Lymph node metastases were detected in 26 patients (41.2%). In our study, no statistically significant difference was found between HPV-positive group and HPV-negative group in terms of metastasis and recurrence.

In our study, no significant difference between HPV and stages in oropharyngeal carcinomas has been recorded. However, there was a significant difference between HPV and stages in oral cavity cancers. In our study, HPV-positive group in oral cavity cancers was seen especially in stages 1 and 2.

Lim et al[34] have not recorded any significant difference in survival between HPV-positive and -negative groups. Ang et al[31] have shown HPV-positive group to have better prognosis than HPV-negative group. In the same study, HPV positivity was found to have a positive effect on survival. Similar results were obtained in the study of Chaturvedi.[35] In our study, it was found that the survival rate of HPV-positive group was statistically significantly better than HPV-negative group in oropharyngeal carcinoma, while there was no significant difference between HPV-positive group and negative group in oral cavity cancer in terms of 3-year survival.

Studies have shown that HPV is associated with head and neck cancers, especially oropharyngeal cancers. In our study, HPV 16 positivity was found as high as 40% in oropharyngeal carcinomas, but this ratio was not found statistically significant. Studies have shown that HPV-associated cancers occurred in younger age groups. But the age distribution in our study is heterogeneous.

The best viral detection method chosen for tumors is still controversial, and both in situ hybridization and PCR are often used. P16 immunohistochemistry is also used to detect HPV infection. Thus, a new marker is required to define the best treatment option for HPV infection. Besides, the presence/absence of HPV infection can be considered as prognostic marker, but its use has not yet been approved. There are still many questions about oral HPV infection.

In the literature, it is seen that the prognosis of HPV-positive cancers is better, and the survival rate is higher. In our study, it was seen that the 3-year survival rate of oropharynx cancer was higher. It was also observed that oral cavity cancers were at earlier stage.

Human papillomavirus-positive cancers' T stage is consistent with the literature but differs from the literature on early T stage in terms of N stage. In the literature, HPV-positive cancers were seen in late N stage, whereas it is in early N stage in our study.

Human papillomavirus-positive oral cavity and oropharyngeal cancers respond better to chemotherapy and radiotherapy than HPV negative.

The limited numerical data and the fact that only HPV P16 markers were examined were accepted as a limitation of our study. A detailed investigation of the relationship between HPV and oral cavity–oropharynx cancers will provide important contributions to the literature.

CONCLUSION

Regarding disease management, we can consider HPV-positive oral cavity and oropharynx cancers as a separate subgroup of HNSCC because of their more positive results. Human papillomavirus-positive oral cavity and oropharyngeal carcinoma patients are typically younger and have a better general health status. In future clinical trials, cancer centers should classify head and neck patients according to HPV status. Regardless of treatment modality, we have an opportunity to investigate treatment strategies that increase survival rates and reduce the rate of lethal side effects. In other words, our general purpose should be to provide high level of life quality and minimal treatment complications. In some studies, this type of treatment strategy seems to be possible for HPV-induced cancers so new studies to be done in this field are required.

We must always emphasize that the best treatment for cancer, especially the main pathogenic agent, is prevention. The importance of vaccination, especially in HPV-related cancers, has been shown in recent years, so we must emphasize the importance of increasing the number of detailed studies that indicate the impact of vaccination on head and neck cancers.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University (Date: April 6, 2015, Decision no: 2015/114).

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

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Original Article

Assessment of Cardiac Autonomic Functions by Heart Rate Recovery Indices in Patients Receiving Chest Radiotherapy

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ABSTRACT

Objective: Although the introduction of radiation therapy for the management of thoracic malignancies has led to a significant improvement in disease-specific survival, this has resulted in the emergence of a new spectrum of cardiovascular disorders induced by radiation injury. Heart rate recovery, as a predictor of cardiovascular events and an indicator of autonomic functions, is measured non-invasively and easily. In the present study, we investigated the effects of mediastinal radiotherapy on heart rate recovery parameters.

Methods: Twenty-one patients were included in this study who were planned to receive chest radiotherapy because of lymphoma or lung cancer. Heart rate recovery parameters were evaluated by treadmill exercise before and after radiotherapy.

Results: We have found decrease in heart rate recovery parameters (26.05 ± 12.54 vs. 19.52 ± 12.28 for HRR1, 39.1 ± 16.15 vs. 32.86 ± 14.83 for HRR2, 42.81 ± 17.66 vs. 38.05 ± 16.14 for HRR3). The higher doses of mediastinal radiotherapy caused significant changes on heart rate recovery parameters. HRR1 changed from 25.70 ± 15.12 to 22.00 ± 14.38 in low dose group and from 26.36 ± 10.41 to 17.27 ± 10.10 in high-dose group ($P < .05$). Attenuation of HRR2 and HRR3 were also more evident in high mediastinal dose group ($P < .05$).

Conclusion: Heart rate recovery parameters were decreased especially in patients who were exposed to more intense mediastinal radiation.

Keywords: cardiac autonomic functions, heart rate recovery, lung cancer, lymphoma, Radiotherapy

INTRODUCTION

Advances in cancer treatment have resulted in a significant improvement in survival in many types of cancer. However, considerable exposure of cardiovascular structures to radiation has been shown to result in cardiovascular adverse effects in the long term.[1] Risk factors for cardiotoxicity associated with radiation therapy include a radiation dose greater than 30 Gy or 2 Gy/fraction, a large volume of cardiovascular structures within the irradiated field, younger age at exposure, a long period following exposure, adjuvant chemotherapy, and co-morbidities (such as diabetes mellitus, hypertension, any other cardiovascular disease).[2] Patients receiving thoracic irradiation for Hodgkin's lymphoma, breast cancer, and lung cancer have risk for cardiovascular adverse effects.[1,3]

Heart rate recovery (HRR) is a relatively inexpensive and very simple diagnostic and prognostic tool that reflects the cardiac autonomic functions, which can be applied in various clinical settings.[4] Heart rate recovery indices show the rate of decline in the heart rate (HR) after the cessation of exercise test and it is defined HR difference between the maximal HR on exercise and the HR during the recovery phase. HRR after graded exercise reflects autonomic activity and predicts cardiovascular events and mortality in various systemic disorders.[4,5]

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Although detection of unfavorable effects of radiotherapy on cardiovascular system in short term may have been beneficial, most adverse effects are noticed in the long-term follow-up. In this study, we aimed to investigate whether thoracic irradiation due to Hodgkin's lymphoma or lung cancer may have an impact on cardiac autonomic functions evaluated with HRR.

METHODS

Among patients who presented to the radiation oncology department between March 2013 and November 2013, 21 patients who were scheduled for mediastinal radiotherapy for lung cancer or lymphoma, in which irradiation field included the heart, were included in the study. Baseline demographic, clinical, and echocardiographic parameters were recorded. Patients underwent detailed physical examination and measurements including height and body weight were obtained. If present, adjuvant chemotherapy was also recorded.

Patients with a history of coronary artery disease, peripheral artery disease, heart failure (left ventricular ejection fraction <50%), acute coronary syndrome, acute or chronic kidney disease, severe valvular heart disease, uncontrolled hypertension, uncontrolled diabetes, and vasculitis were excluded. Furthermore, active smokers and patients with contraindication for exercise stress test were also not included.

Standard 12-lead electrocardiography and transthoracic echocardiography were obtained from all patients. Exercise stress test was performed for evaluation of heart rate recovery at baseline and on the 15th day following radiotherapy.

Blood samples were obtained via the antecubital vein in sterile conditions from the patients during the computer-optimized treatment planning period. Results of routine laboratory tests, including complete blood cell count and serum biochemistry, were recorded.

Three-dimensional conformal radiation therapy was performed in the radiation oncology department. An accurate radiation dose calculation was made. Radiation exposure doses of the left ventricle, right atrium involving sinoatrial node, and paravertebral region involving the autonomic ganglia were determined besides the mediastinal dose. Doses lower than the median dose for each region were defined as low dose of radiation, whereas doses higher than (or equal to) the median dose were defined as high dose. Patients received treatment in the linear accelerator device. Mean radiation doses the patients received throughout the therapy were determined in the treatment planning program.

Main Points

- Considerable exposure of cardiovascular structures to radiation has been shown to result in cardiovascular adverse effects.
- Heart rate recovery (HRR) is a relatively inexpensive and very simple diagnostic and prognostic tool that reflects the cardiac autonomic functions and predicts cardiovascular events and mortality in various conditions.
- Reduce in HRR is significantly more prominent in higher mediastinal radiation dose when compared to lower irradiation dose.

All patients were subjected to exercise stress test using the modified Bruce protocol. 12- lead electrocardiography was recorded at the standard speed of 25 mm/sec during the test. Minimum exercise test duration of 6 minutes and maximal heart rate of 85% of age-predicted maximal heart rate (220- age) were targeted. Maximal exercise was followed by rest phase which lasted at least 3 minutes. Heart rate at maximal exercise; first, second, and third minute of the recovery was noted. HRR1, 2, and 3 were defined as the subtraction of heart rate at maximal exercise from heart rate at the first, second, and third minutes of the recovery phase, respectively.

The study was approved by the ethics committee of Hacettepe University (Date: March 27, 2013, Decision no: GO 13/ 215- 22) and was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Statistical Analysis

Statistical analysis was made using Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, Ill, USA) and Medcalc 11.4.2 (MedCalc Software, Mariakerke, Belgium). Shapiro–Wilk test was used to test the normality of the study population. Numerical variables were expressed as mean ± standard deviation or median (interquartile range, defined as minimum-maximum). Categorical variables were represented as number and frequency. Comparing differences between 2 independent groups when the dependent variable was either ordinal or continuous, but rather normally or non-normally distributed, was done using *t*-test or Mann–Whitney *U* test, respectively. Categorical variables were compared using chi-square or Fisher's exact chi-square test. Differences between before and after radiotherapy were evaluated with paired sample's *t*-test for normally distributed parameters and Wilcoxon signed-rank test for non-normally distributed parameters. Post-hoc analyses regarding the contribution of adjuvant chemotherapy and radiation dose on differences in parameters following radiotherapy, two-way analysis of variance test was done in paired samples. A *P* value <.05 was considered statistically significant.

RESULTS

Twenty-one patients (51.6 ± 16.0 years, 61.9% male) were included in the study. Eight patients (38.1%) were scheduled for radiotherapy for treatment of lymphoma, whereas the remaining 13 patients (61.9%) were being treated for lung cancer. Only 2 patients (9.5%) had a history of hypertension and diabetes mellitus as cardiovascular risk factors. Four patients (19.0%) received adjuvant chemotherapy. Specifically, 1 patient received carboplatin, 2 patients received carboplatin + paclitaxel, and 1 patient received cisplatin + etoposide. Baseline demographic, clinical, and echocardiographic characteristics of the patients are shown in Table 1.

Table 1. Baseline Characteristics of the Patients (n = 21)

Age	51.57 ± 16.01
Gender	
Female (n, %)	8 (38.1)
Male (n, %)	13 (61.9)
Disease	
Lymphoma (n, %)	8 (38.1)
Lung cancer (n, %)	13 (61.9)
BMI (kg/m^2)	27.32 ± 4.91
Hypertension (n, %)	2 (9.5)
Diabetes mellitus (n, %)	2 (9.5)
Drugs	
Beta blockers (n, %)	2 (9.5)
ACE-İnh/ARB (n, %)	1 (4.8)
Echocardiography	
LVEF (%)	61.38 ± 3.79
LVEDD (mm)	46.57 ± 3.41
Adjuvant chemotherapy	
Non-receivers (n, %)	17 (81.0)
Receivers (n, %)	4 (19.0)

When baseline characteristics of the patients were evaluated regarding the type of malignancy, only age and prevalence of adjuvant chemotherapy significantly differed between groups. Patients diagnosed with lung cancer were older [median 62 (40-68) vs. 31 (20-60) years, P = .006]. Adjuvant chemotherapy was seen only in patients with lung cancer (P = .036).

None of the blood pressure parameters, including systolic, diastolic, and mean blood pressure and pulse pressure, showed significant change following radiotherapy A statistically significant change did not occur in heart rate, central systolic, and diastolic blood pressure values following radiotherapy ($P > .05$).

Maximal heart rate (153.4 ± 19.9 vs. 145.4 ± 16.5 bpm, P = .001), HRR1 (26.1 ± 12.5 vs. 19.5 ± 12.3 bpm, P = .001), HRR2 (39.1 ± 16.2 vs. 32.9 ± 14.8 bpm, P = .002) and HRR3 (42.8 ± 17.7 vs. 38.1 ± 16.1 vs., P = .042) parameters were found to be significantly lowered following radiotherapy. However, this was not persistent following the consideration of the forementioned possible confounders ($P > .05$). When heart rate recovery parameters were evaluated regarding presence of adjuvant chemotherapy, only HRR1 was significantly lower in patients who received adjuvant chemotherapy (22.8 ± 10.4 vs. 34.5 ± 8.2 bpm, P = .046). Heart rate recovery parameters before and after radiotherapy is shown in Table 2 and Figure 1.

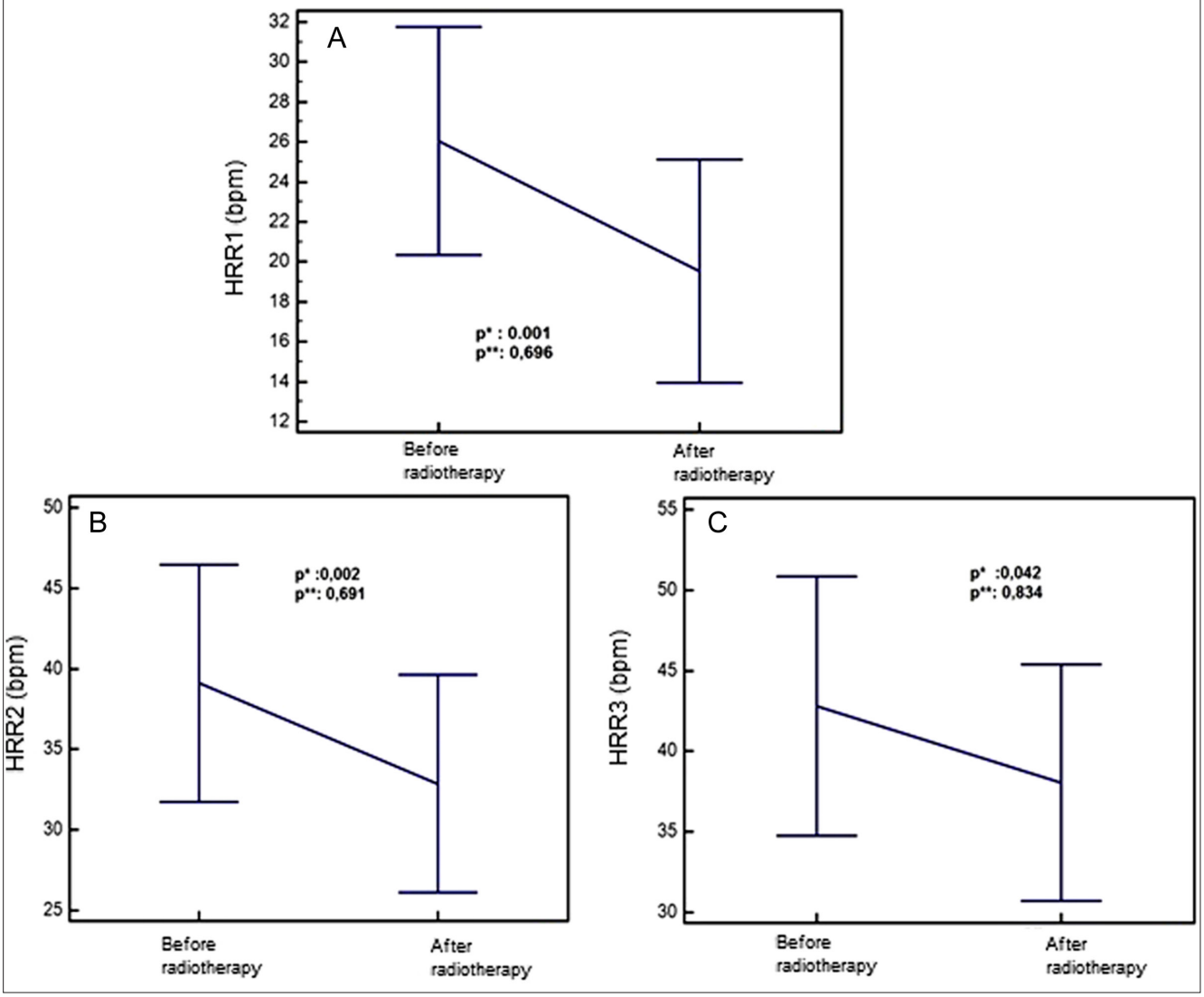
The median radiation doses for mediastinum, left ventricle, atrium, and paravertebral region were 40 Gy, 20 Gy, 30 Gy, and 30 Gy, respectively. Patients who received high-dose radiation to the mediastinum were found to have greater decrease in HRR1 (−9.1 vs. −3.7 bpm P = .001), HRR2 (−9.2 vs. −3.0 bpm P = .003) and HRR3 (−8.5 vs. −0.6 bpm P = .002) when compared to those who received low-dose radiation (Figure 2). Heart rate recovery parameters did not significantly differ between low and high-dose radiation exposure in regions other than mediastinum ($P > .05$). Heart rate recovery parameters regarding irradiation site are shown in Table 3.

Table 2. Heart Rate Recovery Parameters Before and After Radiotherapy (n = 21)

	Before Radiotherapy	After Radiotherapy	P^*	P^{**}
Maximal heart rate (bpm)	153.4 ± 19.9	145.4 ± 16.5	.001*	.518
Maximal heart rate-adjuvant chemotherapy (bpm)			.876	.437
Non-recipient (n = 17)	154.5 ± 21.9	146.7 ± 18.1		
Recipient (n = 4)	148.8 ± 4.9	140.0 ± 5.7		
HRR1 (bpm)	26.1 ± 12.5	19.5 ± 12.3	.001*	.696
HRR1-adjuvant chemotherapy (bpm)			.046*	.046*
Non-recipient (n = 17)	24.1 ± 12.7	18.8 ± 12.9		
Recipient (n = 4)	34.5 ± 8.2	22.8 ± 10.4		
HRR2 (bpm)	39.1 ± 16.2	32.9 ± 14.8	.002*	.691
HRR2-adjuvant chemotherapy (bpm)			.341	.232
Non-recipient (n = 17)	37.5 ± 16.6	32.3 ± 14.7		
Recipient (n = 4)	45.8 ± 14.1	35.3 ± 17.4		
HRR3 (bpm)	42.8 ± 17.7	38.1 ± 16.1	.042*	.834
HRR3-adjuvant chemotherapy (bpm)			.299	.240
Non-recipient (n = 17)	41.8 ± 18.4	38.2 ± 16.3		
Recipient (n = 4)	47.0 ± 15.7	37.3 ± 18.1		

*No interaction was taken into account during the analysis.
**Beta-blockers and type of malignancy were taken into consideration during analysis.
HRR1 heart rate recovery at first minute; HRR2 heart rate recovery at second minute; HRR3 heart rate recovery at third minute.

Figure 1. Comparison of heart rate recovery (HRR) parameters. HRR1 (A), HRR2 (B), and HRR3 (C) show first, second, and third minute HRR parameters, respectively. *No interaction was taken into account during analysis.**Beta-blockers and type of malignancy were taken into consideration during analysis.



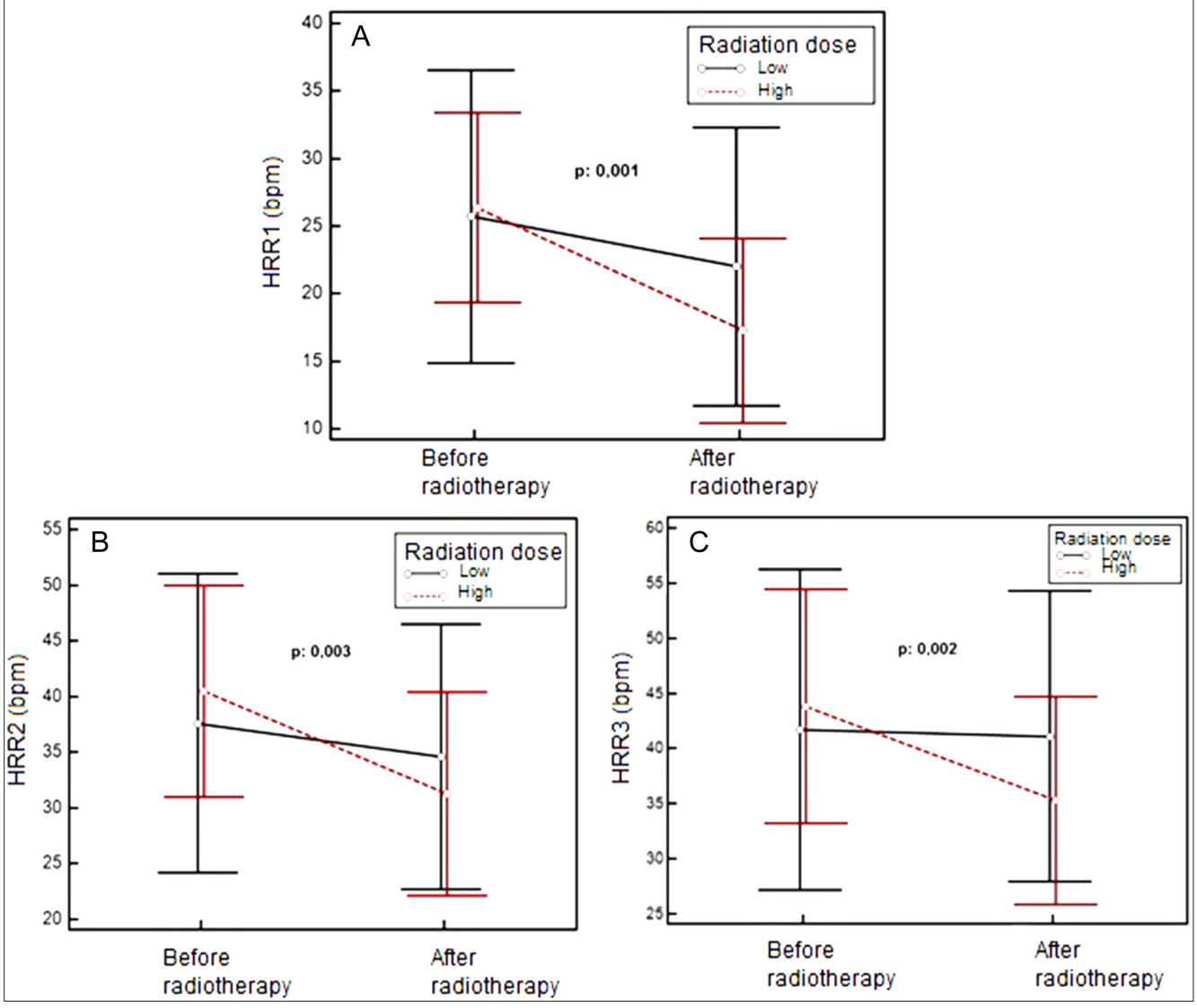
DISCUSSION

This study investigates the impact of thoracic irradiation for Hodgkin's lymphoma or lung cancer on cardiac autonomic function parameters in short term. We investigated the unfavorable effects of radiation therapy by heart rate recovery in short-term follow- up of 15 days.

Abnormal HRR, which is due to sympathetic withdrawal, parasympathetic reactivation, or both, has been reported to be an independent predictor of mortality in previous studies.[6,7] The decline in HR during recovery is principally due to a reactivation of the parasympathetic nervous system, mostly in the early recovery period.[8,9] Inadequate decline in HR immediately after exercise, reflects reduced parasympathetic nervous system activity and this is defined as an attenuated HRR.[10,11] Kannankeril et al[12] demonstrated that sympathetic withdrawal also contributes significantly to early HRR. they suggested that abnormal HRR might be attributable to a defect in sympathetic withdrawal and parasympathetic reactivation or both of them. As these changes correlate with increased risk of death, it was hypothesized that an attenuated HRR would similarly predict an increased risk of death.[7] Cole et al[13] showed that A delayed decrease in the heart rate during the first minute after graded exercise is a powerful predictor of overall mortality, independent of workload, the presence or absence of myocardial perfusion defects, and changes in heart rate during exercise.

However, there is limited data on the impact of cancer treatment on cardiac autonomic functions and most of the data relies on small study population.[13-16] Recently, thoracic radiotherapy has been reported to be associated with autonomic dysfunction, as measured by elevated resting heart rate and abnormal HRR in 263 Hodgkin's lymphoma survivors at a median follow-up of 19 years when compared to control subjects.[17] These

Figure 2. Figure demonstrating the relationship between mediastinal radiation dose and heart rate recovery (HRR). HRR1 (A), HRR2 (B), and HRR3 (C) show first, second, and third minute HRR parameters, respectively.



abnormalities were found to be associated with impaired exercise tolerance, and abnormal HRR predicted increased all-cause mortality in radiotherapy patients.[17]

Our study has demonstrated that HRR1, HRR2, and HRR3 were significantly reduced following radiotherapy. However, when confounding factors were taken into account, this statistical significance was lost ($P > .05$).

When HRR parameters were analyzed regarding the radiation dose, interesting results were obtained. Reduce in HRR1, HRR2, and HRR3 were significantly more prominent in higher mediastinal radiation doses when compared to lower irradiation doses.

Despite autonomic function parameters were expected to be affected by radiation applied at the paravertebral region and atria, no significant effect was observed between radiation dose applied at paravertebral region and atria and HRR.

It is already known that the risk of cardiovascular death is increased following radiotherapy for cancer.[18] At 25 years of follow-up, Schellong et al[19] determined 21% of cumulative cardiac disease incidence in patients with Hodgkin's disease who received mediastinal radiotherapy at the dose of 36 Gy. This risk was reduced to 6% and 5% with doses of 25 Gy and 20 Gy, respectively. Literature data show increased cardiac disease risk with higher mediastinal doses than 30-35 Gy.[20,21]

CONCLUSION

However, since effects related to radiotherapy mostly develop within more than 10 years, studies with short-term follow-up may be insufficient to demonstrate significant cardiovascular

Table 3. Heart Rate Recovery Parameters Regarding Irradiation Site (n=21)

	Irradiation Site	Irradiation Dose	Before Radiotherapy	After Radiotherapy	*P*
Maximal heart rate (bpm)	**Mediastinum**	**Low**	161.4 ± 24.8	153.5 ± 18.8	.696
		High	146.2 ± 10.6	138.0 ± 10.2	
	Left ventricle	**Low**	147.5 ± 16.5	140.1 ± 13.7	.897
		High	158.8 ± 21.8	150.2 ± 18.0	
	Atrium	**Low**	155.5 ± 19.0	146.0 ± 19.5	.580
		High	151.6 ± 21.3	144.8 ± 14.3	
	Paravertebral region	**Low**	160.2 ± 23.2	150.7 ± 20.9	.520
		High	150.7 ± 18.6	143.3 ± 14.8	
HRR1 (bpm)	**Mediastinum**	**Low**	25.7 ± 15.1	22.0 ± 14.4	.001
		High	26.4 ± 10.4	17.3 ± 10.1	
	Left ventricle	**Low**	26.5 ± 15.5	21.8 ± 15.1	.410
		High	25.6 ± 10.0	17.5 ± 9.4	
	Atrium	**Low**	27.4 ± 14.1	21.0 ± 15.3	.627
		High	24.8 ± 11.5	18.2 ± 9.3	
	Paravertebral region	**Low**	27.5 ± 12.0	19.3 ± 14.5	.288
		High	25.5 ± 13.1	19.6 ± 11.9	
HRR2 (bpm)	**Mediastinum**	**Low**	37.6 ± 18.8	34.6 ± 16.6	.003
		High	40.5 ± 14.1	31.3 ± 13.6	
	Left ventricle	**Low**	37.6 ± 19.0	33.4 ± 17.4	.734
		High	40.5 ± 13.8	32.4 ± 12.9	
	Atrium	**Low**	38.5 ± 17.6	31.7 ± 16.1	.664
		High	39.6 ± 15.6	33.9 ± 14.3	
	Paravertebral region	**Low**	43.0 ± 15.8	33.8 ± 18.5	.706
		High	37.5 ± 16.6	32.5 ± 13.9	
HRR3 (bpm)	**Mediastinum**	**Low**	41.7 ± 20.3	41.1 ± 18.4	.002
		High	43.8 ± 15.8	35.3 ± 14.1	
	Left ventricle	**Low**	41.2 ± 21.0	37.3 ± 18.7	.796
		High	44.3 ± 15.0	38.7 ± 14.3	
	Atrium	**Low**	43.2 ± 19.8	37.4 ± 18.4	.720
		High	42.5 ± 16.5	38.6 ± 14.7	
	Paravertebral region	**Low**	49.3 ± 17.3	40.7 ± 20.5	.697
		High	40.2 ± 17.7	37.0 ± 14.8	

HRR1 heart rate recovery at first minute; HRR2 heart rate recovery at second minute; HRR3 heart rate recovery at third minute.

mortality or morbidity data. Our study is remarkable for the fact that unfavorable effects of radiation therapy have been demonstrated in short-term follow-up of 15 days. Early detection of associated subclinical changes may be beneficial for the long-term follow-up and risk stratification.

There are some limitations of our study. First, a larger study population may render the results more reliable. Second, studies with longer follow-up periods are necessary to reveal the long-term effects of radiotherapy. Third, calculation of radiation dose exposure of mediastinal structures separately may help identify the

specific effects of mediastinal structures on cardiovascular endpoints. Finally, our study is a single-center study. Randomized multi-center studies are necessary to further clarify the outcomes of thoracic irradiation on cardiovascular outcomes.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Hacettepe University, (Date: March 27, 2013, Decision no: GO 13/ 215- 22).

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

Peer-review: Externally peer-reviewed.

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Original Article

Clinical Characterictics and Treatments Modalities of Patients with COVID-19 İnfection During the Early Phase of the Epidemic: A Single-Center from Turkey

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ABSTRACT

Objective: The emergence of coronavirus disease 2019 is a major healthcare threat. We aimed to assist in the management of coronavirus disease 2019 infection and contribute to the literature so the hospitals may find the information about our strategies useful in their efforts to reduce the challenges they are facing in this retrospective single-center study.

Methods: We analyzed the data of 1260 laboratory or radiologically confirmed hospitalized cases with coronavirus disease 2019 infection to determine the clinical and epidemiological characteristics. An infectious and a chest disease physician followed all the cases and recorded demographic data, clinical signs, treatment, laboratory, and radiological findings.

Results: The mean age of the patients was 51.96 years old, and 665 (52.7%) were male. The most commonly experienced symptoms at the onset of illness were cough, shortness of breath, myalgia, and fever. Most patients showed normal leucocytes counts, lymphopenia, elevated levels of C-reactive protein, procalcitonin, ferritin, lactate dehydrogenase, and creatine kinase.

Conclusion: Recognizing the changing treatment modalities is especially important for the management of the coronavirus disease 2019 pandemic.

Keywords: clinical, COVID-19 pandemic, medicine, modalities, therapeutics

INTRODUCTION

Severe acute respiratory syndrome (SARS) coronavirus disease-2019 (COVID-19) is the causative agent of coronavirus disease 2019, which was declared a global pandemic by the World Health Organization on March 11, 2020.[1,2] The coronavirus belongs to a family of viruses that can cause various symptoms, including fever, difficulty breathing, and pulmonary infections. These viruses are widespread in animals, but relatively few cases have been known to affect humans.[3,4] From March 11, 2020, the number of infected people increased and COVID-19 spread rapidly throughout Turkey. The science committee of the Turkish Ministry of Health developed guidance on novel coronavirus for healthcare professionals. Algorithms and obligations regarding how to manage the disease were included in the guidance.[5,6] Guidelines for the treatment of this infection may vary from country to country. In general, it is characterized by atypical pneumonia and is usually confirmed by a positive RNA test or computed tomography (CT) of the lung.[7] Different diagnostic techniques, such as serological, molecular, and radiological, can assist health centers in the detection of COVID-19; among others, the radiological method is the most recommended and is able to diagnose the infection quickly and accurately with fewer false-negatives. It is very important to use effective methods for the diagnosis of infections, which is essential for saving patients' lives and preventing the transmission of infection to other people.[8,9]

The majority of the cases show mild symptoms. Rapid progression may occur at the early stage of the disease.[10] To improve the

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prognosis of the disease, early control of viral replication and implementation of host-directed therapy are necessary. As a new infectious disease, it is particularly important to find out its clinical characteristics, mainly in the early stage, which is helping to detect and isolate patients earlier and to minimize its spread.

This retrospective research was conducted during the first 2 months of the current COVID-19 outbreak at a 360-bed state hospital in İstanbul, Turkey the epicenter of the outbreak was in İstanbul, Turkey. In order to manage patient flow and hospital capacity, we provided outpatient care in our hospital for patients with less severe symptoms. All non-urgent elective surgeries, endoscopies, or other invasive procedures were canceled through the peak period to reduce the risk transmission to patients and to provide adequate hospital capacity. We established screening and triage protocols for suspected cases in the emergency unit.

Since the onset of the outbreak, many agents that could have efficacy against COVID-19 have been suggested. The clinical experience of countries will lead to the use of drugs with proven efficacy and safety in the management of the infection. We aimed to report the initial experience with clinical features and the management of patients with COVID-19 infection in Turkey.

METHODS

Hospitalized patients 18 years of age and older due to possible infection of COVID-19 in compliance with the Turkish, Turkey Ministry of Health's General Directorate of Public Health's COVID-19 guide from March 11, 2020, when the first case was reported in Turkish, Turkey until May 11, 2020, were reported in this study. All cases were followed by an infectious and chest disease physician, and demographic data, clinical signs, treatment, laboratory, and radiological findings were recorded at presentation, during the hospital stay, and before discharge. Cases were confirmed either by CT or reverse transcriptase-polymerase chain reaction tests [RT-PCR] performed on nasopharyngeal and oropharyngeal swab specimens. In the very early stages of infection, when the nasopharyngeal swab may still be negative, CT plays a significant role in ultimately diagnosing COVID-19 in highly suspicious patients. Therefore, low-dose CT scanning was performed for the patients before hospitalization. Experienced chest radiologists analyzed all images as compliant or not compliant with COVID-19 pneumonia.[11]

Main Points

- Management of coronavirus disease 2019 pandemic has changed considerably from the beginning until now.
- Hydroxychloroquine has been replaced by specific antiviral agents.
- C-reactive protein, ferritin, D-dimer, lactate dehydrogenase, elevated liver function, and leukopenia are significant initial laboratory findings for clinical progress.
- Age group of pandemic victims is higher at the onset of the pandemic.
- Mask, social distance, and isolation are always important in protection from contamination.

At the onset of the epidemic, patients received hydroxychloroquine [HCQ] as initial therapy, according to Turkish COVID-19 guidelines. Hydroxychloroquine 400 mg was given twice daily for 1 day, followed by 200 mg twice daily for 4 more days. Hydroxychloroquine was usually combined with ceftriaxone (2 g once daily) plus azithromycin (500 mg on D1 followed by 250 mg per day for the next 4 days). Electrocardiograms (ECG) were performed on each patient before treatment and 2 days after initiation of treatment. When the QTc was >500 ms, the treatment was either not initiated or discontinued. Furthermore, during treatment, any drug potentially prolonging the QT interval was discontinued. Favipiravir therapy was started in patients with severe disease who did not respond to HCQ. Symptomatic therapies, including oxygen, were added when needed. Antibiotic treatment was modified in patients who were clinically unresponsive to the initial therapy. When needed, standard blood chemistry was checked. Until then, inpatients already receiving treatment with improved clinical outcomes and effective adherence to treatment were discharged due to a critical need to admit new, untreated inpatients. Patient follow-up continued in outpatient policlinics as much as possible.

Statistical Analysis

It was planned to use multivariate statistical methods instead of univariate statistical methods to increase the internal validity and accuracy of the analysis in the evaluation of the COVID-19 data. Mean and standard deviation [SD] were calculated for continuous variables. The normality of the variables was analyzed using a Kolmogorov–Smirnov test. The Student's *t*-test was used to compare the means between the 2 groups. A chi-square test was used to analyze the differences of drugs in the treatment of COVID-19 and to analyze the frequency of patients who survived or did not survive. The receiver operator characteristic [ROC] curve analysis was used to calculate the diagnostic accuracy as defined by the area under the curve [AUC], being 95% CI. Two-sided *P*-values were considered statistically significant at $P \leq .05$. All statistical analyses were carried out by using R software/programming [version 3.6.2 [2019-12-12] – CRAN].

Ethical Statement

All authors declare that the research was conducted in accordance with the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects." Data collection and analysis of all subjects were approved by the ethics committee of Biruni University (Date: May 28, 2020, Decision no: 2020/40-04).

RESULTS

From March 11 to May 11, 2020, a total of 1260 patients confirmed with either CT findings or RT-PCR tests for COVID-19 were hospitalized. Among all patients, 776 (61.6%) tested positive for COVID-19 RT-PCR on nasopharyngeal and oropharyngeal swab tests. The mean [±SD] age of the patients was 51.96 ± 15.63 years (range: 19_104). Overall, 52.7% (665 of 1260 patients) were male.

Hypertension was the most common comorbidity, affecting 190 (15.1%) of patients with available data. The second most

common comorbidities were diabetes (131 patients, 10.4%) and chronic obstructive pulmonary disease (86 patients, 6.8%). Only 74 patients (5.9%) had a history of cardiovascular disease. Clinical characteristics ranged from an asymptomatic carrier to acute respiratory distress syndrome and multiorgan failure on a large scale. The demographic and clinical characteristics of the patients are shown in Table 1.

The most common clinical symptoms in these patients were cough and shortness of breath, followed by myalgia and fever consistent with the literatüre.

We monitored major laboratory markers from the onset of illness. In survivors, the baseline lymphocyte count was higher than in non-survivors. Levels of C-reactive protein, D-dimer, high-sensitivity cardiac troponin I, serum ferritin, lactate dehydrogenase, creatine kinase, and procalcitonin were clearly elevated in non-survivors compared with survivors throughout the clinical course and increased with disease worsening. Table 2 shows the laboratory findings of the patients on admission.

Oseltamivir was given in 94.6% of all cases. Convalescent plasma therapy was administered to 30.4% of non-survivors, and no transfusion reactions occurred.

The majority (1203, 95.48%) of patients had a favorable outcome and were discharged from our unit. Fifty-seven patients were transferred to the intensive care unit (ICU), of whom 16 improved and were then returned to the ward. Almost 64.91% of non-survivors required invasive mechanical ventilation. We found the mortality rate for COVID-19 cases as 4.52% (Table 3).

DISCUSSION

The most frequently used agents both in Turkey and all over the world for the treatment of COVID19 are HCQ, lopinavir/ritonavir, favipiravir, and remdesivir. Antiviral drugs administered shortly after symptom onset can reduce infectiousness to others by reducing viral shedding in the respiratory secretions of patients. Hydroxychloroquine has already been prescribed to many people for the treatment or prophylaxis of malaria and some rheumatological disorders. Notably, the drug shows antiviral activity in vitro against coronaviruses and specifically, COVID-19.[12] Clinical trials from China and France revealed potential benefits using HCQ, sometimes combined with the macrolide-type antibiotic azithromycin resulting in a more rapid reduction in viral shedding and improved clinical outcomes.[13,14] We performed an ECG before the treatment because of reports about heart complications with this drug in patients with underlying conditions. Patients at risk were hospitalized for ECG monitoring allowing for the early detection and treatment of possible cardiac side effects. The toxicity of HCQ did not pose a major problem in our study. Also, favipiravir is an RNA-dependent RNA polymerase inhibitor and has been shown to be effective in the treatment of influenza and the Ebola virus.[15,16] Favipiravir was given orally. The dose was 1600

Table 1. Demographics and Clinical Characteristics

Characteristics	Non–survivor (n = 57) (%)	Survivor (n = 1203) (%)	All cases (n = 1260) (%)	*P*
Age (mean)	65.74 ± 12.86	51.31 ± 15.45	51.96 ± 15.63	.000
Female/male	24.6/75.4	48.3/51.7	47.2/52.7	.000
Hypertension	21.7	14.8	15.1	.363
Cardiovascular disease	21.7	5	5.9	.001
Chronic obstructive lung disease	8.7	6.7	6.8	.706
Diabetes	30.4	9.3	10.4	.001
Malignancy	4.3	0.7	0.9	.073
Fever	65.2	61.4	61.6	.716
Cough	78.3	82.4	82.2	.615
Sputum production	73.9	64.5	65	.358
Dyspnea	91.3	69.8	70.9	.027
Chest pain	56.5	17.4	19.4	.000
Palpitation	47.8	10	12	.000
Nausea	30.4	57.9	56.4	.010
Diarrhea	17.4	15.2	15.3	.780
Headache	21.7	31.7	31.2	.317

Table 2. Laboratory Findings of Patients Infected with COVID-19 on Admission to Hospital

	Non-survivor n=57 (min-max)	Survivor n=1203 (min-max)	All cases n=1260 (min-max)	*P*
White cell count, $\times10^9$/L	12.918 (0.85-49.01)	6.85213 (1.29-30.84)	7.13 (0.85-49.01)	.000
Neutrophil count, $\times10^9$/L	11.124 (0.63-40.64)	4.506 (0.05-26.78)	4.81 (0.05-40.64)	.000
Lymphocyte count, $\times10^9$/L	1.195 (0.1-4.9)	1.721 (0.3-13.3)	1.69 (0.1-13.3)	.000
Hemoglobin, g/L	11.561 (7-16.1)	13.492 (4.8-19.5)	13.4 (4.8-19.5)	.009
Platelet count, $\times10^9$/L	226.772 (75-1167)	254.164 (52-685)	226.4 (52-1167)	.985
D-dimer, mg/L	6.310 (0.3-35.2)	1.602 (0.19-15.1)	2.03 (0.19-35.2)	.008
Glucose	195.228 (90-523)	128.151 (62-487)	131.58 (62-523)	.000
Urea	94.185 (25-392)	31.391 (7.7-236)	34.4 (7.7-392)	.000
Creatine, μmol/L	1 (0.5-8.4)	1.110 (0.3-8)	0.99 (0.3-8.4)	.606
Aspartate aminotransferase, U/L	74.109 (17-1071)	32.678 (4-929)	34.71 (4-1071)	.040
Alanine aminotransferase, U/L	47.339 (18-472)	31.299 (16-321)	32.11 (16-472)	.093
Sodium, mmol/L	142.536 (127-171)	138.355 (115-152)	138.56 (115-171)	.001
Potassium mmol/L	4.00 (2.6-6.2)	4.27 (2.8-5.9)	3.8 (2.6-6.2)	.217
Creatine kinase U/L	594.806 (29-4585)	166.936 (17-4108)	189.27 (17-4585)	.001
Lactate dehydrogenase, U/L	526.727 (190-1333)	281.301 (77-949)	293.57 (77-1333)	.000
C-reactive protein, mg/L	132.785 (1.24-396)	43.555 (0.1-371)	47.73 (0.1-396)	.000
Procalcitonin ng/ml	4.722 (0.3-9.25)	1.805 (0.12-6.1)	3.42 (0.12-9.25)	.197
Ferritin	996.506 (263-2000)	365.452 (9.6-2000)	392.85 (9.6-2000)	.000

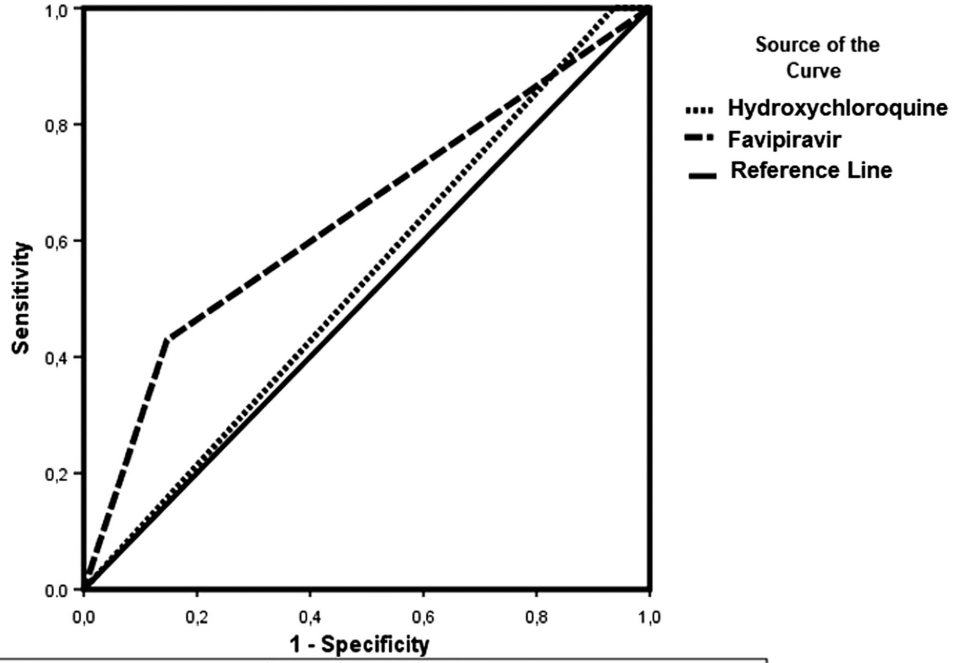
Table 3. Treatments and Outcomes of Patients Infected with COVID-19

Pharmacological Treatments	Non-survivor (n=57) %	Survivor (n=1203) %	All cases (n=1260) %	*P*
Hydroxychloroquine	95.7	96.9	96.8	.738
Oseltamivir	91.3	94.8	94.6	.476
Favipiravir	52.2	7.4	9.7	.000
Azithromycin	69.6	88.3	87.4	.008
Clarithromycin	8.7	16	15.6	.350
Levofloxacin	13	29	28.2	.097
Moxifloxacin	8.7	4.8	5	.398
Ceftriaxone	91.3	96.7	96.4	.180
Piperacillin-tazobactam	39.1	15.2	16.5	.003
Meropenem	26.1	2.6	3.8	.000
Prednisolone	30.4	3.1	4.5	.000
Immune plasma	30.4	0.2	1.8	.000

mg twice daily on day 1 and 600 mg twice daily on days 2-5. If we look at the mortality rates, we can see variations between reports. For example, Guan et al[17] report a death rate of 1.4% while Baud et al[18] report 5.7%. China has resulted in very remarkable results as the patients receiving favipiravir showed cleared viral load in 4 days as compared to 11 days in patients receiving standard care only.[19,20] Favipiravir is in vitro active against COVID-19, and early clinical experience is encouraging in the management of the ongoing pandemic coronavirus. We found the mortality rate for COVID-19 cases to be 4.52%. When we compared mortality rates in our study, the patient group receiving favipiravir was found to be lower than the group receiving HCQ. It has been shown that favipiravir has a protective effect on death (Figure 1). The course of the disease is more modifiable at an early stage, so treatment needs to be started before patients become critically ill. It is understood that antiviral treatment is more likely to have benefits for both influenza and SARS when it is started early during the course of the disease.[16] The outcomes of several randomized controlled trials to test the efficacy of favipiravir for COVID-19 will further identify the role of this drug. Patients infected with COVID-19 are being treated empirically with oseltamivir, but there is little evidence from randomized controlled trials to support the treatment of coronavirus infections with oseltamivir. Oseltamivir, an Food and Drug Admission-approved drug for influenza A and B treatment, inhibits the viral neuraminidase and ultimately prevents the release of viral particles from host cells.[21] In order to detect influenza viruses in respiratory specimens, no diagnostic tests were available, so oseltamivir was administered in 85% of all cases for possible influenza infection in our study.

We also examined the impact of advanced age on mortality rates in our research. A well-known fact now is the rise in mortality with old age for COVID-19. Advanced age has been reported as an independent predictor of death for SARS and the Middle East respiratory syndrome. Early Chinese reports showed that the mortality rate could be 3 times higher in older patients especially those at age over 80.[22] Lvliang Lu et al[23] confirmed that advanced age is associated with mortality in their systematic review. An Italian study reported that COVID-19 has had the greatest impact on those aged over 50, by ICU mortality being 26%, whereas it was 36% for those aged over 65.[24] In our study, we found that the majority of those who did not survive from coronavirus were male patients, older patients, and patients with comorbidities,

Figure 1. Receiver operating characteristic (ROC) curve prediction for favipiravir and hydroxychloroquine in mortality of the patients.



Area Under the Curve					
Variables	Area	SE	p	95%CI	
				Lower Bound	Upper Bound
Favipiravir	0.641	0.070	0.033	0.504	0.778
Hydroxychloroquine	0.532	0.063	0.627	0.409	0.655

ROC Curve

indicating that these patients may have an elevated risk of serious disease or death.

At the time of hospital admission, we analyzed the laboratory data of patients and made a comparison between the patients who died and those who survived in our study. However, in connection, increasingly higher inflammatory markers (C-reactive protein, ferritin, D dimer, lactate dehydrogenase, elevated liver function, and leukopenia) are significant initial laboratory findings in patients who have not survived as in other studies.[25-28] Liver injury in patients with coronavirus infections is often transient and can be directly caused by the viral infection of liver cells.[29] Changes in the number of different blood cells, including leukocytes, lymphocytes, neutrophils, platelets, and hemoglobin, may show the form and severity of the disease.[30]

Limitations

Our study has some notable limitations. Firstly, it was conducted during the global pandemic's early months and the condition was uncertain. It was based upon the limited data available to us as of May 11, 2020. Some cases had incomplete documentation of the exposure history and laboratory testing. This is a single-centered, retrospective analysis and it may be restricted to the critical care resources of the hospital and may not be valid in all other regions. Secondly, some specific clinical data, such as time to the disease onset, was missing. Further research is still required.

CONCLUSION

The clinical features and treatment processes related to COVID-19 pandemic have changed from the beginning until now. Since the beginning of the pandemic isolation, quarantine, social distance and social protection programs have been our choices due to the lack of adequate vaccines and effective treatment. We hope that our patient population will have a better understanding and baseline characteristics, hospital course, and clinical results will give useful information to physicians who are working in a time of exceptional volume and uncertainty.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Biruni University (Date: May 28, 2020, Decision no: 2020/40-04).

Informed Consent: Since our study was a retrospective data study, informed consent was not required.

Peer-review: Externally peer-reviewed.

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Original Article

Emergency Right Hemicolectomy for Pericecal Masses Mimicking Acute Appendicitis: Surgeon's Fearful Dilemma

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ABSTRACT

Objective: Unexpected conglomerated pericecal masses of uncertain etiology encountered in emergency surgery may be indistinguishable, and proper operative strategic management of these cases is a dilemma for digestive system surgeons. Starting from this point, we decided to analyze the patients in whom a right hemicolectomy was performed for the pericecal mass during an appendectomy in our regional hospital.

Methods: Over 8 years between March 2011 and May 2019, 4783 patients who lived in the eastern Mediterranean area underwent emergency surgery for clinical diagnosis of acute appendicitis, and a right hemicolectomy for inflammatory pericecal mass was performed in 44 patients included in this study. Patient records were reviewed for sex, age, preoperative symptoms, preoperative imaging, operation findings, preoperative Complete Blood Count (CBC) and biochemical findings, pathology reports, length of hospital stay, mortality, and any complications encountered.

Results: The histopathological examination revealed that 5 of 44 (11.4%) patients had malignancy while 27 of 44 patients (88.6%) had benign pathologies. All of the malignancies were adenocarcinoma. According to age, there was a statistically significant difference between patients with and without malignancy ($P < .05$).

Conclusion: The pericecal mass in emergency surgery is still a diagnostic and therapeutic dilemma. Hidden appendiceal neoplasm in acute appendicitis is rare but its incidence is higher in patients presenting appendiceal inflammatory mass. On the other hand, most unexpected inflammatory pericecal masses are due to benign pathologies. The choice of the surgical procedure depends on the surgeon's and institute's experience.

Keywords: Appendicitis, cancer, hemicolectomy, pericecal mass

INTRODUCTION

Acute appendicitis is one of the most common gastrointestinal system surgical emergencies worldwide without any doubt.[1] A total of 88% of emergency surgical admissions that require surgery are cases of appendicitis.[2,3] Geographical differences are reported with lifetime risks for appendicitis of 16% in South Korea, 9% in the USA, and 1.8% in Africa.[4,5] Although it is a common disease, obtaining a confident preoperative diagnosis is still a challenge, especially in the elderly population. The unexpected pericecal mass with uncertain etiology occasionally encountered by the surgeon during appendectomy may cause a therapeutic dilemma. The appendiceal mass is generally the result of a walled-off inflammation or infection and represents a pathological spectrum ranging from pericecal phlegmon and abscess to conglomerated solid mass.[6] Various diseases involving the ileocecal region cause pericecal mass, such as severe appendicitis, inflammatory bowel disease, diverticular disease, and malignancy.[7,8] Because benign pericecal masses or cancers can mimic acute appendicitis, sometimes during the operation, the surgeons cannot virtually distinguish the pathology. So, the surgeons are often challenged to determine the pathologic origin of masses.[8] In these circumstances, emergency surgery is associated with a risk of ileocaecal resection or right hemicolectomy. Many reports in the literature have addressed this promiscuousness, and right hemicolectomy has been recommended because of possible malignancy. Most of the limited number of studies were carried out to evaluate the pathologies and surgical management of the pericecal masses in patients with suspected appendicitis.[9-11]

From the above-mentıoned starting points, we decided to analyze the patients retrospectively to present the diversity of the inflammatory pericecal masses in patients with right hemicolectomy

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was performed for the pericecal mass that was detected during an appendectomy for acute appendicitis in our clinic.

METHODS

Ethics

This study was carried out with the permission of the Adana City Training and Research Hospital Ethics Committee (Date: May 22, 2019, Decision No: 446) and has therefore been performed following the ethical standards in the Declaration of Helsinki.

Study Design

In this study, we retrospectively evaluated 4783 patients who underwent emergency surgery for acute appendicitis between March 2011 and May 2019 at the Department of Surgery, Adana City Training and Research Hospital. Forty-four patients who had right-hemicolectomy for inflammatory cecal masses of uncertain etiology were included in our study. The patients who had suspicious or proven for pericecal malignancy preoperatively by the physical, radiological examination, or biopsy were excluded from the study. Patient records were used to identify sex and age. The records were also reviewed, especially for preoperative symptoms, preoperative imaging, operation findings, pre-and postoperative Complete Blood Count (CBC) and biochemical findings, pathology reports, length of hospital stay, mortality, and any complications encountered.

Surgical Technique

Right-hemicolectomy was performed as formal resection of the right colon, including lymphatic drainage along the ileocolic and right colic arteries. If possible, an anastomosis was made by linear stapler or hand saving due to surgeons' preference.

Postoperative Follow-Up

All patients were administered prophylactic antibiotics at induction, and antibiotic treatment was proceeded until postoperative day 3 if there was no severe or mild infective complication. Patients were discharged with a 1-week course of 500-mg paracetamol 3 times daily with suture removal after 1 week. The outpatient consultation was arranged at postoperative month 1 firstly, and postoperative month 3 secondly.

Statistical Analyses

The statistical analyses were performed using Statistical Package for the Social Sciences version 25.0 (SPSS Inc., Chicago, Ill, USA). Chi-square statistical analyses were used for nominal data. The ordinal data and non-parametric numerical data of malignant and non-malignant groups were previously tested for normality by the Shapiro–Wilk test independent samples *t*-test, Mann–Whitney *U* test was used. A *P* value <.05 was considered statistically significant.

Main Points

- The pericecal mass in emergency surgery is a diagnostic and therapeutic dilemma.
- Most of the unexpected inflammatory pericecal masses are due to benign pathologies.
- Malignancy was detected in 11.4% of the patients who were suspected of malignancy due to pericecal mass and who underwent right hemicolectomy.
- When the groups with and without malignancy were compared, age was the major risk factor for malignancy.

RESULTS

Patients and Symptoms

Totally 44 patients underwent a right hemicolectomy procedure; 28 (63.6%) of them were male, whereas 16 (36.4%) were female. The patients included in this study were between the ages of 21 and 86 (mean: 49.59).

Twenty-three of 44 patients had Ultrasonografi (USG) and 25 of 44 had computed tomography (CT) scannings before the surgery. The radiologic examination did not determine whether the processes were malignant or inflammatory (Table 1).

Distribution of Pathologies

All specimens resected were sent to pathologic examination. The histopathologic examination revealed that 5 (11.4%) patients had malignancy while 39 (88.6%) patients had benign pathologies. All of the malignancies were adenocarcinoma. On the other hand, the benign histopathologic diagnosis was perforated plastron appendicitis (n = 22), inflammatory bowel disease (n = 4), cecal diverticulitis (n = 3), mucinous cystadenoma (n = 3), nonspecific active colitis (n = 2), vasculitis (n = 2), tuberculosis (n = 2), and mesenteric fibromatosis (n = 1). The distribution of pathologies showed in Table 2. When malignant and benign groups are compared. According to age, there was a statistically significant difference between patients with and without malignancy ($P < .05$).

Biochemical Tests

There was no statistically significant difference with respect to preoperative blood tests such as white blood cell count (WBC), hemoglobin (Hgb), C reactive protein (CRP), aspartate aminotransferase (AST), and amylase between in patients with and without malignancy ($P > .05$) (Table 3).

Table 1. Preoperative Radiologic Scannings

	n	%
USG		
No pathologic findings	11	25
Acute appendicitis	5	11.4
Complicated appendicitis	7	15.9
Not performed	21	47.7
CT		
No pathologic findings	5	11.4
Acute appendicitis	6	13.6
Complicated appendicitis	14	31,8
Not performed	19	43.2

CT, computed tomography.

Table 2. The Pathologies in Patients

Pathologies	n	%
Perforated plastron appendicitis	22	50
Inflammatory bowel disease	4	9.1
Cecal diverticulitis	3	6.8
Mucinous cystadenoma	3	6.8
Non-specific active colitis	2	4.5
Vasculitis	2	4.5
Tuberculosis	2	4.5
Mesenteric fibromatosis	1	2,3
Adenocarcinoma	5	11,4

Postoperative Follow-Up

The mean length of hospital stay is 9.7 days (5-22 days). The mortality rate was 6.8% (3/44) due to severe comorbidities of the patients and being very elderly. The pathologies detected in 2 of them were benign, whereas the other was a malignant disease.

DISCUSSION

Right lower quadrant abdominal pain is a common presenting symptom in the emergency department. This entity can result from a broad spectrum of conditions, ranging from self-limiting to requiring emergency surgery.[7] Sometimes, inflammatory pericecal masses or cancers may mimic acute appendicitis, and during the operation, the surgeon may not distinguish the pathology.[8] An unexpected conglomerated mass at the ileocecal region may cause a therapeutic dilemma since various diseases involving the ileocaecal region causes pericecal mass, such as perforated appendicitis, inflammatory bowel disease, diverticular disease, and malignancy.[8]

The surgical strategy generally depends on the pathology. On the other hand, it is not always possible to know or predict the nature of the disease, if it is benign or malignant. In daily surgical and emergency settings, the surgeons may not exclude the malignancy, and a radical resection may be necessary.[12-15] A recent questionnaire study performed by Ahmad I et al[9] showed no agreed consensus on the management of appendicecal mass in the Mid-Trent region of England. In the present study, except for a young patient, all of the patients underwent a right hemicolectomy since this is a reasonable procedure in our clinic if the surgeon could not exclude the malignancy. Our study showed that only 11.4% of the patients had malignancy, and the pathology in most of the patients (88.6%) was reported as benign. All specimens resected were sent to pathologic examination. The histopathologic examination revealed that 5 (11.4%) patients had malignancy while 39 (88.6%) patients had benign pathologies. All of the malignancies were adenocarcinoma. The most detected benign histopathologic diagnosis was perforated plastron appendicitis (n = 22, 50%), while inflammatory bowel disease, cecal diverticulitis, mucinous cystadenoma, non-specific active colitis, vasculitis, tuberculosis, and mesenteric fibromatosis were the other benign pathologies detected. When malignant and benign groups are compared. According to age, there was a statistically significant difference between patients with and without malignancy ($P < .05$). Age was an independent factor for malignant pericecal mass. This result is not surprising, so the surgeons should keep a malignancy risk in their minds in elderly patients with periceacal mass.

In the emergency department, ultrasonography (US) is the first choice for investigating the etiology of acute abdominal pain. The diagnostic accuracy of US in patients with right lower quadrant pain is reported as 72%, whereas the CT has a higher diagnostic rate.[14,15] Despite the increased use of CT to evaluate acute appendicitis and diagnose periceacal mass, the number of perforated and complicated cases has been stable in the past 3 decades.[12] In our study, the diagnostic rate of the US was 78.1% while 93.7% for CT. According to our experiences, CT is more accurate to diagnose the pericecal mass but not for distinguishing a benign pathology from a malignancy. There were no statistically significant differences with respect to preoperative serum levels of WBC, Hgb, CRP, AST, and amylase between patients with and without malignancy ($P > .05$).

Table 3. The patients features with and without malignancy

	Malignant (mean)	SD	Benign (mean)	SD	*P*
Age	68.2	±17.21	47.20	±14.17	**.004***
WBC (10^3/μL)	13.36	±4.62	13.14	±4.91	.590**
Hb (g/dL)	11	±1.43	12.62	±1.97	.600*
CRP (mg/dL)	22.17	±14.52	25.12	±23.65	.852**
AST (μ/L)	38	±22.42	27	±14.65	.144*
Total bilirubin	0.6	±0.42	0.23	±0.63	.128**
Amylase (μ/L)	49	±26.62	58.98	±44.01	.914**

*Student t-test.
**Mann-Whitney U test.
WBC, white blood cell; Hb, hemoglobin; CRP, C-reactive protein; AST, aspartate aminotransferase.

The mortality rate of emergency right hemicolectomy varied between 6% and 10% in previous studies.[10-13] In this study, concomitant respiratory and cardiac failure and elderly age were the main reasons for mortalities. The mortality rate was 6.8% in our study, and it was similar to the literature

Although our study gives valuable information about the subject, our study has some limitations. This study was conducted on a group of patients retrospectively who are living in the Mediterranean region. Additionally, the patients were operated on in a single institute. Therefore, for the generalizatıon of our results, further clinical studies with a high number of cases are needed.

CONCLUSION

In conclusion, pericecal mass in emergency surgery is still a diagnostic dilemma. Hidden appendiceal neoplasm in acute appendicitis is rare, fortunately. However, its incidence is much higher in patients presenting appendiceal inflammatory mass. On the other hand, most unexpected inflammatory and conglomerated pericecal masses are due to benign pathologies. The choice of the surgical procedure depends on the surgeon's and institute's experience, and further prospective researches are needed on this topic.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Adana City Training and Research Hospital, (Date: May 22, 2019, Decision no: 35/446).

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

Peer-review: Externally peer-reviewed.

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Declaration of Interests: The authors declare that they have no competing interest.

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Original Article

Relationship of Diet Adherence with Levels of Depression, Anxiety, and Caregiver Burden in Parents of Children with Celiac Disease

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ABSTRACT

Objectives: The aim of this study was to compare caregiver burden and the levels of depression and anxiety among mothers of children with celiac disease who are adherent or non-adherent to a gluten-free diet.

Methods: In this study, 92 patients diagnosed with celiac disease who were regularly followed at the Department of Pediatric Gastroenterology, Çukurova University Faculty of Medicine, and their caregiving mothers were enrolled. Demographic characteristics were captured for both patients and mothers and the Beck Anxiety Inventory, Beck Depression Inventory, and Zarit Burden Interview were completed for caregiving mothers.

Results: There were 69 (75%) mothers in the diet-adherent group and 23 (25%) mothers in the non-diet-adherent group. The caregiving mothers studied had a mean age of 39.6 ± 7.4 years. The mean Beck Anxiety Inventory, Beck Depression Inventory, and Zarit Burden Interview scores of the caregiving mothers were 15.4 ± 8.2, 17.3 ± 9.5, and 37.1 ± 13.5 points, respectively. Compared to the mothers in the diet-adherent group, mean Beck Anxiety Inventory, Beck Depression Inventory, and Zarit Burden Interview scores were statistically significantly higher in the mothers in the non-diet-adherent group (P=.005, .001, .020, respectively).

Conclusions: It is necessary to recognize the burdens that caregivers are exposed to and identify how heavy these burdens are. Mothers of patients who do not comply with gluten-free diet may experience high levels of depression and anxiety and this should be borne in mind while treating celiac disease.

Keywords: anxiety, caregiver burden, celiac, depression

INTRODUCTION

Celiac disease (CD) is defined as a chronic, immune-mediated condition caused by the ingestion of gluten in genetically predisposed individuals, which is characterized by a wide variety of gastrointestinal and/or systemic manifestations, various degrees of inflammatory enteropathy, and high levels of celiac-specific autoantibodies.[1] The global prevalence of CD ranges between 0.5% and 1% with variations in reported rates across countries.[2] In Turkey, CD prevalence has been estimated at 0.47%.[3] Celiac disease results from the interplay between genetic and environmental factors. Although there is ongoing research focusing on the treatment of CD, the only effective treatment continues to be lifelong adherence to a gluten-free diet (GFD). Strict adherence to this diet is important for the prognosis of the disease.[4]

In Turkey, the care for sick individuals is often provided by the parents and it is perceived as a family responsibility. Studies on the assessment of the quality of life in mothers of children with chronic diseases have usually involved children with neurological problems, and there are few studies on mothers of children with CD.[5,6] Moreover, adherence to a GFD may be more challenging in children and it may demand extra effort from their mothers, which can negatively affect them psychosocially.[7,8]

In the present study, we aimed to determine how mothers of children with CD who were compliant or non-compliant with a GFD are affected physically, socially, emotionally, and economically by comparing the quality of life, depression, and anxiety levels of the mothers.

METHODS

In this study, 92 patients diagnosed with CD who were being regularly followed at the Department of Pediatric Gastroenterology clinic and their caregiving mothers were enrolled. The sociodemographic characteristics of the caregiving mothers including age, the length of marriage, total number of children, occupation, education level, and economic status were captured using a study-specific form. Mothers with any mental illness within the

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last 12 months diagnosed either by an expert psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, fifth Edition criteria, on the basis of history or identified through medical chart review, and mothers taking psychotropic drugs were excluded from the study.[9]

Sociodemographic data sheet, the Beck Anxiety Inventory (BAI), and the Beck Depression Inventory (BDI) were completed through face-to-face interviews. Each interview took about 15-20 minutes. Additionally, the adherence of the patients with CD to the diet was assessed using serological CD tests, history, and physical examination. Written consent was obtained from the parents of the patients participating in this study. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki, and ethics approval was obtained from the institutional review board (Date: April 5, 2019, Decision no: 87).

Beck Anxiety Inventory

The BAI was developed by Beck et al.[10] The BAI measures the severity and frequency of anxiety symptoms experienced by the individual. It is a self-assessment tool consisting of 21 items rated on a 4-point Likert scale ranging from 0 to 3 points. The maximum possible score is 63 points. Higher total scores indicate more severe anxiety symptoms in an individual. The reliability and validity of the Turkish version of the scale had been demonstrated by Ulusoy et al.[11] The Cronbach alpha reliability coefficient of the scale was 0.93.

Beck Depression Inventory

The BDI is used to measure the physical, emotional, cognitive, and motivational symptoms experienced in depression. The BDI aims to determine the extent of depressive symptoms objectively. The scale consists of 21 questions with 4 possible responses, and each item is assigned a score from 0 to 3. The items are summed to produce a total depression score which ranges from 0 to 63 points. Higher total scores indicate more severe depressive symptoms. The reliability and validity of the Turkish version of the BDI had been demonstrated by Hisli[12] The Cronbach alpha reliability coefficient of the scale was 0.93.

Zarit Burden Interview

The Zarit Burden Interview (ZBI) was developed by Zarit et al[13] in 1980. The ZBI consists of 22 items that address the impact of caregiving burden on the daily life of caregivers. Each item is rated on a 5-point Likert scale that ranges from 0 (never) to 4 (nearly always). The reliability and validity of the Turkish version of the scale had been demonstrated by Inci and Erdem in 2008, with a Cronbach alpha value of 0.95.[14] The items are summed to produce a total score ranging from 0 to 88 points. The total burden scores are interpreted as little/no burden (0-20), moderate burden (21-40), severe burden (41-60), and very severe burden (61-88). The ZBI items are generally related to social and emotional dimensions and higher total scores indicate greater burden and higher level of distress for the caregiver.[15]

Main Points

- Anxiety and depression in the mothers of children with celiac disease (CD) who require special diet and care are important for the management of the disease.
- The children with CD and their mothers should be provided with psychological support during follow-up.
- The mothers of CD children who are non-compliant with a gluten-free diet have higher levels of depression and anxiety.

Statistical Analysis

The study data were analyzed using the Statistical Package for the Social Sciences software version 22.0 for Windows (SPSS Inc, Chicago, Ill, USA). The Kolmogorov–Smirnov test was used to check whether continuous variables followed a normal distribution. Normally distributed variables were expressed as mean ± standard deviation, while non-normally distributed variables were expressed as median with interquartile range. The categorical variables were presented as percentages. Differences between the 2 groups were analyzed using the Student's unpaired *t*-test or the Mann–Whitney *U* test for parameters with a normal or non-normal distribution. The frequencies of nominal variables were compared using the Fisher's exact test or chi-square test. $P < .05$ was considered statistically significant.

RESULTS

In this study, 92 mothers of patients with CD were included in the study. There were 69 (75%) mothers in the diet-adherent (DA) group and 23 (25%) mothers in the non-diet-adherent (NDA) group. The caregiving mothers studied had a mean age of 39.6 ± 7.4 years, 56.5% of them were primary school graduates, 73.9% were married for an average duration of 16.6 ± 5.9 years, and the mean number of children was 3.1 ± 1.6. The DA group and NDA group were not statistically significantly different in terms of mean age, education status, occupation, length of marriage, and number of children ($P > .05$). With regard to household income, monthly income was less than expenses in 73.9% of the NDA group and 43% of the DA group. There was a significant difference between the 2 groups in terms of monthly income ($P = .038$). Patient age, sex, body weight, height, and disease duration were not significantly different between the 2 groups ($P > .05$) (Table 1).

The mean BAI score was 19.6 ± 9.3 for the mothers in the NDA group and 14.0 ± 7.4 for the mothers in the DA group ($P = .005$). The NDA group had a mean BDI score of 23.1 ± 7.3 and the DA group had a mean BDI score of 15.4 ± 9.4 ($P = .001$). The mean ZDI score was 42.9 ± 9.1 in the NDA group and 35.3 ± 14.3 in the DA group ($P = .020$) (Table 2). When the caregiving burden of the mothers was evaluated based on the ZDI scores, moderate caregiving burden was most common among all mothers (50%). The moderate burden was found in 55.1% of the mothers in the NDA group and 56.5% of the mothers in the DA group reported severe caregiving burden. The difference between the 2 groups in terms of the subdomains of the caregiving burden scale was of borderline significance ($P = .057$)

Table 1. Sociodemographic Characteristics of Patients and Their Caregiving Mothers

Parameters	Total Number of Patients (n=92)	Diet Adherent (n=69)	Non-diet Adherent (n=23)	*P*
Patient's age (month)	131.3 ± 50.0	126.3 ± 49.1	146.6 ± 50.6	.91
Patient's sex				
Female, n (%)	61 (66.3)	46 (66.7)	15 (65.2)	.899
Male, n (%)	31 (33.7)	23 (33.3)	8 (34.8)	
Patient's body weight (kg)	34.3 ± 14.8	33.2 ± 14.4	37.4 ± 15.6	.243
Patient's height (cm)	135.9 ± 22.3	134.4 ± 22.6	140.4 ± 21.0	.266
Disease duration (months)	56.1 ± 25.8	55.2 ± 26.4	58.6 ± 24.4	.588
Adherence to diet, n (%)	92 (100)	69 (75)	23 (25)	
Caregiver age (years)	39.6 ± 7.4	39.5 ± 7.1	39.7 ± 8.6	.917
Length of marriage (years)	16.6 ± 5.9	16.8 ± 5.9	16.3 ± 6.1	.707
Total number of children	3.1 ± 1.6	3.2 ± 1.6	3.0 ± 1.4	.679
Mother's occupation				
Housewife, n (%)	68 (73.9)	51 (73.9)	17 (73.9)	.293
Worker, n (%)	7 (7.6)	7 (10.1)	0 (0)	
Civil servant, n (%)	3 (3.3)	2 (2.9)	1 (4.3)	
Self-employed, n (%)	11 (12.0)	8 (11.6)	3 (13.0)	
Retired, n (%)	2 (2.2)	1 (1.4)	1 (4.3)	
Mother's education status				
Primary school, n (%)	52 (56,5)	41 (59.4)	11 (47.8)	.408
Secondary school, n (%)	17 (18.5)	11 (15.9)	6 (26.1)	
High school, n (%)	16 (17.4)	13 (18.8)	3 (13.0)	
University, n (%)	7 (7.6)	4 (5.8)	3 (13.0)	
Household income				
Income less than expenses, n (%)	47 (51.1)	30 (43.5)	17 (73.9)	.038
Income equal to expenses, n (%)	34 (36.9)	29 (42.0)	5 (21.7)	
Income greater than expenses, n (%)	11 (12.0)	10 (14.5)	1 (4.3)	

Table 2. Levels of Anxiety, Depression, and Caregiving Burden of Caregiving Mothers

Parameters	Total Number of Patients (n=92)	Diet Adherent (n=69)	Non-diet Adherent (n=23)	*P*
Beck Anxiety Inventory	15.4 ± 8.2	14.0 ± 7.4	19.6 ± 9.3	.005
Beck Depression Inventory	17.3 ± 9.5	15.4 ± 9.4	23.1 ± 7.3	.001
Zarit Burden Interview	37.1 ± 13.5	35.3 ± 14.3	42.9 ± 9.1	.020

DISCUSSION

Family members are the ones who provide the greatest support to patients with a chronic conditions and at the same time, they feel that they bear the heaviest burden. This affects the quality of life and mental health of all family members and puts a great deal of stress and pressure on parents. Mothers often feel the obligation to meet all the needs of their sick children and find it difficult to accept the situation.

Celiac disease causes dramatic changes in the daily lives of both the patient and the caregiver and interferes with the habitual routine of the affected individual. Many studies on the caregivers have demonstrated that caregivers experience psychological distress and have a poor quality of life. In a study by Lorenzo et al[7] conducted in Joana de Gusmao Children's Hospital in Brazil, significantly lower quality of life scores were found in the parents of children with CD compared to the parents of children without CD.

It has been reported that mothers of children diagnosed with CD may develop psychological problems such as depression and anxiety along with changes in the quality of life during the course of the disease.[7] In a study by Epifanio et al.[16] a higher level of parenting stress was reported in the parents of children with CD than in the parents of healthy children.

Following a lifelong diet as a treatment modality is a challenging commitment for both patients and their families. Consequently, the rate of noncompliance with the diet is quite high and adherence to a specific diet demands extra effort from the mothers, affecting their psychosocial well-being negatively.[7,8] Celiac disease may cause symptoms of anxiety and depression due to several factors including concerns about adherence to a GFD, high cost of food, problems with meals served at school, limitations in the time to prepare food, and having to eat out.[17] In line with the literature, the mothers of CD children who were noncompliant with a GFD had higher levels of depression and anxiety compared to the mothers of CD children who were compliant with the diet. The development of self-concept and identity in children during the growth period exposes the mother to greater stress due to reasons including refusing the GFD and opposition to the authority, and the physical and mental fatigue that builds over time causes deterioration of the well-being of the caregiver and increases feelings of depression and anxiety. Adolescents non-compliant with a GFD were reported to have a lower overall quality of life, more physical problems, greater burden of illness, and more family problems compared to adolescents who were compliant with a GFD.[18]

We believe that the management of CD should involve the evaluation of the patient and the caregiver together, an early assessment of the risk factors, as well as implementation of measures to eliminate the risks. Various factors associated with the burden on caregivers of patients with chronic conditions have been examined and demonstrated in former studies. These factors include those that are related to the patient and the disease (e.g., age, sex, severity and type of symptoms, disease duration, and treatment), those that are related to the caregiver (e.g., sex, the degree of relationship with the patient, personality traits, socioeconomic, and cultural characteristics), and others (e.g., social support, degree of stigmatization by the community, quality, and accessibility of mental health services).[19,20]

Increased caregiver burden is associated with higher scores for depression and anxiety among caregivers. The burden of caregiving affects the mental health of the patients, and increased caregiving burden affects the quality of life of the caregiver, and this, in turn, results in an increase in the caregiver burden. Studies conducted with mothers of children with chronic illnesses reported mean caregiver burden scores that ranged between 30 and 52 points.[21,22] The caregiver burden scores observed in the present study are consistent with those reported in the literature. Increased caregiver burden has a negative impact on the well-being of caregivers and affects their quality of life adversely. Caregivers may feel overwhelmed and experience anxiety, depression, uncertainty about the future, feelings of inadequacy, and social isolation. Having a child with a chronic condition can cause changes in the physical, emotional, and financial stability of the family and this can take away the ability of the family members to enjoy life, resulting in poor family quality of life. We believe that early social–psychological support should be provided to caregivers to avoid the negative effects of caregiving burden on the lives of caregivers.

The burden of care for a chronic illness also brings along many financial problems, and a study by Arslantas et al[23] concluded that economic costs are perceived as the biggest burden by the caregivers. Removing gluten from the diet may be a challenging task due to several reasons including the high cost of and limited access to gluten-free products, cross-contamination with gluten and prolamins in many marketed products (despite having a "gluten-free label"), unavailability of GFD products, and the presence of a few alternative products such as pure corn starch and rice flour. Increasing monthly allowances for celiac patients and their families and improved access to GFD products seem important to ease the economic burden of CD.

The limitations of our study include the enrollment of only mothers as caregivers, the small sample size, assessment of mental health by looking at symptoms of anxiety and depression only, not determining the factors that cause burden on caregiving mothers, and not comparing the symptoms of psychological problems in the mothers with those in children. Future studies involving comprehensive assessments may help to better understand these relations.

CONCLUSION

Caregiving is a long and challenging process that is associated with restrictions on the daily life of the caregivers and adverse effects on their social interactions. Therefore, it is necessary to recognize the burdens that caregivers are exposed to and to identify how severe these burdens are. Protecting caregivers should be among our primary duties while treating our patients. The quality of care will also be improved when the caregiver is healthy both mentally and physically. We may end up with a new patient population if we fail to acknowledge the difficulties experienced by caregivers.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Çukurova University (Date: April 5, 2019, Decision no: 87).

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Declaration of Interests: The authors declare that they have no competing interest.

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Original Article

Trend of Sex Differences and Predictors of Complications of Cardiac Electronic Device Implantations in the Southeast Anatolian Region of Turkey: An Observational Study

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ABSTRACT

Objective: The comparison of complications after cardiac implantable electronic device procedures has not been studied adequately between both genders. Here, we examined the effect of gender on complications in the Southeast Anatolian Region of Turkey.

Methods: A total of 1640 patients from 3 centers in the Southeast Anatolian Region of Turkey were randomly selected. We compared major adverse cardiac events (clinically significant hematoma, pericardial effusion or tamponade, pneumothorax, and device infection) between genders. Univariate and multivariate analyses were plotted to identify predictors of outcomes between both genders.

Results: The overall rate of major adverse cardiac events was 3.8% (63 of 1640). Major adverse cardiac events occurred in 4.1% (40 of 983) of the men and 3.5% (23 of 657) in the women groups ($P = .557$). The most complications were device-related infection (2.1%) and pneumothorax (1.3%) in both genders. Single- and dual-chamber pacemakers were more implanted in women than in men (11.7% vs. 6.2% and 32.6% vs. 20.1%, respectively, $P < .001$). On the contrary, single- and dual-chamber implantable cardioverter defibrillators were more implanted in men than in women (38.1% vs. 19.6% and 8.5% vs. 4.1%, respectively, $P < .001$). Additionally, warfarin treatment and history of heart failure were found predictors of major adverse cardiac events in multivariable analysis.

Conclusions: This small-scale, real-life patient data revealed no remarkable distinction in terms of complications between both genders. Multinational randomized large-scale cohort trials are required to support our results.

Keywords: Anticoagulants, cardiac devices, cardiac epidemiology, cardiovascular events gender, platelets

INTRODUCTION

In recent years, cardiac implantable electronic device (CIED) procedures with the inclusion of permanent pacemakers (PPM), cardiac resynchronization therapy with pacemaker or defibrillator (CRT-P or CRT-ICD), implantable defibrillator (ICD) have increased exponentially throughout the world.[1] The adverse events due to CIED procedure still remain high despite the improvements in the device or lead technologies and advanced operator experience.[2,3]

Gender differences have been a matter of interest in cardiology lately. In that context, the impact of gender differences was investigated in cardiac procedures such as percutaneous coronary intervention, coronary artery bypass operations, and catheter ablation in atrial fibrillation.[4-6] To date, there are very limited studies that showed the effect of genders on the procedural complications such as rehospitalization, device-related infection, and mortality in CIED implantations.[7-10]

The risk of complications is more likely in women patients because of anatomical barriers such as thinner and smaller vessels, narrower thoracic cavity, and smaller body structure in

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arrhythmia procedures.[11] Also, it has been shown that women have had more complications in ICD procedures.[12]

Here, we present the impact of gender differences on CIED procedures including ICD, PPM implantations, and generator change in the Southeast Anatolian Region of Turkey, and also we explored the incidence of complications of these procedures in a multicenter, small-scale observational study.

METHODS

One thousand six hundred forty patients were randomly selected to reduce selection bias from 3 different hospitals in the Southeast Anatolian Region of Turkey. The study was performed as a retrospective and observational design. De novo implantations of CIED (PPM, CRT-P, CRT-ICD, and ICD) or generator change patients over the age of 18 between January 2010 and 2021 were included. Information about the study was provided both orally and in written form to the patients or their trustees. The study was performed with respect to the Declaration of Helsinki (2013). The study was confirmed by the institution review board (date of approval: March 17, 2022, and number: 74).

Anticoagulation Management

Warfarin therapy was interrupted in all patients until the INR level fell ≤1.7. Surgical procedures were planned if INR fell ≤1.7 in all the centers. Bridging treatment was performed with low molecular weight heparin (LMWH) or unfractional heparin (UFH) if the INR level fell ≤2. Unfractional heparin treatment was interrupted before 4 hours in all procedures and reinitiated 12 hours after implantation. The last dosing of LMWH was performed 12 hours before implantation and reinitiated 12 hours after the implantation. Unfractional heparin or LMWH was given with warfarin until INR ≥2. Additionally, warfarin treatment was continued in patients with previous prosthetic valve thrombosis and a history of ischemic events. In this population, target INR level was maintained between 2 and 3.5.

Non-vitamin K-dependent oral anticoagulants (NOACs) were routinely discontinued 24 hours before the procedure to prevent bleeding. Dabigatran treatment was adjusted according to the patients estimated glomerular filtration rate (eGFR). Non-vitamin K-dependent oral anticoagulant treatment was restarted in the evening of the day of the procedure. Any prior antiplatelet treatment was routinely continued during the perioperative period.

Main Points

- Little is known about the impact of gender on clinical outcomes throughout the pacemaker's surgery.
- The effect of gender difference on clinical outcomes has been a matter of interest in cardiology lately.
- The risk of complications is more likely in women patients because of the anatomical barriers.
- We present the impact of gender differences on cardiac implantable electronic device (CIED) procedures in the Southeast Anatolian Region of Turkey.
- This small-scale, real-life patient cohort of CIED implantation revealed no significant differences in terms of complications between both genders.

Definitions

Clinically significant hematoma (CSH) was defined as hematomas that cause significant swelling and pain at the generator site, cause discontinuation of oral anticoagulant therapy, require the evacuation of the hematoma due to severe pressure, or require a blood transfusion. Pneumothorax, pericardial effusion, and cardiac tamponade were documented by chest x-ray film, computed tomography, or echocardiography as indicated. Pocket infections, lead-endocarditis, and positive blood cultures in 1 or more cultures were considered device-related infections. Infections were described in accordance with previously published guidelines.[13] Cardiovascular disease diagnoses are coded accordingly to the 10th Revision Codes of the International Classification of Diseases (Supplementary Table S1).

Implantation Technique

The punctures were routinely performed with an axillary approach, and the device generator was implanted in the subpectoral fascia. The puncture was planned with subclavian venography under fluoroscopy if the punctures were unsuccessful 3 times. Sandbags were applied to all cases for an hour. A pressure bandage was applied to the patients with signs of swelling and hematoma in the pocket area, and they were kept for at least 1 night. A prohemostatic agent was not applied in any case. Antibiotics were administered to all patients before the procedure to prevent surgical site infections.

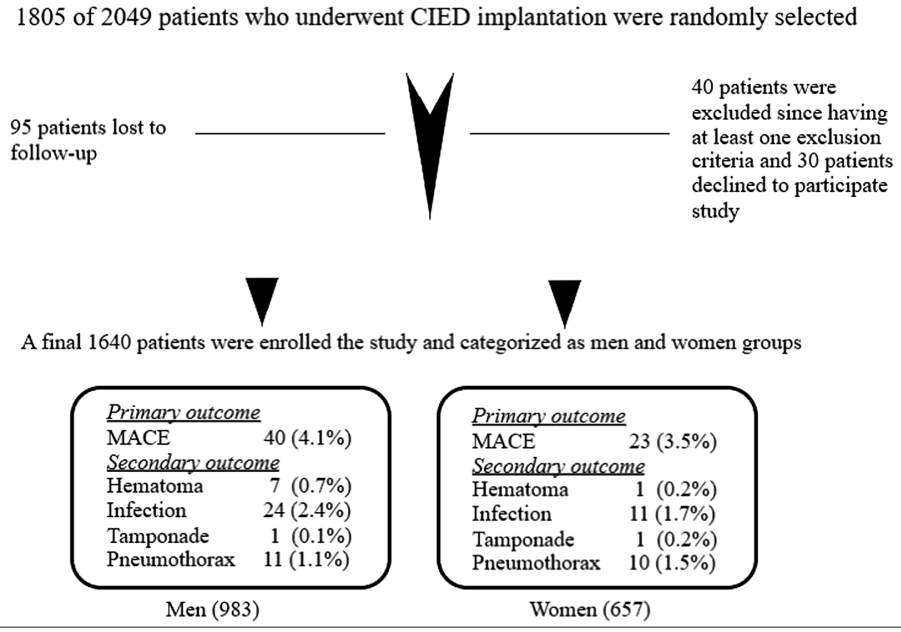
Follow-up and Study Outcomes

Demographic data, clinical history, medications, device implantations, complications, and laboratory data were obtained from electronic hospital records or social security institution registry system. Primary endpoint was accepted as major adverse cardiac events (MACE) with the inclusion of composite of CSH, pericardial effusion or tamponade, pneumothorax and infection related to the device system. Secondary outcomes included each component of MACE. Details of study enrollment (follow-up, outcomes) and study flow chart are shown in Figure 1.

Statistics

The Statistical Package for the Social Sciences for Windows version 25.0 (IBM Corp., Armonk, NY, USA) was used to perform statistical analysis. The distribution of continuous variables was assessed by Kolmogorov–Smirnov test. Continuous variables were indicated as mean ± standard deviation or median (interquartile range). Categorical variables were indicated as percentages and were compared using chi-square test or Fisher's exact test as appropriate. Continuous variables between 2 independent groups were analyzed by Student's *t*-test or Mann–Whitney *U* test as appropriate. Univariable and multivariable analyses for predictors of MACE were applied and also were plotted in a graph. Variables with a $P < .05$ were assumed significant.

Figure 1. Study flow chart.



RESULTS

The study enrolled 1640 patients who underwent CIED procedures from 3 different hospitals in the Southeast Anatolian Region of Turkey. The ratio of women in the total population was 40% (657 of 1640).

We noticed substantial differences in patient baseline clinical characteristics and medications among genders in the whole population (Table 1). Mainly, women were older and had a significantly higher prevalence of Hypertension (HT) and Diabetes mellitus (DM). On the contrary, women had a lower previous history of Coronary artery disease (CAD) and Heart failure (HF). Additionally, antiplatelet drug treatment was more likely higher in men than in women. In contrast to this, edoxaban and dabigatran treatment was more likely higher in women than in men as shown Table 1.

The operation details and procedural complications between genders are shown in Table 2. While single-lead pacemaker, dual-lead pacemaker, and CRT-ICD were more implanted in women, on the contrary, single-lead ICD and dual-lead ICD were less implanted in women. Overall, the MACE occurred in 63 of 1640 patients (3.8%). This was mostly driven by device-related infection (1.3%) and pneumothorax (2.1%). The MACE occurred in 40/983 (4.1%) patients in men as compared to 23/657 (3.5%) women ($P = .557$, Table 2). There were no differences among genders with regard to CSH, pericardial effusion or tamponade, pneumothorax, and device-related infection.

The periprocedural laboratory parameters of patients are represented in Table 3. The men group was positively associated with higher periprocedural white blood cell count, hematocrit, hemoglobin, platelet, urea, creatinine, eGFR, and INR levels and were negatively associated with lower serum albumin, total cholesterol, and triglyceride levels (Table 3).

In the univariable logistic regression analysis, hemoglobin level, receiving warfarin therapy and HF, was found to be predictors of MACE (Table 4). However, gender difference was not a predictor of MACE. In multivariate analysis, HF and warfarin therapy were found to be the predictors of MACE. Moreover, being on warfarin treatment increased approximately 3-fold the risk of MACE (Figure 2).

DISCUSSION

In this multicenter, observational study, we investigated the effect of gender on complications in CIED procedures over an 11-year horizon. According to the results of the study, PPMs are more commonly implanted in women, while ICDs are more commonly implanted in men. The most complications were device-related infection (2.1%) and pneumothorax (1.3%) in both genders, respectively. However, our data demonstrated no remarkable difference in point of procedural complications between the genders.

The management of CIED implantation in women is different from that in men, as women are less likely to undergo a dual-chamber pacemaker and have ICD implantation, even when clinically appropriate. This can be explained by the smaller body structure of the women, co-morbidities, patient preference, and more avoidance of aggressive treatment by female patients.[11]

The number of studies is limited in the literature that indicates the efficacy of gender differences in CIED procedures in a heterogeneous patient cohort with both ICD and PPMs implants. In addition, data relating to gender effects onto pacemaker

Table 1. Clinical Characteristics and Medications of the Patients at Baseline

	Total (n=1640)	Men (n=983)	Women (n=657)	*P*
Age (IQR)	66 (56–73)	65 (55–73)	66 (58–73.5)	***.004***
Body mass index kg/m², IQR	24 (22–25)	24 (22–25)	24 (21–26)	.482
Hypertension, n (%)	719 (43.8)	386 (39.3)	333 (50.7)	***<.001***
Diabetes mellitus n (%)	389 (23.7)	202 (20.5)	187 (28.5)	***<.001***
Atrial fibrillation or flutter, n (%)	148 (9)	84 (8.5)	64 (9.7)	.407
Coronary artery disease, n (%)	1126 (68.7)	748 (76.1)	378 (57.5)	***<.001***
Heart failure, n (%)	1008 (61.5)	677 (68.9)	331 (50.4)	***<.001***
Mechanical prosthesis valve, n (%)	39 (2.4)	25 (2.5)	14 (2.1)	.591
Ejection fraction %, IQR	35 (25–60)	30 (25–60)	50 (30–60)	***<.001***
Time to discharge (days), IQR	4 (3–5)	4 (3–5)	4 (3–5)	.874
HAS–BLED score[a], IQR	2 (1–2)	2 (1–2)	2 (1–3)	.674
Medications, n (%)				
Antiplatelet	997 (60.8)	673 (68.5)	324 (49.4)	***<.001***
ASA	956 (58.3)	637 (64.8)	319 (48.6)	***<.001***
Clopidogrel	183 (11.2)	149 (15.2)	34 (5.2)	***<.001***
Prasugrel	2 (0.1)	2 (0.2)	0	.519
Ticagrelor	12 (0.7)	10 (1)	2 (0.3)	.139
Warfarin	84 (5.1)	52 (5.3)	32 (4.9)	.706
NOAC	119 (7.3)	62 (6.3)	57 (8.7)	.070
Rivaroxaban	81 (4.9)	49 (5)	32 (4.9)	.917
Edoxaban	4 (0.2)	0	4 (0.6)	***.026***
Apixaban	7 (0.4)	5 (0.5)	2 (0.3)	.709
Dabigatran	27 (1.6)	8 (0.8)	19 (2.9)	***.001***
Unfractioned heparin	4 (0.2)	1 (0.1)	3 (0.5)	.308
LMWH	65 (4)	40 (4.1)	25 (3.8)	.788
Bridge therapy	68 (4.1)	41 (4.2)	27 (4.1)	.951

Data are n (%), median (IQR).
[a]HAS–BLED score is an index of the risk of bleeding in patients with atrial fibrillation. HAS–BLED score ≥3 indicating a great risk of bleeding.
ASA, acetylsalicylic acid; LMWH, low molecular weight heparin; NOAC, non–vitamin K oral anticoagulants; IQR, interquartile range.

Table 2. Operative Details and Procedural Complications

	Total (n=1640)	Men (n=983)	Women (n=657)	*P*
Pacemaker, n (%)				
Single–chamber	138 (8.4)	61 (6.2)	77 (11.7)	***<.001***
Dual–chamber	412 (25.1)	198 (20.1)	214 (32.6)	***<.001***
ICD, n (%)				
Single–chamber	504 (30.7)	375 (38.1)	129 (19.6)	***<.001***
Dual–chamber	111 (6.8)	84 (8.5)	27 (4.1)	***<.001***
CRT	454 (27.7)	250 (25.4)	204 (31.1)	***.013***
Generator change	37 (2.3)	27 (2.7)	10 (1.5)	.102
MACE[a], n (%)	63 (3.8)	40 (4.1)	23 (3.5)	.557
CSH, n (%)	8 (0.5)	7 (0.7)	1 (0.2)	.155
Pericardial effusion or tamponade, n (%)	2 (0.1)	1 (0.1)	1(0.2)	1
Pneumothorax, n (%)	21 (1.3)	11 (1.1)	10 (1.5)	.507
Device–related infection, n (%)	35 (2.1)	24 (2.4)	11 (1.7)	.292

[a]MACE included the composite of all clinically significant hematoma (CSH), pericardial effusion or tamponade, pneumothorax, and infection related to the device system.

implantation are hesitant. In the study by Nowak et al.[10] procedural complications were compared in patients who had PPMs implanted. According to the results of this trial, single lead pacemakers were more implanted in women than in men; however, dual lead pacemakers were more implanted in men. In addition, women were more complicated by adverse events such as pneumothorax and pocket hematoma. In our study, sex differences were compared in a more heterogeneous patient population with both ICD and PPMs implants. No significant difference was found regarding the pneumothorax and CSH between both genders, which was quite different from the trial by Nowak et al. Mohammad et al[8] compared the 30-day rehospitalization for cardiac and non-cardiac causes and complications in CIED procedures between both genders. There was no difference between the sexes in terms of all-cause rehospitalization, but cardiac rehospitalization and device-related complications were more common in women. Similar to our study, infections were not significantly different between genders over a 6-year period. In another study, Mohammed et al[7] investigated the difference in complications between genders with all types of CIED procedures. They indicated that women were at an overall higher risk of complications compared with men; however, mortality rates were not found meaningful among genders in a national cohort. In our study, although women are at a higher odds of pneumothorax than men, it was not significant between genders [odds ratio: 1.36 (0.57-3.23), *P*=.479].

Unlike the prior studies, our data certainly indicate that gender is not related to an increased risk of MACE. These differences

Table 3. Perioperative Laboratory Parameters of the Patients

	Total (n=1640)	Men (n=983)	Women (n=657)	*P*
White blood cell count (×10³ µL)	8.8 ± 2.9	9 ± 3	8.7 ± 2.8	***.034***
Hematocrit (%)	41.2 ± 10.5	42.4 ± 12.3	39.3 ± 5	***<.001***
Hemoglobin (g/dL)	13.5 ± 3.8	13.8 ± 1.8	12.9 ± 5.3	***<.001***
Platelets (×10³ µL)	237 ± 76	229.7 ± 76.7	249.5 ± 72.6	***<.001***
Serum albumin, g/dL, IQR	3.7 (3.4–4)	3.7 (3.4–3.9)	3.7 (3.5–4)	***.011***
Urea, mg/dL, IQR	44 (33–57)	45 (35–59)	42 (32–56)	***.001***
Creatinine, mg/dL, IQR	0.92 (0.77–1.15)	0.99 (0.83–1.23)	0.81 (0.7–1)	***<.001***
eGFR (mL/min/L,73m²), IQR	85 (65–101)	88 (67–105)	82 (63–96)	***<.001***
Glucose, mg/dL	137 ± 66	135.7 ± 66.7	140.2 ± 74.3	.198
Preoperative INR, IQR	1.05 (0.99–1.17)	1.07 (1–1.19)	1.03 (0.98–1.12)	***<.001***
Total cholesterol, mg/dL	175 ± 42	167.8 ± 40.8	185.3 ± 43.1	***<.001***
Triglyceride, mg/dL	151 ± 95	147.7 ± 97.4	159.3 ± 101	***.023***
HDL, mg/dL	41.6 ± 11.7	39.7 ± 10.8	44 ± 12.4	***<.001***
LDL, mg/dL	104.5 ± 40.5	100.6 ± 43.6	110.4 ± 33.7	***<.001***

Data are expressed as mean ± standard deviation, or as median (interquartile range) as appropriate. eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; INR, international normalized ratio; IQR, interquartile range.

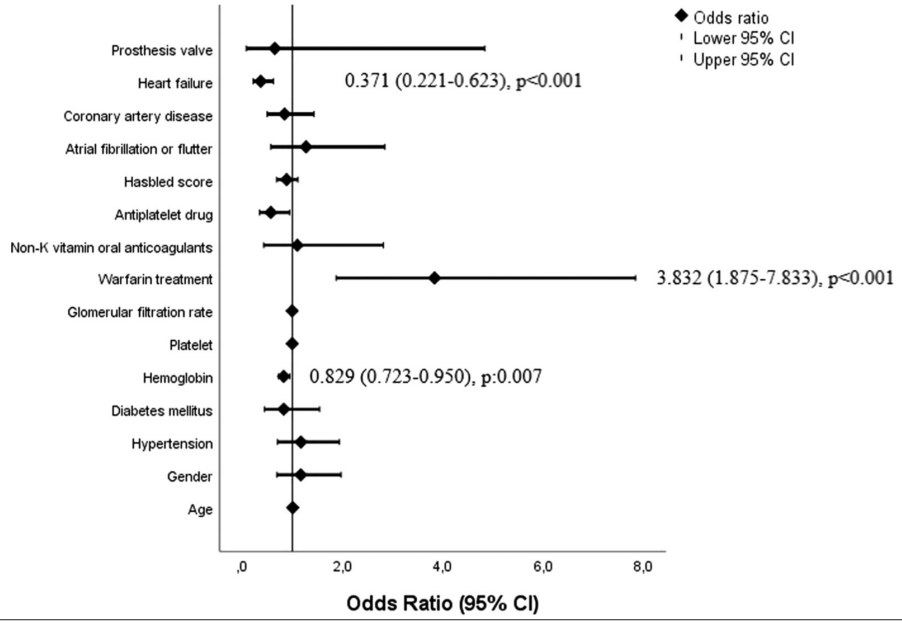
can basically be explained with the ethnicity as with other variables such as body mass index (BMI), anatomical variations, and operator experience. Most of the previous studies have been conducted on the American, European, and Australian populations.[7-10] As far as we know, the present study is one of the original studies to investigate the trends of sex differences and predictors of complications following CIED surgery in the Asian population. Lee et al[14] investigated the temporal trends of CIED procedures in the Asian population. Also, Aktoz et al[15] investigated the effect of gender-specific differences and patient demographics on a cardiac device and pacing mode selection. However, neither of these studies compared the procedural complications between genders. In that context, our study was unique in the Asian population. Another important factor that

Table 4. Predictors of MACE[a] in Univariate and Multivariate Logistic Regression Analysis Model

	Univariate Model		Multivariate Model	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age	1.019 (0.999–1.040)	.065	1.012 (0.993–1.031)	.214
Gender	1.169 (0.693–1.972)	.558		
Hypertension	1.172 (0.708–1.940)	.538		
Diabetes mellitus	0.830 (0.446–1.545)	.558		
Hemoglobin	0.829 (0.723–0.950)	***.007***	0.870 (0.756–1.001)	.051
Platelet	1.001 (0.998–1.004)	.483		
eGFR	0.998 (0.989–1.007)	.628		
Warfarin	3.832 (1.875–7.833)	***<.001***	3.069 (1.436–6.560)	***.004***
NOAC	1.106 (0.435–2.813)	.832		
Antiplatelet drug	0.573 (0.346–0.948)	.030		
HAS-BLED score	0.887 (0.689–1.141)	.349		
Atrial fibrillation or flutter	1.273 (0.569–2.846)	.556		
Coronary artery disease	0.845 (0.498–1.434)	.533		
Heart failure	0.371 (0.221–0.623)	***<.001***	0.420 (0.248–0.713)	***.001***
Mechanical prosthesis valve	0.653 (0.088–4.835)	.677		

[a]MACE included the composite of all clinically significant hematoma, free wall rupture, pneumothorax, and infection related to the device system. eGFR, estimated glomerular filtration rate; OR, odds ratio; NOAC, non-vitamin K oral anticoagulants.

Figure 2. Forest plot graph of predictors of complications.



contributes to CIED complications is BMI. Previous studies have indicated that a lower BMI was related to a higher risk of complications.[16] In the present study, BMI was not significantly different between both genders. Furthermore, Eberhardt et al[17] demonstrated that operation time and complication rate increased with operator experience. Although the experience of one-on-one operators was not evaluated in our study, implantation procedures are mostly performed by experienced electrophysiologists in the existing centers.

As in any study, specific design limitations are also available in the present study. First, our study data does not include details on the indication for CIED procedure and operator experience, and for this reason, we were unable to regulate the differences in these covariates among both genders. Second, we have only focused on major complications and not on all subtypes of complications such as minor bleedings, pericardial effusion without hemodynamic collapse, mild pleural effusion, modest superficial wound infection, and uncomplicated arrhythmias. In summary, minor complications that did not require intervention were excluded. Finally, the study was observational and there is a possibility of unmeasured confounding, and indeed baseline demographic characteristics, medications that are not homogeneous between both genders.

CONCLUSION

This small-scale, real-life patient data revealed no remarkable distinction in terms of complications between both genders. Multinational, randomized, large-scale cohort trials are required to support our results.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Dicle University School of Medicine (Date: March 17, 2022, Decision No: 74).

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.D., K.İ., N.P., K.A.; Design – M.D., B.Y., L.A., A.A.; Supervision – M.D., N.T., T.G.; Funding – None; Materials – M.Ö., K.İ., B.Y., L.A., M.D.; Data Collection and/or Processing – M.Ö., K.İ., B.Y., L.A., M.D., A.A.; Analysis and/or Interpretation – M.D., K.İ., N.P., K.A., B.Y.; Literature Review – M.D., K.A.; Writing – M.D., N.T., M.Ö., K.İ., B.Y., L.A.; Critical Review – N.T., K.A., M.Ö., M.D.

Declaration of Interests: The authors declare that they have no competing interest.

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Review

Subacute Thyroiditis and Graves' Disease Possibly Associated with Sars-CoV-2 Infection: Presentation of Two Cases and Review of the Current Data

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 has been identified as the cause of a pandemic of respiratory illness in Wuhan, China. Coronavirus disease 2019 may cause mild disease with nonspecific signs and symptoms such as fever, cough, myalgia, and fatigue or severe pneumonia with respiratory failure and sepsis. It is not clear whether coronavirus disease 2019 has an effect on the thyroid gland. Evidence support that patients with coronavirus disease 2019 who are followed up in the intensive care unit may develop temporary thyroid dysfunction as non-thyroidal illness syndrome. Until now, 22 cases of subacute thyroiditis and 5 Graves' diseases potentially related to severe acute respiratory syndrome coronavirus 2 infection have been presented in the literature. Herein, we present 2 cases with subacute thyroiditis and Graves' diseases potentially related to severe acute respiratory syndrome coronavirus 2 infection in the context of the review of the literature. Physicians should be aware of the possible relationship between thyroid dysfunction and coronavirus disease 2019.

Keywords: COVID-19, Graves' disease, SARS-CoV-2, subacute thyroiditis, thyroid

INTRODUCTION

Coronavirus disease 2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, has already become a pandemic just a few months after it was first detected in China.[1] The virus penetrates the body via upper respiratory mucous membranes and then spreads to the lungs. After a 2- to 14-day incubation period, most COVID-19 patients develop mild to moderate sickness (fever, cough, myalgia, and fatigue) or viral pneumonia. However, some patients experience serious diseases characterized by respiratory failure, acute respiratory distress, sepsis, myocarditis, and acute renal damage, even multi-organ failure.[2] However, it is not explicitly defined how the endocrine system is affected by this virus although there are some studies showing some endocrine deteriorations in patients with COVID-19. Researchers have looked into the possibility of thyroid dysfunction among the various extra-pulmonary manifestations. For viral and host cell membrane fusion, SARS-receptor CoV-2's-binding domain uses angiotensin-converting enzyme 2 (ACE2) of the host. The pancreas, thyroid, testis, ovary, adrenal glands, and pituitary express ACE2.[2,3] There is no convincing evidence that COVID-19 individuals, whether symptomatic or not, get thyroid dysfunction as a result of infection.

Herein, we present 2 cases with thyrotoxicosis which may be potentially related to SARS-CoV-2 infection in the context of the review of the limited literature on this subject.

CASE 1

A 32-year-old woman presented to the endocrinology outpatient clinic with symptoms of mild fever, cough, fatigue, weakness, palpitations, weight loss, and anterior neck pain radiating to the jaw and ear. In her medical history, she had SARS-CoV-2 infection 3-4 weeks ago; her nasopharyngeal real-time reverse trans criptionpolymerase chain reaction for SARS-CoV-2 was positive, and chest high-resolution computed tomography showed peripheral ground-glass areas which are typical for SARS-CoV-2-related interstitial pneumonia in bilateral lower lobes and right lung superior lobe (shown in Figure 1). Dry cough, fatigue, and weakness were present from the beginning of the COVID-19, but palpitations, weight loss, and anterior neck pain were added to her complaints about 10 days ago before admission.

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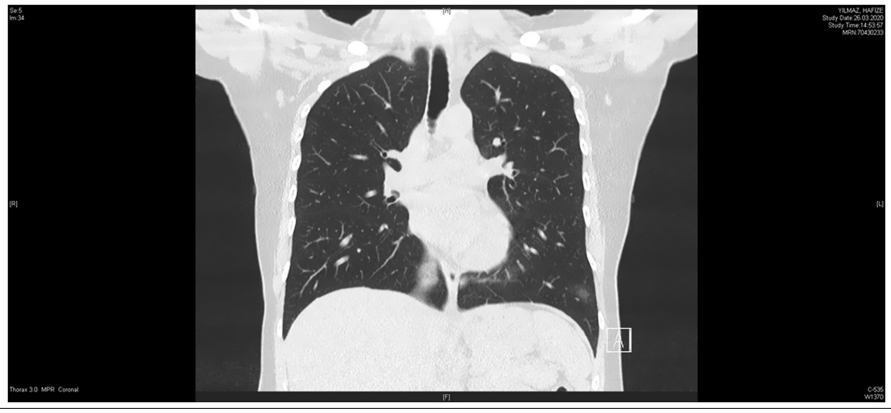
Figure 1. Chest CT shows peripheral ground-glass areas typical of SARS-CoV-2-related interstitial pneumonia in bilateral lower lobes and superior lobe of right lung. CT, computed tomography; SARS-COV-2, severe acute respiratory syndrome-coronavirus-2.



At physical examination, the patient's heart rate was 105 beats per minute, blood pressure was 130/80 mmHg, and the left lobe of the thyroid gland was markedly painful and slightly tender and enlarged on palpation. Her body temperature was 37.5°C. Other systemic examination was unremarkable. Laboratory investigations were as follows: hemoglobin 12.3 g/dL, white blood cell (WBC) 6800, neutrophil 3500, lymphocyte 280, erythrocyte sedimentation rate (ESR) 58 mm/h (<20), C-reactive protein (CRP) 2.58 mg/dL (<0.5), thyroid-stimulating hormone (TSH) <0.004 m IU/L (0.25-4.55), free triiodothyronine (T3) 7.1 pmol/L (3.5-6.5), and free thyroxine (T4) 27.5 pmol/L (11.5-22.7). Thyroid-stimulating immunoglobulin, anti-thyroglobulin (anti-TG), and anti-thyroperoxidase (anti-TPO) antibodies were negative (Table 1). The respiratory viral panel was negative. Thyroid ultrasonography revealed a widespread vascular reduction and several diffuse hypoechoic regions. Subacute thyroiditis (SAT) diagnosis was considered that might be due to SARS-CoV-2 infection. Methylprednisolone treatment was started as 32 mg per day and was gradually tapered every week for 6 weeks. After a few days on methylprednisolone, she noticed a dramatic improvement in her clinical state. The patient is still euthyroid at the third- and sixth-month visits.

CASE 2

A 42-year-old woman was referred to cardiology outpatient clinic with laboratory results compatible with thyrotoxicosis. In her medical history, she had SARS-CoV-2 infection disease 4 weeks ago. She had mild symptoms including fever, muscle pain, and weakness. Reverse transcription-polymerase chain reaction for SARS-CoV-2 was positive. She received symptomatic treatment like paracetamol and hydroxychloroquine. But her husband stayed in the intensive care unit for 10 days due to severe COVID-19. Therefore, the family experienced serious stress during this period. She described palpitations, insomnia, agitation, and weight loss, which she had thought arose from her stressful condition, during the last 2-3 weeks.

Main Points

- Several patients have shown abnormalities in thyroid function after coronavirus disease 2019 (COVID-19).
- There is no convincing evidence that individuals who had COVID-19, whether symptomatic or not, may have thyroid dysfunction as a result of infection.
- Physicians should be aware of the possible relationship between thyroid disease (subacute thyroiditis, Graves' disease, etc.) and COVID-19, which should be observed by prospective studies.

The patient's heart rate was 120 beats per minute, and her blood pressure was 135/90 mmHg. Her clinical examination revealed diffusely enlarged thyroid gland and she had tremor in both hands. She did not have fever or ophthalmopathy, and other systemic examination was unremarkable.

Laboratory results were as follows: WBC 4780, ESR 20 mm/h (<25), CRP 0.4 mg/dL (0.5), TSH < 0.008 mIU/L (0.2-4.5), free T3 level 25.5 pmol/L (3.5-6.5), and free T4 level 56.3 pmol/L (11.5-22.7). Thyroid-stimulating immunoglobulin level was high (2.47 IU/L), and anti-TG and anti-TPO antibodies were negative (Table 1). Thyroid Doppler ultrasound showed diffuse increased vascularity of bilaterally enlarged thyroid lobes (peak systole > 70 cm/s) and parenchyma was heterogeneous. High radioisotope uptake (10%) was obtained with 5 mCi Tc-99 radionuclide thyroid scan (shown in Figure 2).

Graves' disease (GD) was diagnosed as potentially related to directly SARS-CoV-2 infection and/or stress factors. Methimazole (30 mg/day) and propranolol (80 mg/day) treatments were initiated.

Clinical and Research Consequences

Subacute thyroiditis is an inflammatory condition of the thyroid gland that generally manifests as painful thyroid enlargement. Malaise, tiredness, myalgia, arthralgia, and anterior neck discomfort spreading to the jaw and ear are common. A mild to moderate fever is common, with temperatures increasing to over 40°C at times, especially during the night. The disease's peak symptoms and signs begin in 3-4 days and diminish in a week, but

Table 1. Laboratory Results of the Patients

	First Case		Second Case		
Measures	**COVID-19 Period**	**SAT Period**	**COVID-19 Period**	**Graves Period**	**Normal Range**
TSH (µU/mL)	3.66	<0.004	1.2	<0.008	0.25-4.55
FT3 (pmol/L)	NA	7.1	NA	25.5	3.5-6.5
FT4 (pmol/L)	16.23	27.5	15.4	56.3	11.5-22.7
CRP (mg/dL)	1.04	2.58	3.5	0.4	<0.5
ESR (mm/sa)	10	58	45	20	<20
Anti-TPO(IU/mL)	NA	37.2	NA	58	0-60
TgAb (IU/mL)	NA	30	NA	40	0-60
TSI (IU/L)	NA	0.1	NA	2.47	<0.1

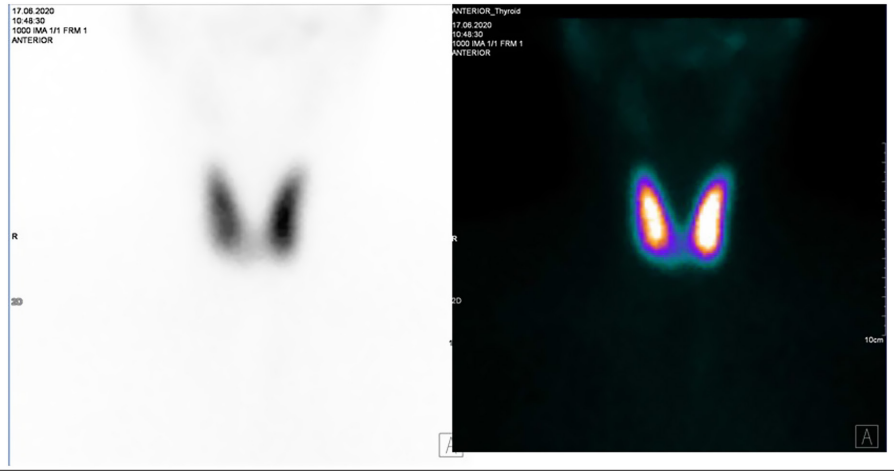
COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FT3, free triiodothyronine; FT4, free thyroxine; TgAb, thyroglobulin antibodies; TPOAb, thyroperoxidase antibodies; TSI, thyroid-stimulating immunoglobulin; SAT, subacute thyroiditis; TSH, thyrotropin; NA, not available.

the onset might take 1-2 weeks and last 3-6 weeks.[4,5] During this pandemic, it has been observed that the clinic presentation of SAT and COVID-19 may overlap making clinical and decision to treat discrimination difficult. Whether COVID-19 may trigger SAT is another matter of debate.

Subacute thyroiditis incidence is 4 times higher in women than in men, and SAT is more frequent between ages 40 and 50 years.[6,7] Several studies showed that susceptibility to the disease and recurrence risk are associated with human leukocyte antigens (HLA) mainly HLA-Bw35 and also HLAB67, HLA-B15/62, and HLA-Drw8.[8] Previous viral infections caused by viruses such as Coxsackie virus, Epstein–Barr virus, adenoviruses, influenza viruses, mumps, measles, and primary human immunodeficiency virus (about 2-6 weeks before SAT) are thought to be triggering factor for SAT.[9,10] Subacute thyroiditis is defined by elevated ESR and CRP levels, typical ultrasound findings including inhomogeneous hypo-echogenic texture with diminished vascularity and laboratory markers of thyrotoxicosis. Symptomatic treatment includes non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids in more severe cases.[4] We did not give NSAIDs to our patient including ibuprofen as it may have suppressive effect on immune response by upregulating ACE2. Paracetamol and methylprednisolone (32 mg per day) were given, and they were progressively tapered every week for 6 weeks. After 6 weeks, she had normal thyroid function and she was symptom-free.

In 2002, it was observed that the SARS epidemic caused some abnormalities in thyroid function.[11,12] Although SARS-CoV was isolated in endocrine organs including parathyroid, pituitary, pancreas, and adrenal gland, it could not be detected in thyroid, testis, ovary, and uterus.[11] Wei et al[12] showed extensive injury to the follicular epithelial cells and the para-follicular cells during autopsies of 5 SARS cases. The breakdown of the follicular epithelium and desquamation of epithelial cells into the follicular lumen were the hallmarks of follicular cell injury. The terminal deoxynucleotidyl transferase-mediated dUPT nick end–labeling (TUNEL) assay confirmed the presence of apoptosis but no inflammatory infiltration or cellular necrosis. Yao et al.[13] on the other hand, looked at pathological changes in individuals who died from SARS-CoV-2 infection by taking minimally invasive autopsies from several organs. They found lymphocytic

Figure 2. Tc-99 radionuclide thyroid scan shows high iodine uptake.



infiltration in the interstitium but no changes in thyroid follicular morphology. Neither immunohistochemistry studies nor PCR analyses detected SARS-CoV-2 in the thyroid gland tissues.[13] Rotondi et al[14] discovered that the ACE2 receptor mRNA is expressed in thyroid follicular cells via direct molecular analysis of surgical samples of thyroid tissue, suggesting them a potential target for SARS-COV-2 invasion.

During this COVID-19 pandemic, first Brancatella et al[15] introduced an 18-year-old woman with SAT diagnosis which occurred after 2 weeks of COVID-19 infection. Prednisone (25 mg/day as the starting dose) was given to the patient, and thyroid function and inflammatory markers of the patient normalized in 40 days. Ippolito et al[16] reported a 69-year-old woman with SAT during the recovery phase of COVID-19 infection following back surgery. Previously, she had a nontoxic nodular goiter and she was diagnosed with thyrotoxicosis during COVID-19. They considered a diagnosis of SAT because the patient responded to steroids but not methimazole.[16] Asfuroglu et al[17] reported a 41-year-old woman with SAT and they suggested that physicians should be aware of screening SAT patients for COVID-19. Ruggeri et al[18] described a 43-year-old woman who developed SAT with thyrotoxicosis 6 weeks after SARS-COV-2 infection. Oral prednisone (25 mg/day as the starting dose) was started on the patient and progressive remission of symptoms and signs and euthyroid status were provided after 4 weeks.[18] Brancatella et al[19] described additional 4 patients with SAT after COVID-19. Twenty-two cases of SAT potentially associated with SARS-CoV-2 infection have been reported to date.[20-28] In a recent review, SAT was found more frequent in women than in men (18 women/4 men). These patients had mild symptoms and signs including fever, myalgia, asthenia, palpitations, weight loss, and anterior neck pain or they were asymptomatic. Beta-blockers, aspirin, and glucocorticoids (prednisone 25-40 mg) were given, and their treatment was gradually discontinued over an average of 3-4 weeks. Despite a short follow-up (35 ± 12 days) period, euthyroid status was achieved after a short duration of subclinical hypothyroidism in most patients.[29]

Graves' disease is an autoimmune illness caused by thyroid autoantibodies that stimulate the production of T4, resulting in hyperthyroidism. The etiology of GD is not clear. Different environmental circumstances (i.e., infections, smoking, stress, radiation, drugs, iodine, and so on) have been suggested to promote GD, particularly in genetically sensitive individuals.[30] Valtonen et al[31] detected evidence of a recent bacterial or viral infection in the serum samples of 36% of patients with newly diagnosed GD, 67% of patients with Hashimoto's thyroiditis, and in only 10% of control subjects.[31] Several research have examined the link between GD and infectious diseases such as Yersinia, Leishmania, Mycoplasma, *Helicobacter pylori*, Foamy Viruses, Parvovirus-B19, Epstein–Barr virus, hepatitis C virus, and retroviruses such as HIV, with different results.[32] Firstly, Mateu-Salat et al[33] reported 2 cases of GD occurring after SARS-CoV-2 infection. Subsequently, Jiménez-Blanco et al[34] described 2 more cases with GD probably related to COVID-19. Pastor et al[35] presented a 45-year-old woman, with a previous history of GD who had been in long-term remission for over 4 years. She was admitted to the emergency department with a thyrotoxic crisis which was most likely caused by COVID-19.

The significance of stress in the development of hyperthyroidism in GD patients is still debated. In cross-sectional studies, stressful life events (SLE) have been shown to be more common in the months before the development of GD.[36] Vita et al[37] evaluated the relationship of SLE with the onset and outcome of GD. Patients with SLE experienced at least one exacerbation or relapse prior to each exacerbation or relapse. The patients who experienced more exacerbations or relapses lived more SLE than the patients with remission.[37] Previously, we showed that the number and impact of negative SLE in GD patients were higher when compared to healthy controls according to the Life Experience Survey.[38] Very recently, we have recommended methimazole and beta-blocker combination for initial therapy and considered dietary changes and radioactive iodine (RAI) treatment unadvisable during the COVID-19 pandemic.[39]

CONCLUSION

In patients who had severe COVID-19 infection, changes in thyroid function may occur potentially related to COVID-19. Thyroid dysfunction has been documented during and after a COVID-19 infection; therefore, some new-onset or recurrent thyroid dysfunction is likely to be linked to a recent SARS-CoV-2 infection. Physicians should be aware of possible relationships between thyroid dysfunction and COVID-19, which should be researched by prospective studies.

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

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – O.C.; Design – O.C.; Supervision I.A.; Resources – materials – data collection – O.C.; Literature search – O.C. I.A.; Writing – O.C., I.A.; Critical review – I.A.

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Letter to the Editor

Brachiocephalic Vein Stenosis in Systemic Sclerosis

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Keywords: Hyperdontia, hypodontia, macrodontia, tooth number anomalies, tooth size anomalies, tooth structural anomalies

Systemic sclerosis (SSc) is a rare disease characterized by widespread collagen deposition resulting in fibrosis.[1] In the literature, central retinal vein occlusion (CRVO)[2-3] and subclavian vein thrombosis[4] were reported as venous involvement in SSc.

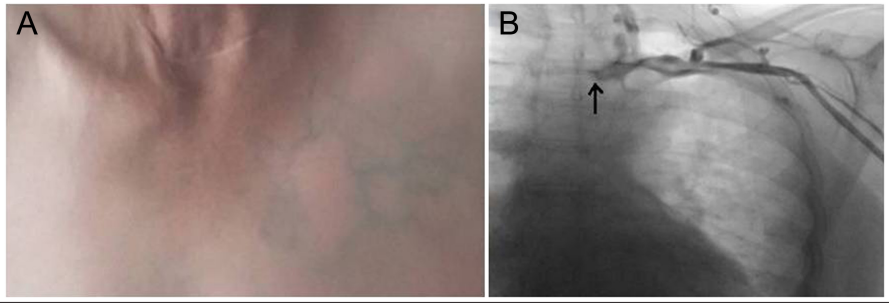
A 65-year-old female presented with complaints of the prominence of vascular structures gradually in the left upper limb. Her medical history revealed that the patient was diagnosed with SSc for 9 months. She had Raynaud's phenomenon, sclerodactyly, proximal sclerosis, and taking methotrexate (10 mg a week), methylprednisolone (12 mg a day), and nifedipine (30 mg/day). On physical examination, she had telangiectasia on the face and vascular structures were prominent in the left upper limb (Figure 1A). Complete blood count, biochemical parameters, and complement were within normal ranges. Anti-topoisomerase-I was positive and rheumatoid factor, anti-cyclic citrullinated peptide, anticardiolipin immunoglobulin M/G direct Coombs test, and double-stranded DNA antibody were negative.

Computerized aortography and upper extremity venography showed stenosis as a thrombus of the left brachiocephalic vein and prominent of the collateral vascular structures (Figure 1B). No hematological malignancy, solid malignancy, heart failure, kidney, or hepatitis disease was detected in the patient. Antiphospholipid antibodies and thrombophilia panel were negative. The thrombosis was thought to be due to old age and corticosteroid use.[4] Resting, the elevation of the limb, and warm compresses were recommended as conservative treatments after the detection of brachiocephalic vein stenosis. Written informed consent was obtained from the patient.

Malik et al[2] reported a case of a 30-year-old male with SSc who developed a sudden decrease of vision in the right eye. He was diagnosed with CRVO. The patient received 3 consecutive intravitreal bevacizumab injections for macular edema. After injections, the best-corrected visual acuity improved from 20/80 to 20/25. Karadžić et al[3] reported a 42-year-old SSc patient with acute deterioration of vision in the left eye. This patient was also diagnosed with CRVO. Berriche et al[5] reported a 56-year-old SSc patient with bilateral subclavian vein thrombosis. Endothelial cell dysfunction which affects the microvasculature system has been mentioned to be the cause of CRVO.[2] This patient was treated with anticoagulant therapy.

In this article, we report a case of SSc presenting with brachiocephalic vein stenosis. To our knowledge, this is the first SSc

Figure 1. (A) Vascular structures prominence in the left upper limb; (B) upper extremity venography of the left upper limb: stenosis of the left brachiocephalic vein and prominence of the collateral vascular structures.



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patient presenting with brachiocephalic vein stenosis as a rare manifestation. Also, the localization is different from the patients presented in the literature. When the prominence of vascular structures develops in patients with SSc, brachiocephalic vein stenosis should be kept in mind.

Informed Consent: Written informed consent was obtained from the patient.

Peer-review: Externally peer-reviewed.

Declaration of Interests: The authors have no conflict of interest to declare.

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Erratum

In the article by Karslıgil and Akdoğan, entitled "COVID-19 Seroprevalence among Healthcare Workers in a University Hospital in Southeastern Turkey" that was published in the June 2021 issue of the European Journal of Therapeutics (Eur J Ther; 27 (2): 106-112, DOI: 10.5152/eurjther.2021.20106), the authors declared that they erroneously forgot to add the financial support of the study and requested a correction.

Author's correction request were evaluated and accepted by the Editorial Board. Thus, the article has been corrected accordingly and updated in the journal's archive. You may access the updated article via the link below.

https://eurjther.com/en/covid-19-seroprevalence-among-healthcare-workers-in-a-university-hospital-in-southeastern-turkey-162638

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