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EDITORIAL

We are proud to publish the fifth issue of our journal, Journal of Health Sciences and Medicine (JHSM), in 2023. Our journal is published 6 issues a year. It is included in many national and international indexes. In the near future, we aim to enter valuable international indexes such as SCI-Expanded, Scopus, ESCI, PubMed and contribute even more to international literature. We would like to thank all the authors who sent their scientific articles to our journal.

Great successes are only possible with good teamwork. We sincerely thank everyone who contributed to the journal at any stage.

Sincerely

Prof. Dr. Aydın ÇİFCİ
Editor-in-Chief

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Evaluation of the readability of consent forms used in cardiovascular surgery clinics

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ABSTRACT

Aims: The readability of the informed consent forms, which are a power of attorney agreement between the physician and the patient, is very important especially in clinics where high-risk procedures such as cardiovascular surgery are performed. In this study, we aimed to determine the readability levels of consent forms, which are frequently used in cardiovascular surgery clinics, according to accepted scales.

Methods: The readability level of the 15 patient consent forms recommended by the Turkish Society of Cardiovascular Surgery to its members; word counts, syllable counts, letter and character counts were calculated using Ateşman and Bezirci-Yılmaz formulas.

Results: Consent forms included in the study were found to be readable at the 11-12th grade level according to the Ateşman scale and at the high school level in the Bezirci-Yılmaz scale. These texts have also been studied at international scales and it has been determined that they are at the level of readability that requires undergraduate education.

Conclusion: Research and data from the Turkish Statistical Institute show that the average year of schooling in our country is 6.5, and the ratio of high school graduates or equivalent to the entire population is 22.3%. We advocate simplifying the informed consent forms recommended by the Turkish Society of Cardiovascular Surgery, from the level that requires high school education to the 6-year education level, which is the average schooling year in Turkey.

Keywords: Informed consent, readability, comprehension

INTRODUCTION

It is very important to inform patients and their relatives about possible complications that may develop after operations in cardiovascular surgery clinics because of the high mortality and morbidity rates of the operations performed. Informed Consent (IC) is accepted as an important element for the medical intervention to be accepted as an intervention in line with laws and medical ethics because of respect for the patient's right to autonomy.[1] In medical interventions, the consent of the patient is an important reason for compliance with privacy law and criminal law. However, the existence of certain elements is important for the consent to be valid. The most important one is the "obligation to inform", which allows the patient to know what s/he consents to, which must be fulfilled.[2] Right at this point, it is necessary to emphasize the importance of the readability level of the informed consent forms, which have the nature of a power of attorney agreement between the physician and the patient.

The readability concept emerged in the USA at the beginning of the 19th century.[3] It is often confused with the concept of legibility. Legibility is determined by characteristics such as the font of the text and the shape of the page. On the other hand, readability is the knowledge of whether a text in any language can be easily followed by the reader(s).[4] In short, readability can be expressed as the level of understanding of a text.

In this study, the purpose was to evaluate the readability levels of informed consent forms, which are used widely in cardiovascular surgery clinics, with the readability scales used in our country and the international literature.

METHODS

The study was carried out with the permission Eskişehir City Hospital Non-invasive Clinical Researches Ethics Committee (Date: 15.02.2023, Decision No: ESH/GOEK

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2023/8). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 15 informed consent forms,[5] which are frequently used in cardiovascular surgery clinics in our country and recommended to the members of the Turkish Society of Cardiovascular Surgery (TSCS), taken from the official site of TSCS were included in the study. The consent forms were evaluated using the Ateşman and Yılmaz-Bezirci scales, which are frequently used for Turkish texts, and the Fresch-Kincaid, Gunning Fog, Automated Readability Index (ARI) scales, which are frequently used in international studies.

Readability Scales

Readability studies first appeared with formulas created according to the word and sentence structure of English and developed in different countries. The readability formulas accepted in the international literature are Flesch-Kincaid (1975), Gunning Difficulty Indicator (1952), and Automated Readability Index (ARI) (1967). After these studies, the study of creating a formula to measure the readability difficulty of Turkish for the first time started with the adaptation of the constants in Flesch's Formula according to the characteristics of Turkish by Ateşman (1997). Bezirci and Yılmaz (2010) analyzed five different studies for English and developed a formula for Turkish that they named "New Readability Value".[6]

The calculation was made by taking the first 100 words of the text over the formula in Atesman Readability Scale. According to this formula, the readability level of a text is determined to be easier as it approaches 100, and more difficult as the readability level approaches 0.[7]

RS=198.825-(40.175.X1)-(2.610.X2)

RS: Readability Score
X1: Number of syllables/word count
X2: Word count/sentence count

The evaluation of the results between 0-100 according to the level of education is shown in Table 1.

Table 1. Education levels corresponding to the scores obtained by Ateşman readability formula[7]

Scores	Education Levels
90-100	It can be read by anyone with an education level of 4th grade and below in primary school.
80-89	It can be read by anyone with a 5-6th grade level education.
70-79	It can be read by anyone with a 7-8th grade level education.
60-69	It can be read by anyone with a 9-10th grade level education.
50-59	It can be read by anyone with a 11-12th grade level education.
40-49	It can be read by anyone with a 12-13th grade level education.
30-39	It can be read by those who have studied undergraduate.
≤29	It can be read by those with a postgraduate education.

The following formula was developed according to the number of words and syllables in the text in the Bezirci-Yılmaz Readability Index.

RS=√AVC × ((H3 × 0.84)+(H4 × 1.5)+(H5 × 3.5)+(H6 × 26.25))

RS refers to readability score, AVC refers to the average number of words, H3 refers to the average number of 3-syllable words, H4 refers to the average number of 4-syllable words, H5 refers to average 5-syllable words, and H6 refers to the average number of words with 6 or more syllables. The scores and education levels according to Bezirci-Yılmaz Readability Scale are shown in Table 2.[8]

Table 2. Education levels corresponding to the scores obtained from the Bezirci-Yılmaz Readability Scale[8]

Scores	Education Levels
1-8	Primary and secondary school
9-12	High school
12-16	Undergraduate
16+	Postgraduate

The computer program developed by Bezirci-Yılmaz was used to calculate the formulas.

$$206.835 - 1.015\left(\frac{\text{total words}}{\text{total sentences}}\right) - 84.6\left(\frac{\text{total syllables}}{\text{total words}}\right)$$

In the Flesch reading-ease test, higher scores indicate material that is easier to read; lower numbers mark passages that are more difficult to read. The formula for the Flesch reading-ease score (FRES) test is:

The Gunning Fog Index Readability Formula, or simply called FOG Index, is attributed to American textbook publisher, Robert Gunning, who was a graduate from Ohio State University.

Grade level=0.4 (ASL + PHW)

ASL=average sentence length (i.e., number of words divided by the number of sentences) PHW=Percentage of hard words[19]

The automated readability index (ARI) is a readability test for English texts, designed to gauge the understandability of a text. Like the Flesch-Kincaid grade level, Gunning fog index, SMOG index, Fry readability formula, and Coleman-Liau index, it produces an approximate representation of the US grade level needed to comprehend the text.[20]

The formula for calculating the automated readability index is given below:

$$4.71\left(\frac{\text{characters}}{\text{words}}\right) + 0.5\left(\frac{\text{words}}{\text{sentences}}\right) - 21.43$$

RESULTS

The scores of all consent forms that were included in the study according to the readability scales are given in Table 3. The endovascular procedures consent form was determined at the readability level that required undergraduate education according to both Ateşman and Bezirci-Yılmaz Scales. It is noteworthy that this value is the most difficult to read among all consent forms.

Table 4 shows the mean scores of the forms on each scale and the level of education that these scores correspond to in the scale. The mean scores of the consent forms on the Ateşman Scale require education at 11-12th grade and are 57.5. According to the Bezirci-Yılmaz Scale, they have an average of 9.15 points and are in a class that requires a high school education. Although the ARI, Flesch-Kincaid, and Fog Scales are considered to be not suitable for Turkish, they were studied for comparison purposes and their readability levels were found to be very difficult and at the undergraduate level in correlation with local scales.

Table 4. Average scores and education levels according to readability scales

	Average scores	Education levels
Ateşman	57,53	11-12th grade level education
Bezirci-Yılmaz	9,15	High school
Gunning Fog	16,84	College senior
Flesch-Kincaid	14,89	College graduate
ARI	22,74	College student

ARI: Automated Readability Index

DISCUSSION

This increased interest in the readability levels of the texts is associated with the concept of moral autonomy, which enables patients to make decisions about themselves. Previous studies conducted to develop patient-centered texts are essential in the new model of clinical relationships based on the "patient-first" concept. Laws regulating human rights in healthcare allow patients to play greater roles in making decisions that affect them. Because, as well as the knowledge and technical competence, healthcare professionals must help patients understand their situation so that they can make decisions. Essentially, the information given must be comprehensible as well as accurate, and sufficient to help patients make decisions.[9,18]

In their study, Yesilyurt et al.[10] introduced the concept of "expected and current schooling year" in 2016, and according to 2016 data, they declared the current schooling year of Turkey as 6.15 and the expected schooling year as 11.03. According to the data of the Turkish Statistical Institute (TUIK) for December 2022, high school graduates or an equivalent school currently constitute 22.3% of the entire population in our country. The rate of illiterate and primary and secondary school graduates is 43% of the entire population.[11]

Although studies conducted on the evaluation of medical texts according to readability criteria have attracted attention in recent years, there are very few studies on cardiovascular operations. As far as we know, the present study is the first for Turkish consent forms.

Table 3. Scores of patient consent forms according to readability scales

	Ateşman	Bezirci-Yılmaz	Gunning Fog	Flesch-Kincaid	ARI
Abdominal aortic, aortailiac and aortafemoral surgery	57,17	9,4	17,45	22,88	14,8
Aortic valve surgery	62,04	7,92	16,06	21,73	13,81
Ascending/Arcus aortic surgery	52,11	10,69	17,42	23,74	15,97
Ascending/descending aortic surgery	57,7	9,3	16,72	22,47	14,83
Pediatric heart surgery	66,59	5,88	14,67	21,21	13,03
Embolectomy	57,71	9,08	17,28	22,67	14,7
Endovascular procedures	39,83	15,63	19,75	26,17	19
Femoropopliteal bypass	62,51	6,93	14,65	22,04	13,98
Intracardiac tumor surgery	61,51	8	16,22	21,89	13,95
Carotid endarterectomy	60,21	8,4	16,66	22,09	14,24
Coronary artery bypass grafting	55,47	9,34	17,32	23,46	15,67
Arteriovenous fistula surgery	58,65	8,52	17,17	22,58	14,7
Mitral valve surgery	61,52	8,02	16,28	21,86	13,96
Peripheral vascular interventions	60	8,63	17,03	22,11	14,27
Thoracic/thoracoabdominal aortic replacement	50,02	11,61	18,03	24,28	16,46

ARI: Automated Readability Index

In the present study, the average education level of the patient information and consent forms recommended by the Turkish Society of Cardiovascular Surgery is 11-12 according to the Ateşman Scale. According to Bezirci-Yılmaz Scale, it was determined that the grade was at least high school level. These scales were also compared with international scales and it was found that the readability level in them is at the undergraduate level.

Similarly, Dural et al.[12] conducted a study on consent forms used in cardiology clinics in 2022 and reported that the readability level of these forms was at the high school level, which is similar to our results. Endovascular interventions consent form was found to be the most difficult to read in the present study and required undergraduate education according to both Ateşman and Bezirci-Yılmaz. In Dural's study, consent for coronary angiography was 12-13 according to Ateşman. It was grade level and had the lowest score (hardest). But no consent form was scored at the undergraduate level.

San Norberto et al.[13] investigated the readability levels of 504 consent forms in different branches of medicine with 5 different readability scales and reported that the readability of consent forms of Spanish surgical specialties was beyond the average education level of the population as a whole. In the same study, it is valuable to say that the only national scientific community that developed consent forms with appropriate readability was Angiology and Vascular Surgery.

In Fischer's study that was published in 2021, 75 separate consent forms were evaluated with 3 scales, one of which was Flesch-Kincaid, and it was found that 2 out of 3 of them did not meet the readability standards accepted in South Africa. The biggest limitation of the studies that were conducted in regions where English was not the mother tongue, including this study, was that these studies were conducted using standard programs without adapting the readability scales to that language because of the nature of the languages.[14,15] It is possible to explain this most simply, although the number of syllables in our language is calculated by the number of vowels in the word, this is not the case in English.[16,17] These are also limitations of our study. For this reason, it is important that our study was conducted with two different scales that were adapted to Turkish.

Of course, patients are not informed only with consent forms before the surgery. Aside from written consent forms, they can have more detailed verbal information from the physicians, and they can even benefit from open sources themselves. However, when emphasizing the importance of readability level, Temur argued that readability and intelligibility were different concepts and both must be evaluated.[15] It can be said that these were the limitations of our study.

CONCLUSION

A total of 15 informed consent forms recommended by the Turkish Society of Cardiovascular Surgery and frequently used in cardiovascular surgery clinics were analyzed by using national and international readability scales, and the average readability education level was found to be 11-12th grade (high school) levels. The current schooling year is 6.5 years in our country. According to TUIK data for 2021, approximately 30% of the population was primary school graduates and 20% was secondary school graduates. Having sufficient and satisfactory information on possible complications before surgery will minimize the legal and moral problems between the patient and the physician in case of possible negativities. For this reason, we advocate that the readability levels of patient information and consent forms must be revised and updated to the 6th-grade level.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission Eskişehir City Hospital Non-invasive Clinical Researches Ethics Committee (Date: 15.02.2023, Decision No: ESH/GOEK 2023/8).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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Original Article

HEALTH SCIENCES
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Miliary cerebral metastases: prevalence and radiological findings

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ABSTRACT

Aims: Miliary cerebral metastases, also known as carcinomatosis encephalitis, represent an unusual form of metastatic disease in the brain. Due to their rarity, limited literature is available, restricting our understanding of this condition. This study aimed to evaluate the prevalence and imaging characteristics of miliary cerebral metastases in a cohort of metastatic patients.

Methods: A retrospective review was conducted on approximately 618 contrast-enhanced MRI scans from patients with metastatic disease who had not undergone surgical intervention or received radiotherapy. Cases of miliary cerebral metastases were identified and analyzed. Demographic data, primary cancer types, non-contrast CT and MRI findings of miliary metastasis cases were evaluated.

Results: Miliary cerebral metastases were identified in 6 out of the 618 metastatic patients included in the study. The radiological features included small, disseminated hyperintense lesions visible on post-contrast T1-weighted images. These lesions were diffusely scattered throughout the brain, predominantly at the grey-white matter junction.

Conclusion: Our findings highlight the rarity of miliary cerebral metastases, supporting the limited cases reported in the literature. These findings underscore the need for increased clinical awareness and further research into this condition. High-resolution, contrast-enhanced MRI plays a vital role in detecting and characterizing miliary cerebral metastases, aiding in their management.

Keywords: Miliary cerebral metastases, carcinomatosis encephalitis, metastatic disease, MRI, brain metastases

INTRODUCTION

Miliary cerebral metastases, or carcinomatous encephalitis, is a rare but severe form of brain metastatic disease.[1,2] This condition, characterized by numerous small, disseminated lesions spread throughout the brain, mimics the miliary spread in certain infectious diseases such as tuberculosis.[1,3] However, these disseminated lesions in the context of brain metastases originate from various primary cancers, including but not limited to breast, lung, and gastrointestinal tumors.[4-9]

Despite the significant burden that brain metastases impose on patients' quality of life and survival, the unique subset of miliary cerebral metastases remains poorly understood.[10,11] The exact incidence of miliary cerebral metastases is uncertain, primarily due to its rarity, and it is thought to represent only a small fraction of all brain metastases. The clinical presentation can often mimic infectious diseases, leading to potential misdiagnosis, delayed treatment, and adversely impacting patient outcomes.[8,12-14]

Furthermore, the treatment and prognosis of patients with miliary cerebral metastases are areas of ongoing research. The response to various treatments varies, including targeted therapies and radiotherapy, and survival outcomes are generally poor.[5,11,18,19] Unfortunately, the existing literature primarily consists of case reports and small case series, limiting our understanding of this complex condition.[1,3-7,12-16,19-22]

Given the diagnostic complexity, potentially severe clinical implications, and the lack of robust, high-quality data, this study aims to provide a more comprehensive understanding of miliary cerebral metastases. Through analysis of our database, we aim to elucidate the demographics, radiological characteristics and prevalence of miliary cerebral metastases, aiming to improve early detection and, ultimately, patient care.

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METHODS

Ethics Statement

Our study was planned retrospectively. The study was carried out with the permission Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date: 22.06.2023, Decision No: 2023-06/57). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was conducted using a retrospective cohort design, reviewing approximately 705 contrast enhanced magnetic resonance imaging (MRI) scans performed in a single institution from January 2016 to May 2023. The cohort included patients diagnosed with metastatic diseases but had not undergone surgical intervention or received radiotherapy treatment.

Inclusion Criteria

Patients included in the study were those with confirmed metastatic disease. In the absence of prior surgical or radiotherapy intervention, miliary cerebral metastases were identified on the MRI scans. Only patients with high-quality MRI scans, devoid of artifacts and with complete medical records, were included in the study.

Exclusion Criteria

Patients were excluded from the study if they had undergone surgical treatment or radiotherapy before the MRI scans (n=43). Patients were also excluded if their MRI scans were of poor quality or incomplete or if their medical records were incomplete or missing (n=12) and with isolated calvarial metastasis (n=32). As a result, 618 brain metastasis cases were included in the study.

In our cohort, miliary cerebral metastases were identified in 6 of the metastatic patients, allowing for a more focused examination of the condition's characteristics and clinical course.

Each patient's demographic information, primary malignancy, and MRI characteristics were meticulously analyzed and recorded. In addition, simultaneous non-contrast computed tomography (CT) images of the patients were also evaluated. This comprehensive approach allowed us to gain significant insights into miliary cerebral metastases' prevalence, manifestations, and imaging characteristics in patients with metastatic disease.

Radiological Technique

All examinations were performed on a 1.5 T MR scanner (Signa Exp, GE Medical Systems) using a 16-channel HNS (head-neck-spine) coil. Before contrast administration, a standard MR examination was carried out including axial T1-weighted images, axial, coronal, and sagittal T2-weighted images as well as axial FLAIR images and DWI. According to the standard protocol, a post-contrast 3D T1W sequence is always required. During the whole MRI examination, the patients were instructed to keep their eyes closed. No sedation or anesthesia was used in any of the patients.

Miliary metastases were identified based on detecting numerous small, disseminated hyperintense lesions on post-contrast T1-weighted images. The lesions were generally less than 2 mm in diameter, uniformly enhancing, and diffusely scattered throughout the brain, often with a predilection for the grey-white matter junction.

Two experienced neuroradiologists blinded to the clinical information independently reviewed radiological data. Regarding discrepancy, a third opinion was obtained from a senior neuroradiologist. This ensured objectivity and minimized bias in the interpretation of imaging findings.

Statistical Analysis

Descriptive statistics were used to summarize demographic and clinical characteristics of the patients. The prevalence of miliary cerebral metastases was calculated as a proportion of the total cohort. Confidence intervals were computed using the exact binomial method. Finally, potential correlations between the demographic characteristics of the patients and the prevalence of miliary cerebral metastases were explored using appropriate statistical tests.

RESULTS

Out of the 618 MRI scans of patients with metastatic disease that were retrospectively reviewed, six patients with miliary cerebral metastases were identified, resulting in an prevelance rate of 0.9% in our study population. Of these six patients, 4 were males, and 2 were females. The age range of these patients was between 38 and 68 years, with a mean age of 57.8 years.

Primary cancer was lung carcinoma in 3 patients (50%), breast carcinoma in 2 patients (33.3%), and malignant melanoma in 1 patient (16.6%). While 2 of 3 patients with primary lung cancer were adenocarcinoma, 1 was small cell lung carcinoma. Of the 2 patients with primary breast cancer, both had invasive ductal carcinoma.

No miliary metastasis pattern was observed in the simultaneous non-contrast computed tomography (CT) examination in any patient. In T2-weighted and FLAIR sequences, all of the lesions were iso-hyperintense signal. Lesions could not be detected on pre-contrast T1-

weighted sequences. While the lesions showed diffusion restriction in 2 of 6 patients (33.3%), restricted diffusion was not observed in the lesions in 4 patients (66.6%).

These demographic and radiological characteristics have been summarized in **Table 1**. Exemplary cases are presented in **Figure 1**.

DISCUSSION

Miliary cerebral metastases are a rare and often underdiagnosed form of brain metastasis. The prevelance rate in our study was determined to be 0.9%, a rate that aligns with the rarity highlighted in the literature. This pattern of metastasis has been reported across various primary cancers, such as lung, breast, and gastrointestinal tumors.[4-9,12-22]

Radiologically, the miliary cerebral metastases were characterized as multiple small, disseminated hyperintense lesions on post-contrast T1-weighted images. The lesions were uniformly enhancing, usually less than 2 mm in diameter, and were diffusely scattered throughout the brain. Predominantly, the lesions were located at the grey-white matter junction.

The clinical presentation of miliary cerebral metastases can often mimic infectious diseases like miliary tuberculosis, leading to potential diagnostic confusion.[8,12] The unique radiological characteristics identified in our study, such as small, uniformly enhancing lesions scattered diffusely throughout the brain, were congruent with those reported in earlier studies.[1,2,15-17,20]

Radiological features of miliary cerebral metastases, both in computed tomography (CT) and magnetic resonance imaging,[21] play a pivotal role in their identification. In CT scans, the nodules are practically invisible without enhancement. When intravenous contrast is administered, a small proportion of the nodules exhibit enhancement and can be subtly identified, underscoring the importance of contrast-enhanced CT in these cases.[15]

Table 1. Demographic characteristics, primary cancer information and Radiologic findings of patients with miliary brain metastases

Patient	Age	Gender	Primary	Subtype	Non-enhanced CT	T2-Signal	FLAIR Signal	Pre-contrast T1	DWI
1	60	M	Lung	Adenocarcinoma	Invisible	Iso-hyperintense	Iso-hyperintense	Invisible	-
2	38	F	Breast	Invasive ductal carcinoma	Invisible	Iso-hyperintense	Iso-hyperintense	Invisible	-
3	58	F	Breast	Invasive ductal carcinoma	Invisible	Iso-hyperintense	Iso-hyperintense	Invisible	-
4	66	M	Skin	Malignant melanoma	Invisible	Iso-hyperintense	Iso-hyperintense	Invisible	+
5	68	M	Lung	Small cell carcinoma	Invisible	Iso-hyperintense	Iso-hyperintense	Invisible	+
6	57	M	Lung	Adenocarcinoma	Invisible	Iso-hyperintense	Iso-hyperintense	Invisible	-

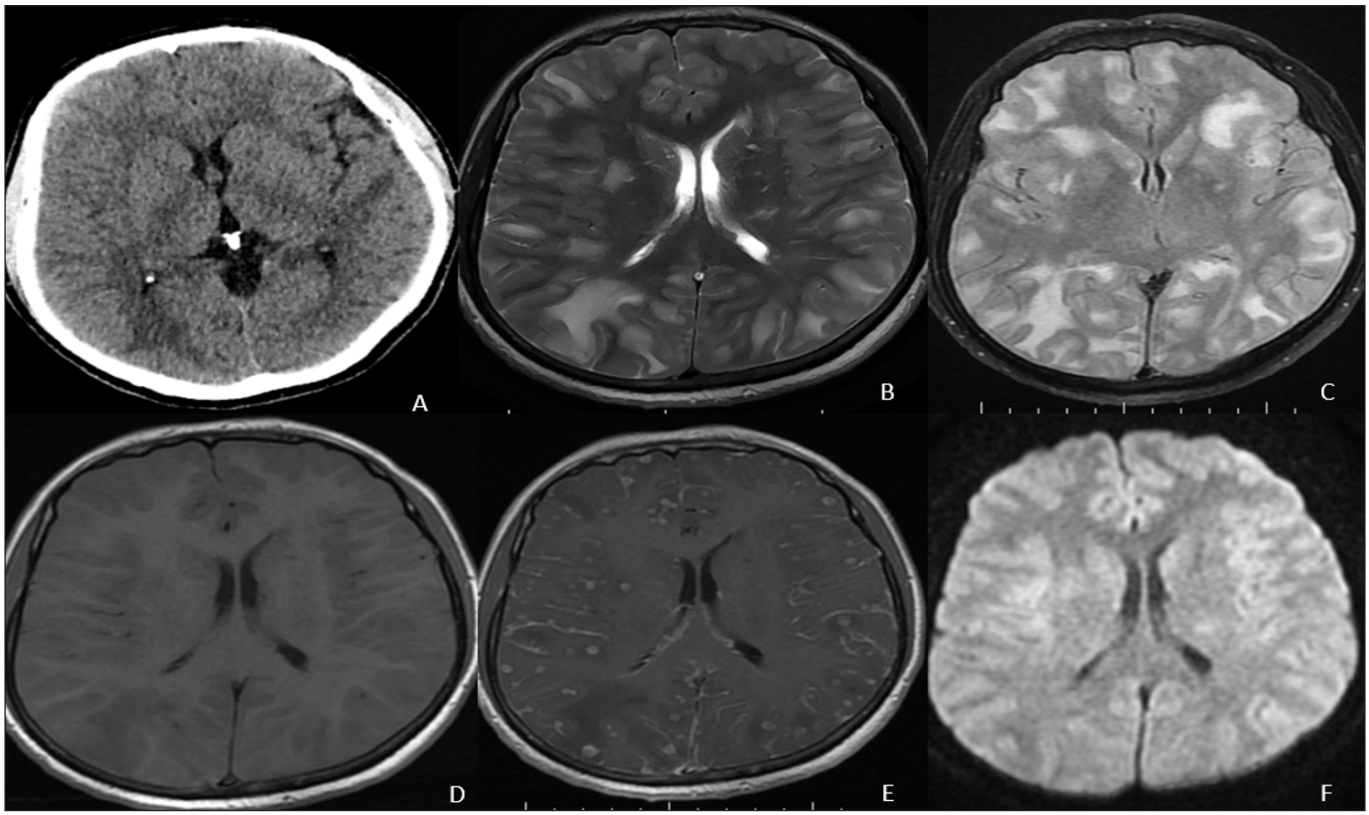

Figure 1: A case of miliary brain metastasis due to lung adenocarcinoma (Patient 1). (A): Axial non-enhanced CT; (B): Axial T2-weighted; (C): FLAIR; (D): Pre-contrast axial T1-weighted; (E): Post-contrast axial T1- weighted; (F): Difusion weighted images.

Regarding MRI, the nodules generally demonstrate mild high signal intensity on T2 and FLAIR sequences and are not observable on T1. The key sequence for demonstrating the extent and distribution of the disease is after contrast administration, where the nodules display avid homogeneous enhancement.[15] Hence, examining MRI images, particularly contrast-enhanced sequences, is critical for detecting miliary cerebral metastases.

Although the characteristic enhancement pattern of miliary brain metastases has been presented with case reports in the literature, few cases of miliary brain metastasis that was hyperintense on T2 and FLAIR sequences and did not show significant contrast enhancement after Gadolinum injection was also presented in the literature.[6,23]

The response and prognosis of miliary cerebral metastases to various treatments, including targeted therapies and radiotherapy, remains poorly understood.[5,11,18,19] In this context, it is crucial to note that most of our patients were untreated before scanning, which further substantiates the necessity for early detection of this rare form of metastasis.

While this study has provided valuable insights into the prevelance, demographics, and radiological features of miliary cerebral metastases, several limitations exist. Firstly, the study's retrospective nature and the small sample size potentially limit the generalizability of our findings to a broader population. Additionally, the study's reliance on medical records could have resulted in incomplete data or reporting bias. Secondly, we could not perform a survival analysis due to the study design, limiting our understanding of the prognostic implications of miliary cerebral metastases. Lastly, the lack of post-mortem histopathological confirmation is a significant limitation in most cases, as it could lead to potential diagnostic inaccuracy.

Given these limitations, future research should prospectively recruit larger cohorts, incorporate survival analysis, and, if possible, perform post-mortem histopathological confirmation to provide more definitive evidence of miliary cerebral metastases.

CONCLUSION

Miliary cerebral metastases, although rare, pose a significant challenge regarding accurate diagnosis and effective management. This study provides an in-depth look into the demographic and radiological characteristics of this unique form of metastasis. Our findings underline the critical role of clinical suspicion and advanced imaging in ensuring timely diagnosis. They also emphasize the urgent need for further research to develop effective therapeutic strategies and to improve our understanding of the prognosis associated with this condition. Enhancing our comprehension of miliary cerebral metastases will enable more personalized patient care and potentially improved patient outcomes.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date: 22.06.2023, Decision No: 2023-06/57).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Quality, reliability, and content assessment of YouTube™ videos associated with aphasia

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ABSTRACT

Aims: Health-related videos on YouTube make it easy to share information on diseases and address a wide audience. However, there is doubt among specialists about their reliability, quality, and whether they contain correct information. The present study aimed to evaluate the quality of the information provided by searching for "aphasia" on YouTube™.

Methods: The results of the YouTube™ search were examined using the keywords "aphasia, Broca, Wernicke, conductive type, transcortical, anomic". A total of 100 videos were identified, containing at least one of the keywords, relevant to the context, having at least 1000 views, published after 2010, in the English language, and shorter than 60 minutes. Modified DISCERN, Global Quality Score (GQS), Video Information and Quality Index (VIQI), and the Journal of American Medical Association (JAMA), rating, viewer interaction, and meta data were used for evaluating the videos. The scores of the scales indicated by The Kruskal-Wallis H Test were compared between the groups according to the video source (news agency, healthcare personnel/specialist, other people). The data were analyzed with Dunn's Test as a post-hoc test. Relational analyses and Spearman's RHO Correlation were used for statistical analyzes. The significance level was taken as $p<0.05$

Results: A total of 59 videos were taken for assessment, 34% of the videos were uploaded by news agencies, 52.5% by healthcare institutions/specialists, and 13.5% by laypeople. Significant differences were detected between GQS scores ($\chi2=8.66$, $p=0.01$) and VIQI ($\chi2=9.87$, $p=0.00$) according to the video sources. Cohen Kappa scores indicating inter-observer agreement were 0.887. The average DISCERN score was 3.74, the VIQI score was 3.64 and GQS score was 3.67 and the JAMA score was 2.59.

Conclusion: The videos about aphasia on YouTube™ were determined to have moderate scores in terms of quality, information accuracy, and reliability. Videos uploaded by healthcare professionals/specialists have higher quality and information accuracy. Especially the news agencies with the highest ratings should be sensitive about publishing accurate information.

Keywords: Aphasia, online video, internet, health, YouTube™

INTRODUCTION

The internet and social media are a part of daily life in today's world. The spread of informative videos about health problems over the internet has become an important tool to increase health awareness in society.[1]

One of the most frequently used social media sites is YouTube™, which was created in 2005 and currently has more than one billion users and provides hundreds of millions of hours of total video watch time each day.[2] YouTube™ is the most popular video-sharing site on a worldwide scale and has over 1 billion hours of views each day with over 30 million medical videos.[3] It is used not only as video storage but also as a social network where users interact with their comments and like to build trust.[4] YouTube™ is also frequently used for health issues.

Online videos make it easy to share information on health issues, reach a wide audience, and can be beneficial in the diagnosis, prevention, and treatment of diseases and improving the quality of life.[5] Recently, some studies have investigated video information in communication disorders focusing on Autism Spectrum Disorder. Kollia et al.[6] and Bellon-Harn et al.[7] analyzed the videos on Autism Spectrum Disorder (ASD). However, no such study has been conducted for aphasia.

Aphasia is an acquired neurogenic language disorder affecting the functioning of the key elements of the language network in the brain, typically the left hemisphere. Varying degrees of impairment are seen in speech, written expression, comprehension, and reading comprehension in aphasia. It is estimated that approximately 100.000-180.000 people in the United States of America suffer from aphasia

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each year.[8] Also, 2-4 million people live with aphasia in the United States.[9] Aphasia is most caused by stroke, but it can also be a result of Traumatic Brain Injuries (TBIs), brain tumors, infections, dementia, or other neurodegenerative diseases. Many YouTube™ channels post videos that offer information on aphasia. Among these, channels such as "National Aphasia Association", "The Aphasia Channel", "American Speech-Language-Hearing Association" health portals such as NHS Choices, Mayo Clinic, and PubMed use YouTube™ social media channels to distribute their content.[10] The videos on these channels were prepared by people who are specialists in aphasia. Madathil et al.[11] reported that videos from government agencies and professional organizations contain reliable and high-quality information.

Videos on aphasia contain information about its symptoms, causes, diagnosis, and treatment and help aphasia patients improve their communication skills. However, the videos on health problems must be supported with accurate information and resources. False or misleading information can cause serious risks to people's health.[9] For this reason, when choosing videos about health problems, one should be careful about the expertise of the authors, information sources of the videos, references, and the accuracy of their contents.[10,12] Investigating the information on YouTube™ to which wide audiences are exposed will help specialists to guide patients to useful, accurate, and accessible information.[13,14] Scales with standard parameters were used in previous studies investigating health-related videos published on YouTube™ to determine the reliability of the contents. The most common of these parameters are the Journal of American Medical Association (JAMA) Criteria, DISCERN, Global Quality Score (GQS), and Video Information and Quality Index (VIQI).

The present study aimed to evaluate the aphasia-related videos in 100 YouTube™ videos on aphasia and its types according to the Journal of American Medical Association (JAMA) Criteria, DISCERN, Global Quality Score (GQS) and Video Information and Quality Index (VIQI) and metadata (e.g. video length, number of views, number of likes and comments), rating, viewer interaction.

METHODS

No human participants or animals were included in the study. Publicly available YouTube videos were analyzed, and for that reason, ethical approval was not needed for other similar YouTube studies.[17,18]

Broca's Aphasia, Wernicke's Aphasia, conductive aphasia, transcortical aphasia, and anomic aphasia types of aphasia were indicated in previous studies.[15] For this study, 6 keywords were determined as "aphasia, Broca, Wernicke, conductive type, transcortical, and anomic". In line with this, the YouTube™ Application Programming Interface (API) was used to search for videos containing queries on aphasia by using the 6 keywords specified on 11.03.2023. Browser history and cookies were cleared, and the Mozilla Firefox browser (Version 62.0.3) was used in private mode to minimize user-targeted search results.

Attention was paid to the fact that the videos were in English and did not have any subject other than aphasia, the upload date was in 2010 and later, and the number of views was 1000 or more. The total duration of the videos that contained the previously mentioned 6 keywords (n=100) was determined as 638 minutes. A total of 41 videos were excluded from the analyses. Video inclusion criteria were determined that it should be in English, be relevant to the title, not be a commercial video, and be no longer than 60 minutes. Two independent speech and language therapists reviewed 59 videos. Inter-observer agreement was evaluated with Cohen's Kappa Coefficient.

The content of each video was categorized and coded. The videos were grouped according to the source of the news agency, healthcare institution/specialist, and other people (e.g., patient relatives). Videos that met the inclusion criteria were analyzed for differences in reliability (DISCERN), quality (GQS), and accuracy of information (VIQI), Journal of American Medical Association (JAMA) criteria, rating, viewer interaction, and metadata, and the correlation between the specified parameters was tested (**Figure 1**).

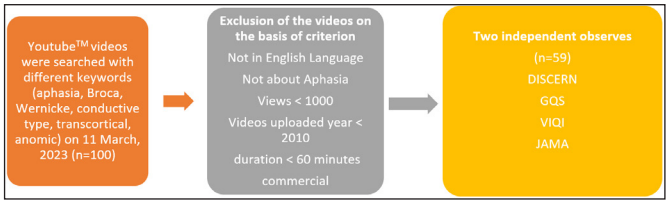

Figure 1. Flow chart of the screening procedure

Video reliability was evaluated by using DISCERN. The modified DISCERN score was used to evaluate clarity, reliability, bias, reference suffix, and areas of uncertainty for information in YouTube™ videos. Each question was scored "Yes" (1 point) or "No" (0 points) ("Are the aims clear and achieved?", "Are reliable sources of information used? (i.e., cited publication, the speaker is a board-certified vascular surgeon)", "Is the information balanced and unbiased?", "Are additional sources of information listed for patient reference?", and "Are areas of uncertainty mentioned?").

The Global Quality Scale (GQS) was used to measure the overall quality of the videos on a 5-point Likert scale (1: Poor, 5: High-Quality). Higher scores indicate better video quality. The items of GQS were: Poor quality, very unlikely to be of any use to patients, Poor quality

but some information present, of very limited use to patients, Suboptimal flow, some information covered but important topics missing, somewhat useful to patients, good quality and flow, most important topics covered, useful to patients, excellent quality and flow, highly useful to patients. Information accuracy, information flow, and quality and precision of the videos were evaluated with the VIQI Scale. A 5-point Likert scale (1: Poor, 5: High-Quality) was used in evaluating the videos with VIQI.

The Journal of American Medical Association (JAMA), which is a system used to evaluate the reliability of health-related online resources, based on 4 criteria (authorship, citation, explanation, and up-to-date status) was used. JAMA criteria are Authorship, Attribution, Disclosure, and Currency and each criterion is graded as "0" or "1".

Finally, meta-data includes the number of views, the length of the videos, and the number of likes, dislikes, and comments. Viewer interaction and rating of the videos were also calculated. Viewer interaction was calculated as follows (Likes - dislikes / total views X 100). The rating was calculated by dividing the number of views by the number of days after uploading and multiplying by 100.[16]

Statistical Method

The statistical software SPSS 26.0 (SPSS Inc., Chicago, IL, USA) was used to analyze different characteristics of aphasia videos. Normal distribution was evaluated by using the Shapiro-Wilk Test. The Kruskal-Wallis H Test, reliability (DISCERN), quality (GQS) and information accuracy (VIQI), JAMA, viewer interaction, rating, and metadata were used to examine whether the videos differed by the video source (news agency, healthcare agency/specialist, and others). In case of significant differences were detected between the groups, Dunn's Test was preferred as the post-hoc test. The Spearman Correlation was performed to examine the correlation between the variables. Analysis results were presented as frequency (percentage) for categorical variables and the significance level was taken as $p<0.05$.

RESULTS

A total of 100 videos were analyzed and 41 were excluded because they did not meet the criteria (non-English languages (n=6), irrelevant to the title (n=4), videos with advertisements (n=6), videos longer than 60 minutes (n=9), and videos with no context, information, or description (n=16). Cohen Kappa scores indicating inter-observer agreement for DISCERN, GQS, JAMA, and VIQI scale scores were 0.822, 0.868, 0.816, and 0.887, respectively. According to the DISCERN scores, 10 of the 59 videos were assessed as excellent, 22 as good, 23 as fair, and 4 as poor. The average DISCERN score was 3.74±1.02 (Figure 2).

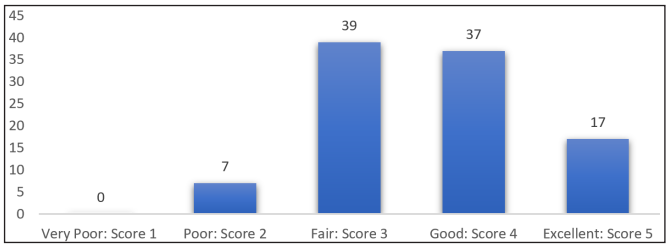

Figure 2. Distribution according to Modified DISCERN scores

JAMA scores ($\overline{x}$=2.59, SD=0.81) were determined as GQS ($\overline{x}$=3.67, SD=0.85), and DISCERN ($\overline{x}$=3.74, SD=1.02). According to the VIQI scores ($\overline{x}$=3.64, SD=0.84), 10 out of 59 videos were found as Scale 5 (high-quality), 22 as Scale 4, 23 as Scale 3, and 4 as Scale 2 (Figure 3).

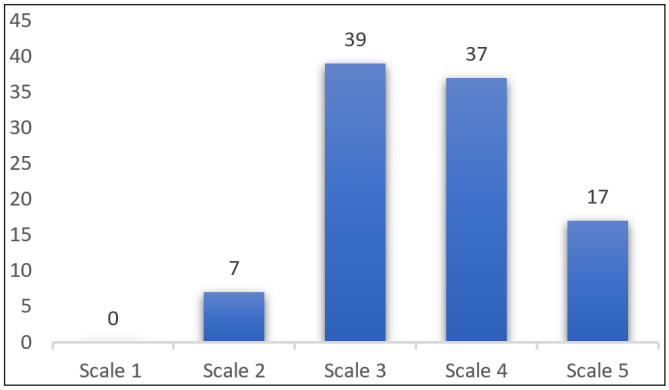

Figure 3. Distribution of videos according to VIQI scores

As seen in Table 1, relational analyzes were made according to the duration of the video, number of views, number of likes, number of comments, the Total DISCERN score, VIQI, GQS, JAMA, rating, and viewer interaction.

Table 1. Correlation between video characteristics and quantitative observations

	Number of views	Modified DISCERN	VIQI	GQS	JAMA
Video duration	-.083	-.053	.338**	.366**	.373**
Rating	.829**	.071	.062	.143	-.148
Viewer interaction	-.184	.107	.314*	.290*	.142
Number of likes	.688**	.000	.103	.180	-.182
Number of comments	.523**	-.051	-.067	.033	.004

Spearman's RHO correlation coefficient, (P <0.05), **Moderate positive correlation, *Weak positive correlation

A total of 34% of the videos were uploaded by news agencies, 52.5% by healthcare institutions/specialists, and 13.5% by laypeople. Significant differences were detected between video source categorization and GQS ($\chi 2$ =8.66, df=2, p=0.01) and VIQI ($\chi 2$=9.87, df=2, p=0.00). The Dunn Post-Hoc Test was used for pairwise comparisons to determine from which groups the significant differences originated as a result of the analysis made with the Kruskal Wallis Test. It was determined that the VIQI scores in the news agency category differed significantly from the healthcare staff/specialist category (p=0.042). GQS scores were also found to differ significantly from

Table 2. Detailed characteristics of classified YouTube Aphasia videos

	News Agency		Healthcare Institution/Specialist		Others	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Duration (sec.)	249.0588	64.89208	216.2000	33.80623	307.5000	66.41160
Number of likes	2242.7647	1694.76636	3471.9500	1676.28064	1325.1250	694.12950
Number of comments	97.2941	78.13999	291.3500	150.86296	190.5000	120.46828
Discern total	3.3750	.41993	4.3588	. 18131	3.5000	.25649
VIQI	3.4000	.15218	4.4088	.18131	3.1250	.35038
GQS	3.4500	.15347	4.1176	. 18947	3.1250	.35038
JAMA	2.5294	.17400	2.6000	.15218	2.1250	.29505
Rating	46619.0588	43970.495	23381.1000	11208.525	2346.7500	1033.29182
Viewer interaction	1.0865	.28090	1.1995	.22698	.6725	.18433

$\bar{x}$: mean, SD: Standard Deviation

the healthcare staff/specialist category according to the news agency category (p=0.050).

The video durations, the number of likes and comments, ratings, viewer interaction scores, and average and standard deviations of the DISCERN, JAMA, VIQI, and GQS scale scores of the groups that were categorized according to the source of the video are given in **Table 2**.

DISCUSSION

Aphasia-related 59 videos were analyzed for reliability (DISCERN), quality (GQS) and information accuracy (VIQI), JAMA, viewer interaction, viewership, and metadata. In the literature, there is no consensus on which of these scales is more precise.[19] For this reason, it was desired to make a more objective assessment by using different scales together. When the correlation analyzes were examined, a highly positive and significant correlation was detected between GQS, DISCERN, and VIQI scores (p=0.00). Considering this finding, it can be thought that the videos that were analyzed with the scales can reach more consistent and reliable results.

The 59 analyzed videos on aphasia were rated as quality on the reliability scale (DISCERN). The question "Is the information presented balanced and unbiased?" had the highest average score among the DISCERN items ($\bar{x}$=0.94). In terms of information accuracy and flow, quality, and precision (VIQI), Scale 3 (39%) was evaluated as fair and Scale 4 (37%) as good. In terms of general quality (GQS), there was a good flow in the total of the videos examined about aphasia and it is considered to be beneficial for patients. In the present study, JAMA scores ($\bar{x}$=2.59, SD=0.81), GQS ($\bar{x}$=3.67, SD=0.85), DISCERN ($\bar{x}$=3.74, SD=1.02), and VIQI ($\bar{x}$=3.64, SD=0.84) scale scores were found to be lower according to scales evolution criterias (1: Poor, 5: High-Quality). It is considered that the videos to be added to YouTube™ about aphasia should increase their credibility in terms of authorship, attribution, explanation, and up-to-date status.

In the present study, it was analyzed whether there were significant differences between the scale scores according to the source of the videos. As a result of these analyses, GQS and VIQI scores showed significant differences between the groups. The GQS and VIQI scores of YouTube™ videos uploaded by healthcare institutions/specialists were statistically and significantly higher than the scores of videos uploaded by news agencies. Madathil et al.[11] reported that videos uploaded by government agencies and professional organizations contained reliable and high-quality information. The finding of the present study supports the study of Madathil et al.

News agencies had significantly lower quality and information accuracy among the three groups. However, it was found that the most watched rate among all groups was in the videos of news agencies. There may be two different reasons for this high rating. The first of these was that the famous actor Bruce Willis was diagnosed with neurodegenerative aphasia due to frontotemporal dementia (FTD) featured in the video titles. Famous people are often used in advertising and media management to attract the attention of social media users.[20] After this world-renowned famous actor was diagnosed with aphasia, aphasia may have been on the agenda more than ever, and for this reason, the rating may be high. Another important factor was that the news agencies that uploaded the videos were international news channels. The high number of subscribers and their international recognition (e.g., BBC News) may have resulted in high ratings. Since the videos uploaded by news channels have high viewership rates, it is recommended that these videos receive support from governmental organizations, associations, educational institutions, and health professionals on aphasia before they are published. In this way, it is thought that they will be more beneficial to aphasia patients.

Considering the viewer interaction scores, the category with the highest interaction rate was healthcare staff/specialists. Similar to the findings of Bilir and Yılancı,[21] in which they analyzed videos about "bruxism", the interaction of specialist videos resulted in more

interaction by viewers than all video sources. One of the reasons this finding was reached may be that the video duration was shorter than the other groups (approximately 3 minutes). The shortness of the duration can be associated with the fact that people who watched the video until the end gave feedback at the end of the video (number of likes: 3471.9500). Compared to other groups, the number of comments in the category of healthcare staff/specialist was also higher (number of likes=291.3500). Topps et al.[22] investigated the relationship between the duration of the videos and attention, participation and comment, stated that the duration of the videos is 5 minutes or more was associated with lower attention and participation scores.

In terms of health-related videos, videos on aphasia are in threat because there are no rules or restrictions on uploading videos, and everyone can easily shoot and upload videos. It was found that the videos on "aphasia" against this threat were mostly uploaded by healthcare staff/specialists (52.5%). This finding shows that there are relatively reliable, accurate, quality videos on YouTube™, at least for aphasia.

One of the limitations of the present study was that it had a cross-sectional design and included videos only in English. In future studies, a comprehensive analysis can be made in different languages by adding other social media platforms. The information acquired from these studies can help people who upload health-related videos to be informed and benefit from this information while producing content. In this way, it will support the public to obtain relatively more useful health information.

CONCLUSION

YouTube™ is a platform where new videos are constantly added and the number of viewers continues to increase. Producing content to increase the quality and accuracy of the information on aphasia videos added to YouTube™, which has the potential to affect large audiences, will increase the benefit of patients in this regard.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval was not obtained as there was no human or animal participation in the study, and the videos were public. Study according to the World Medical Association Declaration of Helsinki, as no patient data or materials were used and all videos used for the study are available on a public social media website (YouTube™).

Informed Consent: There was no human or animal participation in the study and the videos reviewed on YouTube™ were open to everyone. For this reason, it was not necessary to obtain informed consent.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Deep brain stimulation from past to future: research trends and global outcomes with bibliometric analysis during 1980-2022

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ABSTRACT

Aims: We aimed to summarize the intellectual structure of the deep brain stimulation (DBS), to reveal the global productivity, to identify and map the latest trends by analysing the social and structural relationships between the different research components of scientific articles published on DBS.

Methods: 5939 articles on DBS published during 1980 and 2022 were analysed utilized various statistical approaches. Network visualization maps were created to reveal trend topics, citation analysis, and international collaborations. Spearman's correlation analysis was used for correlation investigations. The exponential smoothing predictor was used to determine the article productivity trend.

Results: The most prolific author on DBS was Okun, Michael S. (209 articles) and the most productive institution was the University of Toronto (n=283). The top 3 productive countries were United States of America (n=2371, 39.9%), Germany (910, 15.3%), and United Kingdom (550, 9.2%). From past to present, the most studied topics were Parkinson's disease, subthalamic nucleus DBS, dystonia, globus pallidus, essential tremor, movement disorders, thalamus, functional neurosurgery, neuromodulation, depression, obsessive compulsive disorder, basal ganglia.

Conclusion: The primary trend topics that have been studied more in recent years are tractography, freezing of gait, Parkinson's disease, Parkinson&#8217, Parkinson#apos, autonomy, self, machine learning, non-motor symptoms, functional connectivity, globus pallidus interna, volume of tissue activated, adaptive deep brain stimulation, beta oscillations, medial forebrain bundle, and local field potential. The secondery trend topics were optogenetics, pediatric, frameless, closed-loop DBS, refractory epilepsy, satellite broadcasting, asleep DBS, optimization, biomarker, directional Leeds, nucleus bassals of meynert, personality, authenticity, and anterior nucleus of thalamus.

Keywords: Deep brain stimulation, deep brain stimulator, DBS, research trends, bibliometric analysis

INTRODUCTION

The clinical use of deep brain stimulation (DBS), which presents new therapeutic possibilities for neurological and psychiatric disorders, is one of the most important developments in clinical neurosciences in the last 20 years. DBS is a brain surgery procedure consisting of the implantation to specific targets within the brain of electrodes which allow targeted circuit-based neuromodulation, and the provision of fixed and intermittent electricity from an implanted battery source.[1,2] The basic principle of DBS is to use a small eletrode to transmit electrical impulses to focal brain regions.[2] Since receiving approval from the Food and Drug Administration (FDA) and Conformité Européene (CE), DBS has become a care standard in Parkinson's disease, essential tremor, and dystonia, and it is also being actively investigated for other neurological and psychiatric diseases linked to a pathological circuit, including major depressive disorder, Alzheimer's disease, obsessive-compulsive disease, Tourette syndrome, anorexia nervosa, epilepsy and schizophrenia.[2,3]

Parkinson's disease (PD) is one of the chronic neurological diseases that lead to the most disability and significant levels of loss in quality of life. There are various drugs with which the disease symptoms can be effectively treated, but long-term medical treatment is complicated because of motor complications that develop, generally due to levodopa.[4] Increasing neuronal activity in the subthalamic nucleus (STN) and the pars interna of

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the globus pallidus is thought to be responsible for the impaired motor function in patients with Parkinson's disease.[5] DBS of the STN and pars interna of the globus pallidus is currently the most common therapeutic surgical procedure for PD patients with no response to medical treatment. Neurostimulation has been shown to improve motor activity and daily living activities in advanced PD, but there may be cognitive side-effects such as impulsivity.[4-9]

Together with the emergence of DBS in the treatment of PD and tremor, a natural desire has arisen to attempt DBS for dystonia.[10,11] Some studies have shown that DBS improved mood in treatment-resistant depression and obsessive-compulsive disease.[12] Following those findings, the mechanisms of DBS started to be actively investigated for several different neurological and psychiatric diseases. The history of DBS is an influential example of the interaction between fundamental and clinical research. Current understanding is that DBS activates neurons and regulates pathological activity and expressions in the basal ganglion thalamocortical network, and this improves sensorimotor processing and relieves disease symptoms.[3]

Although the effect mechanisms of DBS have not been fully understood at the cellular, molecular and system level, developments in DBS technology are without doubt expected to expand the scope of application and provide additional clinical and scientific benefits.[1,2] Nevertheless, despite the advantages, DBS continues to be an invasive surgical intervention with low but potentially severe risks, including bleeding and infection.[1,2] It is thought that with new developments such as closed circuit stimuli and optogenetic stimuli, DBS will become a multidirectional treatment strategy.[3]

Bibliometric analysis is a comprehensive method that has become popular in recent years, which uses various statistical approaches to analyse a relatively greater amount of scientific data.[13,14] Bibliometric analyses enable high impact articles to be revealed and show emerging trends of the research subjects, while also contributing to the evaluation of the evolutionary process of a specific area.[15,16] In parallel with the need to analyse the increasing number of publications in literature, many bibliometric studies have been conducted on different subjects.[13-17]

As yet, there is no comprehensive bibliometric study in literature on the topic of DBS. The aim of this study was to summarise the intellectual structure of the topic of DBS by analyzing the social and structural relationships between different research components (countries, institutions, authors, topics) of scientific articles published on the topic of DBS between 1980 and 2022, and to determine the global productivity and recent trends with mapping.

METHODS

This study does not contain any studies with human participants or animals performed by any of the authors. For this type of study ethics committee approval is not required. All procedures were carried out in accordance with the ethical rules and the principles.

Research Strategy

The Web of Science (WoS) Core Collection by Clarivate Analytics database was used to access the articles on DBS. Since articles before 1980 were not indexed in WoS, the search start period was determined as 1980 and the end time was determined as 2022. As a result of the publication search on DBS, all studies with the phrase deep brain stimulation/stimulations, deep brain stimulator/stimulators or DBS were found (Considering that the abbreviation DBS can also be used in different research areas, studies that used the phrase DBS in the title and also the phrase deep brain stimulation/stimulator in the abstract section of the article were included). Repeatability codes for researchers to obtain similar documents (access date: November 15, 2022, search findings may vary depending on different access dates): ((TI="deep brain") AND (TI=stimulation*)) OR ((TI="deep brain") AND (TI=stimulator*)) OR ((TI=DBS) AND (TS=deep brain stimulation*)) OR ((TI=DBS) AND (TS=deep brain stimulator*)).

Statistical Analysis

VOSviewer (Version 1.6.18, Leiden University) software was used for bibliometric network visualizations, mapping of international academic collaborations and citation analysis.[18] Exponential Smoothing estimator, which also takes into account seasonal correction, was used in the Microsoft Excel software to predict the trend of articles in the coming years by using the number of articles published in the past on DBS. The world map was drawn using the open access website (https://app.datawrapper.de). Statistical analyses were performed with SPSS (Version: 22.0, SPSS Inc., Chicago, IL, USA, License: Hitit University) software. Before the correlation analyses, the conformity of the data to the normal distribution was examined with the Kolmogorov-Smirnov test. The relationship between the productivity of the articles on DBS and the Gross Domestic Product (GDP) and GDP per capita values, which are the economic size indicators of the countries, were determined by Spearman correlation analysis (data extracted from the World Bank website[19]). For the statistical significance limit, $P<0.05$ was accepted.

RESULTS

A total of 12511 publications related to DBS, which were indexed in WoS and published between 1980 and 2022, were obtained through the literature review. The

distribution of these publications is Articles (5939, 47.5%), Meeting Abstracts (3591, 28.7%), Review Articles (926, 7.4%), Letters (743, 5.9%), Proceedings Papers (585, 4.7%), and the rest are other publications types (Editorial Materials, Book Chapters, News Item, Book Review, Book, Data Paper, Discussion, Note). Bibliometric analyses were performed with 5939 articles indexed only in the Article publication category out of a total of 12511 publications. 97.3% (n=5780) of these articles were published in English and the rest were published in other languages (German (n=79), Spanish (25), French (18), Polish (11), Hungarian (7), Czech (6), Japanese (4), Portuguese (3), Turkish (2), Chinese (1), Esperanto (1), Italian (1), Korean (1)). Almost all of the articles were indexed in SCI-Expanded (n=5379, 90.6%), Emerging Sources Citation Index (ESCI) (n=313, 5.3%) and Social Sciences Citation Index (SSCI) (n=67, 1.1%).

Research Areas with the Most Published Articles on DBS

The research areas with the highest number of articles published on DBS (research areas with 70 or more articles published) are Clinical Neurology (n=3236, 54.4%), Neurosciences (2211, 37.2%), Surgery (1394, 23.4%), Psychiatry (468, 7.8%), Neuroimaging (434, 7.3%), Engineering Biomedical (251, 4.2%), Medicine Research Experimental (232, 3.9%), Radiology Nuclear Medicine Medical Imaging (187, 3.1%), Multidisciplinary Sciences (183, 3.0%), Psychology (152, 2.5%), Medicine General Internal (141, 2.3%), Behavioral Sciences (95, 1.6%), Rehabilitation (94, 1.5%), Pharmacology Pharmacy (86, 1.4%), Engineering Electrical Electronic (72, 1.2%), and Pediatrics (71, 1.1%) (An article can be found in more than one research area).

Development of Publications by Years and Future Publication Trend on DBS

The line graph showing the distribution of the number of articles published on DBS by years is presented in Figure 1. Since 2022 is not completed, it is not included in the forecast model. In order to determine the number of articles that can be published in 2022 and beyond, our estimation values for the results obtained with the Exponential Smoothing estimator by performing seasonal adjustment are shown in Figure 1. According to the estimation model results, it is estimated that 573 (Confidence Interval %: 530-616) articles will be published in 2022 and 707 (CI%: 560-854) articles will be published in 2026 (Figure 1).

Productive Authors on DBS

The main active authors in the production of scientific papers (with 65 or more articles published) on DBS were Okun MS. (n=209), Foote KD. (162), Lozano AM. (154), Volkmann J. (102), Deuschl G. (90), Visser-vandewalle V. (82), Krauss JK. (81), Limousin P. (78), Aziz TZ. (77), Hamani C. (77), Starr PA. (77), Zrinzo L. (75), Mcintyre CC. (74), Timmermann L. (73), Kuhn AA. (70), and Temel Y. (65), respectively.

Main Productive Institutions that have published the Most Articles on DBS

The most active institution addresses mentioned in more than 100 articles on DBS were University of Toronto (n=283), State University System of Florida (260), University of California System (258), University Toronto Affiliates (256), University of Florida (242), Udice French Research Universities (237), University of London (236), University Health Network Toronto (234), University of Cologne (196), Institut

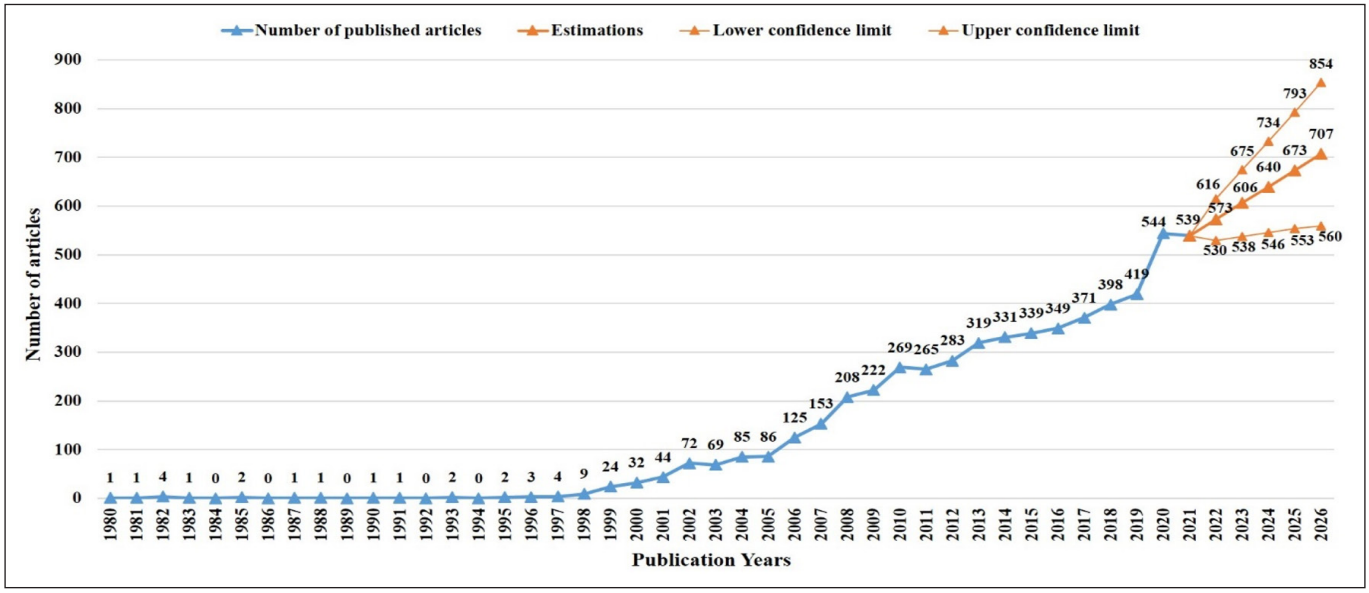

Figure 1. Bar graph showing the distribution of published articles on deep brain stimulation (DBS) over time, with a forecast for the next five years.

National De La Sante Et De La Recherche Medicale (195), University College London (190), Cleveland Clinic Foundation (187), Harvard University (168), University of Oxford (163), Humboldt University of Berlin (160), Free University of Berlin (159), Charite Universitatsmedizin Berlin (155), University of California San Francisco (151), Mayo Clinic (140), University of Amsterdam (128), Centre National De La Recherche Scientifique (127), University of Kiel (122), University of Minnesota System (112), Case Western Reserve University (110), University of Minnesota Twin Cities (110), Harvard Medical School (108), Emory University (106), Baylor College of Medicine (104), and US Department of Veterans Affairs (101), respectively.

Productive Journals on DBS

5939 articles published on DBS were published in 875 different scientific journals. Among these journals, the first 56 most productive journals that have published 20 or more articles on DBS, the total number of citations received by the journals and the average number of citations per article were shown in Table 1.

Productive Countries on DBS

The colour density map showing the distribution of the number of articles by countries and the bar graph showing the top 20 most productive countries were presented in Figure 2. The top 20 most productive countries contributing to the literature on DBS were the United States of America (n=2371, 39.9%), Germany (910, 15.3%), United Kingdom (550, 9.2%), Canada (428, 7.2%), China (420, 7%), France (371, 6.2%), Italy (320, 5.3%), Netherlands (279, 4.6%), Switzerland (202, 3.4%), Japan (188, 3.1%), Spain (176, 2.9%), South Korea (173, 2.9%), Sweden (173, 2.9%), Australia (154, 2.5%), Brazil (114, 1.9%), Belgium (84, 1.4%), Turkey (76, 1.2%), Taiwan (72, 1.2%), Poland (66, 1.1%), and India (57, 1%). Cluster analysis was conducted among 59 countries that published at least two articles from 78 countries that published articles on DBS and whose authors had international collaboration, and the findings are shown in Figure 3.a. According to the cluster analysis findings, it was determined that international cooperation on DBS was divided into 10 different clusters. In addition, international cooperation analysis was carried out among 59 countries and

Table 1. The 56 most productive journals with 20 or more articles on deep brain stimulation

Journals	NA	C	AC	Journals	NA	C	AC
Stereotactic and Functional Neurosurgery	290	6107	21.1	Tremor and other Hyperkinetic Movements	41	238	5.8
Movement Disorders	254	13945	54.9	Journal of the Neurological Sciences	40	684	17.1
Journal of Neurosurgery	209	9272	44.4	Journal of Neuroscience Methods	39	896	23.0
Parkinsonism & Related Disorders	197	4081	20.7	Operative Neurosurgery	39	338	8.7
Neuromodulation	175	2417	13.8	Journal of Neural Transmission	37	705	19.1
Brain Stimulation	132	3415	25.9	Parkinsons Disease	37	360	9.7
World Neurosurgery	130	1642	12.6	Neurological Sciences	36	697	19.4
Neurosurgery	122	5908	48.4	Annals of Neurology	34	3300	97.1
Acta Neurochirurgica	116	2239	19.3	Journal of Neurophysiology	34	2001	58.9
Journal of Neurology Neurosurgery and Psychiatry	99	6855	69.2	European Journal of Neurology	31	848	27.4
Plos One	89	2079	23.4	IEEE Transactions on Neural Systems and Rehabilitation Engineering	31	744	24.0
Frontiers in Human Neuroscience	86	769	8.9	British Journal of Neurosurgery	29	468	16.1
Neurology	84	7944	94.6	Nervenarzt	29	169	5.8
Journal of Clinical Neuroscience	77	1356	17.6	Neuroimage-Clinical	29	586	20.2
Journal of Neural Engineering	77	2543	33.0	Journal of Neuroscience	28	1871	66.8
Journal of Neurology	76	2262	29.8	Neuroethics	28	593	21.2
Frontiers in Neurology	70	323	4.6	Behavioural Brain Research	27	414	15.3
Clinical Neurology and Neurosurgery	65	814	12.5	European Journal of Neuroscience	27	934	34.6
Neuroimage	56	2715	48.5	Deep Brain Stimulation in Neurological and Psychiatric Disorders	26	67	2.6
Brain Sciences	55	271	4.9	Turkish Neurosurgery	26	132	5.1
Clinical Neurophysiology	53	2500	47.2	European Journal of Paediatric Neurology	25	467	18.7
Frontiers in Neuroscience	51	582	11.4	Neuroscience	24	392	16.3
Movement Disorders Clinical Practice	51	332	6.5	Biological Psychiatry	23	4150	180.4
Neurosurgical Focus	50	1345	26.9	Epilepsia	23	1055	45.9
Experimental Neurology	44	1926	43.8	Neurobiology of Disease	22	421	19.1
Journal of Parkinsons Disease	44	349	7.9	Neurologia I Neurochirurgia Polska	21	143	6.8
Scientific Reports	42	366	8.7	IEEE Transactions on Biomedical Engineering	21	581	27.7
Brain	41	4381	106.9	Neuroscience Letters	20	503	25.2

NA: Number of article, C: Number of citation, AC: Average number of citations per article

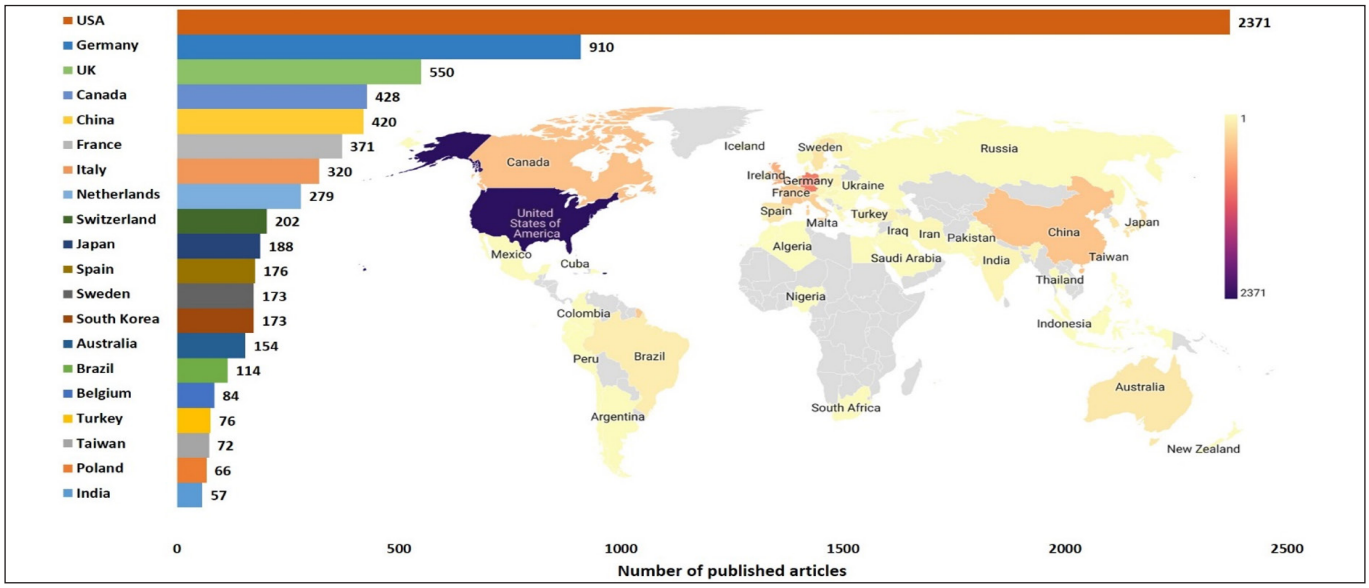

Figure 2. A bar graph showing the top 20 countries with the most published articles on deep brain stimulation (DBS) and a world productivity map showing the distribution of published articles by country.

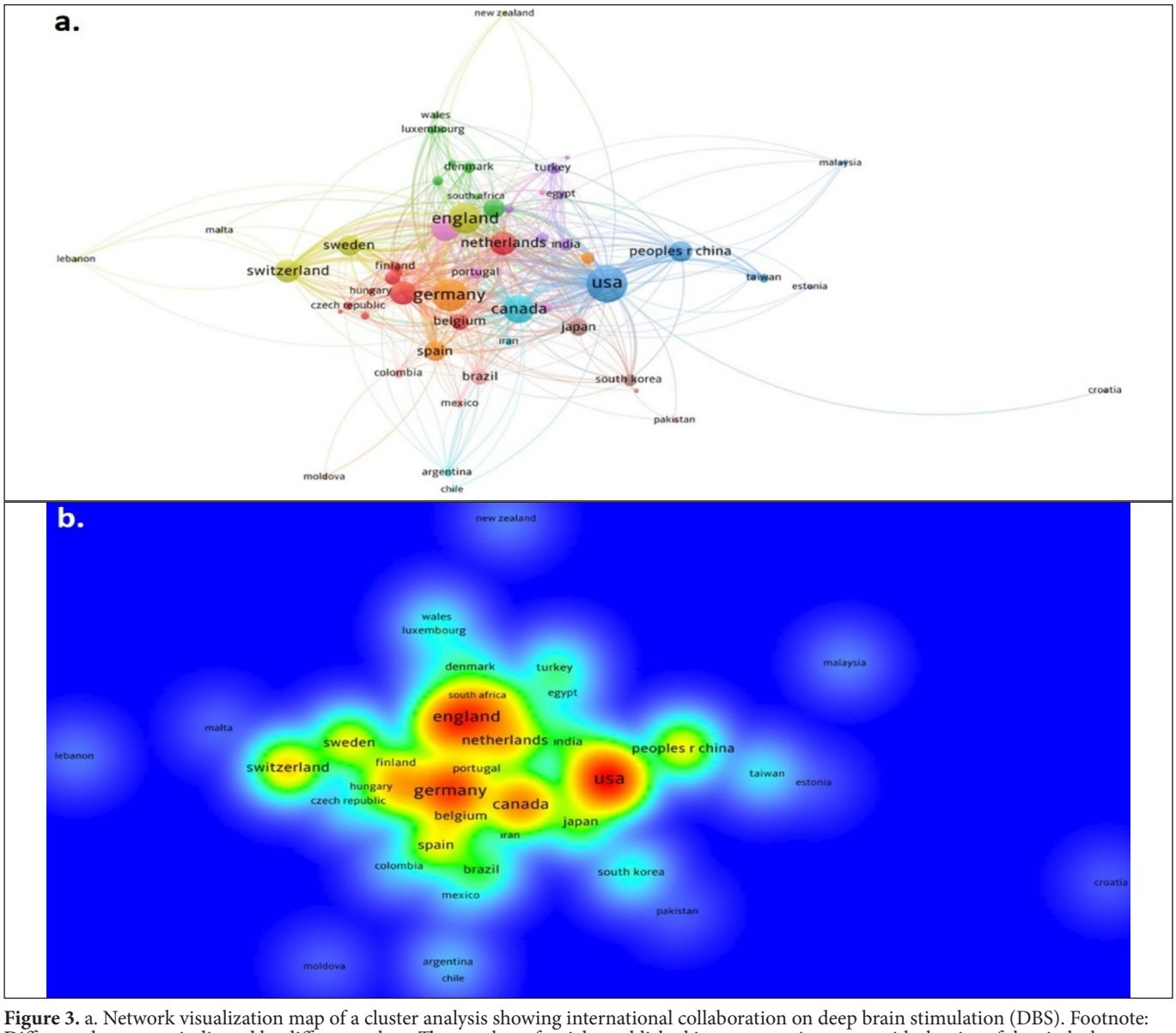

Figure 3. a. Network visualization map of a cluster analysis showing international collaboration on deep brain stimulation (DBS). Footnote: Different clusters are indicated by different colors. The number of articles published in a country increases with the size of the circle. b. A density map displaying the level of international collaboration on DBS among countries. Footnote: The score for the degree of global cooperation goes from blue to red (blue-green-yellow-red).

the scores showing the strength of cooperation were calculated. The cooperation intensity map created based on the calculated cooperation scores is shown in Figure 3.b (Top 15 countries with the highest score for cooperation: USA=994, Germany=701, England=585, Canada=484, France=412, Netherlands=312, Italy=311, Switzerland=298, China=227, Sweden=224, Spain=210, Australia=191, Japan=137, Belgium=126, Brazil=109).

The number of articles published by nations on DBS and their GDP and GDP per capita values were found to be highly statistically correlated (r=0.723, P<0.001; r=0.711, P<0.001, respectively).

Citation Analysis on DBS

The first 25 articles that received the most citations (more than 450 citations) according to the total number of citations out of 5939 articles published on DBS were shared in Table 2 along with the titles, authors, journal names in which they were published, publication years, the total number of citations received by the articles and the average number of citations received per year.

Co-citation Analysis on DBS

There were a total of 74715 publications cited in the references section of all 5939 articles published on DBS.

Table 2. The top 25 most cited articles (more than 450 citations) on deep brain stimulation

No	Article	Author	Journal	PY	TC	AC
1	Deep brain stimulation for treatment-resistant depression	Mayberg HS. et al.	Neuron	2005	2494	138.56
2	A randomized trial of deep-brain stimulation for Parkinson's disease	Deuschl G. et al.	New England Journal of Medicine	2006	1847	108.65
3	Deep-brain stimulation of the subthalamic nucleus or the pars interna of the globus pallidus in Parkinson's disease.	Obeso JA. et al.	New England Journal of Medicine	2001	1154	52.45
4	Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease a randomized controlled trial	Weaver FM. et al.	Jama-Journal of the American Medical Association	2009	1010	72.14
5	Pallidal versus subthalamic deep-brain stimulation for Parkinson's disease	Follett KA. et al.	New England Journal of Medicine	2010	846	65.08
6	Hold your horses: Impulsivity, deep brain stimulation, and medication in parkinsonism	Frank MJ. et al.	Science	2007	752	47
7	Bilateral deep brain stimulation in Parkinson's disease: a multicentre study with 4 years follow-up	Rodriguez-Oroz MC. et al.	Brain	2005	752	41.78
8	Bilateral deep-brain stimulation of the globus pallidus in primary generalized dystonia	Vidailhet M. et al.	New England Journal of Medicine	2005	734	40.78
9	Adaptive deep brain stimulation in advanced Parkinson disease	Little S. et al.	Annals of Neurology	2013	689	68.9
10	Pallidal deep-brain stimulation in primary generalized or segmental dystonia	Kupsch A. et al.	New England Journal of Medicine	2006	659	38.76
11	Subthalamic nucleus deep brain stimulation: Summary and meta-analysis of outcomes	Kleiner-Fisman G. et al.	Movement Disorders	2006	646	38
12	Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression	Lozano AM. et al.	Biological Psychiatry	2008	622	41.47
13	Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression	Schlaepfer TE. et al.	Neuropsycho-pharmacology	2008	612	40.8
14	Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics	Chen S. et al.	Science	2018	588	117.6
15	Cellular effects of deep brain stimulation: Model-based analysis of activation and inhibition	McIntyre CC. et al.	Journal of Neurophysiology	2004	549	28.89
16	Deep brain stimulation of the ventral capsule/ventral striatum for treatment-resistant depression	Malone DA. Jr. et al.	Biological Psychiatry	2009	535	38.21
17	Bilateral deep brain stimulation of the pedunculopontine and subthalamic nuclei in severe Parkinson's disease	Stefani A. et al.	Brain	2007	535	33.44
18	Closed-loop deep brain stimulation is superior in ameliorating parkinsonism	Rosin B. et al.	Neuron	2011	507	42.25
19	Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-resistant depression	Bewernick BH. et al.	Biological Psychiatry	2010	507	39
20	Double-blind evaluation of subthalamic nucleus deep brain stimulation in advanced Parkinson's disease	Kumar R. et al.	Neurology	1998	495	19.8
21	Three-year outcomes in deep brain stimulation for highly resistant obsessive-compulsive disorder	Greenberg BD. et al.	Neuropsycho-pharmacology	2006	490	28.82
22	Deep brain stimulation plus best medical therapy versus best medical therapy alone for advanced Parkinson's disease (PD SURG trial): a randomised, open-label trial	Williams A. et al.	Lancet Neurology	2010	485	37.31
23	A phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease	Laxton AW. et al.	Annals of Neurology	2010	482	37.08
24	Deep brain stimulation of the ventral internal capsule/ventral striatum for obsessive-compulsive disorder: worldwide experience	Greenberg BD. et al.	Molecular Psychiatry	2010	466	35.85
25	Transient acute depression induced by high-frequency deep-brain stimulation	Bejjani BP. et al.	New England Journal of Medicine	1999	463	19.29

PY: Publication year, TC: Total citation, AC: Average citations per year

Among these studies, the 7 most influential studies with more than 350 co-citations were Deuschl et al.[4] (Co-Citation, CC=727), Krack et al.[20] (CC: 594), Weaver et al.[9] (435), Obeso et al.[5] (422), Mayberg et al.[12] (401), Limousin et al.[21] (366), and Benabid et al.[22] (352), respectively.

Past and Current Research Trends on DBS

7020 different keywords were used in all 5939 articles published on DBS. 132 different keywords that were used in 15 or more than 15 different articles from these keywords were presented in Table 3. The map created by the keywords as a result of the clustering analysis is presented in Figure 4. The trend network visualization map, which shows the usage of the keywords in the published articles by years, obtained as a result of the keyword analysis carried out to determine past and current research trends, was presented in Figure 5. The citation network visualization map obtained as a result of the citation analysis performed to reveal the most cited topics and showing the citation numbers of the keywords was shown in Figure 6.

Table 3. The 132 most frequently used keywords in different articles on deep brain stimulation

Keywords	NU	Keywords	NU	Keywords	NU	Keywords	NU
deep brain stimulation (or DBS)	4194	treatment resistant depression	46	psychiatry	27	volume of tissue activated	20
parkinson's disease (PD or parkinson disease)	1816	dopamine	44	targeting	27	connectivity	19
subthalamic nucleus deep brain stimulation (or STN or STN-DBS)	926	functional magnetic resonance imaging (or FMRI)	44	machine learning	26	ethics	19
dystonia	348	gait	43	memory	26	major depression	19
globus pallidus (or interna or internus or gpi)	313	pain	43	autonomy	25	obesity	19
essential tremor (or tremor)	454	neurostimulation	42	brain stimulation	25	parkinson&apos	19
movement disorder (s)	186	neuroethics	41	anorexia nervosa	24	secondary dystonia	19
thalamus	183	infection	38	bradykinesia	24	adverse events	18
functional neurosurgery	181	stimulation	36	chronic pain	24	amygdala	18
neuromodulation	167	outcome	35	dementia	24	pallidal stimulation	18
depression	140	safety	35	meige syndrome	24	pallidum	18
obsessive compulsive disorder	130	dysarthria	34	posterior subthalamic area	24	programming	18
basal ganglia	126	electrical stimulation	34	verbal fluency	24	serotonin	18
magnetic resonance imaging (MRI)	122	pedunculopontine nucleus	33	addiction	23	cerebellum	17
epilepsy	117	seizure (s)	33	electrophysiology	23	chorea	17
complication (s)	100	diffusion tensor imaging	32	magnetoencephalography	23	functional connectivity	17
microelectrode recording (s)	100	electrode	32	motor cortex	23	general anesthesia	17
nucleus accumbens	94	levodopa	32	speech	23	side effects	17
quality of life	94	psychosurgery	32	treatment	23	transcranial magnetic stimulation	17
local field potential (s)	89	brain shift	31	fornix	22	adaptive deep brain stimulation	16
tourette's syndrome (or tourette syndrome)	89	hypothalamus	31	medial forebrain bundle	22	anesthesia	16
cognition (or cognitive function)	80	rat	31	non-motor symptoms	22	beta oscillations	16
stereotactic surgery (or neurosurgery)	76	stereotaxy	31	deep brain stimulator	21	cerebral palsy	16
neurosurgery	65	accuracy	30	meta-analysis	20	hemorrhage	16
ventral intermediate nucleus (or VIM)	58	zona incerta	30	multiple sclerosis	20	mood	16
computational model (or modeling)	54	alzheimer's disease	29	neuropathic pain	20	parkinsonism	16
neuropsychology (or neurophysiology)	53	cervical dystonia	29	pallidotomy	20	prefrontal cortex	16
positron emission tomography (or PET)	51	cluster headache	29	sleep	20	anterior nucleus	15
tractography	51	thalamotomy	29	striatum	20	brain	15
hippocampus	49	parkinson&#8217	28	substantia nigra	20	evoked potentials	15
S disease	47	anxiety	27	thalamic stimulation	20	finite element method	15
high frequency stimulation	46	dyskinesia	27	tics	20	schizophrenia	15
surgery	46	freezing of gait	27	ventral striatum	20	self	15

NU: Number of uses

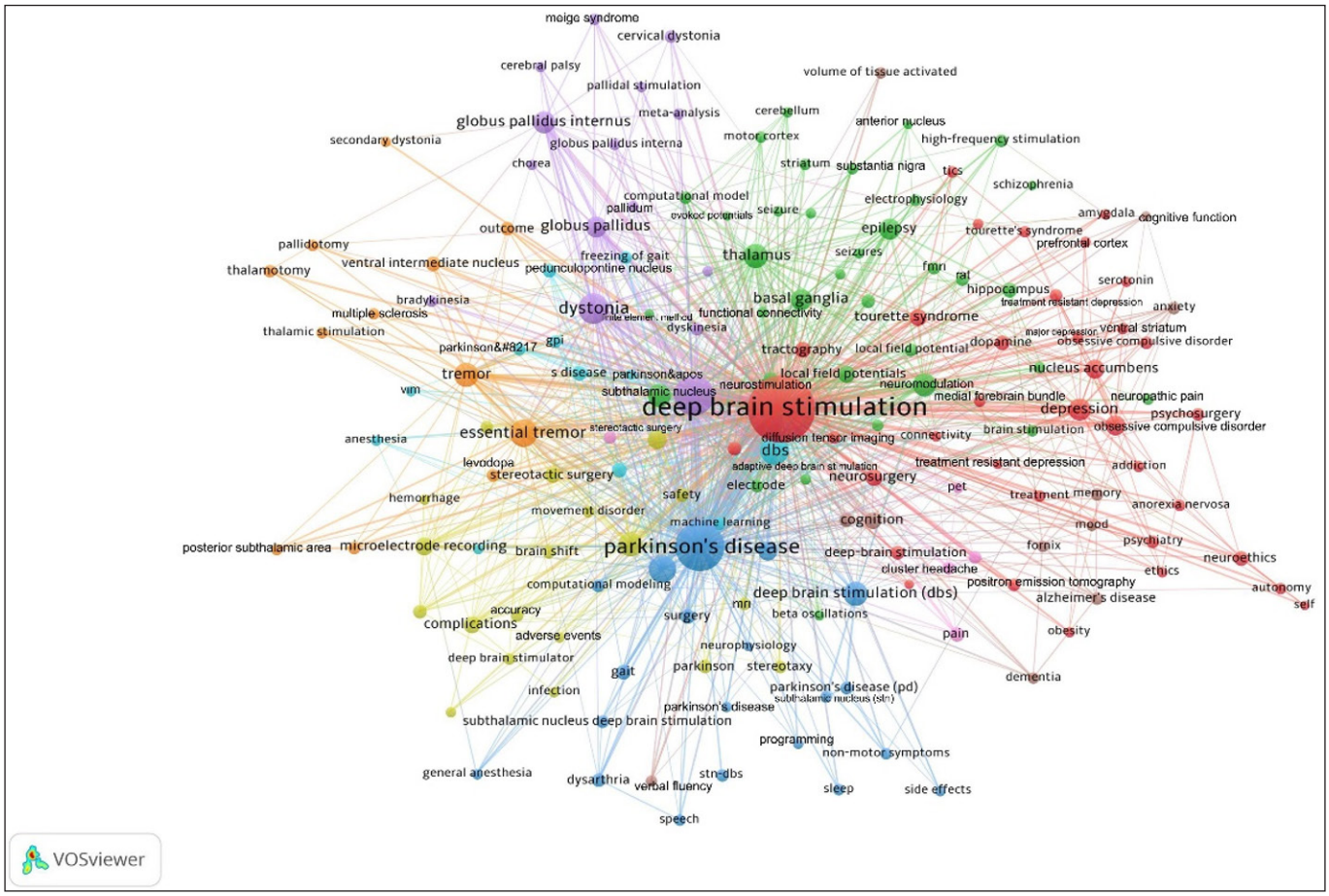

Figure 4. A network visualization map created to show how deep brain stimulation (DBS) topics tend to cluster, based on keyword analysis. Footnote: Different clusters are indicated by different colors. Similar-colored keywords are in the same clusters. The number of keyword uses is indicated by the size of the circle.

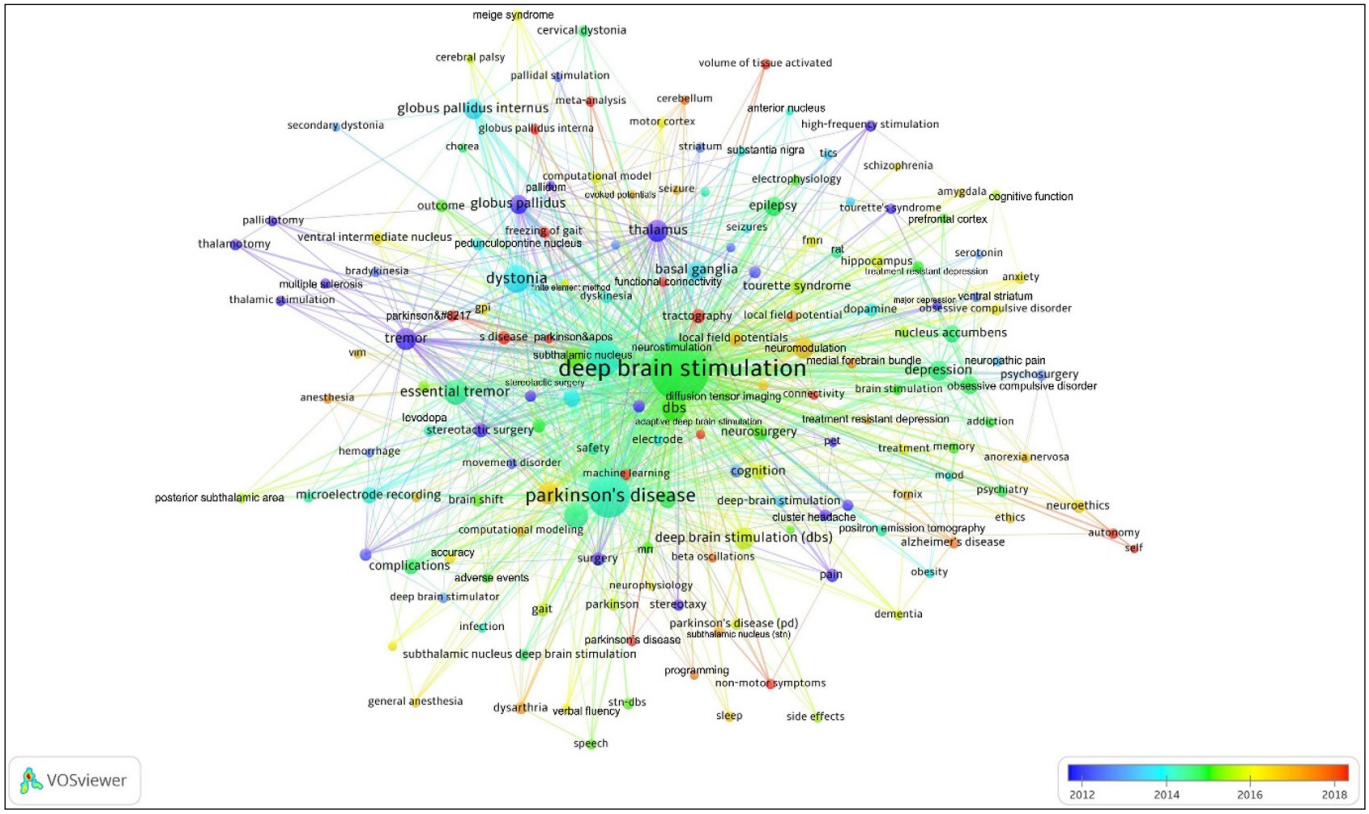

Figure 5. A network visualization map showing past and current trends in deep brain stimulation (DBS) based on keyword analysis. Footnote: The indicator in the figure's lower right corner changes from blue to red (blue-green-yellow-red) as the topics' actuality increases. The number of keyword uses is indicated by the size of the circle.

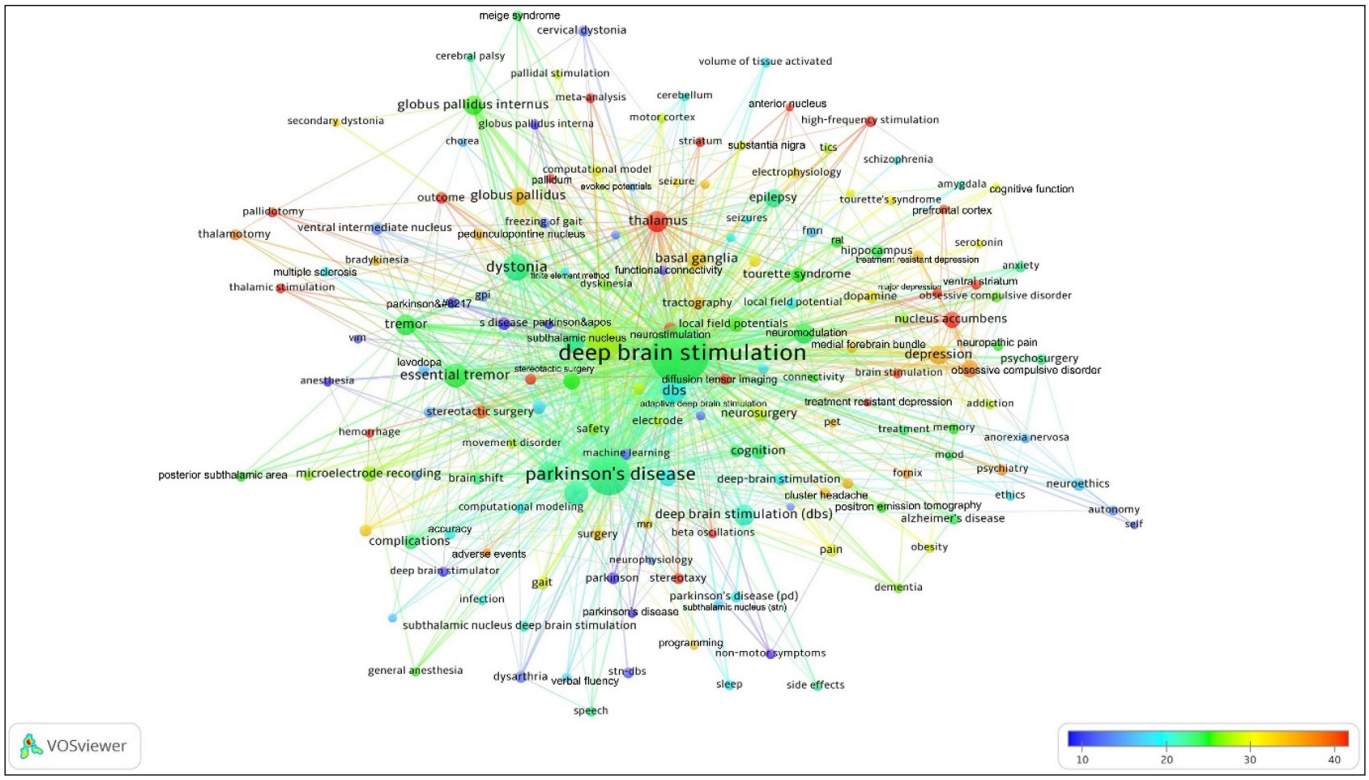

Figure 6. A network visualization map showing citation counts of topics based on keyword analysis in deep brain stimulation (DBS).
Footnote: The indicator in the figure's lower right corner changes from blue to red (blue-green-yellow-red) as the topic receives more citations. The number of keyword uses is indicated by the size of the circle.

DISCUSSION

When the distribution of articles on DBS between 1980 and 2022 was evaluated, there were seen to be 4 different publication trends in the periods of 1980-1998, 1999-2005, 2006-2012, and 2013-2021. A mean of 2 articles per year (range, 1-9) were published between 1980 and 1998. A trend for an increase in the number of articles started in 1999 and mean 59 articles per year (range, 24-86) were published between 1999 and 2005. A second increase trend started in 2006 with mean 218 articles (range, 125-283) published in the period 2006-2012. A third increase was seen to start in 2013, with mean 401 articles (range, 319-544) published between 2013 and 2021. When the 5-year prediction findings were evaluated together with the previous trends, it can be said that from 2022, the increasing trend of scientific productivity on the subject of DBS will continue.

When the article productivity of countries around the world was examined, 17 of the 20 countries (United States of America, Germany, United Kingdom, Canada, France, Italy, Netherlands, Switzerland, Japan, Spain, South Korea, Sweden, Australia, Brazil, Belgium, Taiwan, Poland) contributing the most to literature on DBS were determined to be developed countries and only 3 were developing countries (China, Turkey, India; countries classified as developing but with large economies). A significant correlation at a high level was determined between the number of articles published on the subject of DBS by a country and the GDP and GDP per capita of the country, which showed that the economic size of a country has an impact on the global productivity of articles on DBS. Some previous bibliometric studies in literature on different subjects have also shown significant correlations at moderate and high levels.[13-16] A density map was created based on the total collaboration scores between countries. When this map was interpreted, it was seen that the countries with the most intense cooperation were the USA, Germany, England (in UK), Canada, France, the Netherlands, Italy, Switzerland, China, Sweden, Spain, and Australia. When the common author collaboration was examined, although collaboration was based on geographic proximity in Europe, South America, and the Far East, global collaboration was generally widespread.

The main active journals that contributed the most to the literature on DBS were Stereotactic and Functional Neurosurgery, Movement Disorders, Journal of Neurosurgery, Parkinsonism & Related Disorders, Neuromodulation, Brain Stimulation, World Neurosurgery, Neurosurgery, and Acta Neurochirurgica, respectively. We can state that these journals are more productive for researchers who are preparing to publish on this subject. When the average number of citations per article published by the journals is evaluated (which have published at least 2 articles on DBS), we can say that the most influential journals were the New England

Journal of Medicine (Average number of citations per article=783), Neuron (662), Science (379), Nature Neuroscience (319), Lancet Neurology (246), Archives of General Psychiatry (229), Biological Psychiatry (180), Lancet Psychiatry (167), Neuropsychopharmacology (131), American Journal of Psychiatry (130), Brain (107), Journal of Chemical Neuroanatomy (106), Archives of Neurology (106), Annals of Neurology (97), Nature Medicine (96), and Neurology (95), respectively.

When the total number of citations received by the analysed articles was examined, it was determined that the most influential study with the highest number of citations was Mayberg's et al.[12] study entitled "Deep brain stimulation for treatment-resistant depression" published in Neuron. The second most influential study is Deuschl et al.'s[4] article titled "A randomized trial of deep-brain stimulation for Parkinson's disease" published in the New England Journal of Medicine. The third most effective paper is Obeso et al.'s[5] article entitled "Deep-brain stimulation of the subthalamic nucleus or the pars interna of the globus pallidus in Parkinson's disease" published in the New England Journal of Medicine. The fourth and fifth most influential studies were Weaver et al.[9] and Follett et al.'s[8] articles.

When the average number of citations per year is evaluated, the most influential study is Mayberg et al.[12] The second most influential article is Chen et al.[23] published in Science, titled "Near-infrared deep brain stimulation via upconversion nanoparticle-mediated optogenetics". The third most influential paper is Deuschl et al.[4] The fourth most influential paper is Weaver et al.[9] The fifth most influential paper is Horn et al.[24] published in Neuroimage, titled "Lead-DBS v2: Towards a comprehensive pipeline for deep brain stimulation imaging".

According to the total co-citation numbers of all analyzed articles, the most influential studies were Deuschl et al. [4], Krack et al.[20], Weaver et al.[9], Obeso et al.[5], Mayberg et al.[12], Limousin et al.[21], and Benabid et al.[22] We can suggest that researchers interested in this topic should first read these most co-cited prominent studies.

According to the cluster analysis findings, it was determined that the subtopics used in the DBS main topic were divided into 10 different clusters. The most studied topics from past to present were parkinson's disease, subthalamic nucleus deep brain stimulation, dystonia, globus pallidus, essential tremor, movement disorders, thalamus, functional neurosurgery, neuromodulation, depression, obsessive compulsive disorder, basal ganglia, magnetic resonance imaging (MRI), epilepsy, complications, microelectrode recording, nucleus accumbens, quality of life, local field potentials and tourette's syndrome.

According to the keyword analysis findings carried out to identify trend topics, the most studied trend topics (used in at least 15 different articles in recent years) in the articles published in recent years were tractography, freezing of gait, Parkinson's disease, Parkinson&#8217, Parkinson#apos, autonomy, self, machine learning, non-motor symptoms, functional connectivity, globus pallidus interna, meta-analysis, volume of tissue activated, adaptive deep brain stimulation, beta oscillations, medial forebrain bundle, and local field potential. Also, other trending topics (used in at least 10 different articles in recent years) were Optogenetics, pediatric, frameless, closed-loop DBS, refractory epilepsy, satellite broadcasting, asleep DBS, optimization, biomarker, directional Leeds, nucleus bassals of meynert, personality, identity, authenticity, and anterior nucleus of thalamus. It was also determined that thalamus, thalamus, nucleus accumbens, anterior nucleus, high-frequency stimulation, thalamic stimulation, stereotaxy, outcome, pallidotomy, pallidum, beta oscillations, striatum, ventral striatum, prefrontal cortex, major depression, treatment resistant depression, diffusion tensor imaging, hemorrhage and stereotactic neurosurgery were the most cited keywords among the keywords used in the articles published on DBS from past to present.

From the literature scan performed for this study, there was seen to be no comprehensive bibliometric study on the general subject of DBS. Hu et al.[25] discussed the bibliometric analysis of the 100 most cited articles. Listick et al.[11] evaluated the bibliometric analysis of the 100 most cited articles on DBS treatment of dystonia, and Mishra et al.[26] conducted bibliometric analyses of the 84 most cited articles on the subject of Local Field Potentials in DBS. In the current study, 5939 articles were analysed, so there can be said to be an advantage over other studies in respect of the scope and time period. Another important advantage is the inclusion of different bibliometric approaches such as trend keyword analyses, clusters, correlations, and international collaboration analyses in addition to citation analyses.

A limitation of this study could be considered to be that only the WoS was used in the literature scan. However, it has been emphasized in several studies that the PubMed database is not preferred in bibliometric analyses as citation and co-citation analyses cannot be performed on this.[13-16] In the Scopus database, some low-impact journals are indexed.[14,15] Therefore, compared to other databases, the articles in the WoS are indexed in higher impact journals (the majority in SCI-expanded).[27] In other bibliometric studies in the literature, the WoS has been generally preferred.[13-16,27]

CONCLUSION

We presented a statistical analysis of 5939 articles in this comprehensive research we conducted on DBS, which has an exponential increase in the number of articles in recent years. When the findings of the analysis carried out to determine the trend topics are examined, the primary topics that have been studied more in recent years are tractography, freezing of gait, Parkinson's disease, Parkinson&#8217, Parkinson#apos, autonomy, self, machine learning, non-motor symptoms, functional connectivity, globus pallidus interna, meta-analysis, volume of tissue activated, adaptive deep brain stimulation, beta oscillations, medial forebrain bundle, and local field potential. It can be said that the trending secondary topics in recent years are optogenetics, pediatric, frameless, closed-loop DBS, refractory epilepsy, satellite broadcasting, asleep DBS, optimization, biomarker, directional Leeds, nucleus bassals of meynert, personality, identity, authenticity, and anterior nucleus of thalamus. We have identified the research leadership of Western countries with large economies (especially the USA, European countries and Canada) and China on DBS. Although there are important international collaborations globally, we think that research on DBS in underdeveloped countries should also be encouraged. These bibliometric analyses may contribute to the emergence of new ideas for future studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: This article does not contain any studies with human participants or animals performed by any of the author. For this type of study ethics committee approval is not required.

Informed consent: For this type of study formal consent is not required.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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A detailed analysis of thyroid disorders in autoimmune liver diseases

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ABSTRACT

Aims: Extrahepatic autoimmune diseases are frequently encountered in patients with autoimmune liver diseases (AILD). There is a very limited data in the literature on the incidence of autoimmune thyroid diseases in AILD and the characterization of thyroid diseases in these patients. This study evaluated frequency and clinical features of thyroid disorders in AILD.

Methods: We compared clinical and laboratory data and thyroid ultrasonography findings of 100 patients with AILD and 48 healthy controls.

Results: The frequency of autoimmune thyroid disease and nodularity was higher in the AILD group compared to the control group (34 % vs 12.1 %; p<0.001 and 62 % vs 41.6%; p: 0.023 respectively). The number of nodules per patient was significantly higher in the AILD group than in the control group (1.37±1.3 vs 0.88±1.3; p=0.039). Hypothyroidism was detected in 17 patients (17%) with AILD (10 newly diagnosed). Only one patient had Graves' disease.

Conclusion: In AILD, autoimmune thyroiditis are common. Thyroid pathologies are missed in most patients unless a careful and detailed examination is not performed. Thyroid ultrasonography should be performed in addition to laboratory investigations in the routine follow-up of patients with AILD.

Keywords: Autoimmune thyroid disease, autoimmune liver disease, ultrasonography, nodularity

INTRODUCTION

Autoimmune liver diseases (AILD) is a chronic inflammatory disease that causes damage to hepatocytes or cholangiocytes with immune mediated mechanisms.[1] Autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC) are the most common forms of AILD. Extrahepatic autoimmune diseases are frequently encountered in patients with AILD, because of shared autoimmune pathophysiological mechanisms. Underlying genetic and molecular mechanisms are not fully elucidated for concurrent autoimmune diaseases in patients with AILD. Hashimoto's thyroiditis is the one of the most common extrahepatic autoimmune diseases associated with AILD.[2] There are very limited data on occurrence of other autoimmune diseases in AILD. In these studies, the frequency of concomitant autoimmune thyroid disease in AILD patients ranged from 7% to 34% and diagnosis of autoimmune thyroid disease was based on thyroid autoantibody positivity only.[3-6] There is no study in which thyroid parenchyma and nodularity were evaluated by ultrasound in AILD. The aim of this study was to evaluate the frequency of autoimmune thyroid diseases, thyroid nodularity in patients with AILD.

METHODS

The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committe (Date: 11.06.2018, Decision No: 10-670-18). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Population

One hundred patients with AILD and forty eight healthy control subjects matched for sex and age were included in the study. The diagnosis of AIH was made based on the clinical, laboratory, serological and biopsy findings in accordance with international guidelines. The cholestatic enzyme elevation, anti mitochondrial antibody (AMA) positivity and biopsy findings were taken into account in the diagnosis of PBC. Cases with diagnostic doubt were not

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included in the study. The diagnosis of primary sclerosing cholangitis (PSC) was made based on clinical laboratory and magnetic resonance cholangiopancreatography (MRCP) findings.

Thyroid function tests and ultrasonographic evaluations of the patients and the control group were performed cross-sectionally. Information on concomitant autoimmune diseases and other clinical data of the patients were collected retrospectively. The diagnosis of autoimmune thyroiditis was made according to thyroid ultrasonography findings and thyroid antibodies.

Thyroid Grayscale US, Power Doppler US

All participants were evaluated using high-resolution B-mode grayscale ultrasonography, power doppler ultrasonography (Hitachi EUB 7000 HV machine with 6-13-MHz linear transducer). The nodularity and other US features (thyroid volume, internal blood flow and echogenicity) were assessed. Thyroid volume was calculated as length × width × depth × 0.479 (ml) for each lobe. Parenchymal US patterns were classified into four grades according to hypoechogenicity and heterogeneity degrees (homogeneous, mild heterogeneous, moderate heterogeneous, severe heterogeneous). Hypoechogenicity was determined by comparison of thyroid parenchyma with the echo distribution of surrounding neck muscles, and heterogeneity was defined as any region with an unclear border showing a different echogenicity from other parts of the gland. US-guided fine needle aspiration biopsy (FNA) were performed in eligible patients according to American Thyroid Association (ATA) guideline recommendations.

Laboratory Analysis

Serum thyroid stimulating hormone (TSH), free thyroxine (fT4), and free triiodothyronine (fT3) concentrations were measured by chemiluminescent immunometric assay (Elecsys 170; Roche Diagnostics, Indianapolis, Indiana, USA). Normal range were as follows: TSH: 0.3-4.5 µIU/ml, fT4: 10-22 pmol/l, and fT3: 3-6.5pmol/l. Serum thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) concentrations were measured using a competitive radioimmunoassay (Brahms Dynotest; Brahms Diagnostics, Berlin, Germany).

Statistical Analysis

The results were analysed using SPSS software version 20. Simple descriptive statistics were expressed as means with standard deviations. Categorical variables are expressed as actual numbers and percentages. The frequency distribution of categorical variables between subgroups was compared by the chi-square test. Numerical variables were compared by unpaired t tests. Statistical significance was defined as P<0.05.

RESULTS

The vast majority of the patients were female (91/100; 91%) and the mean age was 55.1±11.8 years in patients with AILD. Of 100 AILD patients, 67 had PBC, 25 had AIH and 8 had PSC. The frequency of autoimmune thyroiditis was higher in patients with AILD compared to controls (34/100; 34 % vs 6/48; 12.1 %; p<0.001) (Table 1). The diagnosis of 21 patients (61 %, 21/34) with autoimmune thyroiditis was made during the study period in AILD group. Hypothyroidism was detected in 17 patients (17%) in the AILD group, and 7 (7 %) of these patients were already taking levothyroxine replacement therapy for hypothyroidism. In 10 patients (2; overt hypothyroidism, 8; subclinical hypothyroidism) the diagnosis of hypothyroidism was made during the study period. Of these newly diagnosed patients, 2 patients with overt hypothyroidism and 4 patients with subclinical hypothyroidism were started on levothyroxine treatment based on clinical evaluation. There was no overt hypothyroidism in the control group and only 2 patients had subclinical hypothyroidism. Thyroid USG examination showed that moderate-markedly heterogeneous echogenicity of the thyroid gland was more common in the AILD group than in the control group (46% vs 14.5%; p <0.001) (Table 2). The frequency of thyroid nodules was significantly higher in the AILD group than in the control group (62 % vs 41.6%; p: 0.023). Furthermore the number of nodules per patient was significantly higher in the AILD group than in the control group (1.37±1.3 vs 0.88±1.3; p=0.039). Fine needle aspiration biopsy was performed in 16 of 62 patients with autoimmune liver disease and thyroid nodules. Cytological examination revealed thyroid malignancy in one patient, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) in three patients and benign lesions in others.

Table 1. Comparison of clinical parameters between AILD and control group.

Parameters	AILD (n=100)	Control (n=48)	p
Sex (male/female)	9/91	5/43	0.771
Age (yrs)	55.1±11.8	52.9±9.2	0.28
TSH (µIU/ml)	2.48±2.18	2.36±1.75	0.75
fT4 (pmol/l)	14.1±2.3	15.1±1.9	0.02
fT3 (pmol/l)	4,3±0.6	4,7±0.55	<0.0001
Concurrent extrahepatic autoimmune diasease (present/absent)	55/45	8/40	<0.0001
Autoimmune thyroiditis (present/absent)	34/66	6/42	0.006

TSH, thyroid stimulating hormone, fT4; free thyroxine, fT3; free triiodothyronine

Table 2. Comparison of ultrasonographic features between between AILD and control group

Parameters	AILD (n=100)	Control (n=48)	p
Thyroid volume (ml)	13.2±10.9	13.3±9	0.982
Thyroid parenchyma			<0.001
Diffuse homogeneous	13	16	
Mild heterogeneous	41	25	
Moderate heterogeneous	30	6	
Markedly heterogeneous	16	1	
Nodularity (present/absent)	62/38	20/28	0.023
Nodules per patient	1.37±1.3	0.88±1.3	0.039

There was no significant difference between patient and control groups in the mean thyroid volume and IgG4 levels. Subgroup analysis showed no significant difference between patients with PBC and AIH in terms of autoimmune thyroid disease (p=0,91). Additional autoimmune diseases other than autoimmune thyroid disease were detected in 30 patients in the AILD group. The most common coexisiting autoimmun disorder was Sjogren syndrome (19%) after autoimmune thyroiditis (Table 3). Other concurrent autoimmune diseases were inflammatory bowel diseases (7%), rheumatoid arthritis (4%), autoimmune skin disease (4%), scleroderma (3%), systemic lupus (3%). Only one patient had graves' disease. Six patients (6%) had concomitant malignant disease (3 patients had papillary thyroid cancer; 2 patients had breast cancer; 1 patients had over cancer). One of the patients with papillary thyroid cancer (PTC) was diagnosed during the study.

Table 3. Frequency of extrahepatic autoimmune diseases in AILD and control group

	AILD (%) (n=55/100)	Control (%) (n=8/48)
*Autoimmune thyroid disease	34 /100 (34)	6/48 (12)
Sjogren's syndrome	19/100 (19)	-
Inflammatory bowel disease	7/100 (7)	1/48 (2)
Rheumatoid arthritis	4/100 (4)	1/48 (2)
**Autoimmune skin disease	4/100 (4)	-
Systemic lupus erythematosus	3/100 (3)	-
Systemic sclerosis	3/100 (3)	-
Type 1 diabetes	1/100 (1)	-
Pernicious anemia	1/100 (1)	-

*Hashimoto thyroiditis and Graves' disease, **Psoriasis, liken planus and vitiligo

DISCUSSION

In our cohort 34% of patients with AILD were diagnosed with autoimmune thyroiditis. The majority of them are newly diagnosed patients (61%). Levothyroxine replacement therapy was started in 6 patients who were not previously diagnosed with autoimmune thyroiditis. In addition, suspicious cytologic findings were detected in three of the patients who underwent thyroid biopsy and one of them was diagnosed with papillary thyroid cancer. In our AILD cohort 55 patients had at least one autoimmune diaseae (55%).

This study mainly focuses on extrahepatic thyroidal autoimmune conditions associated with AILD using single center database. In the literature, there are many studies evaluating extrahepatic autoimmune manifestations of autoimmune liver diseases, and almost all of these studies have shown that the most common concomitant extrahepatic autoimmune disease was Hashimoto thyroiditis. Although it varies from population to population, autoimmune thyroid disease occurs in 10-12% of the population.[7] Generally, more than half of those affected have normal thyroid function tests.

The one of the largest autoimmune liver disease series was published by Muratori and colleagues.[8] In this study, at least one autoimmune disease was detected in 42.3% of 608 AILD patients (327 AIH and 281 PBS). The frequency of autoimmune thyroiditis was 19 % (24% in PBS; 15% in AIH). Wong et al.[3] reported that the prevalence of autoimmune thyroiditis was 18% in 562 patients with autoimmune hepatitis (14.1%; hashimoto thyroiditis, 3.9 %; Graves' disease) and 42% of the patients had at least one autoimmune disease. In another large cohort study by Teufel et al.[2] included 278 patients with AIH, autoimmune thyroiditis was the most common concurrent autoimmune disease (10%). Otherwise 40% of all patients had at least one autoimmune disease. Similar to our results, Floreani et al.[4] detected at least one extrahepatic autoimmune disease in 61% of patients and autoimmune thyroid disease in 23.6% of patients in their PBS cohort of 361 patients. In a recent study by Zeng et al.[9] autoimmune thyroid disease was detected in 113 of 324 (34.9%) AILD patients. In terms of frequency of concomitant autoimmune thyroid disease the most compatible results with our data were obtained in this study. In these large cohorts, the presence of extrahepatic autoimmune diseases was based on retrospective data. Among these studies, there is no study in which thyroid evaluation is as detailed as ours. In most cases, the presence of autoimmune thyroid disease was established based on patients' self-reporting. In our cohort, the higher incidence of extrahepatic autoimmune disease compared to other cohorts could be attributed to advanced thyroid examinations. The possible pathophysiological mechanisms that may explain this relationship were the presence of shared epithelial antigens and autoreactive T cells in the thyroid and liver or the cross-reactivity of thyroid autoantibodies.

In our study, we detected hypothyroidism in 17% of AILD patients. 7 of them were already on levothyroxine therapy. In line with our results, Khoury et al.[10] found that the

frequency of hypothyroidism was higher in AIH patients compared to the healthy control group (17.7% vs. 5%).

This is the first study to perform detail thyroid ultrasound examination besides thyroid function tests in autoimmune liver patients. To the best of our knowledge nodularity is more common in patients with autoimmune thyroiditis. Previous studies have shown that the risk of thyroid nodularity and thyroid cancer increases in autoimmune thyroid diseases. This is attributed to common genetic mechanisms responsible for both clinical conditions.[11] Thus high nodularity rates in the AILD group could be explained by large number of patients with autoimmune thyroid disease in our study.

One of the most striking results of our study was that 6 of the patients with AILD had a malignant disease. In patients with AILD, cirrhosis-related hepatocellular cancers are frequently encountered.[12] In a recent study by Sharma et al.[13] the most common types of cancers other than hepatic cancers in autoimmune liver patients were lymphoproliferative cancers and non-melanoma skin cancers. In accordance with the previous study, Werner et al.[14] showed that lymphoproliferative cancers were most frequently seen after hepatocellular carcinoma in patients with AIH. However, all of the cancers detected in our study were extrahepatic (3 patients had PTC, 2 patients had breast cancer and 1 had ovarian cancer). This higher incidence of extrahepatic cancer may be related to immunosuppressive therapies frequently used in autoimmune liver disease.[15] In our study, two of 3 PTC patients had autoimmune thyroid disease. The relationship between hashimoto thyroiditis and PTC has been widely debated and it is still controversial. In previos studies the association between autoimmune thyroiditis and PTC were based on retrospective pathological studies and several fine-needle aspiration cytology (FNAC) studies.[16,17] In aforementioned pathological studies it was demonstrated that the frequency of PTC increased in hashimoto thyroiditis. In addition increased TSH levels and anti-thyroid autoantibodies (ATA) titers were found to be significant and independent predictive risk for thyroid cancer. Floreani et al.[18], in their large study containing data from 921 primary biliary cholangitis patients from 2 European Centers, 94 patients (10.2 %) had hashimoto thyroiditis, 15 patients (1.6 %) had Graves' disease; 22 patients (2.4%) had multinodular goiter; 7 (0.8%) patients had thyroid cancer. Nowadays, with the widespread use of thyroid ultrasonography, thyroid nodules has been detected more frequently and the prevalence was up to 68% using high-frequency US examination.[19,20] We thought that the higher incidence of nodularity and thyroid cancer in our patients compared to the previous studies may be related to our detailed thyroid USG examinations.

The most important contribution of this study was the initiation of levothyroxine replacement in 6 patients with hypothyroidism who were unaware of the diagnosis. In addition papillary thyroid cancer was detected in one patient and she underwent total thyroidectomy. This demonstrates the usefulness of thyroid ultrasound assessments in patients with AILD as well as routine biochemical tests.

In our study, IgG4 levels were not different in AILD and control groups. The low IgG4 titers in the AILD group were associated with the fact that the majority of AILD patients were under immunosuppressive therapy.

The limitations of our study include the retrospective nature of data collection and lack of other autoantibody levels and detailed clinical features in AILD patients.

CONCLUSION

This is the first study in which detailed thyroid examinations were performed in patients with AILD. We demonstrated that AILD has a strong association with extrahepatic autoimmune diseases especially with autoimmune thyroid disease. Moreover this study showed that thyroid nodularity, thyroid cancer and extrahepatic other cancers are more frequent in patients with AILD. In the light of these findings, it is recommended that in all patients with AILD, besides biochemical procedures, thyroid ultrasonography should be added to the routine examinations.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committe (Date: 11.06.2018, Decision No: 10-670-18).

Informed consent: Written informed consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The effect of COVID-19 pandemic on stroke admissions to a city

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ABSTRACT

Aims: The COVID-19 pandemic has brought about significant changes in healthcare around the world. The idea that hospital admissions due to cerebrovascular diseases may be affected during global epidemics made us do this study. In this study, we aimed to evaluate the effects of the pandemic and curfew restrictions on stroke applications and admissions in Bursa at the local level.

Methods: Our study was conducted by retrospectively scanning the patients who received treatment for cerebrovascular diseases in our hospital between 01.01.2018 and 31.12.2021. The admission dates, ages and International Classification of Diseases (ICD-10) codes of the patients included in the study were recorded. ICD-10 codes were categorized as bleeding (I60/I61/I62), infarct (I63), nonspecific (I64/I67), other (I65/I66/I68/I69) for use in analyses. Patients under the age of 18 and patients with missing file data were excluded from the study. The files of a total of 6997 patients were accessed.

Results: When in this study look at the number of patients by years, it was seen that there were 1326 people in 2018, 1562 people in 2019, 1916 people in 2020, and 2187 people in 2021. It is noteworthy that if we call the years 2018-2019, when there is no COVID-19 pandemic, as prepandemic, and if we call the years 2020-2021, when the COVID-19 pandemic is experienced, as pandemic, the cases due to infarction (I63) increase approximately twice. In the chi-square analysis we performed between categorical diagnoses and years, a statistical difference was found between years and diagnoses ($p<0.001$)

Conclusion: We have raised awareness about improving stroke treatment and the importance of early diagnosis and treatment. Because stroke and its complications affect human life badly. Knowing the increase and decrease of acute stroke in pandemic and non-pandemic periods, we would shed light on the pandemics we will experience in the future.

Keywords: Acute stroke, COVID-19 pandemic, quarantine

INTRODUCTION

The COVID-19 outbreak began at the end of 2019, with the first case in Wuhan.[1] After it was declared as a global epidemic, many measures, including curfews, were introduced by governments in order to reduce the spread of the virus and prevent possible diseases and deaths.[2]

Studies have revealed that there has been a shift from infectious causes to vascular causes, in which non-communicable diseases such as stroke have replaced deaths from communicable diseases and nutritional causes.[2] This effect is probably due to the increase and aging of the world population, as well as the declining mortality rates worldwide in recent years.[3] The most prominent causes of death are vascular, and stroke is now the second leading cause of death worldwide.[4] Ischemic heart disease and stroke together caused 15.2 million deaths (15-15.6 million) in 2015.[4] While ischemic strokes account for the highest number of strokes, most of the global stroke burden, measured in proportion to mortality and mortality and disability-adjusted life years (DALYs), is from hemorrhagic stroke.[5]

The COVID-19 pandemic has brought about significant changes in healthcare around the world. The driving force for this study was the thought that hospital admissions due to cerebrovascular diseases may be affected in global epidemics. In this study, we aimed to evaluate the effects of the pandemic and curfew restrictions on stroke applications and admissions in Bursa at the local level.

METHODS

The study was carried out with the permission of Bursa City Hospital Ethics Committee (Date: 04/01/2023, Decision No: KAEK 2023-1/4). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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Our study was conducted by retrospectively scanning the patients who received treatment for cerebrovascular diseases in our hospital between 01.01.2018 and 31.12.2021. The admission dates, ages and International Classification of Diseases (ICD-10) codes of the patients included in the study were recorded. ICD-10 codes were categorized as bleeding (I60/I61/I62), infarct (I63), nonspecific (I64/I67), other (I65/I66/I68/I69) for use in analyses. Patients under the age of 18 and patients with missing file data were excluded from the study. The files of a total of 6997 patients were accessed. Six patients were excluded because they were younger than 18 years of age.

Statistical Analysis

The findings of the study are evaluated using 'The Jamovi project (2021), Jamovi (Version 2.0.0) [Computer Software]. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used while evaluating the study data. The Mann-Whitney U test was used for comparisons between two groups of quantitative variables without normal distribution. Pearson chi-square test and Fisher Exact test were used to compare qualitative data. One-way Anova test and Kruskal-Wallis test were used to investigate whether there was a significant difference between more than two independent groups according to the arithmetic mean. Post-hoc Tukey and Tamhane tests were used to investigate which groups caused the significant difference between the groups. Pearson test was used for parametric variables and Spearman test was used for nonparametric variables during correlation analysis. Linear regression was used to estimate the variation in hospital admissions for stroke in our hospital. Statistically, $p<0.05$ was considered significant at the 95% confidence interval.

RESULTS

This stud have shown the total number of emergency admissions, the total number of applications due to stroke, and the incidence of stroke among all admissions during the years 2018 and 2021 included in our study (Table 1).

Table 1. 2018-2021 Incidence of stroke

Years	Stroke referral	Total emergency referral	Stroke rate(%)
2018	1326	4.038.754	3.2
2019	1562	4.517.149	3.4
2020	1916	3.571.055	5.3
2021	2187	4.557.953	4.8

A total of 6991 patients were included in the study. The mean age of the patients was calculated as 69 ±15.8. When we look at the number of patients by years, it was seen that there were 1326 people in 2018, 1562 people in 2019, 1916 people in 2020, and 2187 people in 2021. It is noteworthy that between 2018 and 2019, when there was no COVID-19 pandemic, and between 2020 and 2021, when the COVID-19 pandemic was experienced, the cases caused by infarction (I63) increased by approximately 2 times (Figure 1). When this study look at the categorical diagnostic distributions of all cases included in the study, patients with intracranial bleeding (I60/I61/I61) were 15%, intracranial infarction (I63) cases were 17.9%, bleeding or infarct was not differentiated (I64, I67) 44.9% and other (I65, I66, I68, I69) constitute 22.2% of the cases. In the chi-square analysis we performed between categorical diagnoses and years, a statistical difference was found between years and diagnoses ($p<0.001$) (Table 2). In the Linear regression analysis between the cases by years, there was no statistical difference between 2018 and 2019 ($p=0.507$), while a statistical difference was found between 2018 and 2020 and 2021 in terms of the number of cases ($p<0.001$, $p<0.001$) (Table 3).

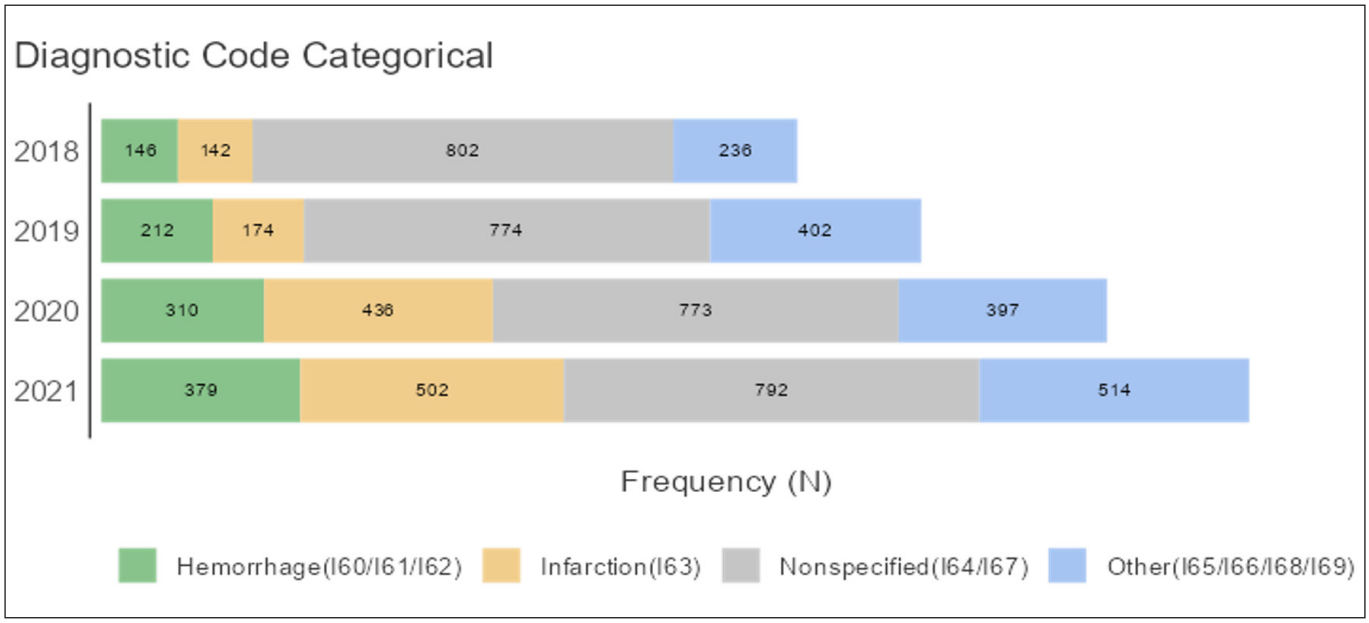

Figure 1. Distribution of categorical diagnoses by years

Table 2. Chi-square analysis of categorical diagnoses by years os stroke

Date	Diagnostic Code Categorical				Total	χ^2=309, p<0.001
	Hemorrhage (I60/I61/I62)	Infarction (I63)	Nonspecified (I64/I67)	Other (I65/I66/I68/I69)		
2018						
Observed	146	142	802	236	1326	
Expected	199	238	596	294	1326	
% within row	11.0 %	10.7 %	60.5 %	17.8 %	100.0 %	
2019						
Observed	212	174	774	402	1562	
Expected	234	280	702	346	1562	
% within row	13.6 %	11.1 %	49.6 %	25.7 %	100.0 %	
2020						
Observed	310	436	773	397	1916	
Expected	287	344	861	425	1916	
% within row	16.2 %	22.8 %	40.3 %	20.7 %	100.0 %	
2021						
Observed	379	502	792	514	2187	
Expected	328	392	983	485	2187	
% within row	17.3 %	23.0 %	36.2 %	23.5 %	100.0 %	
Total						
Observed	1047	1254	3141	1549	6991	
Expected	1047	1254	3141	1549	6991	
% within row	15.0 %	17.9 %	44.9 %	22.2 %	100.0 %	

Table 3. Estimation of linear regression of hospital admissions by stroke subtypes between 2018 and 2021

Predictor	Estimate	SE	t	p
Intercept[a]	28.507	0.0264	107.992	< .001
Date				
2019 - 2018	0.0238	0.0359	0.664	0.507
2020 - 2018	-0.1946	0.0343	-5.668	< .001
2021 - 2018	-0.1918	0.0335	-5.733	< .001

DISCUSSION

In a study published in 2019, the number of strokes over the age of 20 was reported to be 7 million annually and its prevalence was 2.5%.[6] In a study, the rate of stroke among all emergency admissions made during a 2-year period was reported to be less than 1%.[7] In our study, among all emergency applications, it was observed that acute stroke cases were between 3.2-3.4% before the pandemic, and increased by 4.8-5.3% in the last two years with the pandemic. Our stroke application rate, which is above the literature, may be due to the difference in genetic and environmental factors. We think that this finding, which is a very important stimulus for our country, should primarily be examined in new research and new health policies, as the prevalence of stroke patients in the world.

In this study examining acute stroke applications from several countries on 54366 people, it was reported that acute stroke applications decreased after the declaration of the pandemic compared to the pre-pandemic.[8] In a study known from the USA on 3556 patients, findings were reported that acute stroke admissions decreased but mortality increased with the COVID-19 outbreak.[9] In a study conducted with a narrow population of 328 patients, it was observed that despite the decrease in mild acute stroke admissions by 38%, there was an increase in cases of severe acute stroke by 21%, and there was no difference between pre-pandemic and post-pandemic stroke diagnoses.[10] In a population of 89 patients in China, it has been reported that acute stroke cases have a 1 hour delay in reaching the hospital compared to the pre-pandemic period, and the number of acute stroke cases has decreased compared to the pre-pandemic.[11] In a study examining the effect of the COVID-19 epidemic on acute strokes in Southern Europe, it was reported that the application time was 30 minutes delayed compared to the pre-pandemic period, and the rate of admission for acute stroke decreased by 25% compared to the pre-pandemic period.[12] In a study conducted in Southern Brazil, it was reported that there was a significant decrease in mild acute stroke admissions before the pandemic, but there was no significant change in the number of patients presenting with intraparenchymal and subarachnoid hemorrhage in severe acute stroke cases.[13] It has been reported in a study that no significant difference was observed between the pre-pandemic and acute stroke applications in the first 3 months of 2020 with the pandemic, but a significant decrease was observed in acute stroke applications between the period after 2020 and the pre-pandemic period.[14] In a study conducted with the participation of 18 centers from 7 countries in South America, although a decrease was observed in acute stroke applications compared to the pre-pandemic period, when the countries participating in the study

were examined, an increase in acute stroke cases after the pandemic was similar to our study in a sociodemographic and socioeconomic country like Mexico, similar to Turkey seen.[15] In many studies reported from around the world, researchers attributed the decrease in acute stroke applications compared to the pre-pandemic period in the study results to the curfews due to COVID-19, and to the perception that if they apply to the hospital, they may encounter COVID-19 microorganism more likely in the hospital due to inpatients due to COVID-19. In our study, contrary to the literature, the increase in acute stroke cases compared to the pre-pandemic; It can be attributed to the higher rate of use of hospital emergency services compared to other countries, the perception of people to get rid of isolation by applying to the hospital due to the release of the curfew in the pandemic on the condition of going to the hospital, and the fact that Turkish people do not think that the risk of transmission will increase in the hospital as a result of the fatalistic understanding. We find it natural that the picture revealed by COVID-19 causes coagulation disorders and vascular problems occur, and the increase in acute ischemic stroke cases with the decrease in mobility during the isolation period.

Study Limitations

Limitations of this study are the inability to enter the subtypes because the diagnosis of stroke/TIA/ICH in hospitals was obtained using hospital ICD codes. Centers contributing to this data have hospital information systems to track stroke admissions; therefore, the data from this analysis are correct. Details on patient-level data, including demographic information, stroke subtypes, and clinical outcomes, were not collected because they were not the focus of the study. As in all other studies, the frequency of stroke may have been low due to our data, the frequency of admission at each center and during the study period, and the individual fear of the epidemic. The definition of the pandemic period may differ from country to country, as the epidemic begins and peaks at different times in different places.

CONCLUSION

Stroke will continue to be the reason for hospital admissions, increasing its importance in the future with the prolonged life expectancy and aging world population. We think that it is important to examine stroke, which is a vascular cause of death, during pandemic periods in our world where there is always the suspicion of encountering a pandemic again. With the right health policies, early applications of stroke to health institutions can be triggered and encouraged, even under the pressure of the pandemic. As in every disease, early admission to the health center will prevent many complications, including morbidity, mortality and prolongation of the rehabilitation process.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Bursa City Hospital Ethics Committee (Date: 04/01/2023, Decision No: KAEK 2023-1/4).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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The relationship between osteoporosis and non-dipper hypertension in postmenopausal women

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ABSTRACT

Aims: Previous studies have shown association between hypertension and osteoporosis, however has not been evaluated alterations of circadian blood pressure patterns. The present study investigated the effects of osteoporosison the circadian blood pressure assessed by ambulatory blood pressure monitoring.

Methods: 68 patients (mean age: 61.3±8.7 years, 34 dipper and 34 non-dipper patients) with postmenopausal hypertension (HT) were prospectively enrolled in this study. Ambulatory blood pressure monitoring was performed on all patients. We measured bone mineral density (BMD) and T-score by dual X- ray absorptiometry at the lumbar spine. The receiver operating characteristics (ROC) curve was used to demonstrate the sensitivity and specificity of lumbar spine BMD, optimal cut - off value for predicting non-dipper hypertension (NDHT).

Results: T-score and BMD in non-dipper patients were lower than the dipper patients. There was a significant correlation between the rate of fall in night of mean blood pressure and both lumbar spine T-score and lumbar spine BMD (r= 0.330 and P =0.006 vs r=0.322 and P =0.007, respectively). A lumbar spine BMD <0.944 g/cm² measured by DXA had a 64.7% sensitivity and 64.7% specificity in predicting non-dipper hypertension at ROC curve analysis.

Conclusion: Low lumbar spine BMD is an independent and strong predictor of non-dipper hypertension. Osteoporosis may be an indicator of cardiovascular risk. Therefore, osteoporotic patients should be treated more aggressively.

Keywords: Menopause, non-dipper hypertension, osteoporosis

INTRODUCTION

Hypertension (HT) is a common disorder throughout society and it represents an important risk factor for cardiovascular diseases.[1] It is known that the blood pressure measured by ambulatory blood pressure monitoring (ABPM) predicts the cardiovascular event risk better than blood pressure measured in office.[2] As is already known, blood pressure has a circadian rhythm and this rhythm can be non-invasively assessed by ABPM. Among patients with essential HT, almost 25% also have non-dipper hypertension (NDHT).[3] The pathophysiological mechanism underlying NDHT is not yet fully understood. It is known that the rate of target organ damage among patients with NDHT is higher than the rate in patients with dipper hypertension (DHT).[4]

Osteoporosis is one of the causes of morbidity and mortality in the elderly. In women, the incidence of both osteoporosis and HT increases with menopause. HT was shown to be more common among osteoporotic women than non-osteoporotic women. This was explained by the elevation in the incidence of arterial stiffness and the levels of osteoprotegerin that occur concomitant to osteoporosis.[5] Interestingly, previous studies have demonstrated increased osteoporosis incidence in hypertensive patients and some even argued that HT is a risk factor for fractures.[6] Increased levels parathyroid hormone (PTH),[7] osteoclastic activity of the renin-angiotensin-aldosterone system[8] and decreased serum ghrelin levels were responsible for this situation.[9]

Although there are several studies[3,9,10] demonstrating the relationship among menopause, HT and osteoporosis in women, no study has been performed thus far to assess the relationship between osteoporosis and ambulatory blood pressure. The purpose of this study is to determine the effects of osteoporosis in postmenopausal women on circadian blood pressure assessed by ABPM.

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METHODS

The study was carried out with the permission of Erzurum Province Training and Research Hospital Ethics Committee (Date: 04.02.2011, Decision No: 417). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Population

A total of 68 consecutive women patients with postmenopausal HT, who were admitted to the cardiology department of our hospital, were prospectively evaluated. Patients fulfilling the following criteria were included in the study: (i) being post-menopausal; (ii) no previous diagnosis of osteoporosis; and (iii) hypertensive and/or antihypertensive drug use. The exclusion criteria were: coronary or peripheral artery disease, heart failure, valve disease, arrhythmia, chronic diseases (renal, hematologic, inflammatory, hepatic, malignancy), thyroid dysfunction, alcohol use, secondary HT, medication associated with bone metabolism (e.g anti-osteoporotic, steroid, hormone replacement therapy, statin and warfarin), history of bone fractures and inconvenient sleep durations (<6 h or>12 h).

Ambulatory Blood Pressure Monitoring

Twenty-four hour ABPM was conducted by a Tracker NIBP2 device (del Mar Reynolds, Hertford, UK). The average 24-hour, daytime and nighttime blood pressures (systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP)) were evaluated in all patients. An appropriate cuff size was selected for each subject. Daytime was defined as 07:00-23:00 h and nighttime was defined as 23:00-07:00 h. Measurements were performed at 15-and 30-minute intervals during the day and night, respectively. Measurements were not considered valid if SBP was <70 mmHg or >250 mmHg, DBP <40 mmHg or >150 mmHg, or PP < 10 mmHg. The patients sleeping and awake times were recorded and total sleep duration was calculated. The nocturnal fall rates of SBP, DBP and MBP were calculated according to the following formula:[1,11] the rate of fall in night (%) = (daytime BP - nighttime BP) × 100/ daytime BP). Patients with 10-20% rate of fall in night of MBP were classified with DHT (n=34), while patients with<10% were classified with NDHT (n=34).[12]

Measurement of Bone Metabolism By Dual X Ray Absorptiometric (DXA)

Bone metabolism was measured using a whole body DXA scanner (Hologic QDR-2000, Bedford, MA, USA). Bone mineral density (BMD) was measured in all patients at the lumbar spine (L1-4) with a coefficient of variation of 0.8% and <1.2%, respectively. Bone mineral density (g/cm²) for each subject was calculated by dividing the amount of bone mineral content by the projected area of the region of interest. Results were also expressed as a T-score (defined as the number of standard deviations [SDs] below the mean value of young women) and as Z-score (defined as the number of SDs below the mean for women of the same age). Women with a T-score < -2.5 were considered osteoporotic.[8]

Data Sources and Definitions

Blood pressure was measured at the clinics on the day of admittance and the following week, and the mean value of these two measurements was taken as the office blood pressure. Patients were accepted as hypertensive if the following were present: 1) systolic pressure was >140 mmHg and/or diastolic pressure was > 90 mmHg in office; 2) current use antihypertensive medication; or 3) average 24 h BP value above 130/80 mmHg by ABPM.[2] Neck circumference (NC) was measured at the midpoint of the neck between the mid-cervical spine and the mid-anterior neck 0.5 cm below the laryngeal prominence.[13] Waist circumference (WC) was measured in a standing position after normal expiration, midway between the lower rib margin and the iliac crest.[14] Transthoracic echocardiography for left ventricular mass index and epicardial fat thickness was performed using a system V (Vingmed, GE, Horten, Norway) with a 2.5-MHz phased-array transducer. Left ventricular mass index was calculated by the recommended formulas in the literatüre.[15] Epicardial fat thickness was measured on the free wall of the right ventricle at the end-diastole using both parasternal long and short axis views on M-mode echocardiography in three cardiac cycles.[16] A woman was considered to be postmenopausal if she did not report having a menstrual period within the past 12 months (provided that it is not associated with a gynecological disease).[3] Metabolic syndrome was diagnosed according to the National Cholesterol Education Program Adult Treatment Panel III criteria. Any patient can be diagnosed with Metabolic syndrome when any three of the following criteria are present:[17]

High WC, whose thresholds depend on populations and countryspecific definitions (≥102 cm and ≥88 cm for European men and women respectively);[18]

- Blood TG ≥ 150 mg/dl;
- Blood HDL cholesterol < 40 mg/dl in men and
- Blood fasting glucose ≥ 100 mg/dl.

Statistical Analysis

Quantitative variables were expressed as mean value ± SD, and qualitative variables were expressed as percentages (%). A comparison of parametric values between two groups were performed by means of two-tailed student's t-test. Categorical variables were compared by the likelihood-ratio $\chi 2$ test or Fisher's exact test. Pearson's correlation coefficients examined the degree of association between examined variables. A P value <0.05

was considered as significant. The receiver operating characteristics (ROC) curve was used to demonstrate the sensitivity and specificity of lumbar spine BMD, optimal cut-off value for predicting NDHT in patients, and postmenopausal HT. The effects of different variables on NDHT was calculated in the univariate analysis for each. The variables for which the unadjusted p value was <0.10 in the logistic regression analysis were identified as potential risk markers and included in the full model. The model was reduced by using backward elimination multivariate logistic regression analyses, and potential risk markers were eliminated using likelihood ratio tests. Confidence interval (CI) was 95%. All statistical studies were carried out with SPSS package program (version 15.0, SPSS, Chicago, Illinois, USA)

RESULTS

Baseline characteristics are shown in Table 1. The NDHT group was older than DHT group. The rate of ≥ five years of antihypertensive drug use was higher the NDHT group (P=0.04). While 95.5% of the patients was using an antihypertensive drug, the ratio of using more than two drugs (combination therapy) among these patients was 5.8%.

Table 1. Baseline characteristics of study patients

	Dipper hypertension (n= 34)	Non-dipper hypertension (n=34)	P value
Age, y	58.8±6.6	63.3±10	0.03
Height, cm	154.2±7	153.7±6.9	0.74
Weight, kg	78.9±9.7	78±14.8	0.77
Neck circumference, cm	34.7±2.1	34.8±2.2	0.91
Waist circumference, cm	79.7±11.8	83.2±17.5	0.32
Diabetes mellitus	6 (17.6)	8 (23.5)	0.54
Hyperlipidemia	19 (55.8)	23 (67.6)	0.31
Family history for hypertension	17 (50)	10 (29.4)	0.12
Current smoker	3 (8.8)	2 (5.8)	0.64
Metabolic syndrome	32 (94.1)	28 (82.3)	0.13
Hypertension time, y	5 ±4.4	6.1±4.4	0.28
Medication time for hypertension			0.04
<5 Year ≥5 Year	20 (58.8) 14 (41.1)	12 (35.2) 22 (64.7)	
Only β-blocker use	4 (11.7)	5 (14.7)	0.72
Only ACE-I use	12 (35.2)	7 (20.5)	0.17
Only ARB use	11 (32.3)	12 (35.2)	0.79
Only diuretics use	1 (2.9)	0 (0)	0.31
Only CCB use	4 (11.7)	5 (14.7)	0.72
Combined drug use	1 (2.9)	3 (8.8)	0.3
Left ventricular mass index, g/m²	79±17.4	79.7±18.6	0.88
Epicardial fat thickness, mm	5.8±1.6	5.6±1.7	0.66

Mean values (SD) and % (n) are reported for continuous and categorical variables, respectively. ACE-I: Angiotensin Converting Enzyme Inhibitor, ARB:Angiotensin Receptor Blocker, CCB: Calcium Channel Blocker

With respect to baseline laboratory status (Table 2), there was no significant difference lipid profile, glucose level, uric acid and mean platelet volume (MPV) between groups. The red cell distribution width (RDW) value was significantly higher in non-dipper patients than in the dipper patients.

Table 2. Laboratory findings of patients

	Dipper hypertension (n= 34)	Non-dipper hypertension (n=34)	P value
Creatinine, mg/dl	0.6±0.1	0.7±0.2	0.27
Glucose, mg/dl	128.2±67	113.3±34.3	0.25
Total cholesterol, mg/dl	212.2±38.5	200.5±46.1	0.25
LDL cholesterol, mg/dl	129.2±47.8	120.6±46	0.45
HDL cholesterol, mg/dl	46±14.5	48.5±24.4	0.61
Triglycerides, mg/dl	154.5±66.6	152.8±60.1	0.91
Hemoglobin, g/dl	13.7±0.8	13.5±1.3	0.4
RDW,%	10.9±0.63	11.4±0.8	0.01
MPV,fl	8.1±1	8.1±0.97	0.99
Uric acid, mg/dl	4.1±0.8	4.6±1.4	0.09

Mean values (SD) are reported for continuous variables. LDL: Low-density lipoprotein, HDL: High-density lipoprotein, RDW: Red cell Distribution Width, MPV: Mean Platelet Volume

The results of ABPM and DXA for both groups are shown in Table 3. With respect to ABPM, there was no significant difference in SBP and DBP (day, night, 24 h). Total sleep duration was similar between the two study groups. Non-dipper patients had a longer duration of menopause than dipper patients and osteoporosis was more common in this patient group (50% vs 20.5%; p=0.01, respectively). Lumbar spine BMD was lower in non-dipper patients.

Table 3. The results of ABPM and DXA of patients

	Dipper hypertension (n= 34)	Non-dipper hypertension (n=34)	P value
Sleep time, h	8±1.2	8.2±1.6	0.74
Average day-time SBP, mmHg	138.9±11.3	134±17.6	0.27
Average night-time SBP, mmHg	122.9±11.5	130.6±19.6	0.05
Average 24-hour SBP, mmHg	134.2±10.5	133.7±18	0.8
Average day-time DBP, mmHg	92.3±10.4	89.5±15.4	0.38
Average night-time DBP, mmHg	76±8.5	79±10.6	0.14
Average 24-hour DBP, mmHg	87.6±9.1	84±10.6	0.15
Average day-time MBP, mmHg	108.4±10.9	105.9±13.1	0.39
Average night-time MBP, mmHg	91.2±9.7	96.5±13.2	0.06
Average 24-hour MBP, mmHg	104.1±12.2	100.6±12.7	0.25
Nocturnal fall in SBP, %	11.1±3.9	3.8±3.6	<0.0001
Nocturnal fall in DBP,%	17.9±6	7.7±7.4	<0.0001
Nocturnal fall in MBP,%	16.2±3.7	4.5±4.3	<0.0001
Office SBP, mmHg	139.4±12.3	140.5±19.1	0.76
Office DBP, mmHg	79.6±6	80.9±8.6	0.48
Office heart rate, beats/min	81.7±8.1	81.4±10.6	0.9
Post-menopauosal time,y	10.6±7.9	15.7±10.33	0.02
Osteoporosis	7 (20.5)	17 (50)	0.01
Lumbar spine BMD, gr/cm²	1±0.13	0.91±0.11	0.004
Lumbar spine T score, SD	-1.5±1	-2.2±0.94	0.003

Mean values (SD) and % (n) are reported for continuous and categorical variables, respectively. ABPM: Ambulatory Blood Pressure Monitoring, DXA:Dual X-ray Absorptiometric, SBP: Systolic Blood Pressure, DBP:Diastolic Blood Pressure, MBP: Mean Blood Pressure, BMD: Bone mineral density

There was a significant correlation between the rate of fall in night of MBP and both lumbar spine T-score and lumbar spine BMD (r= 0.330 and P=0.006 vs r=0.322 and P=0.007, respectively). Interestingly, the rate of the fall in night for SBP and both BMD and T-score was not significantly correlated (Table 4, Figure 1).

Table 4. Correlation (r) between dansitometric features of bone's and the rate (%) of fall in night of BP's

		r value	P value
MBP	Lumbar spine T-score	0.330	0.006
	Lumbar spine BMD	0.322	0.007
SBP	Lumbar spine T-score	0.208	0.09
	Lumbar spine BMD	0.204	0.09
DBP	Lumbar spine T-score	0.203	0.09
	Lumbar spine BMD	0.204	0.09

Abbreviations: MBP: Mean Blood Pressure,SBP:Systolic Blood Pressure,DBP: Diastolic Blood Pressure, BMD:Bone mineral density

The ROC curves of lumbar spine BMD for predicting NDHT are shown in Figure 2. Lumbar spine BMD <0.944g/cm² measured had a 64.7% sensitivity and 64.7% specificity in predicting of NDHT. When we divided the study population into two groups (Table 5) according to the <0.944g/cm² lumbar spine BMD cut-off value used in the ROC analysis, NDHT and age were significantly higher in the low lumbar spine BMD group. While the low spine BMD group had lower height and NC, they had higher weight and WC. Office and daytime DBP was lower in the low lumbar spine BMD group, while there was no difference between the groups with respect to other blood pressures. The rate of fall in night of MBP was significantly lower in low lumbar spine BMD group; however this significance was not observed for SBP or DBP. Echocardiographic findings indicating LV remodeling were similar between the two groups, whereas epicardial fat thickness significantly increased in the high lumbar spine BMD group.

An unadjusted p value < 0.10 in the univariate analysis was identified as a potential risk marker for NDHT and was included in the full model. Age >65 y, hypertension duration (>5y), glucose, HbA1c, RDW, high uric acid (≥6 mg/dl), weight, low lumbar spine BMD, post-menopausal time, height, NC, WC and epicardial fat thickness were analyzed using a multivariate logistic regression model. At the multivariate analyses, hypertension duration (>5 y), RDW, high uric acid (≥6mg/dl) and low lumbar spine BMD (odds ratio=6.13, 95% confidence interval:1.2-30.6, p=0.02) were still independent predictors of NDHT (Table 6).

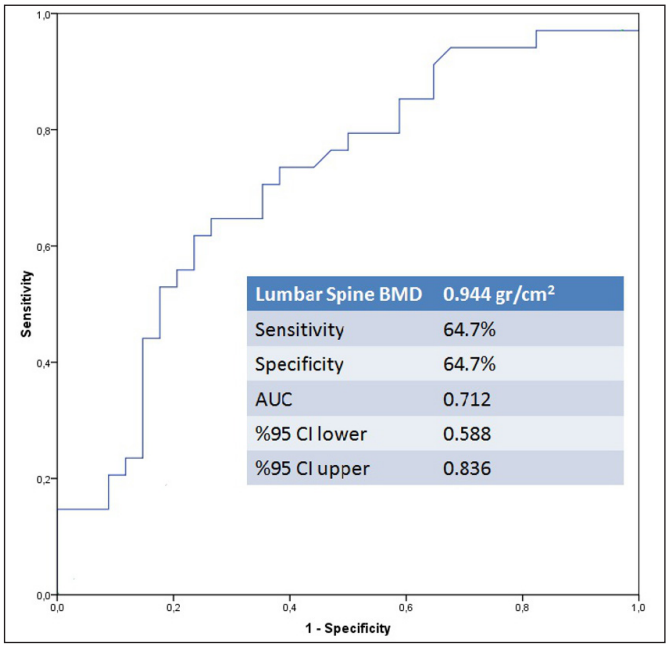

Figure 2. The receiver-operating characteristic (ROC) curve of lumbar spine BMD for predicting non - dipper hypertension (BMD: Bone mineral density, AUC: area under curve, CI: confidence interval)

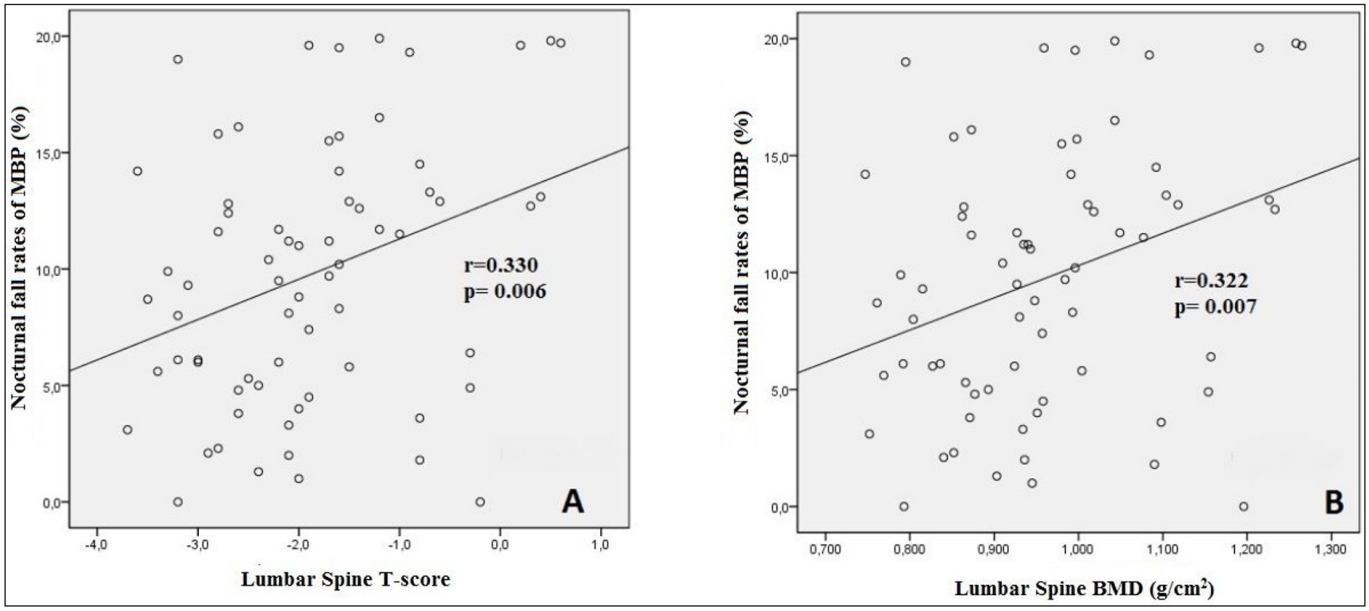

Figure 1. Relation of the rate (%) of fall in night of MBP with lumbar spine T - score (A) and lumbar spine BMD (B) in a scatter figures (MBP: Mean blood pressure, BMD: Bone mineral density)

Table 5. The results of patients stratified by lumbar spine BMD

	High lumbar spine BMD (n =34)	Low lumbar spine BMD (n= 34)	P value
Age, y	56.7±5.7	65.4± 9.1	<0.001
Diabetes mellitus	9 (26.4)	5(14.7)	0.23
Current smoker	2 (5.8)	3 (8.8)	0.64
Family history for hypertension	17 (50)	10 (29.4)	0.22
Hypertension time,y	6.1±4.7	5.2±4.1	0.47
Medication time for hypertension			0.33
<5 Year ≥5 Year	16 (47) 18 (53)	19 (55.8) 15 (44.1)	
Height, cm	155±7	152.3±7.3	0.04
Weight, kg	74.2±12	82.7±11.6	0.004
Neck Circumference, cm	35.4±2.2	34±1.7	0.007
Waist Circumference, cm	80.6±9.5	90.9±11.2	<0.001
Only Diuretics use	0 (0)	1(2.9)	0.31
Only ACE-I use	10 (29.4)	9 (26.4)	0.78
Only ARB use	11 (32.3)	12 (35.2)	0.79
Combine drug use	1 (2.9)	3 (8.8)	0.3
Average day-time SBP, mmHg	140.1±11.7	133.6±16.9	0.07
Average night-time SBP, mmHg	127.8±16	125.8±17.1	0.62
Average 24-hour SBP, mmHg	136.5±11.8	131.5±16.8	0.16
Average day-time DBP, mmHg	91.7±10.1	86±11.7	0.03
Average night-time DBP, mmHg	79±9.6	76.7±9.8	0.34
Average 24-hour DBP, mmHg	88±8.9	83.7±10.6	0.07
Average day-time MBP, mmHg	106.4±10.7	104.9±13	0.46
Average night-time MBP, mmHg	94.9±11.9	93±11.8	0.53
Average 24-hour MBP, mmHg	105.1±12.3	99.6±12.3	0.07
Office SBP, mmHg	141.3±14.4	138.6±17.5	0.48
Office DBP, mmHg	82.4±6.3	78.1±7.9	0.01
Nocturnal fall in SBP, %	8.4±6.3	5.8±5	0.06
Nocturnal fall in DBP,%	14±9.1	10.5±6.7	0.07
Nocturnal fall in MBP,%	13±6.3	7.8±5.6	0.001
Non-dipper hypertension	12 (35.2)	22 (64.7)	0.01
Lumbar spine T score, SD	-1.04±0.79	-2.7±0.51	<0.001
Post-menopauosal time,y	8.4±7.1	17.9±9.2	<0.001
Left ventricular mass index, g/m²	78.1±18	80.6±17.9	0.58
Epicardial fat thickness, mm	6.2±1.8	5.3±1.2	0.02

Mean values (SD) and % (n) are reported for continuous and categorical variables, respectively. ACE-I: Angiotensin Converting Enzyme Inhibitor, ARB:Angiotensin Receptor Blocker, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, MBP: Mean Blood Pressure, BMD:Bone mineral density, RDW: Red cellDistribution Width, MPV: Mean Platelet Volume

Table 6. Effects of various variables on non-dipper hypertension in univariate and multivariate logistic regression analyses

Variables	Univariate OR	% 95 CI	P value	Multivariate OR	% 95 CI	P value
Age	1.067	1.003-1.135	0.04	-	-	-
Age >65 years	2.012	0.703-5.76	0.193	-	-	-
Hypertension time (>5 y)	2.96	1.104-7.942	0.03	4.542	1.126-18.325	0.03
Glucose	0.994	0.984-1.005	0.25	-	-	-
Hemoglobin A1c	0.805	0.469-1.379	0.42	-	-	-
RDW	0.28	0.103-0.78	0.016	4.809	1.161-19.912	0.03
Uric asit (>6 mg/dl)	7.07	0.803-62.311	0.07	27.89	1.32-121.82	0.03
Weight	0.994	0.95-1.033	0.77	-	-	-
Low lumbar spine BMD	3.36	1.2-9.08	0.017	6.13	1.2-30.6	0.02
Post-menopauosal time	1.063	1.006-1.123	0.02	-	-	-
Neck circumference	1.013	0.8-1.27	0.91	-	-	-
Height	0.98	0.92-1.059	0.74	-	-	-
Waist circumference	1.03	0.98-1.08	0.32	-	-	-
Epicardial fat thickness	0.52	0.2-9.3	0.66	-	-	-

OR: odds ratio, CI: confidence interval, RDW: red cell distribution width, BMD:Bone mineral density

DISCUSSION

This study includes three major findings in patients with postmenopausal HT: i) osteoporosisis more common in patients with NDHT; ii) NDHT frequency is higher in patients with low lumbar spine BMD; and iii) lumbar spine BMD <0.944 g/cm² is an independent risk factor for NDHT.

Impairment in the circadian rhythms of bone tissue and blood pressure as seen with menopause increases the incidence of osteoporosis and HT.[3,5] These two diseases are associated with mortality and morbidity.[6] Physiologically, the osteoblastic and osteoclastic activities of the bones are in an equilibrium and osteoclastic activity is greater during the night than during the day.[19]

The most interesting finding of this study is that NDHT was more frequent the patients with low lumbar spine BMD, which to our knowledge, this is the first study to report an association between osteoporosis and NDHT. This clinical coexistence may be explained by four pathophysiological mechanisms that blunted the nocturnal decline in blood pressure as well as increase in bone loss during nighttime. First, PTH; the physiological rhythmic secretion of this hormon which is associated with HT is impaired in osteoporotic patients.[20,21] In patients with low BMD reaches peak levels between 02.00-11.00 a.m. compared to patients with normal BMD.[7] Secondly; plasma melatonin and ghrelin levels, which are physiologically expected to be elevated during nighttime, however have reduced serum levels in osteoporotics.[9,22] In addition to their osteoblastic properties, these two hormones are also known to have positive effects on cardiovascular tissues and it was demonstrated that a decrease in serum levels of these hormones is a risk factor for NDHT.[9,22] In patients with excessive sodium consumption, a negative correlation was noted between urine sodium/creatinin ratio and BMD. As known, excessive sodium consumption is one of the possible causes of NDHT.[10] Fourthly, autonomous system disorders worth mentioning. Hyperactivation of the sympathetic system during the night is known to increase bone resorption and the frequency of NDHT.[23] Due to the above mentioned four reasons, on one hand osteoclastic activity is maintained and on the other hand, a decrease in blood pressure will be inadequately during the night.

Gunebakmaz O et al.[24] demonstrated that increased RDW levels in patients with NDHT and they have shown a negative correlation between the rate of nocturnal fall in SBP and RDW. However, Afsar B et al.[25] determined that a high level of uric acid is an independent marker of NDHT. Although no relation between osteoporosis and RDW levels has been reported thus far in the literature, it is argued that high uric acid levels may be protective against osteoporosis.[26] Our findings showed that the uric acid levels were similar between the two groups in this study. We have observed that RDW and high uric acid level may be among the independent markers of NDHT. Although the relation between RDW or high uric acid level and hypertension has not yet been completely understood, this may be considered to be associated with the increased inflammatory response and oxidative stress in this group of patients.[24,25]

In the current study, we observed that patients with high lumbar BMD had increased NC and epicardial fat thickness; according to the literature[27] this condition has the closest clinical association with obstructive sleep apnea. Sforza E et al.[13] demonstrated that in patients with obstructive sleep apnea, apnea-related intermittent hypoxia induces bone remodeling process. Our findings indicated that patients with high lumbar BMD had high NC and epicardial fat thickness may be associated with obstructive sleep apnea.

Limitations of the Study

Several limitations should be taken into consideration while assessing the results of this study. First, only a small number of patients from one center were enrolled. However, in the case of postmenopausal women, the patient ratios for HT and osteoporosis were similar (50% and 50%, respectively) with previous studies.[3,9,25] Secondly, parameters that are biochemical markers of bone turnover (such as calcium) and plasma renin activity, PTH, ghrelin, and melatonin, all of which effect bone turnover, were not assessed in this study. Thirdly, keeping in mind that the HT induced osteoporosis develops in the target organ damage and activity of the renin-angiotensin-aldosterone system, we might have underestimated the prevalence of osteoporosis because of some of the patients were using antihypertensive medications. Further information can be provided on this subject by extensive studies performed on large patient populations.

CONCLUSION

In patients with postmenopausal HT, the decrease in lumbar spine BMD increases the frequency of NDHT. Therefore, the detection of osteoporosis in postmenopausal hypertensive women may be a predictor of cardiovascular risk.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erzurum Province Training and Research Hospital Ethics Committee (Date: 04.02.2011, Decision No: 417).

Informed consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Utility of immature granulocyte count in differentiating between pyelonephritis and cystitis in pediatric patients

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ABSTRACT

Aims: Immature granulocyte (IG) is an easily accessible and inexpensive test that can be measured in hemogram parameters without additional analysis. It can be used in differentiation because of its practical applicability. This study aimed to investigate the role of IG count and inflammation-related complete blood count (CBC) parameters in differentiating between cystitis (CYS) and pyelonephritis (PYL) in pediatric patients.

Methods: This retrospective cross-sectional study analyzed data from 79 pediatric patients (40 with PYL and 39 with CYS) who presented at a hospital pediatric outpatient clinic between January 2020 - February 2021. In addition to clinical symptoms and signs, laboratory and urinalysis results were evaluated. Laboratory analyses focused on IG count, IG percentage, and all hemogram parameters.

Results: No significant demographic differences were observed between the PYL and CYS groups ($p>0.05$). IG counts, and C-reactive protein levels significantly differed between the two conditions ($p<0.001$). IG count was identified as an independent factor for distinguishing between PYL and CYS, with a sensitivity of 89.5% and a specificity of 92.6% ($p<0.0001$).

Conclusion: The IG count, an easily accessible and cost-effective test, is valuable in differentiating PYL and CYS ($p<0.001$). This finding holds promising implications for the prompt and accurate diagnosis of these conditions in pediatric patients.

Keywords: Immature granulocyte, C-reactive protein, pyelonephritis, cystitis

INTRODUCTION

Urinary tract infections (UTIs) are among the common diseases in early childhood, even though the findings or symptoms may vary according to demographic conditions.[1] Due to long-term sequelae in children, it is essential to distinguish between upper and lower UTIs.[2,3] In the diagnostic evaluation of infection, when it is limited to the bladder, it may cause pyelonephritis (PYL). If the infection spreads to the kidneys, we encounter the clinic of cystitis (CYS).[4] Although the long-term damage of CYS is limited, the situation may be more troublesome for pyelonephritis.[5] Because it increases the risk of renal scarring, which can lead to proteinuria and chronic kidney disease in the long term.[6,7] Although the clinical diagnosis of acute pyelonephritis is based on symptoms such as fever and pain associated with pyuria, urine culture, and blood tests support the diagnosis.[8,9] Screening 99mTc-dimercaptosuccinic acid (DMSA) is beneficial but has a high cost and radiation exposure.[10]

C-reactive protein (CRP) and procalcitonin are commonly used parameters for laboratory evidence of pyelonephritis.[11] Although it is the cheapest and most accessible method, its specificity and sensitivity are insufficient.[12] Today, immature granulocyte (IGs), especially in the routine evaluation of neonatal sepsis and associated with many inflammatory events, nine can be used in UTI differentiation in correlation with CRP. It can be preferred to CRP because the hemogram device analyzes it without requiring additional analysis, such as CRP.[13] The IG count, calculated by counting the cells in the area above the neutrophil granulocytes, is the combination of promyelocyte, myelocyte, and metamyelocyte cells that mature with myeloid cells. It can be helpful for prompt diagnosis and discrimination of UTIs.[9,14]

Immature granulocyte is an easily accessible and inexpensive test that can be measured in hemogram parameters without additional analysis and can be used

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in differentiation because of its practical applicability. The study investigates the role of the IG count and inflammation-related CBC parameters in CYS and pyelonephritis and whether these parameters are helpful in the differentiation of CYS and PYL.

METHODS

The study was carried out with the permission of Kastamonu University Clinical Researches Ethics Committee (Date: 21.04.2021, Decision No: 2021/KAEK-143-89). The ethics committee exempted the current research coordinator and participants from the need to get any informed consent due to the retrospective design. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This cross-sectional retrospective research analyzed 79 patients in 2 groups, including 40 PYL and 39 CYS with homogeneous distribution in terms of age and gender, who applied to the hospital pediatric outpatient clinic between January 2020 - February 2021.

Patient Collection

We analyzed the parameters including clinical signs, general laboratory and urinalysis results in PYL (n:40) and CYS (n:39). As seen in the flowchart (Figure 1), according to power analysis (α:0.05 | β:0.10 | Power:0.80), the study needs 72 participants at least. All pediatric patients aged 0-18 were included in the study, and the mean age was 44.1±42.5 for all the participants. Acceptance criteria for the study are: being in the pediatric age group and having PYL or CYS. Exclusion criteria for research are the history of systemic disease (e.g., diabetes, autoimmune), long-term or recent use of drugs, bacterial infection other than UTI, hematological/oncological disease, and lack of hospital record. We confirmed the diagnosis of PYL and CSY at the first admission according to the guidelines: There were symptoms such as fever, dysuria, abdominal pain, and costovertebral/abdominal tenderness during the examination. We evaluated hemogram, urinary culture, radiological evaluation records, and ultrasonography/voiding cystourethrography.

Laboratory Analysis

The laboratory results were obtained using the first sampling data after the admission of all the participants. The IG count, IG percentage (IG count divided by WBC×100), and all the hemogram parameters were analyzed using an XN1000 SYSMEX (Kobe, Japan). The CRP and other routine biochemistry parameters were determined using the DxC 700AU Beckman Coulter Autoanalyzer (Beckman Coulter Co., United States). We analyzed all the variables, including CRP, leukocyte esterase, leukocyte count, IG, neutrophil, platelet, lymphocyte count, and demographic data in both groups.

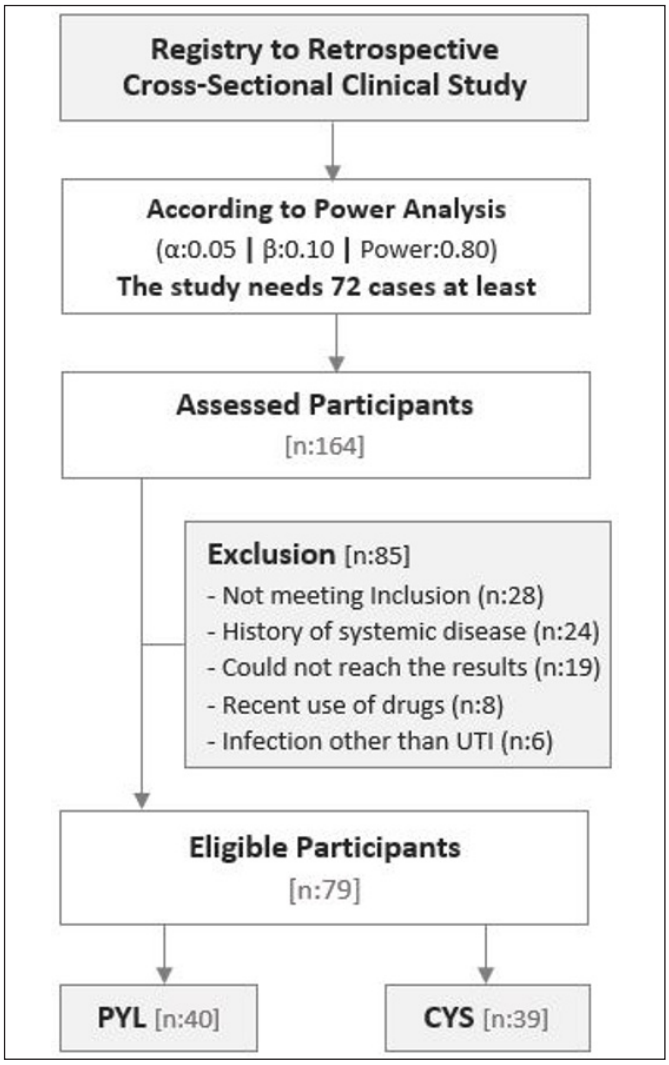

Figure 1. The flowchart presenting the selection of pyelonephritis and cystitis

Statistical Analysis

We used an MS-Windows 64bit-based SPSS program for data evaluation in the study (version 25, USA). The cut-off for significance is a 2-sided-p-value less than 0.05. While Mann-Whitney U was used for continuous data pairwise comparisons, we applied the Chi-square test to compare categorical data. According to the result values, we shared the median and interquartile ranges if the variables are numeric and frequency and percentage if the variables are categorical; In the regular distribution analysis, we used the Kolmogorov-Smirnov test for the suitability of numerical variables. We designed a logistic regression model to determine independent parameters in the differentiation of PYL and CYS. Associations between the IG count and other parameters were assessed using Spearman correlation analysis. Variables were tested using multivariate linear regression analysis to identify the essential independent factors influencing IG count. To evaluate the predictive ability of the PYL from CYS, we used a Receiver operating characteristic (ROC) for IG, CRP, neutrophile, and leukocyte.

RESULTS

All data, including demographic results, were similar in PYL and CYS (p>0.05). The mean age was 43.9 (29.6-58.3 months) and 44.5 (30.6-58.4 months) in PYL and CYS, respectively. Fever was detected in 42% of the patients on examination, while the most common presenting symptoms included dysuria (36.2%), abdominal pain (13.5%), and vomiting (12.1%). There were more urinary leukocytes in PYL than in CYS (p=0.041). No difference was seen in a comparison of PYL and CYS variables in terms of gender (p=0.919), urine bacteria (p=0.156), culture (p=0.513), urine nitrite (p=0.16), and leucocyte esterase (p=0.299). In comparing blood parameters, immature granulocytes, and CRP differed regarding PYL vs. CYS (Table 1).

Table 1. Comparisons of blood parameters according to the groups

Variables	Pyelonephritis	Cystitis	P value
Age, months	43.9±44.8	44.46±40.4	0.959
Leucocyte esterase, U/ml	159±295.2	57±119.3	0.354
C-reactive protein, mg/L	90.2±89.82	4.32±11.9	0.0001
WBC, 10^3/uL	13.6±4.52	8.35±4.37	0.0001
Red blood cell, 10^6/uL	4.57±0.58	4.72±0.49	0.217
Hemoglobin, g/dl	11.7±1.43	12.26±1.85	0.155
Hematocrit, %	35.4±4.34	37.03±5.04	0.154
Neutrophil, 10^3/uL	7.87±3.91	3.27±2.68	0.0001
Lymphocyte, 10^3/uL	4.34±3.07	4.14±2.01	0.743
Monocyte, 10^3/uL	1.22±0.7	0.69±0.54	0.0001
Eosinophil, 10^3/uL	0.16±0.23	0.21±0.14	0.284
Basophil, 10^3/uL	0.049±0.026	0.041±0.025	0.318
IG, 10^3/uL	0.073±0.076	0.021±0.023	0.0001

Abbreviations. IG: Immature granulocyte, WBC: White Blood Cell

There were significant correlations between IGs and the parameters, including eosinophil (r:-0.242; p=0.036), basophil (r:-0.278; p=0.016), WBC (r:0.494;p<0.001), CRP (r:0.705; p<0.001), neutrophil (r:0.459; p<0.001). We designed a logistic regression model to determine the effectiveness of IG in differentiating PYL and CYS. The model Nagelkerke's R square was 51.4%. This model could detect PYL with 92.5%. Moreover, IG was an independent factor in differentiating PYL and CYS (Table 2). Dominant parameters for predicting PYL, IG (p<0.0001), and CRP (p<0.0001). Leucocyte esterase (p=0.331) did not show significance in the ROC analysis (Table 3). The AUC for the CRP, IG, leukocyte, and neutrophil were 0.946 (95% CI: 0.891-0.999), 0.957 (95% CI: 0.9173-0.997), 0.861 (95%CI: 0.772-0.951), and 0.859 (95% CI: 0.768-0.951), respectively. The IG count's cut-off value as a PYL predictor was 0.031, with an 89.5% sensitivity and 92.6% specificity value (Figure 2).

Table 2. Logistic regression for differentiating pyelonephritis and cystitis

Variables	B	Wald	P value	Exp (B)	95% CI	
					Lower	Upper
IG, 10^3/uL	-76.918	16.744	0.0001	0.0001	0.00001	0.0001
Sex, male	0.853	1.027	0.311	2.348	0.450	12.235
Age, months	-0.008	1.126	0.289	0.992	0.976	1.007
Constant	2.623	12.054	0.001	13.774	-	-

Abbreviations. IG: Immature granulocyte, CI: Confidence interval, Exp(B): the odds ratio.

Table 3. The ROC analysis for predicting pyelonephritis against cystitis

Variables	AUC	SE	P value	95% CI	
				Lower	Upper
C-reactive protein, mg/L	0.946	0.027	0.0001	0.892	0.999
Leucocyte esterase, U/ml	0.565	0.067	0.331	0.435	0.696
WBC, 10^3/UL	0.861	0.046	0.0001	0.772	0.951
NEUT, 10^3/UL	0.859	0.046	0.0001	0.768	0.949
IG, 10^3/UL	0.957	0.020	0.0001	0.917	0.997

Abbreviations. IG: Immature granulocyte, WBC: White Blood Cell, NEU: Neutrophile, CI: Confidence interval, AUC: Area Under Curve, SE: Standart error.

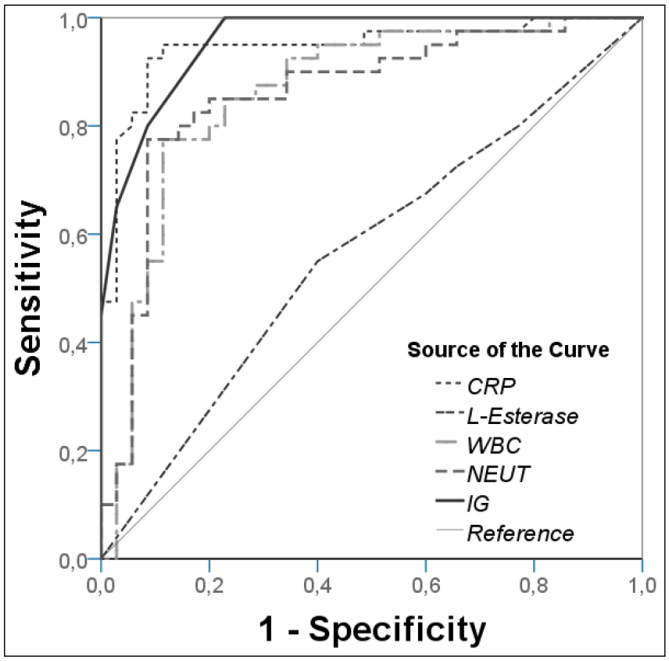

Figure 2. The ROC analysis for discrimination of pyelonephritis from cystitis

DISCUSSION

Knowing the long and short-term clinical importance of the distinction between PYL and CYS and seeking a diagnostic marker out of necessity, we conducted a detailed IG analysis for these two diseases regarding cost and practical applicability. This first study presented that IG count can cost-effectively meet all needs in distinguishing these two diseases.

Today, DMSA screening is considered the gold standard for diagnosing PYL.[10] DMSA is accessible in almost all centers in Turkey, hence using the immature granulocyte

count for a patient's acute management in provincial hospitals is appropriate. In addition, as a costly diagnostic tool, biomarkers such as CRP are also regarded as valuable in determining the presence of PYL.[11] It is noteworthy to remember that the level of CRP can increase in many other conditions, such as viral infection, non-infectious inflammatory diseases, and myocardial infarction.[15] CRP test in UTIs was investigated alone or in combination with other parameters in several studies regarding its predictive value.[16] According to Biggi et al.[17] and Kotolula et al.[18], CRP's specificity and sensitivity in PYL were 63-98% and 5-92%, respectively. Guven et al.[19] examined the diagnostic efficacy of CRP in diagnosing PYL and found that the cut-off of 2, 5, and 10 mg/dl showed a sensitivity of 67%, 68%, and 73%, respectively.

Although CRP and CBC are comprehensive tests in the diagnosis and follow-up of infections, the IG is a newly evaluated marker to potentially detect inflammation or bacterial infection. The appearance of IGs in peripheral blood indicates an early response to infection, and inflammation, especially in inpatients other than the neonate and pregnant population.[20] Detecting IG quickly and reliably provides a new diagnostic opportunity for related diseases. Nierhaus et al.[21] showed the diagnostic power of IGs on discrimination between infected and uninfected patients with systemic inflammatory response syndrome at 89.2% sensitivity and 76.4% specificity. They also found that IG is a more potent predictive marker than CRP and interleukin.[6] In a similar article, Henriot et al.[22] showed that the number of IGs in children with viral or bacterial infections was significantly higher than in healthy children.

Few studies on the relationship between UTI and IG have shown evidence to be very useful in bacterial infections. Incir et al.[23] in a study with 55 patients and 47 controls, presented evidence that IG count is a readily available measure that can be used in conjunction with the CRP value and other indicators in managing UTIs. Yoon et al.[24] investigated the role of the delta neutrophil index, which shows the ratio of IG to total neutrophil count in infants with febrile UTI-associated bacteremia. Significantly higher values were observed compared to infants with UTI and without bacteremia. In the present analysis, IGs, CRP, and hematologic infection markers increased in both groups, consistent with these two studies. However, the increase rate exhibited a positive divergence in the form of a more substantial and tremendous increase in IG values for PYL than CYS.

Incir et al.[23] found 65.45% sensitivity and 65.96% specificity of the IG count to predict a UTI above the 0.03 cut-off value.Lee et al.[25] reported a sensitivity value of <50% for IG as a predictor of UTI and a moderate specificity ranging from 70% to 90%. In contrast, another study by Park et al.[26] examining the number of IGs in patients with UTIs has reported high sensitivity values of 82% to 95%. This highly significant difference in susceptibility may be related to the study populations' adult age or systemic infections such as bacteremia or sepsis in selected patients. In the present study, IG presented an independent factor in differentiating PYL and CYS. IG and CRP were the dominant parameters for prediction in the ROC analysis. The cut-off value of the IG count as a predictor of PYL was 0.031, with an 89.5% sensitivity and 92.6% specificity value.

The strength of the present study is that, to our best knowledge, it is novel research to evaluate the diagnostic value of IG count in the differentiation of PYL and CSY in children. Homogeneous demographic data and detailed analysis of patient data are valuable. The main limitation of the research is that we examined PYL with clinical, laboratory, and ultrasonographic findings. The number of patients was relatively limited to perform the two-group analyses. Sensitivity and specificity values will be more accurate for the number of IGs calculated with a more significant number of patients.

CONCLUSION

Immature granulocyte is an easily accessible and inexpensive test. It helps differentiate PYL from CYS because it has practical applicability instead of Crp and related hemogram parameters. Our results strongly support that IG count can meet all needs cost-effectively in the differentiation of PYL and CSY, and its routine application will provide physicians with much comfort in practice.

Abbrevation: IG: Immature granulocyte, CBC: Complete blood count, CYS: Cystitis, PYL: Pyelonephritis, UTIs: Urinary tract infections, DMSA: 99mTc-dimercaptosuccinic acid, CRP: C-reactive protein, WBC: White Blood Cell, ROC: Receiver operating characteristic, AUC: Area under the curve.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kastamonu University Clinical Researches Ethics Committee (Date: 21.04.2021, Decision No: 2021/KAEK-143-89)

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Is rheumatoid arthritis a neglected comorbidity in neurofibromatosis type 1?

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ABSTRACT

Aims: Neurofibromatosis type 1 (NF-1) is a relatively rare disorder with autosomal dominant inheritance. Despite current reports highlighting the association between NF-1 and some rheumatic diseases (e.g., systemic lupus erythematosus, juvenile idiopathic arthritis, ankylosing spondylitis, and antiphospholipid antibody syndrome), the literature seems to have missed focusing on its relationship with rheumatological disorders. Hence, the present study attempted to explore definite NF-1 molecular genetic mutation in association with accompanying rheumatic diseases, particularly rheumatoid arthritis.

Methods: The patients (n=23) aged 18 years who were diagnosed with NF-1 genetic mutation between 2010-2022 in the medical genetics department of our university were recruited for medical examination regarding rheumatic disorders in our rheumatology outpatient clinic.

Results: There were a total of 23 patients in this study, 14 (60.9%) males and 9 (39.1%) females, with a mean age of 27.4±9.2 years (18-51 years). As a result, 4 (17.3%) patients were diagnosed with rheumatoid arthritis (RA), 3 with seropositive RA, and one with seronegative RA. Of the diagnoses, two were established RA, and two were early RA. All patients with RA had a positive metacarpophalangeal joint (MCP) squeeze test and experienced pain in bilateral hands and wrists and morning stiffness for more than 45 min.

Conclusion: While the community prevalence of RA is about 1%, it is noteworthy that we detected RA in 17.3% of our patients. In the follow-up of patients with NF-1, routine examinations for pain in bilateral hands and wrists, morning stiffness over 45 minutes, and positivity of the MCP squeeze test are thought to allow early diagnosis of RA and, thus, relevant therapies.

Keywords: Neurofibromatosis type 1, arthritis, rheumatoid arthritis, autoimmune disease, joint

INTRODUCTION

NF encompasses NF type-1 (NF-1), NF-2, and schwannomatosis, which exhibit a range of clinically and genetically diverse characteristics. Accounting for about 90% of cases, NF-1 is detected in one in 3,000 live births regardless of sex. It has an autosomal dominant inheritance, but half of the patients often have a family history, while it develops due to de-nova mutations in the other half.[1] It was previously reported that the clinical diagnosis of NF-1 in about 95% of patients appears with a probable pathogenic variant.[2] The NF1 gene, located in the 11p12 region of chromosome 17, encodes a GTPase activating protein, a tumor suppressor protein called neurofibromin.[3] Neurofibromin, on the other hand, is a negative regulator of the Ras-mitogen-activated protein kinase signaling pathway, which regulates cell growth and proliferation. Therefore, inactivation of neurofibromin leads to hyperactivation of these pathway mediators and tumor formation.[4]

The most patent finding of NF-1 may be congenital café-au-lait spots. It is also accompanied by malignancies (e.g., lisch nodules, peripheral neurofibroma, optic glioma, brain stem gliomas, malignant peripheral nerve sheath tumors, leukemia, and gastrointestinal stromal tumors) and many other cardiovascular, orthopedic, and psychiatric comorbidities.[5,6] In this sense, patients with NF-1 may need to be followed up in several medical departments (e.g., dermatology, general surgery, nephrology, endocrinology, neurology, physiotherapy,

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ophthalmology, orthopedics, and psychiatry), as NF-1 is directly and indirectly associated with many comorbidities 1, which makes patient follow-up and management challenging and leads to some disorder-related issues underestimated. In the literature, few case reports/series reported NF-1 to be coexistent with varied autoimmune disorders (e.g., multiple sclerosis, systemic lupus erythematosus (SLE), membranous glomerulonephritis, IgA nephropathy, mixed connective tissue disease, juvenile idiopathic arthritis (JIA), ankylosing spondylitis (AS), antiphospholipid antibody syndrome (APS), autoimmune hemolytic anemia, bullous pemphigoid, vitiligo, and Graves' disease).[7-11]

In our rheumatology practice, it was deemed a remarkable observation that three different NF-1 patients applied with complaints of pain and stiffness in their hands, and one patient was diagnosed with seropositive rheumatoid arthritis (RA). To date, only two case reports on the coexistence of NF-1 and RA have noted some interesting adverse reactions in NF-1 patients during RA treatment.[12,13] Reckoning on our observations and previous research, we attempted to investigate the prevalence of RA and other rheumatological disorders that can be missed as lesser-known comorbidities in NF-1 patients.

METHODS

This study was approved by the Medical Faculty of Afyonkarahisar Health Sciences University Clinical Researches Ethics Committee (Date: 03.03.2023, Decision No: 2023/131). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Figure 1 presents a flowchart for inclusion criteria. We excluded those without an NF1 mutation in the molecular genetic analysis and under 18 years. While we recruited members of the same family who were followed up at our faculty, it was not the case for their non-followed-up relatives. Accordingly, we performed this study with 23 patients with a definite diagnosis of NF-1 in our tertiary center between January 2022-2023. The diagnosis of RA was decided upon the RA classification criteria proposed by the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) in 2010.[14] We diagnosed early RA and established RA according to the ACR Rheumatoid Arthritis Guidelines.[15] According to this guide, if disease or symptom duration was <6 months, it was defined as early RA, and if ≥6 months, it was defined as established RA.

At the initial visits, we carefully performed anamnesis and physical examination of the patients and noted down symptom durations in symptomatic patients and arthritis patterns in those with arthritis (number, localization, and distribution of affected joints). Moreover, we inquired all patients about the presence of rheumatological disorders or complaints in their relatives. Next, considering the patients' clinical findings, we ordered pertinent imaging tests, as well as laboratory tests for complete blood count (CBC) and biochemical profile, complete urinalysis, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and all autoantibodies including rheumatoid factor (RF; nephelometric, normal <14 IU/ml), anti-cyclic citrulline peptide (anti-CCP; ELISA, normal <17 U/ml). Blood samples were also studied for HLA-B27 and anti-nuclear antibody (ANA) (indirect immunofluorescence method, titration, and pattern), hepatitis B and C virus, and brucella (endemic to the research place). Moreover, the patients' complaints urged us to order radiographs of the hands, feet, sacroiliac joints, and the affected joints. We also performed ultrasonography (USG) in patients with arthritis and arthralgia in bilateral hands and wrists.

In this descriptive study, we recorded all the data in patient files and retrospectively analyzed the data in the hospital database. While presenting categorical variables as percentages and frequencies, we express continuous variables as means and standard deviations (range, min-max). All statistical analyses were performed on SPSS 26.0.

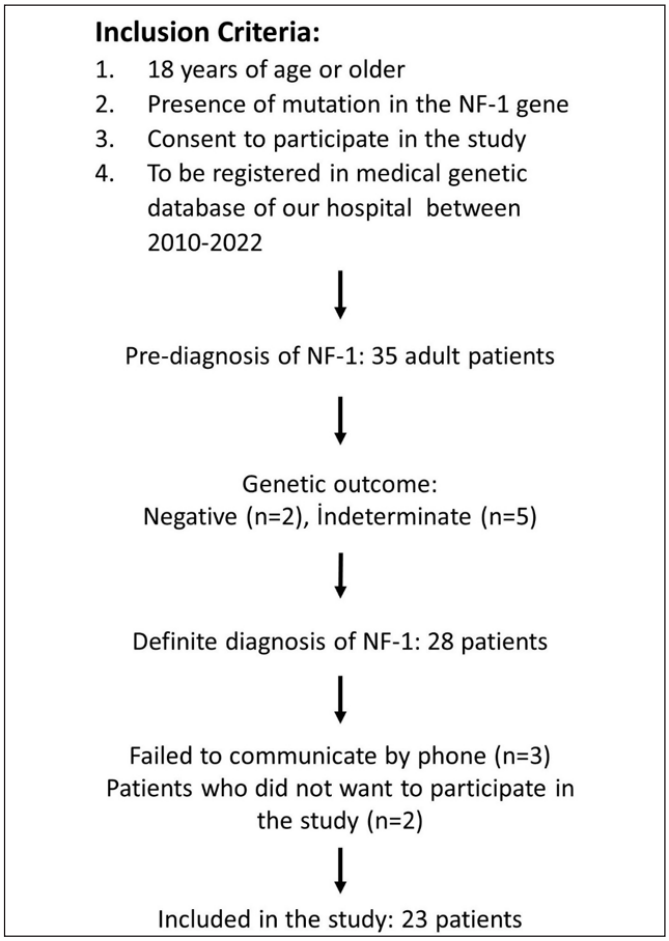

Figure 1. Inclusion flow diagram
Abbreviations: NF-1: Neurofibromatosis Type-1

RESULTS

There were a total of 23 patients in this study, 14 (60.9%) males and 9 (39.1%) females, with a mean age of 27.4±9.2 years (18-51 years). The patients' clinical and genetic characteristics pertinent to NF-1 are presented in **Table 1**. All patients had heterozygous mutations. The pedigree analysis showed no family history in only 2 (8.7%) patients, and they were considered to have a de-novo mutation. The cases recruited from the same family were Case 5 and Case 6 (siblings), Case 14 and Case 9 (siblings), Case 10 and Case 11 (mother-child), and Case-16, Case 18, and Case 20 (mother-two children) (**Supplementary Table**).

Table 1. NF-1 patients' sociodemographic, clinical, and genetic characteristics

Sex; n (%)	
Male	14 (60.9%)
Female	9 (39.1%)
Age, years; M±SD (min-max)	27.5±9.2 (18-51)
Clinic features associated with the NF-1	
Family history of positivity; n (%)	21 (91.3%)
Café au lait; n (%)	23 (100%)
Neurofibroma; n (%)	9 (39.1%)
Lisch nodule; n (%)	2 (8.7%)
Epilepsy; n (%)	3 (13.0%)
Ataxic Gait; n (%)	1 (4.3%)
NF-1-associated genetic features †	
Pathogenic	12 (47.8%)
Likely pathogenic	10 (43.5%)
Uncertain significance (VUS)	1 (8.7%)

NF-1: Neurofibromatosis Type-1, VUS: Variant of uncertain significance
† Mutations are considered "pathogenic, likely pathogenic, uncertain significance, likely benign, and benign" within the framework of the Mutation Classification defined by the American College of Medical Genetics.[31]

Table 2 displays the clinical and genetic characteristics of the patients with RA. Our first case, Case 3, had stiffness and pain in bilateral hands for about two hours in the mornings for 24 months and swelling in the last six months. In his physical examination, we discovered polyarthritis with symmetrical involvement of the proximal interphalangeal (PIF) joints of bilateral hands and arthralgias in both wrists and metacarpophalangeal (MCP) joints. Moreover, he had a positive bilateral MCP squeeze test. We also obtained the following laboratory findings: RF=129 IU/ml, anti-CCP=86 U/ml, sedimentation=49 mm/hr, and CRP 9.3 mg/dl. While the hand radiography showed periarticular osteoporosis and soft tissue swelling, the hand and wrist USG revealed joint capsule enlargement, synovial hypertrophy, and bone erosions on the joint surfaces in the metacarpophalangeal joints (**Figure 2**). The patient was diagnosed with seropositive RA upon the ACR/EULAR 2010 criteria.

Three out of four NF-1 patients with RA were males

Table 2. Clinical, laboratory, and genetic characteristics of NF-1 patients with RA

Case ID	Sex	Age (years)	Complaints (Symptom duration)	Morning stiffness duration	Physical Examination Findings	MCP Squeeze Test	Laboratory findings†	Genetic features associated with the NF-1 ‡	NF-1 major findings and dysmorphic features	Another affected family member	Diagnosis
Case 3	Male	35	Pain and stiffness in bilateral hands (24 months)	120 min	Symmetrical polyarthritis of PIP and MCP joints of bilateral hands	Positive	ESR: 49 mm/h CRP: 9,3 mg/dl RF: 129 IU/ml Anti-CCP: 86 U/ml	NF1 c.1392+1G>T Likely pathogenic	Multiple café-au-lait spots, neurofibromas, facial dysmorphic features	2 daughters, 2 maternal uncles	Established seropositive RA
Case 9	Male	29	Pain, swelling, and stiffness in bilateral hands and wrists (12 months)	90 min	Arthritis of the 2nd and 3rd PIP joints of bilateral hands and wrists	Positive	ESR: 35 mm/h CRP: 11,2 mg/dl RF: 81 IU/ml Anti-CCP: negative	NF1 c.109_110delGA p.Glu37Alafs*29 Likely pathogenic	Multiple café-au-lait spots, neurofibromas, facial dysmorphic features	2 brothers, father, paternal grandmother	Established seropositive RA
Case 17	Male	44	Pain and stiffness in bilateral hands, wrists, and knees (5.5 months)	60 min	Arthritis in the 2nd, 4th, 5th PIP joints of bilateral hands and wrists. Arthralgia in bilateral knees	Positive	ESR: 29 mm/h CRP: 8,1 mg/dl RF: negative Anti-CCP: 102 U/ml	NF1 c.4537 C>T p.Arg1513X Pathogenic	Multiple café-au-lait spots, neurofibromas, rhabdomyosarcoma, Lisch nodules, facial dysmorphic features	Son, sibling, mother	Early seropositive RA
Case 22	Female	26	Pain and stiffness in bilateral wrists (5 months)	45 min	Arthritis in the right wrist. Arthralgia in right hand (2nd,4th PIPs), left hand (2nd,3rd PIPs), and left wrist.	Positive	ESR: 17 mm/h CRP: 6,2 mg/dl RF: negative Anti-CCP: negative	NF1c.3852_3854delAAT p.Ile 1284del CD147163 Pathogenic	Multiple cafe au lait spots	Mother	Early seronegative RA

MCP: Metacarpophalangeal; PIP: Proximal interphalangeal; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; RF: Rheumatoid factor (≥14 IU/ml positive); Anti-CCP: Anti-cydic citrullinated peptide (≥17 U/ml positive); NF-1: Neurofibromatosis type-1; RA: Rheumatoid arthritis, † In addition to routine examinations, we performed tests for HLA-B27 and anti-nuclear antibody (ANA) (indirect immunofluorescence technique), hepatitis B and C, and brucella (endemic to the research place). ANA and HLA-B27 tests were negative in 4 patients with RA. ‡ Mutations are considered "pathogenic, likely pathogenic, uncertain significance, likely benign, and benign" within the framework of the Mutation Classification defined by the American College of Medical Genetics[31]

Supplementary Table

Case ID	Age	Gender	Mutation	ACMG clasification	NF1 Major Findings	Dysmorphic Features	Zygosity	Is There Another Affected Individual?	Relationship Status	Labortory Data
1	30	Man	NF1 c.2531 T>G p.L844R	Likely pathogenic	Multiple cafe au lait spots, neurofibromas, unilateral hearing loss	Long face, broad forehead, deeply set eyes, broad eyebrows, thick eyebrows, long palpebral fissures, prominent antitragus, long ears, narow nasal bridge, fullness paranasal tissue, deep philtrum, exaggerated Cupid's Bow	Heterozygous	Aunt	None	No significant positivity in laboratory data
2	30	Man	NF1 c.2990+5 G>A	Pathogenic	Multiple cafe au lait spots	Long face, cheekbones prominence, broad chin, deeply set eyes, narrow nasal ridge, deep philtrum, exaggerated Cupid's Bow, thin lower lip wermillon	Heterozygous	Father	None	No significant positivity in laboratory data
3-RA	35	Man	NF1 c.1392+1G>T	Likely pathogenic	Multiple cafe au lait spots, neurofibromas	Brachycephaly, frontal balding, long face, prominence cheekbones, long chin, deeply set eyes, hypotelorism, sparse eyebrow, prominent antitragus, thick ala nasi, low insertion columella, narrow nasal bridge, smooth philtrum	Heterozygous	2 daughter, 2 maternal uncle	None	ESR: 49 mm/h; CRP: 9.3 mg/dl; RF: 129 IU/ml; Anti-CCP: 86 IU/ml
4	43	Man	NF1 c.6955 C>T p.Q2319*	Pathogenic	Multiple cafe au lait spots, neurofibromas	Long face, malar flattening, prominent nasolabial fold, broad chin, deeply set eyes, thick eyebrows, telecanthus, enlarged nares, wide nasal base, wide nasal bridge, deep philtrum, exaggerated Cupid's Bow, thin lower lip wermillon	Heterozygous	Mother, 1 sibling	None	No significant positivity in laboratory data
5	19	Man	NF1 c.910 C>T p.R304*	Pathogenic	Multiple cafe au lait spots, seizure, neurdevelopmental delay, lisch nodules	Malar flattening, thick eyebrows, telecanthus, thick ala nasis, bulbose nose, long philtrum, thick lower lip vermillon, thick upper lip vermillon	Heterozygous	Father, grandfather, uncle	Sibling with case 6	No significant positivity in laboratory data
6	37	Man	NF1 c.910 C>T p.R304*	Pathogenic	Multiple cafe au lait spots, neurofibromas	Long face, narrow face, prominence cheekbone, tall chin, thick eyebrows, low hanging columella, wide nasal base, thick upper lip vemillon, thick lower lip vermillon	Heterozygous	Father, grandfather, uncle	Sibling with case 5	No significant positivity in laboratory data
7	51	Woman	NF1 c.4084C>T p.R1362*	Pathogenic	Multiple cafe au lait spots, neurofibromas, sarcoma excision from arm	Long face, cheekbones prominence, malar flattening, broad chin, tall chin, deeply set eyes, downslanted palpebral fissures, high insertion columella, malaligned philtral ridges	Heterozygous	1 daughter, 1 son and maternal grandmother	None	No significant positivity in laboratory data
8	18	Man	NF1 c.6772C>T p.R2258*	Pathogenic	Multiple cafe au lait spots, ataxic gait	Malar prominence, deeply set eyes, sparse eyebrows, infraorbital creases, upslanted palpebral fissures, ptosis, thick ala nasi, wide nasal bridge, wide nasal ridge, deep philtrum, exaggerated Cupid's Bow	Heterozygous	2 brother, mother, maternal uncle, maternal grandmother	None	No significant positivity in laboratory data
9-RA	30	Man	NF1 c.109_110delGA p.Glu37Alafs*29	Likely pathogenic	Multiple cafe au lait spots, neurofibromas	Full cheeks, midface prominence, tall chin, downslanted palpebral fissures, wide nasal base, thick lower vermillon	Heterozygous	2 brother, father, paternal grandmother	Sibling with case 14	ESR: 49 mm/h; CRP: 9.3 mg/dl; RF: 129 IU/ml; Anti-CCP: 86 IU/ml

Supplementary Table

Case ID	Age	Gender	Mutation	ACMG clasification	NF1 Major Findings	Dysmorphic Features	Zygosity	Is There Another Affected Individual?	Relationship Status	Labortory Data
10	46	Woman	NF1 c.1541_1542delAG p.Q514Rfs*43	Pathogenic	Multiple cafe au lait spots	Broad chin, tall chin, smooth philtrum, thin lower lip vermillon	Heterozygous	1 son	Mother of case 11	No significant positivity in laboratory data
11	21	Man	NF1 c.1541_1542delAG p.Q514Rfs*43	Pathogenic	Multiple cafe au lait spots, short stature	Midface prominence, pointed chin, tall chin, wide spaced eyes, upslanted palpebral fissures, telecanthus, overfolded helix, narrow nasal ridge, exaggerated Cupid's Bow	Heterozygous	Mother	Son of case 10	No significant positivity in laboratory data
12	21	Man	NF1 c499_502delITGTT p.C167fs10	VUS	Multiple cafe au lait spots, Macrocephaly, Regressive autism	Regressive autism	Heterozygous	1 couisin	None	No significant positivity in laboratory data
13	18	Woman	NF1 c.5439dupA p.Q18113fs*27	Likely pathogenic	Multiple cafe au lait spots	No features	Heterozygous	De Novo	None	No significant positivity in laboratory data
14	23	Man	NF1 c.109_110delGA p.Glu37Alafs*29	Likely pathogenic	Multiple cafe au lait spots, neurofibromas	Triangular face, full cheeks, midface prominence, pointed chin, downslanted palpebral fissures, wide nasal base, thick lower vermillon	Heterozygous	2 brother, father, paternal grandmother	Sibling with case 9	No significant positivity in laboratory data
15	19	Woman	NF1 c.1655_1657delTTC	Pathogenic	Multiple cafe au lait spots	No features	Heterozygous	De Novo	None	No significant positivity in laboratory data
16	45	Woman	NF1 c.5675delA p.K189fs*12	Likely pathogenic	Multiple cafe au lait spots	No features	Heterozygous	2 daughter	Mother of Case 18 and Case 20	No significant positivity in laboratory data
17-RA	44	Man	NF1 c.4537 C>T p.Arg1513X	Pathogenic	Multiple cafe au lait spots, neurofibromas, rhabdomyosarcoma , lisch nodules	Prominent supraorbital ridges, cheekbones prominence, deeply set eyes, prominent antihelix stems, protruding ears, macrotia, low insertion columella	Heterozygous	Son, sibling, mother	None	ESR: 29 mm/h; CRP: 8.1 mg/dl; RF: negative; Anti-CCP: 102 IU/ml
18	20	Woman	NF1 c.5675delA p.K189fs*12	Likely pathogenic	Multiple cafe au lait spots, Epilepsy	No features	Heterozygous	Mother, 1 sibling	Case 16's daughter, Case 20's sister	No significant positivity in laboratory data
19	29	Man	NF1 c.1541_1542del (p.Gln514fs)	Pathogenic	Multiple cafe au lait spots, thousands of Neurofibromas	Painful Neurofibromas	Heterozygous	Mother and 1 sibling	None	No significant positivity in laboratory data
20	19	Woman	NF1 c.5675delA p.K189fs*12	Likely pathogenic	Multiple cafe au lait spots, seizure	No features	Heterozygous	Mother and 1 sibling	Case 16's daughter, Case 18's sister	HLA-B27 positivity; no clinical signs of spondyloarthropathy
21	23	Man	NF1 c.3011_3011delA N1004Ifs*8	Likely pathogenic	Multiple cafe au lait spots, developmental delay	Broad forehead, short chin, prominet antihelix stem, serpenginous antihelix stem, wide nasal base, wide mouth	Heterozygous	Father, paternal uncle, paternal grandmother	None	No significant positivity in laboratory data
22-RA	26	Woman	NF1 c.3852_3854delAAT p.lle 1284del CD147163	Pathogenic	Multiple cafe au lait spots	No features	Heterozygous	Mother	None	ESR: 17 mm/h; CRP: 6,2 mg/dl, RF: negative; Anti-CCP: negative
23	30	Woman	NF1 c.2023_2034 insC	Likely pathogenic	Multiple cafe au lait spots	No features	Heterozygous	Mother	None	ANA positive in a dense fine mottled pattern at a titer of 1/80. Anti DFS-70 was positive. No clinical findings

*The patients in the blue filled row were diagnosed with rheumatoid arthritis.

aged between 26-44 years. All of them had stiffness in bilateral hands and wrist joints in the mornings for over 45 minutes and arthritis and/or arthralgia in bilateral hands and/or wrists. Then, we decided on seropositive RA for three patients (75%) and seronegative RA for 1 (25%). While two had established RA (50%), the other two were diagnosed with early RA (50%). Besides, we could not detect intra-articular neurofibroma in the ultrasonographic examination of any patient with RA (Figure 2).

All patients diagnosed with RA, except for Case 22, had neurofibromas accompanying café-au-lait spots and dysmorphic facial features. Unlike the others, we detected lish nodules and rhabdomyosarcoma in Case 17 (Supplementary Table). Yet, none of the patients with RA had a de-novo mutation, and their relatives did not have a history of any concomitant rheumatic disorder. RA Despite diagnosing Case 9 with RA, it was not the case for his 23-year-old brother, Case 14.

In the 30-year-old female patient (Case 23), the ANA by the indirect immunofluorescence technique was positive at a titer of 1/80 with a dense fine-spotted pattern. Anti-DFS-70 also was positive in the immunoblot test. She had no features of connective tissue disease. Yet, our 19-year-old female patient had HLA-B27 positivity and no clinical signs of spondyloarthropathy.

Case-22 commenced hydroxychloroquine treatment since preparing for pregnancy. We started methotrexate treatment in the other three patients with RA due to the absence of contraindications. In addition, the patients were started on low-dose steroid bridge therapy and underwent tapering. No adverse effects were encountered in these patients, with a follow-up period of 3 months to 1 year until January 2023. Since being rhabdomyosarcoma in remission, Case 17 has been followed closely in the medical oncology department.

DISCUSSION

To date, diverse hypotheses have been proposed regarding an unusual aspect of NF-1, susceptibility to autoimmunity. Since neurofibromin is involved in the development and regulation of T cells, lymphocytic proliferation develops as a consequence of unregulated reticuloendothelial system (RAS) activity in T cells in NF-1 patients.[16]

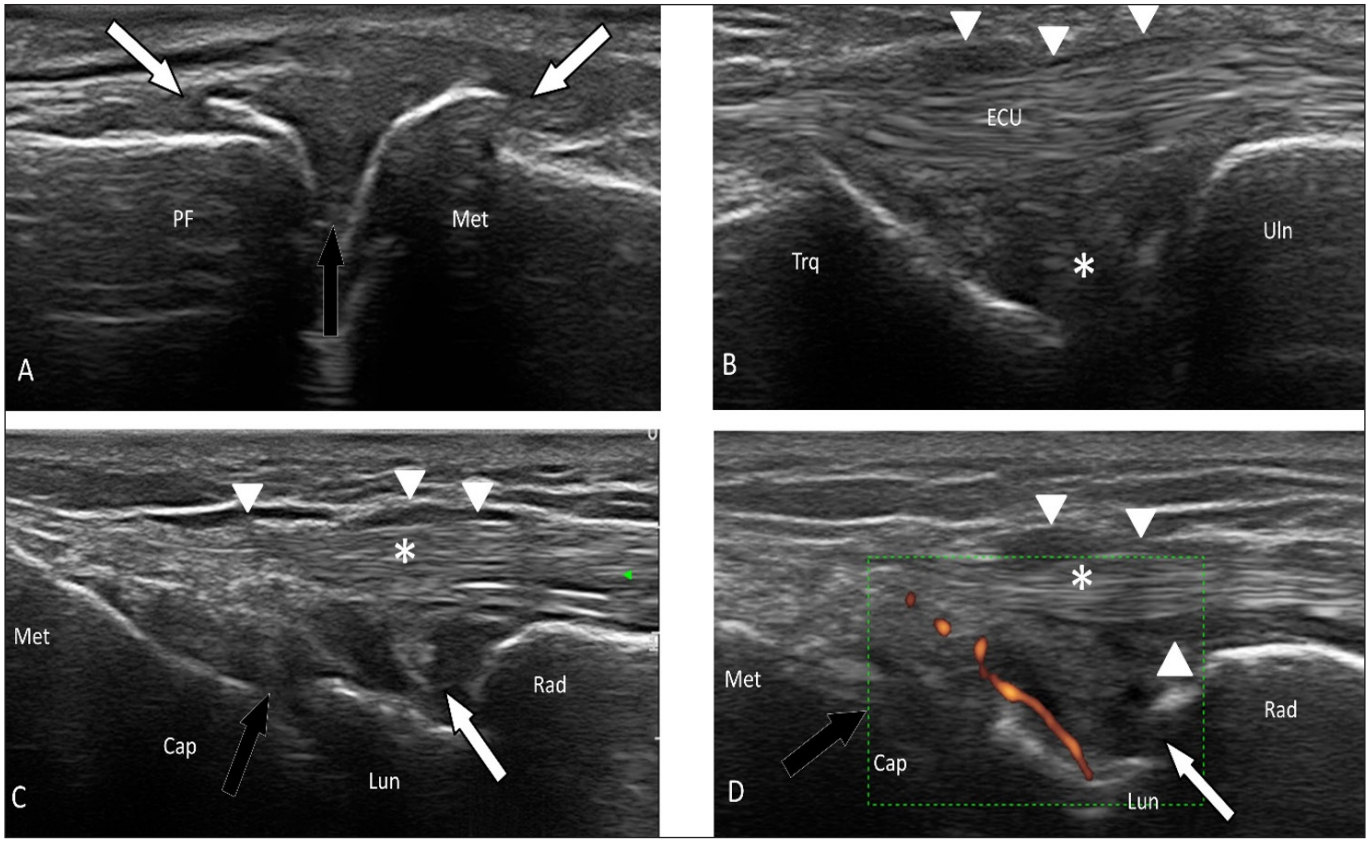

Figure 2. Ultrasonography findings of bilateral hands and wrists of patients with RA
Figure A (Case 3): Metacarpophalangeal joint (black arrow). Enlargement of the joint capsule, synovial hypertrophy, and bone erosions on the joint surfaces (white arrows).
Figure B (Case 9): Ulnocarpal joint (asterisk). Synovial hypertrophy and effusion in the joint space and tenosynovitis around the extensor carpi ulnaris (ECU) tendon (white arrowheads).
Figure C (Case 17): Radiocarpal joint (white arrow) and midcarpal joint (black arrow). Synovial hypertrophy and effusion in the joint space and tenosynovitis in the fourth compartment extensor tendon (white arrowheads). Asterisk: fourth extensor compartment tendon
Figure D (Case 22): Radiocarpal joint (white arrow) and midcarpal joint (black arrow). Increased Doppler activity in the joint space and tenosynovitis in the fourth compartment extensor tendon (White arrowheads). Asterisk: fourth extensor compartment tendon.
Abbreviations: Rad: Radius; Uln: Ulna; Lun: Lunate; Cap: Capitate; Met: Metacarpal; Trq: Triquetrum; PF: Proximal Phalanx.

Previously, loss of the immunosuppressive effect of neurofibromin was designated to be a possible hypothesis for the association of NF-1 with autoimmune anomalies. Moreover, mice studies on the link between neurofibromin deficiency and autoimmunity emphasized that immune dysregulation contributes to the development of myeloid leukemias, lymphoproliferative diseases, and autoimmune disorders.[17,18] Another possible mechanism for autoimmunity involves free DNA released from proliferating cells and triggering an antigenic response. Thus, free DNA can be detected in NF-1 patients and systemic autoimmune diseases.[19,20]

RA is known to be the most prevalent cause of chronic autoimmune inflammatory arthritis, developing as a result of the complex interactions of genes and environmental factors and ending up with an impaired immune tolerance and synovial inflammation in characteristic symmetrical joints.[21] Besides, the literature host two interesting case reports pointing to the association of RA and NF-1. Despite being diagnosed with NF-1 at the age of 5, a 45-year-old female patient, who was followed up with additional seropositive RA for about 20 years and had no increased neurofibromas for the last 25 years, developed a diffuse cutaneous neurofibroma eruption on her arms, body, and face six weeks after tofacitinib 10 mg/day treatment. It was also reported in the study that cutaneous neurofibromas regressed within months following the discontinoatioın of tofacitinib treatment.[12] Tofacitinib is an inhibitor that inhibits the JAK3 pathway the most, and JAKs may also affect other signaling pathways through a process called intracellular crosstalk.[22] Since tofacitinib potentially affects the RAS pathway through crosstalk, the same study speculated that it may have caused the development of diffuse neurofibromas in the patient without neurofibromas for 25 years.[12] Drago et al.[13] reported that a 78-year-old female patient with RA developed dermatomal nodular with compatible histopathology with neurofibroma in the right thoracic region six months after infliximab treatment. The patient without a family history was considered segmental neurofibromatosis.[13] As evident in the two cases above, it can confidently be asserted that anti-TNF and JAK inhibitors utilized in rheumatoid arthritis therapy may aggravate NF-1 findings or lead to atypical NF-1 clinics by affecting the pathways in the pathogenesis of NF-1. Therefore, these case reports specifically point to the possible unusual side effects of biologic drugs in the treatment of autoimmune diseases accompanying NF-1.

While the previous research often reports the prevalence of RA between 0.49% and 1% in Türkiye 23-25, we diagnosed 4 (17.3%) out of 23 NF-1 patients with RA. In this respect, this finding can be considered noteworthy for the relevant literature. Initiating RA treatment immediately after the diagnosis seems critical to prevent long-term sequelae and complications.[26] Despite negative RF or anti-CCP antibodies in the early period of RA, about 80% of the patients have RF and/or anti-CCP positivity in the later stages. We were also able to diagnose 2 of the NF-1 patients with early-stage RA and 2 with established RA thanks to our awareness following our clinical observations, which may imply that rheumatological disorders can be overlooked in NF-1 patients. Even NF-1 follow-up guidelines do not often emphasize that rheumatological complaints should be questioned.[27] Therefore, our findings suggest that rheumatological complaints should also be explored in NF-1 follow-ups. Even without inflammatory arthritis, NF-1 patients may experience muscle and joint pain due to the nature of the disease. Plexiform, especially nodular neurofibromas, can cause joint pain if localized close to the joint area. Scoliosis, pseudoarthrosis, long bone dysplasia, and other bone lesions may also cause pain in patients with NF-1. In an NF-1 patient, it can be challenging to tell whether the pain is due to the nature of the disease or the development of RA.[1,3,4] In these challenging situations, morning stiffness in the joints for more than 45 minutes and high CRP should warn clinicians about RA. Besides, since being a convenient, practical, and cheap technique, we recommend utilizing the MCP squeeze test as a RA screening technique.

In addition to RA, different inflammatory arthritis can be observed in patients with NF-1. For example, Till et al.[8] reported a 3-year-old male patient presenting with monoarthritis in the right knee. What made this case stand out was that while the etiology of arthritis was being investigated, the patient was diagnosed with NF-1 upon noticing typical skin lesions.[8] Gundogdu et al.[9] reported a 43-year-old male patient with NF-1 who was diagnosed with AS due to inflammatory low back pain, HLA-B27 positivity, and bilateral grade 3/4 sacroiliitis findings on a sacroiliac radiograph.[9] Despite HLA-B27 positivity in a 19-year-old female patient in our patient group, she had no significant clinical manifestation of spondyloarthritis. The prevalence of HLA-B27 in the general population is about 8%, and AS develops in only 1-2% of HLA-B27-positive patients.[28] It should also be noted that intra-articular neurofibromas are also involved in the differential diagnosis when an NF-1 patient presents with arthritis. In the case report by Saidane et al.[28] a 33-year-old female NF-1 patient suspected of septic sacroiliitis had articular plexiform neurofibromas in the juxta exhibiting extensive invasion of the right sacroiliac joint and soft tissue on magnetic resonance imaging.[29] Accepted as the prototype of autoimmune disorders in NF-1, SLE is often reported to be more prevalent than RA that it accompanies.[30] In our inquiry about SLE and other autoimmune connective tissue disorders, our patient

group had no clinical findings, such as a history of miscarriage or thrombosis, oral aphthae, photosensitivity, malar or discoid rash, alopecia, sicca symptoms, and Raynaud's phenomenon. Despite having ANA and Anti DFS-70 positivity, our 30-year-old female patient had no clinical features of autoimmune connective tissue disorder.

Our research delves into uncharted territory by investigating the prevalence of rheumatoid arthritis and other inflammatory rheumatic disorders in patients with NF-1. Nevertheless, a few limitations must be considered when interpreting the findings. The major drawbacks of this study may be its retrospective, single-center nature and relatively small sample size due to the rarity of the disease. It may, therefore, be challenging to generalize the findings to different populations.

CONCLUSION

Overall, we think RA prevalence in NF-1 patients may be higher than in the general population. In the follow-up of patients with NF-1, routine examinations for pain in bilateral hands and wrists, morning stiffness over 45 minutes, and positivity of the MCP squeeze test are thought to allow early diagnosis of RA and, thus, relevant therapies. It should also be noted that the presence of NF-1-related neoplasms and adverse reactions to be confronted during treatment may complicate the management of RA among these patients. Thus, there is a pressing need for more extensive research to gain deeper insights into the prevalence and relationship of RA among NF-1 patients, as well as safety data regarding the treatment of rheumatological conditions in this particular patient group.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Researches Ethics Committee (Date: 03.03.2023, Decision No: 2023/131).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Analysis of consultations requested from the tertiary intensive care unit and response times: a retrospective study

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ABSTRACT

Aims: It is aimed to evaluate the effectiveness of the consultations and response times requested from the Intensive Care Unit on the cost.

Methods: This study was conducted retrospectively in a 16-bed anesthesia intensive care unit (ICU) between 02.01.2019 and 30.12.2019. Patient information from the hospital data system was analyzed. Accordingly, demographic data, hospitalization diagnoses, departments for which consultation was requested, times of request, response times, and the average cost per day of a patient were investigated.

Results: It was determined that consultation was requested from a total of 522 patients, 223 (43%) of the patients were female, 299 (57%) were male, and the age range was 18-98 (mean age 57). It was found that the average consultation response time in all departments was 4.09 hours outside of working hours and 3.54 hours during working hours. There was no significant difference in the response time of consultations between internal and surgical departments. The daily cost of one patient in the ICU was found to be 2380.39 ₺.

Conclusion: Failure to promptly respond to the requested consultations in the intensive care unit may cause delays in patients' treatment and their discharge to the service. This situation increases the patient's length of stay and causes the intensive care units not to be used effectively and correctly. However, it can also increase morbidity and cost.

Keywords: Intensive care unit, consultation, response time, morbidity, cost

INTRODUCTION

Intensive care unit (ICU); it is a multidisciplinary unit with high operating costs, equipped with advanced technology devices that are privileged in terms of patient care, monitoring of vital signs for 24 hours, treatment and follow-up of patients who are at risk of losing or losing some or all of their vital functions.[1] Interdisciplinary communication and treatment approaches are often required to diagnose and treat patients admitted to the ICU. Comprehensive critical care support (Comprehensive Critical Care Outreach, 3CO) has emerged in the last two decades, and its multidisciplinary approach in the ICU provides a safe and rapid approach to acutely unidentified critical patients.[2] In order to approach a case holistically, it is inevitable for more than one clinical medicine discipline to work together.

Consultation, derived from the Latin word "consultatio," is the meeting of two or more physicians who are experts in different branches at a patient's office and making a joint evaluation of that patient upon the request of the patient or his family or the need of the treating physician, in the face of a not fully elucidated case or a disease that is difficult to diagnose. Can be defined as.[3] In this case, the "consultant physician" is the physician from whom the patient is consulted and whose knowledge and opinions are sought.

ICU provides consultation services in our country according to the regulation published by the Ministry of Health and each institution's configuration and possibilities.[4] Due to the high demand for intensive care units, evaluating intensive care beds and their resources efficiently and effectively is essential.[5]

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Consultations are one way of doing this, and there are several reasons. During the visit, the intensive care team asks for consultation from other services. Many departments may request consultations during the perioperative period, such as surgical branches for patient follow-up, infectious diseases for infection treatment, oncology for various oncological diseases, and nephrology consultation for hemodialysis needs.[2]

After the completion of intensive care treatments, consultation is also required for the discharge or transfer procedures of patients who do not indicate hospitalization. After the decision to transfer the patients from the intensive care unit to another service (palliative care, a lower level intensive care unit, inpatient services) is made, the opinion of the relevant branch is taken.

Our aim in this study is to determine the response times to the desired consultations regarding treatment planning or discharge to the service while being treated in the intensive care unit and to evaluate the cost of delays, if any.

METHODS

The study was carried out with the permission of Dokuz Eylül University Non-interventional Clinical Researches Ethics Committee (Date: 26.10.2020, Decision No: 2020/26-45). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. We retrospectively reviewed the patients hospitalized in the 3rd level anesthesia intensive care unit with 16 beds and over 18 years of age between 02.01.2019 and 30.12.2019. patients were evaluated. Our study is a cross-sectional analytical study. All consultations requested from the anesthesia intensive care unit within the specified period were evaluated by examining the electronic records in the hospital information management system. All patients over 18 treated in the intensive care unit who completed or met the criteria for leaving the intensive care unit and were consulted for transfer to another service were included in the study.

Pediatric patients under the age of 18, drug consent consultations, consultations requested for repetitive consultations, and physiotherapy purposes were determined as exclusion criteria from the study. The demographic data of the patients, age, gender, and hospitalization diagnoses, from which internal or surgical service the requested consultations were made, whether the requested time was in or out of working hours, and the response times were recorded in the www.veritopla.biz.tr program, which is the data collection base of our department. The identity information of the patients was not used in the study. The departments for which consultation was requested were divided into internal and surgical departments according to their areas. internal departments were accepted as dermatology infectious diseases, chest diseases, cardiology, neurology, mental health and diseases radiology, endocrinology, gastroenterology, hematology, nephrology, and oncology clinics. surgical departments; neurosurgery, general surgery, ophthalmology, thoracic surgery, cardiovascular surgery, otorhinolaryngology, orthopedics and traumatology, plastic and reconstructive surgery, and urology were accepted as clinics.

In addition, the average daily cost of a patient in the anesthesia intensive care unit was calculated. While making the calculation, the daily ICU cost of a patient was subtracted. All laboratory tests requested and/or applied from the patient during the day, imaging methods, drug costs, blood components, consultation costs, tertiary intensive care unit hospitalization costs, percutaneous tracheostomy performed in the hospital, pleural drainage, central venous catheter application, tube thoracostomy, etc. The direct medical cost covering the costs of the applications was calculated as Turkish lira (₺) from the Social Security Institution (SGK) perspective. Direct medical costs (service fee, drug cost, material cost, etc.) were calculated by dividing the total cost by the total number of days invested, using the invoice information of the patients in the Probel Hospital Information Management System (Probel HBYS).

Statistical Analysis

The study's data were transferred to the SPSS 24.0 (Windows, Chicago, IL, USA) program. Categorical data were expressed by frequency and percentage, while continuous data were represented by mean, standard deviation, median, minimum, and maximum. Categorical data were compared with the Chi-square test. The conformity of the consistent data to the normal distribution was tested with the Shapiro-Wilkonson test. When comparing the means, the t-test was used for data with normal distribution, and the Mann-Whitney U test was used for data unsuitable for normal distribution. The significance level was accepted as $p < 0.05$.

RESULTS

In light of the data obtained by retrospectively examining our hospital database, it was determined that 522 patients were consulted. When the demographic data of the patients were examined, it was seen that 223 (43%) were female and 299 (57%) were male, and the age range was 18-98 (mean age 57). In the gender analysis of the requested consultations, no gender difference was found between the male and female gender.

When the consultations requested are classified according to the diagnosis groups, the most consultation is respiratory diseases (24.7%), circulatory system diseases

(23%), and digestive system diseases (18.2%) are the third (Table 1). The number of consultations requested from internal departments was higher than in surgical departments.

Table 1. Classification of consultations according to their diagnosis

	Number (n=522)
The respiratory system	129 24.7
The circulatory system	120 23.0
Digestive system	95 18.2
Neurology	73 14.0
Trauma	62 11.9
Oncology and hematology	27 5.2
Endocrine-nutrition	8 1.5
Suicidal	6 1.1
Other	2 0.4
Total	522 100.0

It was found that the average consultation response time in all departments was 4.09 hours outside of working hours and 3.54 hours during working hours. There was no significant difference between internal and surgical departments regarding response times. When the timing of the consultations was analyzed, there was no significant difference between internal and surgical departments in terms of in and out of working hours (p>0.05) (Table 2).

When the consultation response times were analyzed according to departments, a significant difference was found between the response times of cardiology, plastic& reconstructive surgery, and chest diseases compared to other departments (p>0.05) (Table 2).

One-day tertiary anesthesia intensive care cost of a patient; Dividing the available total (4,931,409.33) by the total number of days hospitalized (2071,6835), it was found to be 2,380,39 ₺ (Table 3).

Table 3. Anesthesia intensive care unit cost table

	(₺)
Service fee	1.884.697,08
Medication fee	2.453.575,13
Material cost	593.137,12
Grand total	4.931.409,33
Cost per patient per day	2.380,39

DISCUSSION

Our study analyzed the consultations requested in the anesthesia intensive care unit for one year. The number of consultations requested from the internal departments was higher than the surgical departments. As the diagnostic group, consultations for respiratory diseases were first. It was found that the average consultation response time in all departments was 4.09 hours outside of working hours and 3.54 hours during working hours. There was no significant difference between internal and surgical departments regarding consultation response times. However, it was determined that the consultation response times of the cardiology, chest diseases, and plastic and reconstructive surgery clinics were significantly different regarding the response times of the requested consultations during working and non-working hours.

Table 2. Distribution of consultations by units

Desired Unit	During working hours (n=577)	During working hours Mean+SD	Out of hours (n=544)	Out of hours Mean+SD	p
Department of Neurosurgery	29	(5.19±7.51)	28	(4.56±3.52)	0.51
Department of Dermatology	8	(12.22±11.85)	10	(6.28±8.68)	0.20
Department of Endocrinology	1	(.8333±)	3	(4.56±1.830)	0.50
Department of Infectious Diseases	305	(2.73±2.72)	154	(2.76±2.63)	0.25
Department of Gastroenterology	8	(12.22±11.85)	24	(4.21±2.90)	0.36
Department of General Surgery	32	(3.80±3.81)	59	(4.76±4.46)	0.10
Department of Thoracic Surgery	12	(2.79±2.77)	14	(4.91±6.82)	0.25
Department of Chest Diseases	23	(4.02±7.01)	32	(4.51±5.32)	0.015
Department of Eye Diseases	6	(4.41±4.341)	10	(4.51±5.32)	0.43
Department of Hematology	5	(14.86±25.85)	11	(3.84±1.120)	1.00
Department of Cardiovascular Surgery	10	(4.90±3.60)	19	(3.07±2.61)	0.14
Department of Cardiology	34	(6.32±5.16)	45	(3.68±3.50)	0.019
Department of Ear Nose Throat Diseases	7	(3.54±2.94)	21	(3.68±3.50)	0.09
Department of Nephrology	32	(2.31±2.04)	29	(3.21±4.22)	0.50
Department of Neurology	32	(5.77±7.275)	32	(3.26±3.20)	0.82
Department of Oncology	3	(2.36±.035)	3	(6.26±2.69)	0.10
Department of Orthopedics and Traumatology	8	(7.68±6.21)	15	(5.43±3.376)	0.55
Department of Plastic and Reconstructive Surgery	7	(3.74±2.17)	8	(11.24±5.57)	0.021
Department of Radiology	3	(6.28±2.65)	5	(11.24±5.57)	1.00
Department of Mental Health and Diseases	4	(4.46±3.03)	10	(4.12±1.76)	0.84
Department of Urology	8	(3.90±5.19)	12	(4.12±1.76)	0.57

All values were presented as percent or mean±standard deviation (SD).

According to the guideline containing the decisions for admission and discharge to the ICU published in 2019, it is recommended that the consultations requested from the ICU be answered by the relevant consultant physician within 30 minutes, 7 days a week, 24 hours a day, by coming to the intensive care unit and, if necessary, by making two visits a day.[6] According to the consultation procedure updated by our university in 2017, the consultation response mentioned among the physician's responsibilities should be quick and effective.[7] The consultant physician should evaluate the patient within 30 minutes at the latest in the emergency consultations requested from the emergency services. However, our ICU within our hospital has the same status as other inpatient services and has no priority regarding consultation response time. According to the same consultation procedure, the consultations requested from the services must be evaluated within the working hours within 24 hours.

Since the consultations requested from the ICU are within the scope of inpatient service, there may be problems in terms of functioning; we think it would be appropriate to consider ICU consultations as an emergency service. While the consultations requested from the ICU are answered promptly, treatment plans should be evaluated with the intensive care unit and the consultation officer.[8] Rapid consultation and joint evaluation reduce critically ill patients' mortality and their ICU stay.[5] However, it was observed that the consultation response time in our unit was far beyond these times.

It is essential to work multidisciplinary in the operation of the ICU, seek consultations from various services when needed, and answer these consultations as soon as possible. Consultations are requested for very different and vital situations, such as the infectious status of the patients, the need for hemodialysis, psychiatric evaluation, possible operation decision, and evaluation of the patient in terms of follow-up in the postoperative period. In a study that performed a descriptive analysis of neurological consultations in non-neurological ICUs to determine the frequency of various neurological complications and to evaluate the diagnostic yield, therapeutic effects, and prognostic utility of these consultations, it was found that 48% of patients had treatment change following neurological consultation and was beneficial for their prognosis.[9]

Consultation response time is of fundamental importance in infectious processes such as sepsis. The last published sepsis guideline states that the most appropriate antimicrobial should be started in sepsis patients within the first hour.[10] Delayed antimicrobial therapy is associated with a significantly increased mortality risk. In another study, each hour of delay in antibiotic treatment was found to decrease the chance of survival by 7.6%.[11] In support of this situation, some studies advocate 24/7 accessibility to the infectious diseases specialist in the ICU and emphasize the importance of meeting face-to-face daily.

Therefore, delayed consultation response in infectious processes may increase the morbidity and mortality rates of the cases.[12] In order to increase the rational use of antibiotics, reduce the cost of treatment and reduce antibiotic resistance, the authority to prescribe antibacterial, antifungal, and antiviral agents has been granted to infectious diseases and clinical microbiology specialists (EHU) under the heading of antibiotic prescribing rules in the Health Practice Communiqué (SUT) published by the Social Security Institution since 2005.[13] Antimicrobials in SUT; are divided into three groups those that do not have restrictions, those that do not require approval up to 72 hours for their prescription, and those that require absolute Infectious Diseases and Microbiology specialist approval.

However, considering the inpatient profile in the 3rd level ICU, prescribing following the guidelines by intensive care specialists, who also have Infectious Diseases rotation in the specialty medicine core training program, may contribute to the reduction of morbidity and mortality, especially in septic patients, so that there is no delay in the treatment of complicated patients. In addition, it is essential to use early warning systems in critical laboratory results for patient safety and to perform imaging procedures as soon as possible.[12] In this context, legal regulation may benefit the intensive care specialist to prescribe antibiotics, antiviral and antifungal drugs.

Early and timely consultation is crucial in transferring critically ill patients to the service. It has been reported that delayed admission from the ICU to the ward increases the length of hospital stay and causes significant morbidity and mortality.[14] The ICU decisions guide recommends that the patient goes to the service within 4 hours after the ICU officer decides to leave the patient.[15] In our unit, due to the length of the response time for out-of-hours consultations and the fact that the patient is expected to go to the service during the daytime, the hours of going to the service follow the guideline.

Another dimension of delayed admission of critically ill patients to the service is the inability to use hospital and country resources effectively. ICUs consume 20% of hospital expenditures and 1% of gross domestic product.[16] Due to the high demand for intensive care, intensive care beds, and resources must be utilized efficiently and effectively.[17] Consultation from the relevant branch is requested When transferring patients from intensive care to another service (palliative care, a lower-level intensive care unit, inpatient service). Delayed response to the

desired consultation causes prolonged patient stays in the ICU, increased morbidity and mortality, and additional costs.[16]

The daily cost of an intensive care bed is 6-8 times higher than that of a service bed.[18] In a study by Yıldız et al.[19] the total cost of 20 patients who could not be removed from the intensive care unit due to delayed consultation or other reasons for 1 year was 6058±12676.63 ₺ (min:160).-max: 58780). In our study, when a patient's one-day tertiary anesthesia intensive care cost was calculated, it was found to be 2,380.39 ₺. Due to the high costs, the unnecessary occupation of intensive care beds due to delayed response to consultation not only puts an extra burden on the health system but also increases the morbidity and mortality of the patients. Considering the number of intensive care beds in Turkey, we can predict that the cost can reach high figures.

Dimitra Karabatsoua et al.[20] in a study they conducted in a 7-bed 3rd-level intensive care unit in 2016 in Greece, the average daily cost was calculated as 573.[18] Euros, and they stated that the shortening of the stay in the intensive care unit and the decrease in the duration of mechanical ventilation caused a significant decrease in total costs. Dasta et al.[21] stated that interventions that shorten the length of stay in the intensive care unit cause significant reductions in the total cost of inpatients. Lefrant et al.[22] found the total daily cost of 104 patients in intensive care in France to be 1425±520 € (95% CI=1323-1526 €) and strongly correlated the average cost of ICU with the duration of care per bed performed by human resources.

For these and similar reasons, delay in responding to the requested consultations may adversely affect the treatment of patients, cause prolonged morbidity and mortality, and ultimately impose a severe burden on the health system.

CONCLUSION

Due to the multidisciplinary study, consultation is requested from the patients hospitalized in our intensive care unit for different reasons. The response time of the consultations is important both in terms of making the best follow-up and treatment plans for the patients and not disrupting them, and in terms of the cost to the health system of the patients who cannot be taken to the service outside the patient. In our study, although the cost of patients treated in the intensive care unit was calculated, the cost of delayed consultations was not calculated. For this purpose, further studies are needed.

We aim to investigate the causes of delays in consultation response time based on this study, develop preventive strategies (SMS model, etc.) and conduct new studies investigating both the clinical and cost consequences of this delay and ensure that intensive care is effective, efficient and less costly contribute to its use.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dokuz Eylül University Non-interventional Clinical Researches Ethics Committee (Date:26.10.2020, Decision No: 2020/26-45).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Assessing the effects of asthma attack simulation on cognitive, psychomotor, and affective learning in nursing students: a randomized controlled study

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ABSTRACT

Aims: Asthma is a global health problem. Nursing students, who play a key role in managing asthma attack, should be capable of recognising and responding to asthma symptoms. This research aimed to assess the repercussions of asthma attack simulation training on nursing students cognitive, psychomotor, and affective learning domains.

Methods: A randomised controlled trial was used in this study. Fourth-year nursing students with no prior simulation training experience were recruited. This research randomly divided participants into two distinct groups: a simulation group, consisting of 53 members, and a control group, with 62 members. Each group received 100 hours of standard training, and only the simulation group received 210 hours of asthma attack simulation training instruction based on Bloom's taxonomy the following day. A knowledge questionnaire was used to evaluate nursing students' cognitive learning on asthma attacks right after theoretical training and three months afterwards. The Objective Structured Clinical Examination was used as a standardised evaluation instrument to evaluate students' psychomotor learning, and the emotional learning, empathy, motivation, self-efficacy, and anxiety levels of nursing students were assessed using a Likert scale ranging from 1 to 10 three months after their theoretical training.

Results: Asthma attack cognitive, psychomotor, and emotional learning of nursing students in the Simulation group improved after the intervention compared to the control group ($p<0.05$).

Conclusion: Simulation-based training demonstrates potential efficacy in enhancing nursing students' cognitive, psychomotor, and affective learning related to asthma attacks.

Keywords: Asthma attack, education, learning, nursing students, simulation

INTRODUCTION

Asthma is a global health problem characterised by chronic airway inflammation that causes coughing, wheezing, shortness of breath, and chest tightness.[1] According to GINA (2017) and Asthma Diagnosis and Treatment Guide (2020), asthma is prevalent, impacting 334 million people worldwide and 3.5 million people in Turkey alone.[1,2] According to the Network (2014), an estimated 14% of the world's 2.2 billion children have asthma.[3] To address the global burden of asthma, nurses should be trained and knowledgeable in current asthma management requirements. As Hanson et al.[4] pointed out, the lack of understanding among nurses could negatively affect the effectiveness of asthma management. Their research found a link between nursing proficiency levels and recognising asthma symptoms and administering treatment on time. In addition, Harrington et al.[5] demonstrated that nurses play an essential role in reducing asthma symptoms. As a result, it is critical to adopt instructional approaches that support the development of this skill in the education of student nurses.

Simulation is the recreation of multiple tasks, relationships, phenomena, equipment, behaviour, or cognitive activities in real-world environments.[6] It provides students a risk-free and authentic learning environment to apply gained knowledge, explore possibilities, and improve psychomotor abilities in a safe setting.[7] Simulation-based education, which utilises high-fidelity models, has gained recognition in nursing education because it enhances the acquisition of knowledge, critical thinking, clinical skills, and performance.[8] Previous research has shown that high-fidelity simulation improves students' critical thinking abilities,[9] knowledge,[10] clinical reasoning,[8]

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and self-confidence while simultaneously reducing anxiety.[11,12] As a result, incorporating simulation-based training is critical, particularly in nursing education, which requires clinical competence.[13]

Nursing education should seek to accomplish high-level learning objectives to provide nurses with the ability to effectively prepare for asthma attack management. In this context, using Bloom's taxonomy, which emphasises achieving advanced learning goals, can be beneficial in developing simulation-based training and scenarios. Bloom's taxonomy thus enables learning outcomes at six levels, from the lowest level of recall to the highest level of knowledge formation and evaluation.

Previous research has shown that education planned with Bloom's taxonomy leads to high-level learning outcomes.[14,15] Bloom's taxonomy-based scenarios allows nursing students to use their educational, perceptual, and psychological learning experiences. It enables students to develop essential skills such as critical thinking, evaluation, problem-solving, decision-making, and data analysis. Using high-fidelity simulators in simulation scenarios provides nursing students safe, monitored environment to learn knowledge, skills, and reasoning.[16]

This study aimed to investigate the effectiveness of Bloom's taxonomy-structured simulation scenario in enhancing nursing students' comprehension of asthma attacks. High-fidelity simulation was employed to support nursing students in identifying asthma attacks symptoms, making informed treatment decisions according to asthma severity, and evaluating treatment outcomes. The primary objective was to assess the effects of simulation training on nursing students' cognitive, psychomotor, and affective learning outcomes related to asthma attacks.

METHODS

The study was carried out with the permission of by Bursa Uludağ University Faculty of Medicine Clinical Researches Ethics Committee (Date: 02.06.2021, Decision No: 2021-7/11). Oral consent was obtained from all volunteered students. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

H1: Simulation training improves cognitive learning in asthma attacks more than the control group.

H2: Simulation training improves psychomotor learning in asthma attacks more than the control group.

H3: The simulation training enhances the affective learning of asthma attacks to a greater extent than in the control group

Study Design and Participants

This study was an experimental (three-blind) design. Nursing students were randomly allocated to one of two groups in this study: Simulation Group (n=53) and Control Group (CG) (n=62). One hundred and thirty volunteer students formed the sample. The fourth-year nursing students (n=168) at a university in Bursa between July and November 2021 with no prior experience in simulation training were recruited and randomly assigned to one of two groups. Criteria for inclusion of nursing students: To take public health nursing courses. The sample size for the study was 53 individuals in each group (simulation group and control group) and 106 individuals in total for an effect size of 0.550 for the primary hypothesis with 0.80 power and 0.05 Type I error (G*Power Version 3.1.9.7 statistical software). The sample size was calculated using the study's results with a similar methodology to this study.[17] The investigators employed the medicres randomly assigned person-to-group software for student randomisation. In total, 165 students were randomly designated, with 65 in group A for simulation, and 65 in group B as the control.) (https://e-picos.com.tr/apps/calculation/rags).

Due to research dropouts, the study was completed with 115 students who participated in the simulation (n=53) and control (n=62) groups. Randomization was conducted after the initial assessment, so the instructor was unaware of the group assignments of the students during the initial assessment. The students were unaware of which group they belonged to.

Procedure

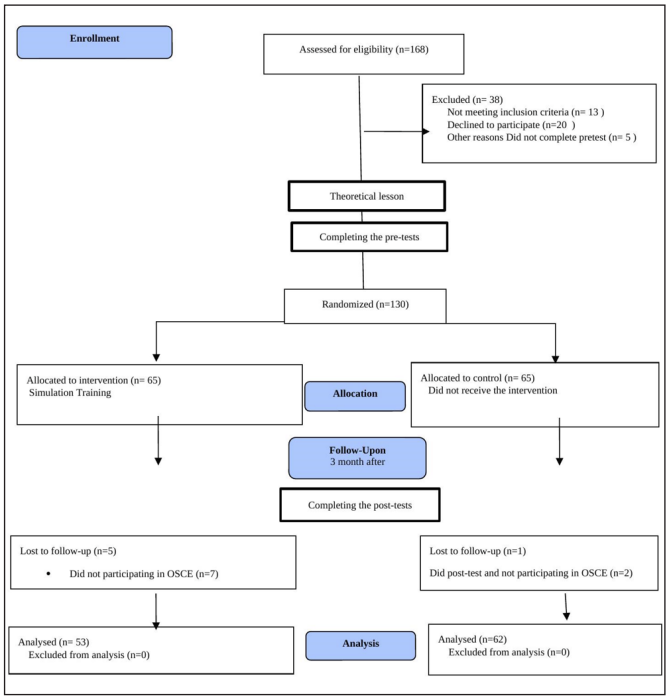

Figure 1. The flow diagram of the study

Theoretical Training

Asthma attacks management (pathophysiology, asthma attack symptoms, drug administration steps and drug effect evaluation) was theoretically given to nursing students who took the public health nursing module (50 minutes).

The researcher showed a video of three films to each nursing student. The first video describes the symptoms of asthma; the second video demonstrates mild, moderate, and severe asthma attacks; and the third video demonstrates the methods for administering a metered dose inhaler for an asthma attack and evaluating the medication's results. Each video lasted 10 minutes. After viewing the film, questions were answered for 20 minutes (50 minutes).

Completing The Pre-tests

Following the instruction, the asthma knowledge questionnaire and the Objective Structured Clinical Examination (OSCE) were used to assess pre-test knowledge and skills.

Randomisation

Nursing students who completed the knowledge test and the ability to apply an OSCE were divided into the simulation group (65) and control group (65) by a simple random sampling method.

Simulation Training Planning

The asthma attack simulation scenarios were formulated in accordance with Bloom's taxonomy and global clinical simulation benchmarks.[14,15,18]

- **The first level/stage (remember):** Recall the definition of asthma, clinical criteria and asthma attack classification, common asthma medications/ interventions, and asthma diagnostic tests.
- **The second level (understand):** Understand the clinical presentation spectrum by classifying the forms of asthma and their relationship to the underlying cause, for example, by classifying the severity of an asthma attack and changes in vital signs.
- **The third level (apply):** Utilise your expertise to identify the issue and implement the proper rapid response for each type of attack.
- **The fourth level (analyse):** Analyse the evidence for the best treatment techniques in various circumstances and use clinical outcomes to differentiate different asthma exacerbations.
- **The fifth level (evaluate):** Assess the validity of invasive monitoring and its impact on outcomes or short-acting beta-agonists, ipratropium bromide, and magnesium types.
- **The sixth level (create):** Update new information, such as new diagnostic measures or prognostic indicators.

A simulated training scenario involving a 17-year-old patient with allergic asthma was developed. Nursing students received instruction about the scenario. The script was sent the day before was stated that it would be taped. On a high-fidelity simulator (ARES), nursing students were asked to assess asthma attacks (diagnosis, diagnostic), intervention, and referral criteria. The instructor was a facilitator in each session, monitoring the nursing students' behaviour and responding as needed" A nursing student dedicated 55 minutes to the simulation, which consisted of nine iterations of a 5-minute briefing, a 10-minute simulation, and two 40-minute debriefing sessions. The overall simulation process took 210 minutes to complete.

The Briefing (5 Minutes)

The high-reality simulator was introduced to the nursing students. The objectives of the scenario, the expectations, and the role of the facilitator were all explained.

Goals

i. Nursing students' recognition and categorisation of asthma attack symptoms,
ii. Understanding asthma attacks according to the asthma class,
iii. Implementing the intervention based on the severity of asthma symptoms,
iv. Distinguish among asthma conditions utilising the asthma guideline,
v. Evaluation of the asthma intervention delivered in accordance with the asthma guidelines,
vi. New diagnostic measurements and information are being developed in order to validate new markers.

A Case Study

Ada, a 17-year-old woman, visits the outpatient clinic with symptoms of asthma and cough. Ada complains of continuous coughing and breathing difficulties to the attending nurse (voiced as a voiceover).

Expectations of nursing students based on the simulated scenario.

First Nursing Student

Step one: Nursing students' recognition and classification of asthma attack symptoms and diagnosis of an asthma attack (Level 1 of the Bloom Taxonomy).

Second Nursing Student

Step two: To understand clinical intervention in an asthma attack by classifying it according to the severity of the attack based on vital sign changes (Level 2 of the Bloom Taxonomy).

Third Nursing Student

Step three: To apply the intervention to the severity of the symptoms for each type of asthma attack (Level 3 of the Bloom Taxonomy).

Fourth Nursing Student

Step four: Analyse various asthma conditions in accordance with the asthma guide and distinguish between various asthma conditions (Level 4 of the Bloom Taxonomy).

Fifth Nursing student

Step five: Evaluation of the patient's response to the asthma intervention as suggested by the asthma guidelines (Level 5 of the Bloom Taxonomy).

Sixth Nursing Student

Step six: New diagnostic measurements provide new information for validating new markers (Level 6 of the Bloom Taxonomy).

Intervention

The high reality simulator has adapted the scenario to reflect the onset of mild, moderate, and severe asthma attacks. The case was read aloud, and the scenario was played out on the simulator. The nursing students were separated into two groups. Each scenario involved 6-7 nursing students, performed nine times for each nursing student to experience. As nursing students were engaging with the scenario, their peers in the debriefing room were concurrently analysing the performance of others in the scenario enactment.

When the nursing students arrived, the simulation started with a 5-minute briefing. The simulation's learning objectives, expectations, a brief scenario description, the protocol for each scenario phase, roles, outpatient room, High-fidelity simulator (ARES), and resources were all discussed. A break was provided when all nursing students in each group had finished the simulation. The trainer then conducted a 40-minute Defibring session, which was held in two Defibring sessions. In the debriefing session, the Gather, Analyse, Summarise (G.A.S.) method was used.19 The nursing students actively participated in a reflective discussion during the educational session, encompassing an assessment of their performance in the asthma attack simulation. Topics addressed included self-evaluations of strengths and weaknesses, identification of potential areas for improvement, peer evaluations, and subjective experiences throughout the educational intervention.

For the content validity of the intervention program curated by the investigator, the insights of nine experts were sought (comprising two chest specialists, five nurses, and two simulation specialists). With a scope valence index of 0.84 and the minimum scope valence ratio being 0.75, the findings affirm the content validity of the intervention program.

Post-test

An evaluation was carried out three months after the training to evaluate the nursing student's skills, their perceptions of the learning process, and their performance on the asthma knowledge test.

Data Collection

Knowledge Test

The researcher (N=1) developed the knowledge test by conducting a comprehensive literature review and seeking expert opinions from chest disease specialists(N=4) and nurses working in clinical settings (N=4). The test content encompassed various aspects, including identification of asthma attack symptoms (3 questions), drug selection decision-making (2 questions), comprehension of drug administration protocols (10 questions), and evaluation of treatment outcomes (5 questions). Each correct answer was assigned a score of 1, resulting in a maximum test score of 20 and a minimum score of 0 The analysis of knowledge pertaining to asthma care content retains its validity, as indicated by a scope valence index of 0.88 and the lowest scope valence ratio recorded at 0.75.

Asthma Attack Skills Assessment

The Objective Structured Clinical Examination (OSCE) assessed nursing students' ability to manage asthma attacks. The OSCE form comprised ten phases covering various aspects of asthma assessment and intervention. The form's content included students' ability to identify asthma (1 point), classify asthma (1 point), diagnose asthma (1 point), correctly diagnose an asthma attack (1 point), comprehend clinical interventions for asthma (1 point), implement asthma interventions effectively (1 point), recognise various types of asthma (1 point), evaluate the outcomes of asthma interventions on the patient (1 point), and make appropriate decisions regarding continuation (1point) or elimination of asthma interventions (1 point). The researcher assessed each step by assigning a rating of either successful (1) or unsuccessful (0). This evaluation yielded a minimum score of 0 and a maximum score of 10.

In order to establish the content validity of the OSCE form developed by the researcher, the perspectives of nine experts were gathered, consisting of two academic nurses, three clinical nurses, and four pulmonologists. The obtained metrics, with a scope valence index of 0.86 and a minimum scope valence ratio of 0.75, provide affirmation of the content validity of the OSCE form. The OSCE was evaluated by two standardized patients and conducted in two outpatient clinics to assess the students' performance.

Each clinic setting was allocated a researcher and a standardised patient (totalling two clinical settings). The researcher trained an instructor for the OSCE assessment of students. The instructor assisted students with their OSCE assessment. Each student was given two minutes to read the case and five minutes to accomplish the skill. The researcher examined the records to confirm that the OSCE forms completed for each nursing student were accurate. Throughout an internship, skills were assessed. The OSCE form was filled out immediately following the theoretical instruction at the simulation centre and three months later during the skill practice.

Students' Perception of the Process

The nursing students' perceptions of the standardised patient were evaluated based on four key parameters: motivation, critical thinking abilities, self-awareness, and empathy. For each parameter, students were required to use a Likert-type rating system ranging from 1 to 10.

Data Analysis

Data analysis was conducted using the SPSS 23 software package. The Mann-Whitney U test, Wilcoxon signed rank test, and Chi-square test were utilised for statistical data evaluation. Importantly, the statistical analysis was carried out in a blind manner, meaning the analyst did not know the group assignments of the nursing students.

RESULTS

The mean age of the students in the simulation group was 22.18±2.01 years, and the average age in the control group was 21.74±1.36 years. The proportion of female students in the groups was 70% and 80% (Table 1). Gender and age were similar between the groups.

Table 1. Descriptive characteristics of students in groups

Descriptive characteristics	Simulation group (n=53) n %	Control group (n=62) n %	Test statistics	p value
Age (mean±sd)	22.18± 2.01	21.74± 1.36	1435.50*	0.223
Gender				
Female	42 80	44 71	1.038**	0.308
Male	11 20	18 29		

Abbreviations: sd: standard deviation, * Mann-Whitney U Test, **Chi square test.

While the pre-test knowledge scores of the students in the simulation group were 7.24±2.15, the post-test knowledge scores were 14.27±2.91, and there was a statistically significant difference (p<0.05).

The post-test knowledge scores of the students in the simulation group were 6.95±2.08 higher than the pre-test knowledge scores of 12.52±3.06 (Table 2). (Table 2). There was a statistically significant difference between the groups in the pre-test and post-test knowledge scores (p<0.05) (Table 2).

Table 2. Average scores of students' knowledge and OSCE skill levels.

	Simulation group (n=53) mean±sd	Control group (n=62) mean±sd	Test statistics	p value
Cognitive				
Knowledge test (0-20)				
Pre-test	7.24±2.15	6.95±2.08	1509.50*	0.734
Post-test	14.27±2.91	12.52±3.06	1071.00*	0.001
Test Statistics p-value	-6.22 <0.001**			
Psychomotor				
OSCE (0-10)				
Pre-test	1.41±2.09	1.24±2.22	1495.50*	0.340
Post-test	7.16±1.92	6.04±1.73	1069.50*	0.001
Test Statistics p-value	-6.29 <0.001**			

Abbreviations: sd: standard deviation, *Mann-Whitney U Test, **Wilcoxon signed ranks test

While the pre-test skill scores of the students in the simulation group were 1.41±2.09, the post-test skill score was 7.16±1.92, and there was a statistically significant difference (p<0.05). While pre-test skill scores of the students in the control group were 1.24±2.22, their post-test skill scores were 6.04±1.73, and there was a statistically significant difference (p<0.05) (Table 2). There was a statistically significant difference between the groups in the pre-test and post-test skill scores (p<0.05) (Table 2).

The perceived motivation scores (7.24± 1.74), critical thinking scores (8.90± 1.06), empathy scores (8.0 5± 1.23), self-awareness scores (8.00± 1.19) of the students in the simulation group were higher than the perceived motivation scores (6.00±1.87), critical thinking scores (7.95±1.20), empathy scores (7.48±1.32), self-awareness scores (7.25±0.92) of the students in the control group (p<0.05) (Table 3).

Table 3. Evaluation of the perception of the Process by the students

Affective competences	Simulation group (n=53) mean±sd	Control group (n=62) mean±sd	Test statistics	p value
Motivation	7.24±1.74	6.00±1.87	1037.50*	0.001
Empathy	8.05±1.23	7.48±1.32	1183.50*	0.008
Critical thinking skill	8.90±1.06	7.95±1.20	874.00 *	<0.001
Self-awareness	8.00±1.19	7.25±0.92	1054.00*	0.001

Abbreviations: sd: standard deviation, *Mann-Whitney U Test

DISCUSSION

Cognitive learning (asthma knowledge scores), psychomotor learning (OSCE scores), and affective learning (perceived self-awareness, perceived motivation, perceived empathy, and critical thinking scores) scores of the simulation group were higher compared to the control group. Study results support our hypothesis.

This study found that simulation is a better method of teaching asthma attacks than control. Previous studies have found simulation to be effective for teaching the management of asthma exacerbations.[20,21] The simulation scenario prepared according to Bloom's taxonomy for the simulation group in this study provided nursing students with complete learning in cognitive, psychomotor and affective domains.

Nursing students in the simulation group had a higher level of knowledge, which we measured to determine cognitive competence in asthma attack management, compared to nursing students in the control group. We believe that this difference in the level of knowledge is due to the structure of the simulation scenario in line with Bloom's taxonomy, which allows for the synthesis of information and learning through the simulation experience. Furthermore, reflective thinking in the defibring part of the simulation training allowed nursing students to create knowledge.

The skill levels of administration, which we measured to determine psychomotor competence in asthma attack management, were higher in the simulation group compared to the control group. We believe that this difference is due to the structure of Bloom's taxonomy and simulation that enables holistic cognitive, psychomotor, and affective learning. This is because simulation sessions enable the integration of multiple information at cognitive, psychomotor, and sensory levels, thereby gaining the necessary competencies.[22]

Self-awareness, motivation, and empathy were higher in nursing students in the simulation group than in the control group. A similar study found that the simulation method is more motivating in learning bronchial asthma than the classical method.[23] These results show that perceived motivation is an essential factor positively affecting learning.[23] For this reason, the simulation should be included in nursing training.

In the study, Alamrani et al.[24] found that critical thinking skills in nursing students remained similar in the traditional and simulation group. Simulation with high reality increased nursing students' critical thinking skills.[9] In this study, the students' necessary thinking skills in the simulation group were higher than those in the control group. As a result, we think that the scenario prepared using Bloom's taxonomy increases critical thinking by allowing nursing students to experience asthma attacks with high reality simulation, make decisions in necessary conditions and be essential in notification sessions. The simulation teaches nursing students how to think in the face of an acute situation. It allows you to remember old information in an unexpected case and make new quick decisions to solve the problem.[25]

Limitations of the Study

One notable strength of this research lies in the construction of the simulation scenario, which aligns with Bloom's taxonomy and effectively promotes learning in the cognitive, psychomotor, and affective domains. This study shows the results of nursing students at a university. The small sample size of this study limits the generalisation of the findings. One of the other limitations of this study is to see the effect of cognitive, psychomotor and affective domain assessments, and they were measured three months after the training rather than immediately after the training. Meanwhile, both groups could not be influenced by other places.

CONCLUSION

Learning gains could be measured objectively thanks to the scenario prepared according to Bloom's taxonomy. The level of cognitive knowledge could be measured objectively with the knowledge test; the psychomotor level could be measured objectively with OSCE; and the affective level could be measured objectively by scoring students' motivation, empathy, and self-awareness. These three domains were measured in the acquisition of asthma attack proficiency and found simulation e more effective than control in learning asthma attack management, which is important in nursing. Nursing students determined that simulation is more motivating, increasing self-awareness, developing empathy, and gaining critical thinking skills compared to control. According to this study's conclusions, we suggest using simulation in nursing education in the future.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of by Bursa Uludağ University Faculty of Medicine Clinical Researches Ethics Committee (Date: 02.06.2021, Decision No: 2021-7/11).

Informed consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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A six-year retrospective evaluation of odontogenic infections in pediatric patients requiring hospitalization

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ABSTRACT

Aims: Odontogenic infections in pediatric patients are common conditions which need to rapid treatment because of the progression of the infection into deep facial spaces. This study aimed to investigate the treatment modalities and clinical findings of hospitalized patients because of odontogenic infections.

Methods: The study sample was collected from patient's medical records. Demographic data, clinical findings, hospital length, source of the infection and laboratory findings were compared.

Results: 330 patients were included and the study competed with 111 girls (34%) and 219 boys (66%) with a mean age of 6.81±3.25 years (min:1-max:17). 173 (%52) patients were treated for buccal space infection. The average duration of hospital stay was 2.69±0.78 days (min: 1, max: 6) in all cases and hospital stay was statistically higher in boys than girls (p=0.019). The duration of hospitalization was statistically higher in patients with fossa canina infection (p<0.001). 267 patients (%81) received ampicillin/sulbactam combined with metronidazole. The average day of the extraction of the causative tooth was the second day of the hospital stay. There was a positive correlation between length of hospital duration and CRP levels (p<0.001).

Conclusion: Odontogenic infections with facial cellulitis are generally seen in boys under six years old. The upper face is the most affected side with a rate of 72%. Intravenous penicillin and metronidazole treatment and early dental extraction with surgical drainage are necessary for rapid resolution of the infection.

Keywords: Odontogenic infections, pediatric, hospitalization, hospital stay, abscess

INTRODUCTION

Odontogenic infections are caused by bacterial invasion of the tooth and its surrounding soft and hard tissues and they generally originate from pulpal necrosis with bacterial invasion of peri radicular tissue or from deep periodontal pockets and pericoronitis leading to the formation of purulent collections.[1] Depending on the location and severity of the infection, the symptoms might be moderate to severe. Pain, swelling, and redness of the affected area are the most typical symptoms. Odontogenic infections can worsen breathing and swallowing difficulties, face cellulitis, fever, and other symptoms.[2] However, the inflammation process can advance and expand into deep facial regions if the primary source is not eradicated.[3] Multiple serious complications, including airway obstruction, cavernous sinus thrombosis, mediastinitis, eye globe affections, brain abscess, sepsis, and organ failure have been reported.[1-3]

Maxillofacial infections require early diagnosis and treatment. It depends on the severity of the infection and its location. Controlling and removing the causative agent is the primary goal.[4] Mild infections can often be treated with antibiotics alone, while more severe infections may require surgical intervention with drainage and hospitalization. Hospital care is indicated for patients who spread the infection to the parapharyngeal, peritracheal, and deep facial spaces, airway compromises and rapidly progressing cases with a fever higher than 38°C.[3,4] Children with facial infections commonly respond to antibiotic therapy alone. Moreover, rapid dental extraction with antibiotic therapy helps to shorten the length of hospital stay. The main purpose of this study was to perform a retrospective assessment of odontogenic infections in pediatric patients requiring hospitalization and to compare the clinical differences and treatment modalities.

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METHODS

Sample Collection

This retrospective study was approved by Erciyes University Clinical Researches Ethics Committee (Date: 06.02.2019, Decision No: 2019/113). The study sample was collected from patient's medical records in the hospital database of the Oral and Maxillofacial Surgery Department. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Hospitalized patients between the ages of 0-18 diagnosed with facial cellulitis of odontogenic origin between 2013-2019 years were included in the study. Cases of infections from unreported sources or with insufficient information were disregarded. Demographic parameters such as age, gender, and medical compromises were recorded. Detailed clinical findings such as the source of infection, region(upper or lower jaw), and affected spaces, a month at the time of disease onset, antibiotic usage before hospital visit, fever, and required treatment including total antibiotic usage during hospital care, antibiotic regimen, length of stay was investigated. Surgical interventions such as extraction and additional drainage, and anesthetics methods during surgical procedures (local or sedation/general) were recorded. WBC, lymphocyte, neutrophil, platelet counts, hemoglobin, erythrocyte sedimentation rate (ESR), and C- reactive protein (CRP) levels during the hospital care were obtained from patient records.

Statistical Analysis

Shapiro-Wilk's test, q-q plots, and the histogram were all used to evaluate the data's normality. The Levene test was used to analyze the homogeneity of the variance. For quantitative data, one-way analysis of variance (ANOVA) or Kruskal-Wallis tests were used to assess group differences; for qualitative data, Fisher exact test was used. Data values were expressed using mean±standard deviation, median (1^{st}-3^{rd} quartiles), or frequencies(percentages). Analyses were conducted using TURCOSA (Turcosa Analytics Ltd. Co., Turkey, www.turcosa.com.tr). Statistical significance was defined as a p-value 5%.

RESULTS

The study sample consisted of 361 patients hospitalized due to odontogenic facial cellulitis. 31 patients were excluded from the study because of insufficient data in patient records and laboratory findings. The study competed with 111 girls (34%) and 219 boys (66%) with a mean age of 6.81±3.25 years (min: 1-max: 17). More than half of the patients (54%) were individuals under 6 years of age. 35 patients had additional systemic conditions including epilepsy, FMF, diabetes, and asthma. Facial cellulitis originated from upper teeth in 168 patients (51%) and lower teeth in 162 (49%) patients. 173 patients were treated for buccal lodge abscesses and these patients constituted approximately 52% of all cases. Besides, there were 87 patients with submandibular abscesses (26%) and 64 (19%) patients were treated because of fossa canina abscess. 6 patients were hospitalized because of vestibular/lingual abscess formation. (Table 1). Most cases were hospitalized at 9^{th} (n=40) and 12^{th} months (n=40). The distribution of patients by month is shown in Chart. 228 patients (69%) had no history of using any antibiotics before hospitalization and 95 patients(29%) had used oral antibiotics before admission to the hospital. The initial admission to the hospital was considered the 1st day of the hospitalization. The average duration of hospital stay was 2.69±0.78 days (min: 1, max: 6) in all cases and hospital stay was statistically higher in boys than girls (p=0.019). According to the type of infection, the duration of hospitalization was statistically higher in fossa canina patients (p<0.001). There was no statistical difference between hospital stay and causative agents (upper or lower teeth, p=0.068). Similarly, there was no statistical difference between patients according to the usage of antibiotics before hospitalization (p=0.701) (Table 2).

Table 1. Descriptive statistics of study variables

Variables	Statistics
Sex, n (%)	
Female	111 (34)
Male	219 (66)
Age, (years)	
mean±sd	6.81±3.25
M (min-max)	6 (1-17)
Origin of infection	
Upper teeth	168 (51)
Lower teeth	162 (49)
Space, n (%)	
Buccal	173 (52.0)
Submandibular	87 (26.0)
Fossa canina	64 (19.0)
Vestibular/lingual	6 (3.0)
Hospital stay (days)	
mean±sd	2.69±0.78
M (min-max)	3 (1-6)

The average day of the extraction of the causative teeth was the second day of the hospital stay. It was observed that patients received an average of 4 doses of parenteral antibiotics before tooth extraction and 3 doses after the extraction. 44 patients (13%) received parenteral ampicillin/sulbactam, 267 (81%) received ampicillin/sulbactam combined with metronidazole, and 17 patients (5%) were treated with clindamycin because of penicillin allergy.

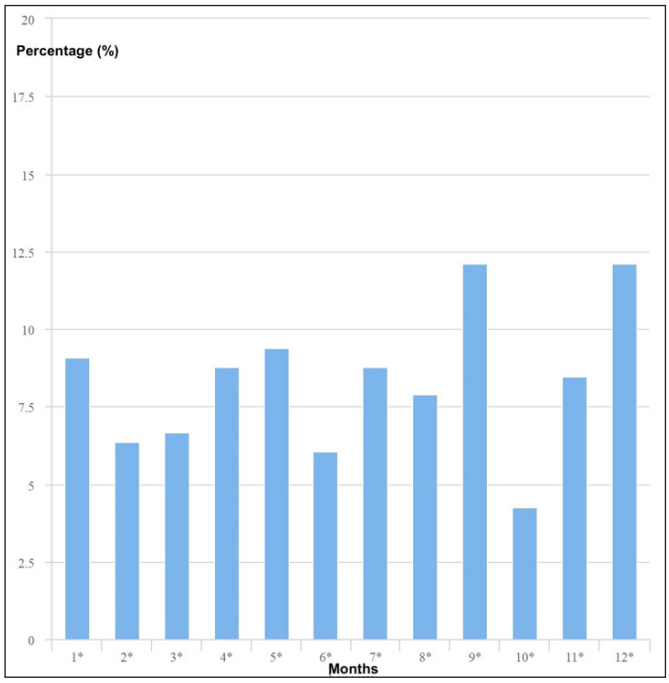

Chart. Incidence of infection by months

Tooth extraction was performed in 66 patients with local anesthesia and sedation was used in 264 patients. An average of 2 (1-3) teeth were extracted from each patient. There was no need for additional drainage of pus formation in 300 patients, while additional intraoral or extraoral drainage was performed in 30 patients, and a Penrose drain was inserted. The drain was removed on the second day after the tooth extraction. Fever over 380 was observed in 81 (25%) patients before tooth extraction and post-operative fever was seen in 40 patients (12%). When the relationship between the duration of hospital stay and the presence of preoperative fever was evaluated, it was determined that the duration of hospital stay was statistically higher in patients with preoperative fever than in those without (p<0.001). Similarly, the length of hospital stay was statistically higher in patients who had postoperative fever than in patients without postoperative fever (p= 0.004).

The average CRP, ESR, and WBC levels were 21.59 (10.9-52.2), 20 (13-29), and 10.5 (8.7-13.8) in all patients respectively and there were no statistical differences according to sex (p>0.05). When the levels of CRP, ESR, and WBC were compared to the causative agent, CRP levels were found statistically higher in infections associated with lower teeth (p=0.014). There were no statistical differences according to the type of infections (p>0.05). Besides, CRP, ESR, and WBC levels were statistically higher in patients who had a preoperative fever (p<0.05). CRP and ESR were statistically higher in patients who had postoperative fever after tooth extraction but WBC count was not different. (Table 2). Also, it was observed that there was a positive correlation between length of hospital duration and CRP levels (p<0.001). Other laboratory findings including lymphocyte, neutrophil, platelet counts, and hemoglobin, were compared according to the sex and type of infection and there were no statistical differences (Table 3).

Table 2. Comparisons of hospital stay and infection parameters with clinical findings

	Length of hospital stay (days)	CRP (mg/L)	ESR (mm/s)	WBC
Sex				
Girls	2.5±0.78	20.4 (9.4-45.0)	22 (13.5-29)	10.5 (8.6-13.8)
Boys	2.76±0.77	23.3 (12.7-55.2)	19.5 (12-29.25)	10.6 (8.7-13.7)
P value	0.019	0.228	0.455	0.773
Causative agents				
Upper teeth	2.76±0.78	20.4 (9.3- 45.4)	20 (13- 29)	10.5 (8.6- 13.9)
Lower teeth	2.61±0.76	23.3 (14.0- 55.6)	21 (12-30)	10.7 (8.8- 13.6)
P value	0.068	0.014	0.529	0.966
Type of infection				
Buccal space	2.5±0.78	19.8 (10.2- 45.6)	18 (13-26)	10.4 (8.3- 13.1)
Fossa canina	2.9 ±0.76	31.6 (11.3- 56.1)	22 (13-31)	10.5 (8.9- 15.1)
Others	2.77±0.72	22 (12.8-63.0)	21 (12-32)	10.9 (9.3- 13.6)
P value	<0.001	0.157	0.529	0.275
Antibiotic usage before hospitalization				
No (n=228)	2.67±0.79	17.9 (10.1- 50.2)	20 (13- 28)	10.6 (8.8- 13.7)
Yes (n=102)	2.74±0.74	30.3 (17.6-56.1)	20 (11- 35)	10.8 (8.6- 13.8)
P value	0.701	0.114	0.529	0.263
Pre-operative fever				
No (n=249)	2,5 ±0.71	19.7 (10.0- 42.3)	20 (12- 27)	10.2 (8.6- 12.6)
Yes (n=81)	3.1±0.80	46.2 (16.5-69.9)	21 (15- 35)	12.7 (9.2- 15.3)
P value	<0.001	<0.001	0.007	<0.001
Post-operative fever				
No (n=290)	2.6±0.74	21.3 (10.6- 49.9)	20 (12-28)	10.5 (8.7-13.8)
Yes (n=40)	3.0±0.91	37.1 (16.3-106. 0)	26 (15-36)	10.4 (9.2 -13.7)
P value	0.004	0.003	0.047	0.870

mean± standard deviation, Median(1st-3rd quartiles), Mann Whitney U, Kruskal Wallis, Student T test,

Table 3. Evaluation of laboratory findings in different study groups

	Lymphocyte count	Neutrophil count	Platelet count	Hemoglobin (mg/dl)
Total	2.48 (1.7-3.3)	6.9 (5.1- 9.5)	321.5 (267.5 -384.5)	12.8 (12.1-13.6)
Sex				
Girls	2.6 (1.9-3.3)	6.7 (5.1- 9.5)	321 (263-378)	12.8 (12.3- 13.6)
Boys	2.3 (1.6- 3.4)	7.1 (5.1-9.3)	322 (269- 387)	12.8 (12- 13.6)
P value	0.161	0.984	0.959	0.792
Type of infection				
Buccal space	2.5 (1.6- 3.1)	6.6 (5.0-8.6)	325 (264-387)	12.7 (11.9- 13.3)
Fossa canina	2.7 (2.1- 3.7)	6.5 (5.0- 10.4)	319 (270- 374)	12.7 (12.1- 13.8)
Others	2.1 (1.6 -3.4)	7.7 (5.5-10.1)	320 (268- 384)	13.3 (12.3- 13. 8)
P value	0.132	0.161	0.807	0.252

DISCUSSION

Pediatric patients frequently experience oral infections, especially those who are under the age of six. Odontogenic infections spread more quickly in children than in adults due to the presence of tooth germs, bone development centers, and more cancellous bone with wider medullary spaces.[4] It is challenging for clinicians to create a comprehensive management strategy for these individuals because of the anatomical heterogeneity.[5] The prevalence of odontogenic infections is more common in males sex than females with the range of 58%-65%.[6,7] In this study, most of the patients consisted of the male population (66%) and 54% of the patients were under 6 years similar to the literature. In addition, the length of hospital stay was statistically higher in boys.

Upper face infections are more common in pediatric patients and young children are more likely to have a maxillary buccal infection.[8,9] In our study, buccal space was the predominant site of the infection and 237 patients (72%) had upper face infection. The symptoms of upper-face infections are more severe include periorbital cellulitis, cavernous sinus thrombosis, systemic meningitis, and sepsis.[5] Lower face infections can spread to the deep neck and leading Ludwig angina which occurs because of dental origin with a rate of 75%-90%.[5,10] Cellulitis must be aggressively treated with intravenous antibiotics in order to clear up the infection quickly and with the least amount of morbidity as possible. The first choice of antibiotics for odontogenic infections is penicillin alone or in combination with metronidazole.[7,8] If the child is allergic to penicillin, clindamycin, cefazolin or ceftriaxone can be used.[1] A retrospective analysis of odontogenic infections treated with hospitalization showed that penicillin with metronidazole is mostly prescribed antibiotics but various can be used.[7] Odontogenic infections tend to be mixed with a variety of Gram-positive and Gram-negative organisms, with anaerobic organisms, and most infections respond well to empirical penicillin treatment.[11] It is generally accepted that ultimate antibiotic treatment should be started according to the results of sensitivity tests and cultures. The acquisition of cultures and sensitivity data, it was reported, does not appear to be therapeutically helpful and did not directly result in any antibiotic or other therapy adjustments.[6] In this study, we started the empiric antibiotic treatment with penicillin and metronidazole in 81% of the patients and repeat resolution was observed within 2 days and all patients in this study were discharged uneventfully.

Clinical signs can be various in patients with facial cellulitis and most of the patients admit to the hospital because of facial swelling and fever. The body temperature and WBC count of pediatric patients are higher than those of adults, according to an epidemiologic review comparing infections of the craniofacial region in children and adults.[12] Preoperative clinical signs and blood parameters reflect the patient's recovery status. It is accepted that higher CRP and the N/L ratio are related to the length of hospital stay, total antibiotic dosage, and recovery status.[13,14] According to Peters et al.[15] the location of the infection and the underlying medical condition are the best indicators of how long a patient will need to stay in the hospital. In this study, we observed that patients who had facial cellulitis of fossa canina lodge and preoperative high body temperature have more hospital stays than others. Additionally, it was observed that, as the CRP level increased, the length of hospital stay also increased. It is thought that the increase in the length of stay in the hospital is related to the high fever due to the high CRP level.

Intravenous antibiotic therapy with drainage is the most effective treatment modality in maxillofacial infections. Carter et al.[16] reported that surgical interventions under general anesthesia were required in 46% of patients with odontogenic infections. Previously, it was believed that the time of surgical drainage should be performed after the complete resolution of the swelling, and the extraction of the tooth generally be postponed to a later time in a subacute or chronic phase of the infection.[17,18] But recent studies focus on early surgical interventions for treatment. Treviño-Gonzalez et al.[19] showed that delay of tooth extraction of more than three days is

associated with an increased rate of mediastinitis, intensive care unit stay (ICU), and length of ICU stay. A delayed dental extraction was linked to a hospital stay of more than 7 days, according to findings from a different study.[20] Heim et al.[17] concluded to the removal of infection focus on surgical drainage in the early stage leads to the lowest length of hospital stay. In our clinic, we generally performed early extraction of the causative tooth and surgical drainage with intravenous antibiotic treatment and in this study, surgical interventions were performed within two days and 80% of the patients were treated under sedation or general anesthesia.

The limitations of this study are mostly due to its retrospective design. Data on complications follow-ups and blood culture and sensitivity are insufficient. Comparisons according to the different antibiotic treatment modalities are lacking due to the insufficient sample size. More clinical findings in different groups with more patients could contribute to the exact inferences.

CONCLUSION

Odontogenic infections with facial cellulitis are commonly seen in pediatric patients especially in boys under six years old. The upper face is the most affected side with a rate of 72%. Intravenous penicillin and metronidazole treatment and early dental extraction with surgical drainage are beneficial for rapid resolution of the infection with minimal morbidity.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Clinical Researches Ethics Committee (Date: 06.02.2019, Decision No: 2019/113).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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A scientometric analysis and visualization of Pott's disease; 2000-2021

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ABSTRACT

Aims: Spinal tuberculosis, or Pott's disease, is a bacterial infection of the spine, which is primarily brought on by the bacterium Mycobacterium tuberculosis. In this study, Pott's disease-related research papers from the Web of Science database were examined scientometrically. The study's time frame is between 2000 and 2021. To shed light on the trends and advancements in research on Pott's disease, bibliometric techniques are used in the analysis.

Methods: A literature search on spinal tuberculosis was carried out in November 2022 using the WOS search engine. Using specific retrieval keywords, the search covered the years 2000 through 2021. Titles, document types, publication years, author details, affiliations, keywords, funding sponsors, journal names, abstracts, and citations were examined. The data was further processed for both quantitative and qualitative analysis using VOSviewer (1.6.18).

Results: A total of 892 papers from 2000 to 2021 were examined based on search parameters. 430 of these were released as Open Access articles. 81.0% of them were in the Science Citation Index Expanded. The publications came from 77 different nations, China dominated research on Pott's disease with 306 papers, followed by India with 147 and the United States with 86. The investigation revealed a rising trend in recent years, pointing to a rise in interest in Pott's disease. Research fields like neurology and orthopedics made important advances. Notably, 7.7% of the research' funding for Pott's disease publications came from the Chinese National Natural Science Foundation. The leading publishing journal has become the European Spine Journal.

Conclusion: The data shows that China has played a significant role in Pott's disease research, followed by India and the United States. The survey also reveals a growing need for PD research, notably in the disciplines of orthopedics and neurology.

Keywords: Pott's disease, Scientometrics analysis, spinal tuberculosis

INTRODUCTION

Spinal tuberculosis (TB) is the term for TB that affects the vertebra.[1,2] The disease is popularly known as Pott's disease (PD).[3] The Pott name may be attributed to British surgeon Sir Percival Pott's monograph from 1779, which described TB infection of the spine.[4]

The main cause of human tuberculosis is Mycobacterium tuberculosis.[1,2] This disease has a long history; 9000-year-old Egyptian mummies had spinal TB characteristics.[1,2] Spinal TB is the most prevalent type of osteoarticular TB, with 50% of all cases.[5] PD primarily affects the lower thoracic and upper lumbar regions; involvement of the cervical and upper thoracic regions is less common.[6,7] Although uncommon, PD can have serious effects, including as persistent deformities and neurological abnormalities, if it is not detected and treated promptly.[8]

The exact incidence and prevalence of PD are unknown due to underreporting.[3] Given that the disease frequently affects young persons during their most productive entire working life, early detection and diagnosis are crucial. Although it is fortunately still rare, there have been case reports of PD that is multidrug resistant. PD is still quite uncommon in the developed world and is typically diagnosed in people who have immigrated from or have spent a lot of time in endemic nations.[9] Since the beginning of the Human Immunodeficiency Virus (HIV) era, the chance of acquiring PD has grown. Over 90% of all new tuberculosis infections are centered in sub-Saharan Africa and Southeast Asia.[10] Preventing long-term morbidity, deformity, and impairment from the condition requires early identification and treatment.[9,10]

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The purpose of this study was to provide a scientometric analysis of research papers on PD that were taken from the Web of Science (a Thomson Reuters Company) database and covered the years 2000 through 2021. This analysis made use of the bibliometric methodology to provide a more thorough understanding of the development of PD-related research. Furthermore, it made it easier to identify potential future focal points that could become well-known areas of investigation in the field of PD research. Additionally, this study may examine the dominance in terms of the most popular countries, journals, and affiliations.

METHODS

As it is not a human or animal study there is no need for ethical approval. All procedures were carried out in accordance with the ethical rules and the principles.

A literature search was performed in the WOS search engine in November 2022. The fact that the data were taken from open databases eliminated the need for ethical approval. We took "tuberculosis, spinal"[MeSH Terms] OR Spinal Tuberculoses OR Spinal Tuberculosis OR Tuberculoses, Spinal OR Pott's Disease OR Disease, Pott's OR Potts Disease OR Pott Disease OR Disease, Pott OR Pott's Paraplegia OR Tuberculous spondylitis OR vertebral Tuberculosis (Title)" as topical retrieval keywords and time span limited to 2000-2021.

The titles, document types, years of publication, names of authors, affiliations, keywords, funding sponsors, names of publishing journals, abstracts of each record, and citations within the WOS publications were saved as TXT files and imported into Microsoft Office Excel 2019 (Los Angeles, CA, USA).

All document types were selected in this study.

Two authors independently checked the data collection and entry.

The WOS website was utilized to retrieve the literature data for this study.

Data Analysis and Visualization

The Web of Science txt data were imported into VOSviewer (1.6.18 for Microsoft Windows) (https://www.vosviewer.com) developed by Nees Jan van Eck and Ludo Waltman. Both a quantitative and qualitative analysis of the data was performed. For bibliometric analysis, The VOSviewer was used to create the visualization maps. The H-index were added to the table for a more complete scientometric results analysis.

Microsoft Office Excel 2019 was used for tabulation. The percentage and frequency values were given for data expression. Advanced statistical analysis not used.

RESULTS

A total number of 1298 publications published between 1970-2022. 892 of these were published between 2000-2021. We only analyzed these 892 publications according to the search criteria. 430 of the documents were published as Open Access. %81.0 of them were published in the Science Citation Index Expanded (SCI-EXPANDED) index and %17.9 of them in Emerging Sources Citation Index (ESCI) index (Table 1). There were 844 records in these publications, the majority of which were in English. There were also 21 publications in French, 14 in Spanish, 5 in Turkish, 3 in Portuguese, 2 in German, 1 in Indonesian, Japanese, and Korean, in addition to the other languages. The fact that these publications are distributed internationally demonstrates how popular and engaged the topic under study is throughout the world. A total 68.2% of these documents were articles (Table 2).

Table 1. Web of science index

Web of Science Index	Record Count	% of 892
Science Citation Index Expanded (SCI EXPANDED)	723	81.0
Emerging Sources Citation Index (ESCI)	160	17.9
Conference Proceedings Citation Index - Science (CPCI-S)	40	4.4
Social Sciences Citation Index (SSCI)	14	1.5
Arts & Humanities Citation Index (A&HCI)	3	0.3
Book Citation Index - Science (BKCI-S)	3	0.3

Table 2. Document types

Document Types	Record Count	% of 892
Article	609	68.2
Book chapters	3	0.3
Book review	1	0.1
Correction	2	0.2
Early access	2	0.2
Editorial material	83	9.3
Letter	72	8.1
Meeting abstract	63	7.1
Proceeding paper	4	0.4
Retracted publication	1	0.1
Retraction	2	0.2
Review article	57	6.3

The publications examined in this study came from 77 different nations, representing a wide range of geographic origins. The People's Republic of China topped the list of publishing nations with 306 publications, followed by India with 147 publications and the United States of America with 86 publications. These countries were identified through the analysis of publications in the field of PD. Other noteworthy contributors included the following: Turkey, with 54 publications, England, with 37 publications, South Africa, with 27, France, with 21, Pakistan, with 19, Spain, with 16, and Germany, with 14.

These findings demonstrate the disparities in scientific output and research interest in PD among different countries, with China and India significantly advancing the body of knowledge on this topic.

The distribution of research output over time is shown by analyzing publications in the field of PD. Recent years show a higher level of research activity, such as 2021 with 95 publications and 2020 with 80 publications. This upward trend in publications, which began in 2017 with 67 publications and has continued since then, points to an increase in attention being paid to research into and management of PD. Recent years have seen an increase in scientific contributions, which may reflect a growing understanding of the importance of PD and efforts to improve knowledge and treatment options for this condition (**Figure 1**, **Table 3**).

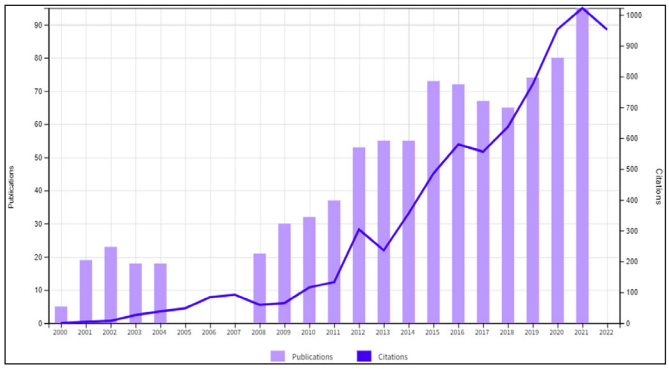

Figure 1. The citations and documents on PD between 2000-2021.

Table 3. Number of documents by years

Publication Years	n	% of 892
2021	95	10.6
2020	80	8.9
2019	74	8.2
2018	65	7.2
2017	67	7.5
2016	72	8.1
2015	73	8.1
2014	55	6.1
2013	55	6.1
2012	53	5.9
2011	37	4.1
2010	32	3.5
2009	30	3.3
2008	21	2.3
2004	18	2.0
2003	18	2.0
2002	23	2.5
2001	19	2.1
2000	5	0.5

The publications selected for this study's analysis came from a diverse range of 61 different research fields. With 224 publications, orthopedics had the most among these research specialties, closely followed by neurosciences and neurology with 210 publications. With 157 and 147 publications, respectively, general internal medicine and surgery also demonstrated significant contributions. Other noteworthy research areas with 56 publications each were experimental medicine and infectious diseases. Additionally, radiology, nuclear medicine, medical imaging, rheumatology, pediatrics, and the respiratory system each contributed 49, 33, 29, and 29 publications, respectively. This broad range of research fields demonstrates the interdisciplinary nature of the PD research and its relevance to numerous medical and scientific fields.

National Natural Science Foundation of China (NSFC) funded 7.7% of the studies and this funding agency was the main funder of PD publications.

Numerous prestigious publishing publications made significant contributions to the subject of Pott's disease research. Notably, the European Spine Journal, which has 33 papers on this subject, has become a well-known venue. With 30 articles, International Orthopaedics came in second, while World Neurosurgery also made a strong showing with 27 publications. In addition, 23 articles about Pott's illness were published in the journal Medicine. Additionally, by publishing 20 articles each about this medical disease, the Asian Spine Journal and the International Journal of Clinical and Experimental Medicine both performed key roles. The most publishing journals were summarized in **Table 4**.

Table 4. Most publishing journals on Pott disese

Journals	Record Count	% of 892
European Spine Journal	33	3.7
İnternational Orthopaedics	30	3.3
World Neurosurgery	27	3.0
Medicine	23	2.5
Asian Spine Journal	20	2.2
International Journal of Clinical and Experimental Medicine	20	2.2
Spine	14	1.5
Bmc Musculoskeletal Disorders	13	1.4
Journal of Orthopaedic Surgery and Research	13	1.4
Spine Journal	12	1.3

*Showing 10 out of 383 entries

The research productivity and impact of PD publications varied among different countries. China exhibited the highest number of publications, with 306 articles, and also achieved a substantial number of citations, totaling 2,978. The average number of citations per document was 9.73, and the H index, a measure of both productivity and impact, was 27. India followed with 147 publications and 1,574 citations, resulting in an average of 10.71 citations per document and an H index of 19 (**Table 5**).

Table 5. The research productivity and impact of most publishing countries

Country	Number of publications	Number of citations, total	Number of citations, per document	H index
China	306*	2978*	9.7	27*
India	147	1574	10.7	19
The USA	86	941	10.9	17
Turkey	54	614	11.3*	11
England	37	176	4.7	8

*Shows the higher number

Figure 2 shows the overlay visualisation of mostly cited countries on PD. The distribution of citations throughout time is depicted by colors. The countries with the most publications are represented by the biggest bubbles. Greater numbers of citations transferred between various nations are shown by thicker gray linkages between bubbles (Figure 2).

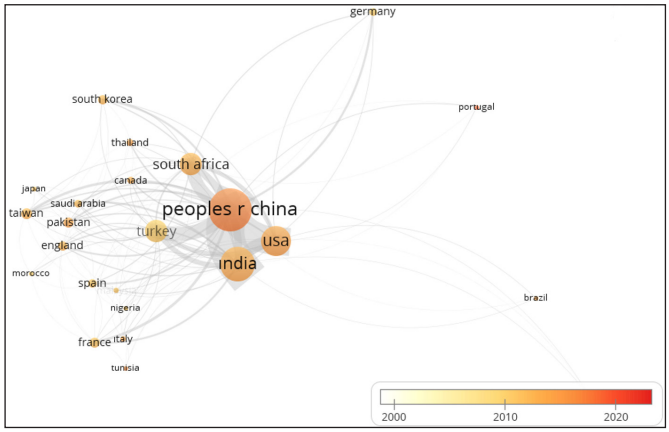

Figure 2. Overlay visualisation of mostly cited countries on PD

Figure 3 shows the overlay visualisation of co authorship between countries by years between 2000-2021. The colors depict the distribution of citations throughout time. The countries with the most publications are represented by the biggest bubbles. Stronger links between bubbles imply that those nations have worked together and co-authored articles.

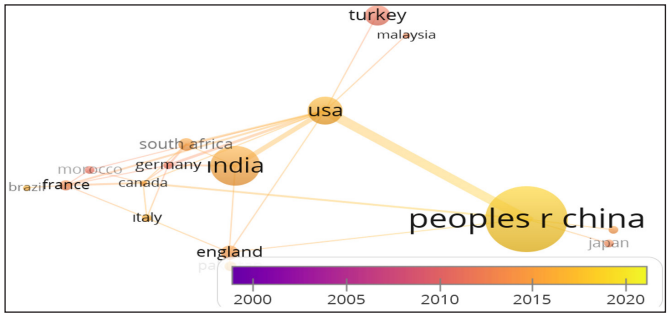

Figure 3. Overlay visualisation of co authorship between countries by years between 2000-2021

The keyword analysis is shown in Figure 4. In the figure, keywords that are frequently used together in publications are depicted by collaborative colors. The biggest bubbles correspond to the keywords that were used the most in the publications under study. This analysis aids in determining the prevalence of particular keywords in the topical research as well as patterns of keyword co-occurrence.

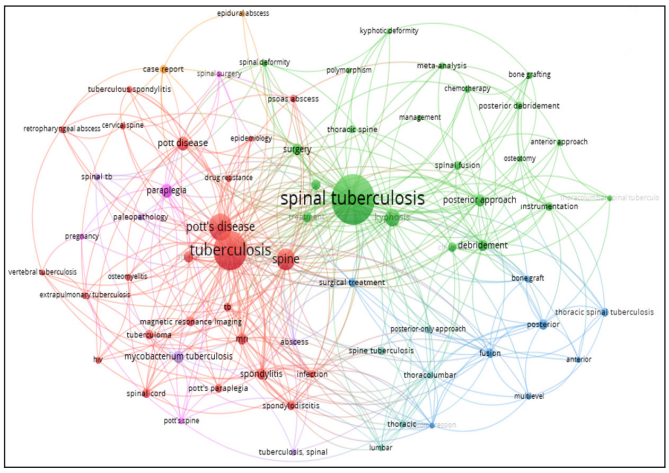

Figure 4. Keyword analysis

DISCUSSION

Based on the scientometric analysis provided, it is evident that research on spinal tuberculosis (Pott's disease) has gained significant attention in recent years. Spinal tuberculosis is the most common form of extrapulmonary tuberculosis, and an increase in tuberculosis cases is observed due to factors such as the rapid growth of the world population and increased migration.[11] A study conducted in China between 2001 and 2016 showed an increasing annual incidence of spinal tuberculosis.[12] Similarly, in our study, we also observed an increase in research related to spinal tuberculosis.

Our study, which focused on studies between 2000 and 2021, shows that PD has remained important in the last 20 years. There is still an increasing number of studies as we approach the present that supports this trend.

A significant portion of the studies have been conducted in China, India, the USA, and Turkey, with 94.6% of them being published in English. The majority of publications were in English, but there were also contributions in various other languages, indicating the international significance of the topic. Nearly 50% of these studies were carried out by orthopedic and neurological disciplines. The rise in the number of studies conducted by these two disciplines might be indicative of the neurological complications caused by spinal tuberculosis and the common clinical symptoms such as spinal deformities, spinal tenderness, and back pain.[3,13]

In a previous similar study by Wang et al.[14] covering the years 1994 to 2015 in the WOS database, 1558

papers were found, and by January 2016 these papers had accumulated 16,152 citations. With 15.1% of articles, 22.3% of citations, and an H-index of 33, the United States was in first place. In terms of articles, citations (815), and H-index (13), China came in third. The literature on spinal tuberculosis indicated a slow progression. The quality of articles needs to be raised despite China's increasing output, and the USA continues to dominate research. This study suggests that "bone fusion" is a new area of interest. In our study, a significant portion of the studies have been conducted in China, India, the USA, and Turkey. Also, India and China are the leading countries in terms of the number of publications on spinal tuberculosis, ranking third and fourth in terms of citation frequency and first and second in terms of h-index. This indicates the need for improvements in the quality of articles coming from China and India. While the USA ranks third in terms of the number of publications, it ranks second in citation frequency and third in h-index. Turkey ranks fourth in terms of the number of publications, following China, India, and the USA, but it ranks first in terms of citation frequency. This increase in citation frequency seems to be the result of high-quality publications in recent years.

The"Spine" journal has previously published a significant portion of the studies related to spinal tuberculosis.[14,15] In our new study, we found that most of the research on spinal tuberculosis was published in European Spine Journal, International Orthopaedics, World Neurosurgery, Medicine, and International Journal of Clinical And Experimental Medicine. This suggests that future studies on Pott's disease are likely to be published in these journals as well.

South Africa is among the top 10 countries with the highest incidence of spinal tuberculosis in the world.[16-18] However, the low number of publications and low citation rate indicate the need for both qualitative and quantitative research in the region.

Limitations

This study is a single database study and also the study's time span was 2000-2021. The main goal of this study, which was to understand advancements within the last two decades, is at the core of the justification for not including data before the year 2000. It's possible that a similar study to the one you've suggested will be developed in the future with a different focus and may make use of different databases. By incorporating a wider temporal range and utilizing alternative data sources, such an endeavor could produce insightful findings that would ultimately contribute to a more thorough investigation of the subject.

CONCLUSION

Our analysis shows that China still leads in terms of the number of publications, but improvements in the quality of their research are needed. Turkey has shown an increase in high-quality publications in recent years. Overall, this scientometric analysis highlights the importance and growing interest in research on spinal tuberculosis. It also points out the significant contributions of certain countries and the need for international collaboration to tackle the challenges associated with PD effectively. The results of this analysis serve as a valuable resource for researchers, policymakers, and healthcare professionals working in the field of spinal tuberculosis.

The improved prognosis associated with prompt identification and treatment in spinal tuberculosis cases highlights the importance of early diagnosis, a current research focus, according to an extensive review of relevant literature on the disease. The complex relationship between vaccination and tuberculosis is highlighted by new perspectives, underscoring its crucial function as a catalyst for further research into the subject of spinal tuberculosis. There have been investigated a number of diagnostic modalities, including clinical presentation, laboratory assays, surgical methodologies, and imaging methods. Furthermore, to prevent cases of misdiagnosis, a thorough understanding of differential diagnostic criteria is essential.

ETHICAL DECLARATIONS

Ethics Committee Approval: As it is not a human or animal study there is no need for ethical approval.

Informed consent: As it is not a human or animal study there is no need for written informed consent.

Referee Evaluation Process: Externally peer reviewed.

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Original Article

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Characteristics and effects of headaches on quality of life in individuals with epilepsy in Çorum province of Turkey

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ABSTRACT

Aims: Varying headache prevalence rates have been reported in individuals with epilepsy in the literature. This study was investigate the frequency and types of headaches in individuals with epilepsy, and the impact of headaches on the clinical features and quality of life in this patient group.

Methods: 150 individuals with epilepsy, 83 female and 67 male, were included in the study sample by random sampling method. Headaches were primarily defined as pre-ictal, ictal, and post-ictal headaches according to the temporal relationship with seizures. Headaches that were not temporally related to seizures were defined as inter-ictal headaches. Types and features of participants' headaches were evaluated using the headache questionnaire consisting of 35 questions. Individuals' quality of life was evaluated using the Quality of Life in Epilepsy Inventory (QOLIE-10).

Results: Of the 150 participants included in the study, 73.33% had generalized, 20% focal, and 6.66% combined generalized focal epilepsy, and 41.33% had accompanying headache complaints. Of the participants with headaches, 35.48% were male and 64.51% were female. Inter-ictal headache, which was detected in 72.58% participants, was the most common type of headache. Tension and migrainous type headaches were more common among participants with inter-ictal headache, whereas migrainous type headaches were more common among participants with pre- and post-ictal headaches. There were statistically significant differences in the scores obtained from all three subscales of QOLIE-10 inventory between the participants with and without headache. Accordingly, quality of life was worse in the headache group than in the headache-free group ($p<0.05$).

Conclusion: Although clinicians focus more on diagnosis and treatment in the context of epilepsy, the treatment of comorbidities is also important. In this context, it is likely that accurate diagnosis of headaches in individuals with epilepsy and treating headaches along with epilepsy will significantly reduce the burden of disease in this patient group.

Keywords: Headache, migraine, tension headache, epilepsy, quality of life

INTRODUCTION

Diverse epidemiological findings have emerged from various studies on the prevalence of headaches among individuals diagnosed with epilepsy. Migraine, a frequent comorbidity of epilepsy, has been the focus of numerous research investigations. We aimed to explore the frequency and classification of headaches among individuals with epilepsy, as well as the impact of headaches on the clinical characteristics and quality of life in this population. This study was conducted in the Çorum Province of Turkey, focusing on patients attending neurology outpatient clinics.

METHODS

This prospective, single-centre study involved patients with epilepsy assessed for headaches at the neurology outpatient clinics of Hitit University Erol Olçok Training and Research Hospital and initiated after Hitit University Faculty of Medicine Clinical Researches Ethics Committee (Date: 16.03.2023, Decision No: 2023-33), and adhered to the ethical guidelines established by the Declaration of Helsinki. All the participants provided informed consent.

The Kolmogorov-Smirnov test assessed the normal distribution of data, while the chi-square test, Pearson's correlation test, and Spearman's correlation rho coefficient were used for statistical analyses. All analyses were two-tailed, with p-values ≤0.05 indicating statistical significance.

Headache characteristics of patients with epilepsy were evaluated according to the International Headache Classification. Individuals with secondary headaches, symptomatic epilepsy, and intellectual disabilities were

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excluded. Ultimately, the sample consisted of 150 epilepsy patients (83 females and 67 males) attending neurology outpatient clinics, who were selected through random sampling. Headaches were categorized as pre-ictal, ictal, and post-ictal based on their temporal relationship with seizures, whereas headaches unrelated to seizures were classified as inter-ictal. Pre-ictal headaches were defined as those commencing within 24 hours before a seizure and lasting until seizure onset. Ictal headaches coincided with other seizure symptoms, and post-ictal headaches developed within 3 hours after a seizure, resolving within 72 hours. Epilepsy diagnoses followed the International League Against Epilepsy classification criteria, while migraine diagnoses followed the International Headache Society criteria.[1-3] Seizure types were determined based on electroencephalographic (EEG) findings and the patient's medical histories. Clinical and demographic data were collected, and headache types and characteristics were assessed using a 35-question headache questionnaire. The Quality of Life in Epilepsy Inventory (QOLIE-10), comprising 10 items, was used to evaluate the participants' quality of life.

The QOLIE-10, which measures the quality of life, features three subscales: effects of epilepsy, mental health, and role functioning. Higher QOLIE-10 scores signify a lower quality of life. Analyses compared the headache and headache-free groups and their respective subgroups.

RESULTS

A total of 150 participants were included in the study, with 110 (73.33%) having generalized epilepsy, 30 (20%) having focal epilepsy, 10 (6.66%) having generalised focal epilepsy, and 62 (41.33%) reporting concurrent headache complaints. The average age of the sample was 35.2±17.9 years. The study population comprised 90 (60%) married and 60 (40%) single individuals. The average age of epilepsy onset in the study sample was 15±10.4 years, while the average duration of epilepsy was 11.8±7.9 years.

The headache group's mean age was 32±10.3 years. Among the 62 epilepsy patients with headaches, 22 (35.48%) were male and 40 (64.51%) were female; 14 (22.58%) had focal onset, 45 (72.58%) had generalised, and 3 (4.83%) had combined generalised and focal epilepsy. In the headache group, the mean duration of epilepsy was 10.8±5.1 years, and the mean age of epilepsy onset was 14.03±10.3 years. Forty-five (72.58%) patients received monotherapy, while 17 (27.41%) received polytherapy. Levetiracetam was the most commonly prescribed antiseizure medication in the headache group, followed by carbamazepine, valproic acid, lacosamide, topiramate, and zonisamide. In this group, the patients' headaches continued while they were using antiepileptic therapy. None of the patients were receiving prophylactic treatment other than the use of analgesics.

Inter-ictal headache was the most prevalent headache type, occurring in 45 (72.58%) participants, followed by post-ictal headache in 11 participants and pre-ictal headache in six participants. No participants reported ictal headaches.

Patients with pre- and post-ictal headaches more frequently experienced migrainous-type headaches. Consequently, migraine headaches were identified in four patients with pre- and five patients with post-ictal headaches. Only three patients with pre-ictal headaches reported visual aura.

In the inter-ictal headache group, 20 (44.44%) patients experienced tension-type headache, 14 (31.11%) had migraine without aura, 5 (11.11%) had migraine with aura, 4 (8.88%) had mixed-type headache, 1 (2.22%) had cluster headache, and 1 (2.22%) had paroxysmal hemicranias headache. No significant differences were found between the subgroups in the headache group in terms of age, epilepsy duration, epilepsy onset age, seizure and epilepsy types, and EEG findings.

The average headache duration in the overall study group was 5.1±4.3 years. A majority (64.51%) of epilepsy patients with headaches were female. The average number of headache days in the last month in the headache group was 3.2±0.9 days. Paracetamol was the most commonly used medication for headaches (27.41%). Other simple analgesics used by the patients included naproxen, flurbiprofen, and etodolac.

Thirty patients (48.38%) experienced compressive or heavy headaches, and 28 (45.16%) reported throbbing headaches. Patients typically described migraine headaches as occurring in the frontal region of the head and/or around the eyes, regardless of left or right side. The average visual analog scale score in the headache group was 5±3.8. Twelve (19.35%) patients reported dizziness and unsteadiness during headaches. Nine patients described visual aura, and three patients reported sensory aura. Furthermore, 18 (29.03%) patients described allodynia as the most severe headache, with 12 (66.66%) complaining of mild allodynia. Ten (16.12%) patients believed fasting, 15 (24.19%) attributed stress, and 14 (22.58%) reported poor sleep as triggers for both seizures and headaches.

In the interictal headache group, 15 (33.33%) patients stated that headaches limited at least one day of their school, work, study, or social life; ten (22.22%) patients experienced headaches almost daily; ten (22.22%) patients attempted to sleep of their headaches; and 25 (55.55%) patients used analgesics for headache relief. None of the patients had medication overuse headache.

Factors such as sex, marital status, epilepsy type, and treatment modalities did not significantly affect the quality of life. However, patients with an income at or below the minimum wage level had significantly higher total QOLIE-10 scores than those above the minimum wage, indicating a positive correlation between income and quality of life. Statistically significant differences were observed in the scores obtained from all three QOLIE-10 subscales between patients with and without headaches. Comparing the headache and headache-free groups revealed statistically significant differences in all three dimensions of the QOLIE-10. Consequently, the quality of life was worse in the headache group than in the headache-free group ($p<0.05$). In the headache group, there was no statistically significant relationship between income level and headache.

In the headache group, no significant difference in quality of life was observed between subgroups with and without a temporal relationship with seizures. However, in cases with inter-ictal headaches, QOLIE-10 scores were higher, albeit not significantly, in the tension headache group than in the migraine group, indicating that the quality of life in tension headache patients was worse among individuals with epilepsy. The findings are summarized in **Table 1**.

DISCUSSION

Epidemiological findings indicate that individuals with epilepsy have an increased propensity for headaches, including migraine. These headaches may manifest before, during, or after a seizure, and seizures and headache episodes are often not simultaneous. The pathophysiological connections between epilepsy and headaches are intricate and remain incompletely understood.[4] A multicenter study conducted in Turkey involving 809 epileptic participants aged 6-40 found that 62.8% experienced headaches.[5] Regarding headache types, interictal headaches and migraines were most prevalent among patients with epilepsy. Headaches, including migraines, are reported in 29.5% of epilepsy patients and are among the most frequent comorbidities after anxiety and mood disorders.[6] In 2018, headaches were detected in 47.6% of epilepsy patients, with tension-type headaches being the most common, followed by migraines with and without aura, stabbing headaches, cluster headaches, and other primary headaches.[7] Research on genetic and clinical features common to the etiopathogenesis of epilepsy and headaches has emphasised the imbalance of inhibitory and excitatory neurotransmitters.[8-12]

The migraine comorbidity in epilepsy has been extensively examined.[13,14] The prevalence of migraine-like headaches in patients with seizure-related headaches varies among studies.[15,16] A substantial association has been reported between migraine-like headaches and interictal migraines in seizure-related headaches.[17] Consistent with numerous studies in the literature, the present investigation found no significant relationship between sex, age, education level, marital status, epilepsy duration, seizure type, and QOLIE-10 scores. Although some studies, like this one, did not identify a relationship between epilepsy onset and duration and quality of life, others reported a decline in quality of life as the duration of epilepsy increased.[18-20] The negative correlation between low-income levels and quality of life found in this study aligns with the findings of other studies.[21, 22]

Stigmatisation is a factor that influences the quality of life of individuals with epilepsy. A stigmatisation study involving patients with epilepsy in Norway revealed that 56% felt stigmatised, with 70% internalizing or experiencing stigma at least once.[23] While the present study could not examine all factors, it sought to

Table 1. Demographic, clinical characteristics, and quality of life scores in epilepsy patients with and without headache

		QOLIE-10 average score	Effects of epilepsy	Mental health	Role functioning
Gender					
n=150	Female	27.20±8.90	8.78±4.14	8.7±2.56	9.06±4.30
p>0.05	Male	26.04±10.09	8.46±4.54	8.61±2.69	9.71±4.33
Marital status					
n=150	Single	27.65±9.10	9.09±4.14	8.43±2.44	10.13±4.45
p>0.05	Married	26.17±9.73	8.09±4.06	8.19±4.16	9.16±4.52
Income status					
n=150	Minimum wage and below	28.20±9.50	9.26±4.19	8.94±3.02	10.20±4.38
p<0.05	Above the minimum wage	25.04±7.32	8.17±3.93	8.23±1.59	8.64±3.30
The presence of headaches					
n=150	Headache-free group	22.00±7.80	7.10±3.59	7.55±2.95	7.50±3.48
p<0.05	The group with headaches	26.94±9.22	8.57±4.33	9.03±2.39	9.43±4.00
Interictal headache group					
n=45	Tension-type headache	26.31±10.06	8.68±4.38	8.01±2.45	9.46±4.03
p>0.05	Migraine	25.01±7.37	7.72±3.46	8.58±2.00	8.44±3.75

emphasise the diminished quality of life in epilepsy patients with headache. These disorders share numerous characteristics, and frequent recurrence in this population negatively affects quality of life. Therefore, it is crucial to consider shared etiopathogenesis when both disorders coexist and to plan treatments accordingly. Prophylactic treatment appropriate to headache was started for all patients in the headache group participating in the study. The absence of medication overuse headache in the study can be explained by the fact that epilepsy patients avoid excessive analgesic use because they think it may affect their current antiepileptic treatment.

Several limitations of this study should be considered when interpreting its results.

Study Limitations

1. A relatively small sample size may have reduced the generalizability of our findings to a broader population of patients with epilepsy. A larger sample size would enhance the statistical power and the external validity of the results.
2. The study was conducted at a single center, which may have introduced selection bias and limited the diversity of the patient population.
3. Study did not examine the effect of seizure frequency on the quality of life in detail.
4. Absence of comprehensive mental state evaluations may have limited our understanding

CONCLUSION

In the context of epilepsy, clinicians often prioritise diagnosis and treatment of the primary condition, while the management of comorbidities, such as headaches, may receive less attention. The findings from this study emphasise the importance of accurately diagnosing and addressing headaches in individuals with epilepsy. By considering the complex relationships between epilepsy, headaches, and quality of life, a comprehensive treatment approach can be developed that addresses both the primary condition and its comorbidities. This integrated approach has the potential to significantly reduce the burden of disease in this patient population, ultimately improving their overall quality of life and well-being.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Faculty of Medicine Clinical Researches Ethics Committee (Date: 16.03.2023, Decision No: 2023-33).

Informed consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

HEALTH SCIENCES
MEDICINE

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The effect of COVID-19 vaccines on thyroid function and thyroid autoimmunity

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ABSTRACT

Aims: There have been reports about various thyroid autoimmune events after SARS-CoV-2 vaccinations. There is limited data on the extent to which vaccines for COVID-19 are effective on thyroid autoimmunity. This study investigates how COVID-19 vaccination affects thyroid antibodies and functions in individuals without any thyroid disease history.

Methods: The study evaluated individuals aged 18-65 with no previous COVID-19 history or thyroid disease who had at least two COVID-19 vaccine doses (CoronaVac + Pfizer-BioNTech or Pfizer-BioNTech alone) between October 2021 and October 2022. All participants' thyroid hormone (free triiodothyronine, thyroid-stimulating hormone, and free thyroxine) and thyroid autoantibody (anti-thyroglobulin, antithyroid peroxidase, and TSH receptor antibody) levels were measured.

Results: The study included 92 individuals in total. Thyroid functions and antithyroid antibody levels were found to be in the normal range before the implementation of the SARS-CoV-2 vaccine. Of the study participants, 42 received the Sinovac + BioNTech vaccine, and 50 received the BioNTech vaccine alone. While a decrease in st4 value was observed only in the BioNTech group after vaccination (p=0.007), thyroid dysfunction was not observed in any participant. After vaccination, TRAB positivity was observed in one participant, ANTI-TPO positivity in six participants, and ANTI-TG positivity in eight participants. No statistically significant antibody positivity was detected. No participants with antibody positivity displayed thyroid dysfunction.

Conclusion: Although some positivity in terms of antithyroid antibodies was observed after COVID-19 vaccination, this antibody positivity did not have a statistically significant level, and thyroid dysfunction was not detected in any participant. The COVID-19 vaccine is safe for thyroid function and autoimmunity.

Keywords: COVID-19, Sinovac, BioNTech, anti-thyroid peroxidase, anti-thyroglobulin, TSH receptor antibody

INTRODUCTION

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has severely impacted the entire world. With the COVID-19 pandemic causing serious mortality and morbidity, various types of vaccines were developed in a relatively brief period to protect against the disease.[1]

The rapid development of SARS-CoV-2 vaccines and rapid community vaccination has led to a decrease in the severity and spread of the disease. In addition to the tremendous social benefits offered by vaccination, some inflammatory and autoimmune side effects were also observed with vaccination, and these side effects were followed closely.[2]

Some cases reported following SARS-CoV-2 vaccination include various thyroid disorders related to autoimmune and inflammatory mechanisms, like Graves' disease, autoimmune hypothyroidism, and subacute thyroiditis.[3] In order to increase vaccine response, most vaccines contain adjuvants. They can also trigger autoimmune and inflammatory adverse effects in genetically predisposed individuals by activating autoimmune cascades.[4]

Currently, inactivated virus vaccine (CoronaVac) and the mRNA vaccine (BNT162b2) are administered in Turkey. However, there is limited data as to the effects of vaccines against COVID-19 on thyroid autoimmunity. To increase our knowledge on this subject, we evaluated

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thyroid antibodies and thyroid functioning following the implementation of COVID-19 vaccines in individuals without a previous history of autoimmune thyroid disease.

METHODS

The study was carried out with the permission of by Başkent University Non-interventional Clinical Researches Ethics Committee (Date: 17.11.2021, Decision No: 21/439). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All the participants gave their written informed consent.

Volunteers aged 18-65 years who were admitted to Başkent University Faculty of Medicine Endocrinology and Metabolic Diseases Clinic between 01.10.2021 and 01.10.2022 were included in our study. The inclusion criteria in our study were the following: having serum thyroid autoantibodies tested before and obtaining a negative result, having been vaccinated against COVID-19, and having had the vaccination at least one and at most six months ago. The exclusion criteria included having a history of COVID-19 infection and a history of autoimmune thyroid disease, being currently on or having previously used thyroid hormone replacement or antithyroid medication, having pregnancy, having an active malignancy, receiving active chemotherapy, taking immunomodulatory drugs or tyrosine kinase inhibitors that may affect the thyroid function test, having another vaccination (such as human papillomavirus, influenza, pneumonia, and hepatitis B vaccination) in the last 6 months, serious active infection, radiation therapy to the neck and being on drugs that can alter thyroid function tests like amiodarone and steroids.

All the participants were tested for any of the thyroid autoantibodies before administering the first COVID-19 vaccine dose, and they were found to be negative. Moreover, they all displayed normal thyroid functioning in tests. The participants' demographic data and any information about their diseases, if any, were recorded. All the participants completed at least two COVID-19 vaccine doses as per the recommendations. Vaccines were administered to participants as CoronaVac (Sinovac) + Pfizer-BioNTech or simply Pfizer-BioNTech.

Blood was collected from all participants in order to measure the levels of free thyroxine (fT4), serum thyroid stimulating hormone (TSH), thyroid peroxidase antibody (anti-TPO), free triiodothyronine (fT3), TSH receptor antibody (TRAB), and thyroglobulin antibody (anti-TG). The levels of serum TSH, TRAB, fT3, and fT4 were measured using a chemiluminescent immunoassay using Abbott Alinity I (Abbott Diagnostic, IL, USA). Serum anti-TG and anti-TPO levels were detected using a chemiluminescent immunoassay with a Beckman Coulter Access 2 analyzer (Beckman Coulter Inc., CA, USA). The reference ranges were 0.35-4.94 mU/L, 0.7-1.48 ng/dl, and 1.58-3.91 ng/dl for TSH, fT4, and fT3, respectively. An anti-TPO level > 5.61 IU/ml, an anti-TG level >4 IU/ml, and a TRAB level >1.5 IU/L were considered positive.

Statistical Analysis

The study data were analyzed using IBM SPSS Statistics 25.0 package software (IBM Corporation, Armonk, NY, USA). The Kolmogorov-Smirnov test was implemented in order to determine whether continuous numerical variables had a normal distribution, and the Levene test was used to assess the homogeneity of variances. Continuous numerical variables were given as median (25^{th} percentile-75^{th} percentile) or mean±standard deviation. On the other hand, categorical variables were stated as the number of cases and as a percentage. Student's t-test was used to assess the significance of the variance between the groups with regard to mean values. In terms of continuous numerical variables for which the assumptions of parametric test statistics were not met, the significance of inter-group variance was investigated by the Mann-Whitney U test. When analyzing categorical data, the data analyzed were evaluated through Fisher's exact probability test in cases where at least 25% of the cells had an expected frequency of less than 5 in 2 × 2 cross tables. Furthermore, the $\chi 2$ test with continuity correction was implemented if the expected frequency was between 5-25. The Wilcoxon Sign test was implemented in order to find out whether the groups displayed statistically significant differences with regard to biochemical measurements before and after vaccination. Multivariate linear regression analysis was applied in order to ascertain whether the type of vaccine administered had a statistically significant modifying effect on post-vaccine anti-TPO measurements when adjusted for age, gender, and pre-vaccine anti-TPO measurements. For each variable, 95% confidence intervals and regression coefficients were calculated. A p-value of <0.05 was accepted as statistically significant unless stated otherwise. On the other hand, the Bonferroni correction was applied to inspect Type I errors in all possible multiple comparisons.

RESULTS

The study sample included 92 participants. Prior to the implementation of the SARS-CoV-2 vaccine, thyroid functions, and antithyroid antibody levels were found to be in the normal range. Among the sample, 42 received Sinovac + BioNTech, and 50 received BioNTech alone. The participants with Sinovac + BioNTech had a statistically significantly higher mean age than those with BioNTech

alone (p=0.009). The percentage of males was statistically significantly higher, and the percentage of females was statistically significantly lower in the Sinovac + BioNTech group than those with BioNTech alone (p=0.024). However, no statistically significant difference was detected between the groups concerning smoking history, obesity, concomitant diseases, autoimmune disease, and thyroid disease in the family (p >0.05) (Table 1).

Table 1. Demographic and clinical characteristics of the participants by groups

	Total (n=92)	Sinovac + BioNTech (n=42)	BioNTech (n=50)	p value
Age (years)	43.6±12.3	47.2±12.2	40.6±11.6	0.009†
Sex				0.024‡
Female	74 (80.4%)	29 (69.0%)	45 (90.0%)	
Male	18 (19.6%)	13 (31.0%)	5 (10.0%)	
Smoking	33 (35.9%)	16 (38.1%)	17 (34.0%)	0.850‡
Obesity	15 (16.3%)	8 (19.0%)	7 (14.0%)	0.712‡
Comorbidities				
Diabetes mellitus	20 (21.7%)	12 (28.6%)	8 (16.0%)	0.229‡
Hypertension	16 (17.4%)	10 (23.8%)	6 (12.0%)	0.225‡
Hyperlipidemia	6 (6.5%)	4 (9.5%)	2 (4.0%)	0.406¶
Coronary artery disease	2 (2.2%)	2 (4.8%)	0 (0.0%)	0.206¶
Chronic kidney disease	2 (2.2%)	1 (2.4%)	1 (2.0%)	>0.999¶
Family history of thyroid disease	31 (33.7%)	15 (35.7%)	16 (32.0%)	0.878‡
Autoimmune disease	12 (13.0%)	8 (19.0%)	4 (8.0%)	0.209‡

† Student's t-test, ‡ Continuity corrected $\chi 2$ test, ¶ Fisher's exact probability test.

The groups did not show any statistically significant difference with regard to TSH levels before and after vaccination (p=0.459, p=0.743, respectively). Pre-vaccine and post-vaccine TSH levels were similar in participants that had BioNTech alone and Sinovac + BioNTech (p=0.576, p=0.923, respectively). Finally, compared to pre-vaccination, the groups did not have any statistically significant difference regarding changes in the TSH level after vaccination (p=0.815) (Table 2).

The ST4 levels in the groups were not statistically significantly different before and after vaccination (respectively; p=0.377, p=0.952). The ST4 levels showed a statistically significant decrease after vaccination in participants with BioNTech alone (p=0.007). In participants who had Sinovac + BioNTech, pre- and post-vaccination ST4 levels were close (p=0.197). Finally, the study did not reveal any statistically significant difference between the study groups with regard to the change in ST4 levels following vaccination (p=0.490) (Table 2).

Regarding the pre-and post-vaccination anti-TPO levels, the groups showed no statistically significant differences (p=0.469, p=0.909, respectively). There was a statistically significant increase in anti-TPO levels following vaccination in participants who had BioNTech alone (p=0.010). In patients who had Sinovac + BioNTech, pre- and post-vaccination anti-TPO levels were statistically similar (p=0.993). Finally, no statistically significant differences were detected between the groups with regard to the change in anti-TPO levels following vaccination (p=0.153). When adjustments were made for age, gender, and pre-vaccination ANTI-TPO measurements, post-vaccination Anti-TPO levels remained higher in participants who had BioNTech alone compared to those with Sinovac + BioNTech (B=2.835, 95% CI: 0.034 - 5.635, p=0.047).

Table 2. Biochemical measurements according to groups and follow-up times

	Before vaccination	After vaccination	p value†	Changing
TSH				
Sinovac+Biontech	1.73 (0.912.48)	1.66 (0.89-2.74)	0.923	-0.03 (-0.36-0.42)
Biontech	1.85 (1.14-2.56)	1.44 (0.93-2.90)	0.576	-0.07 (-0.79-0.46)
p-value ‡	0.459	0.743		0.815
fT4				
Sinovac+Biontech	0.93 (0.78-1.03)	0.88 (0.82-0.93)	0.197	0.00 (-0.10-0.01)
Biontech	0.95 (0.87-1.04)	0.90 (0.81-0.93)	0.007	-0.05 (-0.14-0.04)
p-value ‡	0.377	0.952		0.490
Anti-TPO				
Sinovac+Biontech	0.69 (0.07-1.30)	0.55 (0.30-0.90)	0.993	0.00 (-0.63-0.50)
Biontech	0.40 (0.20-1.00)	0.60 (0.30-1.00)	0.010	0.01 (-0.10-0.52)
p-value ‡	0.469	0.909		0.153
Anti-TG				
Sinovac+Biontech	0.90 (0.90-0.90)	0.90 (0.90-0.90)	0.892	0.00 (0.00-0.00)
Biontech	0.90 (0.90-0.90)	0.90 (0.90-0.90)	0.225	0.00 (0.00-0.00)
p-value ‡	0.714	0.389		0.512

Descriptive statistics were expressed as the median (25th percentile-75th percentile). † These results were considered statistically significant for $p < 0.025$, according to the Wilcoxon sign test, the Bonferroni correction, and between pre- and post-vaccination comparisons within the groups. ‡ For comparisons made within each follow-up time, the results were considered statistically significant for $p < 0.025$, according to the Mann-Whitney U test and the Bonferroni correction. For comparisons of changes following vaccination, the result was considered statistically significant if $p < 0.05$.

The pre- and post-vaccination anti-TG levels were not statistically significantly different in the groups (p=0.714, p=0.389, respectively). The pre- and post-vaccination anti-TG levels were statistically similar in participants who had BioNTech alone and participants who had Sinovac + BioNTech (p=0.225, p=0.892, respectively). Finally, as shown in Table 2, there were no statistically significant differences between the groups regarding the change in anti-TG levels following vaccination (p=0.512).

According to Table 3, the BioNTech group and Sinovac + BioNTech group did not show any statistically significant difference with respect to the percentage of individuals positive for Anti-TG, TRAB, and Anti-TPO after vaccination (p > 0.999, p=0.457, and p=0.684, respectively).

Table 3. Comparison of the two groups in terms of post-vaccination Anti-TPO, Anti-TG, and TRAB positivity

	Sinovac+Biontech (n=42)	Biontech (n=50)	p value†
Anti-TPO positivity	2 (4.8%)	4 (8.0%)	0.684
Anti-TG positivity	4 (9.5%)	4 (8.0%)	>0.999
TRAB positivity	1 (2.4%)	0 (0.0%)	0.457

† Fisher's exact probability test.

DISCUSSION

There have been numerous reports of the development of case-base autoimmune thyroid disease after COVID-19 vaccination. However, there are a minimal number of studies on the risk of developing autoimmune thyroid disease following vaccination against COVID-19. This study investigated the changes observed in thyroid function and antithyroid antibodies after COVID-19 vaccination in people without a history of thyroid disease.

Our study observed no overt thyroid dysfunctions following either type of COVID-19 vaccine (BNT162b2 and CoronaVac). There were no significant changes in post-vaccination TSH levels. While the group that received BioNTech alone showed a statistically significant decrease in the st4 levels, in general, the st4 levels did not differ statistically significantly following vaccination across the groups. Although TRAB positivity was observed in one participant, anti-TG positivity in four participants, and anti-TPO positivity in six participants following vaccination, all the patients were euthyroid. In addition, thyroid antibody positivity was not found to be statistically significant. Our results showed no clinically significant thyroid autoimmune disease due to COVID-19 vaccination.

In a recent study conducted on 72 healthy people in Greece, although a slight decrease was observed in values of TSH, total triiodothyronine (T3), and total thyroxine (T4) after vaccination with BioNTech for COVID-19, all values were within normal limits, and no thyroid dysfunction was observed.[5] In another study involving 215 people in China, after COVID-19 vaccination (60% BNT162b2; 40% CoronaVac), TSH values did not change, but fT4 slightly increased and fT3 slightly decreased. Abnormal thyroid function was observed in only three patients after vaccination, and none of these were clinically overt.[6] Also, in a prospective study conducted in China, 36 (6.38%) of 564 individuals who participated in the study with normal thyroid function at baseline developed thyroid dysfunction following the implementation of the COVID-19 vaccine (BBIBP-CorV and CoronaVac).[7] As a result, our study is concordant with the literature, and we can state that no clinically significant thyroid dysfunction developed after vaccination with both CoronaVac and BioNTech.

In a recent prospective study conducted in China, none of the 545 recipients who were negative for antithyroid antibodies before vaccination developed an abnormal antibody result following COVID-19 vaccination.[7] In another study in China, after BNT162b2 and CoronaVac vaccines were administered to 215 participants, anti-Tg and anti-TPO titers moderately increased following vaccination, but anti-TPO/Tg positivity did not show any statistically significant difference. A greater increase in anti-TPO titer was observed after BNT162b2.[6] Another study evaluated the TRAB levels of 231 Graves' patients after administering the inactivated COVID-19 vaccine. Increasing TRAB levels were detected following vaccination.[8] In our study, some participants were positive for some of the thyroid autoantibodies, but this antibody positivity was not statistically significant, and thyroid dysfunction was not detected in any participants.

Although at least 5 billion people were vaccinated against COVID-19 until Dec 21, 2022,[9] post-vaccine thyroid-related autoimmune and inflammatory diseases have only been reported on a case-by-case basis. Since vaccination is widespread worldwide, the possibility of coincidental occurrence of these detected thyroid cases within a period should not be ignored.

In a review of COVID-19 post-vaccination thyroid autoimmune inflammatory diseases, 52 cases of subacute thyroiditis, 22 cases of Graves', and six cases of silent thyroiditis were reported after vaccination. The mean age was 45-46 years, and the symptoms started in an average of 9-15 days. Most of the patients were women.[10] Similarly, another case series presentation reported one subacute thyroiditis, one Graves' disease, and one silent thyroiditis case after COVID-19 vaccination.[3] Two cases of Graves' disease and one case of hypothyroidism were reported in another case series after the COVID-19 vaccination. It was stated here that these patients had a family history of thyroid disease, and it was reported that the family

history could be a sign of genetic predisposition.[11] Similarly, in the case series of 51 patients evaluated for post-COVID-19 thyroid autoimmune conditions, the majority of the cases were Graves' patients, female, and did not have a pre-existing thyroid disease.[11]

Among our participants, when those with positive post-vaccination antibody titers were examined individually, it was found that one participant with TRAB positivity was a female with a family history of thyroid disease, and anti-TPO positivity was found in a total of six participants who were all female. Moreover, four of them had a history of thyroid disease in their families. Of the eight participants who were found to be anti-TG positive, seven were female, and half had a family history of thyroid disease. Based on these findings, it can be suggested that females and those with a family history of thyroid disease may be at a higher risk for post-vaccination thyroid autoimmunity.

It is thought that the adjuvants in the vaccines affect thyroid autoimmune and inflammatory events that develop after COVID-19 vaccination.[11,12] It is possible to find adjuvants in various types of vaccines, and they are utilized to augment the vaccination response. Events involving various autoimmune conditions due to adjuvants were defined as autoimmune/inflammatory syndrome (ASIA) by Shoenfeld and Agmon-Levin in 2011.[13] Aluminum is one of the most commonly used adjuvants in vaccines. Aluminum increases proinflammatory cytokines by affecting the immune system itself.[14] In addition, adjuvants can cause ASIA by affecting the immune balance and triggering the activation of B lymphocytes in genetically predisposed individuals, leading to a wide variety of autoimmune events, including autoimmune thyroid disease.[14,15]

Previous studies have obtained data showing that vaccines other than those against COVID-19 also affect thyroid autoimmunity. Among the studies performed, thyroid autoimmune events have also been reported following vaccinations against human papillomavirus (HPV), Hepatitis B, influenza, and Bacille Calmette-Guerin (BCG). However, these reported cases did not hinder the benefit of vaccination, and whether these side effects developed purely by chance over time has been an issue of debate.[16-19]

Our study had certain limitations. One of these was that the pre-vaccination anti-TPO levels of all the participants were measured, but this was not the case for anti-TG and TRAB levels. In addition, long-term thyroid function tests of participants with positive antibody titers were unavailable. In addition, only those without pre-existing thyroid disease were included in the study. How vaccination affects people with a history of thyroid disease and those who use thyroid medication could not be evaluated. Finally, COVID-19 PCR or IgG testing was not performed to exclude asymptomatic COVID-19 cases before the study was included in the study.

CONCLUSION

COVID-19 vaccination resulted in a statistically insignificant rate of positivity in antithyroid antibodies. However, there was no clinical reflection of this positivity, and none of the participants showed any clinically significant thyroid dysfunction. Our results demonstrate that COVID-19 vaccination does not have a severe clinical side effect on the thyroid. COVID-19 vaccination can proceed safely concerning thyroid autoimmunity.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of by Başkent University Non-interventional Clinical Researches Ethics Committee (Date: 17.11.2021, Decision No: 21/439).

Informed consent: All patients signed and free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluation of the relationship between digital mammography radiation dose and patient age, breast volume and density

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ABSTRACT

Aims: To determine the average radiation dose values in patients who underwent routine screening mammography in our hospital, establish the relationship between breast density and volume, and investigate other factors affecting radiation dose.

Methods: Screening bilateral mammography was retrospectively evaluated within the specified period of 2 months. Patient age, breast density ratio, mammographic size of the breast, calculated breast volume, tube voltage, current, exposure time (ms), compression force (kg), compression thickness (mm), and radiation dose (mGy) given in each projection were recorded separately for each patient. According to the BI-RADS, breast densities classified as types A-B were considered non-dense, while types C-D were considered dense breasts. The 75th percentile dose value (mGy) was chosen as the cutoff for high dose group. Logistic regression analyses were used to examine the factors affecting radiation dose.

Results: 1720 mammograms from 430 patients were studied. 276 (64.2%) breasts were non-dense, while 154 (35.8%) breasts were dense. The mean total breast volume was 595±334 ml, compression thickness was 36.5±12.0 mm, and radiation dose was 2.04±0.75 mGy. There was a negative correlation between radiation dose and age (r=-0.330, p<0.001), while a positive correlation was found between radiation dose and breast volume (r=0.514, p<0.001), kV (r=0.608, p<0.001), mAs (r=0.912, p<0.001), exposure time (r=0.820, p<0.001), compression thickness (r=0.629, p<0.001) and strength (r=0.084, p<0.001). In the regression analysis conducted excluding technical parameters, age, breast volume, density, and compression thickness all influence radiation dose, with compression thickness having the greatest effect, followed by breast volume, age, and finally breast density.

Conclusion: The most important factors influencing radiation dose are technical parameters such as tube voltage, current and exposure time. However, apart from technical parameters, compressed breast thickness is the most affecting factor, followed by breast volume, age, and least of all, breast density, in affecting radiation dose.

Keywords: Mammography, breast, radiation dosage, diagnostic imaging, breast density, diagnostic screening programs

INTRODUCTION

Breast cancer is the most common cancer in women, both in Turkey and worldwide.[1,2] The mortality rate in patients with breast cancer can reach up to 30.7%, accounting for 11.6% of total cancer deaths.[3-5] Furthermore, it is stated that a woman has a 12% risk of developing breast cancer throughout her lifetime.[5,6] Due to its high prevalence and mortality rates, early diagnosis of breast cancer is crucial. For this purpose, screening programs for breast cancer have been developed in many developed countries for women aged 40 and above. These programs aim to detect the disease at an early stage and reduce mortality and morbidity. There are studies that demonstrate a decrease in mortality from breast cancer through these screening programs.[7]

Mammography is the most commonly used imaging technique for breast cancer screening due to its low cost, accessibility, ease of application, and high sensitivity.[8] In most mammography procedures, two different projections, craniocaudal (CC) and mediolateral oblique (MLO), are routinely performed for each breast. This method is based on the use of ionizing radiation for imaging. The use of imaging techniques that involve ionizing radiation is becoming increasingly widespread. This situation has raised concerns about the risk of radiation exposure.[9,10] While the radiosensitivity of each tissue varies, it is known that radiation exposure increases the incidence of many cancers.[11] The radiation weighting factor for the breast has been determined to be 0.12. It is known to be a radiosensitive tissue, and it represents

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12% of the total radiation damage that occurs in the case of homogeneous radiation exposure throughout the body.[12] In breast cancer screening programs, radiation exposure to each breast in two different projections every year after the age of 40 raises concerns about radiation exposure in some women in the screening age group and causes avoidance of screening mammography.[13,14] However, it has been reported in the literature that screening mammography alone has led to a decrease in breast cancer-related mortality in the United States, and the benefit obtained from early detection of breast cancer outweighs the risk of breast cancer associated with radiation exposure.[15,16]

Understanding the factors that affect radiation exposure to the breast and the radiation dose in mammography used in routine screening programs is important for controlling radiation dose. The radiation dose absorbed by the breast can vary widely depending on various factors. There are studies in the literature that examine mammography doses and the influencing factors. These studies have indicated a positive correlation between breast compression thickness, body mass index, and breast radiation dose. Although studies on the relationship between breast density and dose are limited, there are studies indicating higher dose exposure in dense breasts. In order to generate high-quality images in denser breasts, it may be necessary to use more X-rays, which can result in higher radiation exposure.[5] Additionally, it is stated that women with denser and larger breasts may have a higher radiogenic risk.[5]

To assess a patient's radiation-induced exposure, the average glandular dose (AGD) is evaluated and expressed in mGy (milligray). AGD represents the dose received by the compressed tissue of the breast. Compression is applied to reduce the thickness and radiation exposure of the breast. The volume of the breast is obtained by measuring the breast dimensions in two planes and calculating it with compression thickness. It has been reported that young women with denser and larger breasts are at a higher risk for radiation-induced secondary breast cancer due to higher radiation exposure. In the literature, it has been reported that the average reported dose per mammography image ranges from 1.1 to 2.2 mGy, while it varies between 2.0 and 5.4 mGy for each breast.[5,17-19] In radiation protection, the fundamental principles used to minimize the risk of radiation exposure are correct justification, dose optimization, and dose limitation methods.[17,20,21] Therefore, it is recommended to keep the dose applied to the breast during mammography as low as reasonably achievable (ALARA) without compromising image quality. The assessment of mammography doses and the investigation of factors affecting radiation dose are crucial for evaluating the procedure and ensuring compliance with national and international diagnostic reference levels.

The aim of this study is to determine the average radiation dose values in patients who underwent routine screening mammography in our hospital, establish the relationship between breast density and volume, and investigate other factors affecting radiation dose. In this way, it is also aimed to raise awareness about radiation exposure and to emphasize dose control.

METHODS

The study was designed retrospectively and was approved by the ethics committee of our hospital (Date:05/07/2023, Decision No:2023-KAEK-79). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

The retrospective evaluation of bilateral mammography examinations performed for screening was conducted at our hospital's mammography unit using a single device (Giotto IMS, Bologna, Italy).

The inclusion criteria were determined as follows: being 40 years of age or older, being female, having a Breast Imaging Reporting and Data Systems (BI-RADS) assessment result of category 1 or 2 (negative or benign findings), and having adequate quality images in bilateral craniocaudal (CC) and mediolateral oblique (MLO) projections.

The exclusion criteria for the study included a history of mastectomy, breast-conserving surgery, or radiotherapy treatment, BI-RADS assessment categories of 3, 4, 5, or 6, being a male patient, having breast implants, missing one of the bilateral CC and MLO projections, or having other types of mammography images such as spot compression or magnification.

In the determined two-month period, 120 images of 30 patients due to BI-RADS 3,4,5, 44 images of 22 patients due to previous mastectomy, 2 images of 2 patients due to only one projection and on a single breast, a total of 54 patients 166 images were excluded from the study. These patients were not included in the study.

Consequently, a total of 1720 mammography images from 430 patients were included in the study.

Data Collection

The images were retrospectively reviewed using the same model workstation (Giotto IMS, Bologna, Italy) as the mammography device. Patient age, breast density ratio according to American College of Radiology (ACR) breast imaging-reporting and data system (BI-RADS), mammographic size of the breast, kV, mA, exposure time (ms), compression force (kg), compression thickness (mm), and radiation dose (mGy) given in each projection were recorded separately for each patient.

The density of the breast according to ACR BI-RADS was evaluated in four categories. The breast density was classified as follows: 0-25%: category A, 25-50%: category B, 50-75%: category C, and 75-100%: category D. The images were visually evaluated by a radiologist with 10 years of experience, and a consensus was reached by comparing the density result determined according to ACR BI-RADS in the patient's previous report written in the Hospital Information System. The final decision was made by a 10-year-experienced radiologist who evaluated the images, taking into account the report in the hospital system. Breast densities classified as types A-B were considered non-dense, while types C-D were considered dense breasts.

Mammographic breast size measurements were performed on the workstation by measuring the anterior-posterior (AP) and mediolateral (ML) dimensions of the breast in the CC projection. In conjunction with breast compression thickness, the formula ($\pi/4 \times$ AP measurement $\times$ ML measurement $\times$ compression thickness), as described by Kalbhen et al.[22] was used to calculate breast volume.[23] For MLO images, the AP and CC dimensions of the breast were measured, and using the same formula, the total volume of the breast and axilla was calculated. The measurement procedure is shown in Figure 1.

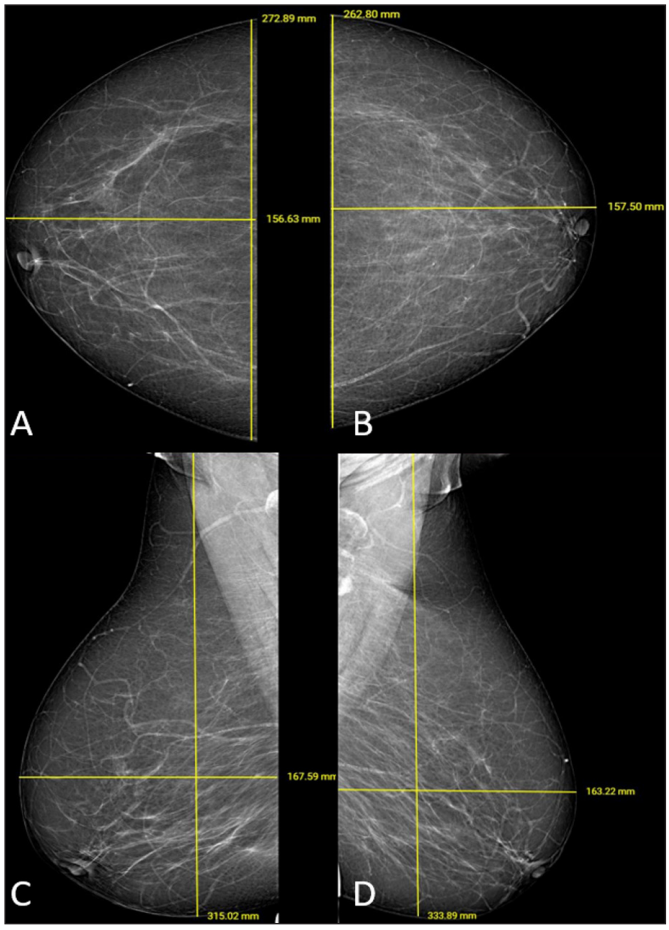

Figure 1. Breast measurements. A, B. Measurement of anteroposterior and mediolateral diameters in craniocaudal (CC) images, C, D. Measurement of anteroposterior and craniocaudal diameters in mediolateraloblic (MLO) images.

The radiation dose exposure mentioned in the study represents the total radiation exposure dose calculated by the mammography unit in the specified workstation for both CC and MLO images. The safe limit for a single projection mammogram, as stated by both the Food and Drug Administration (FDA) and the International Commission on Radiological Protection (ICRP), is 3 mGy in terms of Mean Glandular Dose (MGD).[12] Additionally, some diagnostic reference level studies utilize the 75th and 95th percentile values. In this study, the 75th percentile value was chosen as the cutoff for high dose, classifying images with doses higher than the 75th percentile as high dose, and those with lower doses as low dose.

Statistical Analysis

For all analyses, IBM SPSS 26.0 (NY, USA) statistical software was used. Descriptive statistics were presented as mean±standard deviation for numerical data, and counts and percentages for categorical data. The normality of the data was assessed using the Kolmogorov-Smirnov test, and it was determined that none of the variables followed a normal distribution. Therefore, nonparametric tests such as Mann-Whitney U test for group comparisons and Spearman correlation analysis for correlation analyses were used. Logistic regression analyses were used to examine the factors influencing radiation dose, including patient age, breast density, and compression thickness, both with and without the technical parameters associated with the device. The results of the regression analysis were presented with odds ratios and 95% confidence intervals. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 430 patients' mammography images, comprising 1720 images in total, were included in the study. These images consisted of an equal number (n=430) of right and left breasts, including both CC and MLO views. The mean age of the patients was 54.6±10.7 years. According to the ACR BI-RADS breast density categories, 126 (29.3%) breasts were classified as type A, 150 (34.9%) as type B, 114 (26.5%) as type C, and 40 (9.3%) as type D. When type A-B breasts were considered as non-dense and type C-D breasts were considered as dense, 276 (64.2%) breasts were classified as non-dense, while 154 (35.8%) breasts were classified as dense (Table 1).

The mean total breast volume was 595±334 ml, with the right breast having an average volume of 586±334 ml and the left breast having an average volume of 603±344 ml. When including the axillae in the MLO view, the mean volume for both sides was calculated as 899±488 ml, with the right side calculated 877±489 ml and the left side calculated 921±499 ml (Table 2).

Table 1. Breast densities of patients according to ACR BI-RADS classification

BI-RADS density	N	%
Non-dense	276	64.2%
Type A breast	126	29.3%
Type B breast	150	34.9%
Dense	154	35.8%
Type C breast	114	26.5%
Type D breast	40	9.3%
Total	430	100%

Table 2. Breast volume measurements results from CC and MLO projections on the right and left breast

Volume measurements (ml)	N	Mean	Standart Deviation
Right breast volume (CC)	430	586	334
Left breast volume (CC)	430	603	344
Average breast volume (CC)	860	595	334
Right breast and axilla volume (MLO)	430	877	489
Left breast and axilla volume (MLO)	430	921	499
Average breast and axilla volume (MLO)	860	899	488

CC: Craniocaudal, MLO: Mediolateraloblique

The mean compression thickness was 36.5±12.0 mm, the mean kV value was 27.1±1.6 kV, the mean mAs value was 94.8±29.5 mAs, the mean exposure time was 697±212 ms, the mean compression force was 14.8±3.1 kg, and the mean radiation dose was 2.04±0.75 mGy (Table 3). The distribution of these measurements in the right and left breast CC and MLO projections is shown in Table 3.

In the comparison made between the dense and nondense breast groups based on breast density, statistically significant differences were found in radiation dose for each projection, total radiation dose and all mammography technical parameters ($p<0.05$) (Table 4).

According to the results of the correlation analysis, there was a negative correlation between radiation dose and age, while a positive correlation was found between breast volume, kV, mAs, exposure time, compression thickness and strength (Table 5, Figure 2).

Due to the data not suitable a normal distribution, univariate and multivariate logistic regression analyses could not be performed. Using the cut off value of 2.6 mGy obtained from the 75th percentile in our study, all images

Table 3. Distribution of the obtained data values in the right and left breast CC and MLO projections

	Right CC (Mean±SD)	Left CC (Mean±SD)	Right MLO (Mean±SD)	Left MLO (Mean±SD)	Total (Mean±SD)
AP Mesaurements (mm)	101.0±26.5	102.9±26.6	109.8±26.5	110.1±26.4	106.0±26.8
ML Measurements (mm)	204.4±25.3	206.6±27.3	244.8±24.5	246.9±24.2	225.7±32.4
Compression Thickness (mm)	33.6±10.9	33.5±11.2	38.7±11.9	40.3±12.4	36.5±12.0
Breast Volume (ml)	586±334	603±344	877±488	921±499	747±450
Voltage (kV)	26.7±1.3	26.7±1.4	27.4±1.6	27.6±1.7	27.1±1.6
Tube Current (mAs)	87.6±27.3	90.1±27.3	102.2±29.2	99.4±31.5	94.8±29.5
Exposure Time (ms)	657±209	669±203	744±207	717±216	697±211
Compression Pressure (kg)	14.7±3.0	15.0±3.1	14.9±3.0	14.7±3.3	14.8±3.1
Radiation Dose (mGy)	1.84±0.65	1.87±0.63	2.24±0.76	2.23±0.87	2.04±0.76

CC: Craniocaudal, MLO: Mediolateraloblique, AP: Anterioposterior, ML: Mediolateral, SD: Standart Deviation

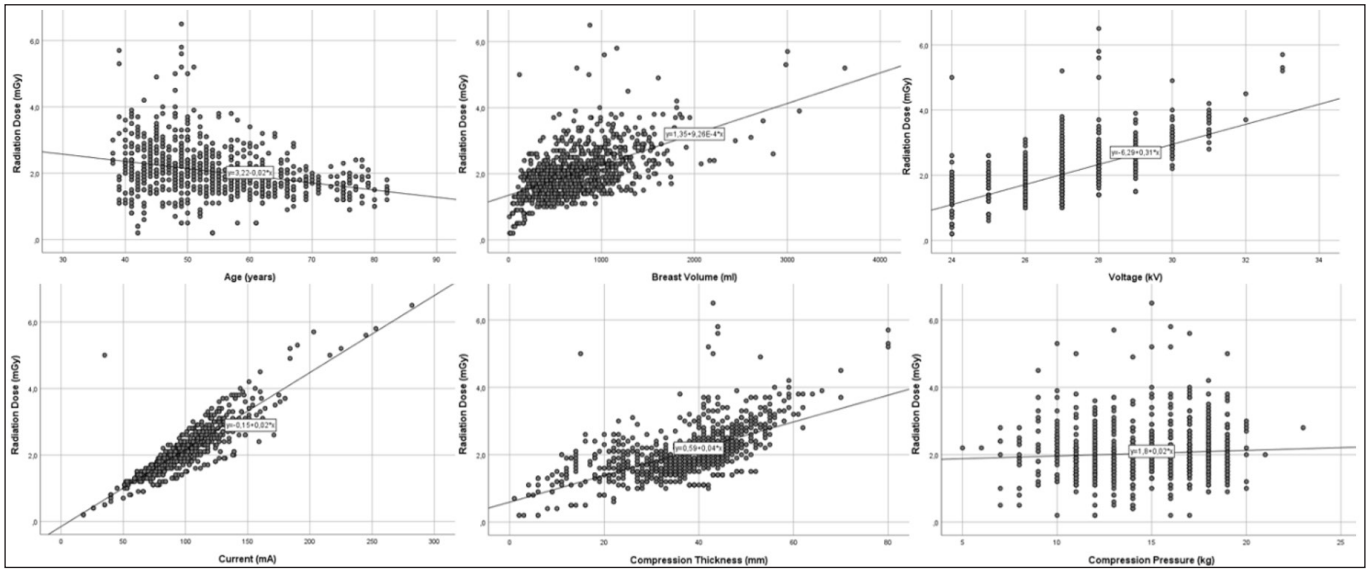

Figure 2. Correlation graphs between radiation dose (mGy) and age, breast volume, voltage, current, compression thickness and compression pressure, respectively. There is a negative correlation between age and radiation dose, and a positive correlation is observed in other graphs. It is seen in the graph that there is a weak positive correlation between the compression pressure and the radiation dose.

were divided into two groups: high dose (≥2.6 mGy) and low dose (<2.6 mGy) exposures. Thus, binary logistic regression analysis was conducted. The comparison of the values between the high dose and low dose groups is shown in Table 6, and the results of the regression analysis are shown in Table 7. In the initial regression analysis, technical parameters related to the automatic exposure of the device, such as mA, kV, exposure time, and compression force, were included. In the second analysis, only age, breast density, and compression thickness were included, excluding these technical parameters (Table 7). According to the results of the regression analysis, among the technical parameters, tube voltage has the most significant impact on increasing radiation dose, followed by tube current. Exposure time contributes to a lesser extent to the increase in radiation dose. Compression pressure, on the other hand, did not have an effect on radiation dose in our study. In the regression analysis conducted excluding technical parameters, age, breast volume, compression thickness, and breast density all affect radiation dose, with compression thickness having the greatest effect, followed by breast volume, age, and finally breast density (Table 7).

Table 4. Comparison of radiation dose measurements and technical parameters in dense and non-dense breast groups

	N	Non-Dense Breast (Mean±SD) (n=1104)	Dense Breast (Mean±SD) (n=616)	P Value
Voltage (kV)	1720	27.4±1.5	26.5±1.5	<0.001
Tube Current (mAs)	1720	90.5±21.2	102.4±39.2	<0.001
Exposure Time (ms)	1720	658±136	766±291	<0.001
Breast Volume (ml)	1720	864±467	537±325	<0.001
Age (years)	430	58.7±10.6	47.2±5.6	<0.001
Compression Thickness (mm)	1720	39.0±11.1	32.2±12.3	<0.001
Compression Pressure (kg)	1720	14.9±3.1	14.6±3.0	0.015
Radiation Dose (mGy) (for each projection)	1720	2.00±0.64	2.12±0.93	0.001
Total Radiation Dose (mGy)	430	7.99±2.14	8.48±3.12	0.028

*Mann-Whitney U test, SD: Standart Deviation

Table 5. Correlation analyzes between radiation dose with age and mammographic parameters

	Non-Dense (n=1104)		Dense (n=616)		Total (n=1720)	
	r	p	r	p	r	p
Age/dose*	-0.449	<0.001	-0.137	0.001	-0.330	<0.001
Breast volume/dose*	0.645	<0.001	0.545	<0.001	0.514	<0.001
Tube voltage/dose*	0.783	<0.001	0.485	<0.001	0.608	<0.001
Tube current/dose*	0.938	<0.001	0.893	<0.001	0.912	<0.001
Exposure time/dose*	0.845	<0.001	0.798	<0.001	0.820	<0.001
Compression thickness/dose*	0.817	<0.001	0.508	<0.001	0.629	<0.001
Compression pressure/dose*	0.058	0.046	0.136	0.001	0.084	<0.001

*Spearman correlation test

Table 6. Comparison of radiation dose measurements and technical parameters in high dose and low dose groups

	Low dose < 75th percentile (Mean±SD)	High dose ≥ 75th percentile (Mean±SD)	p value
Image (n=1720)	1272 (74%)	448 (26%)	
Radiation Dose (mGy) (for each projection)	1.70±0.41	3.01±0.68	<0.001
Total Radiation Dose (mGy)	7.55±1.80	11.85±3.14	<0.001
Age (years)	56.5±11.1	49.0±7.0	<0.001
Voltage (kV)	26.6±1.2	28.4±1.6	<0.001
Tube Current (mAs)	82.8±19.3	128.7±27.1	<0.001
Exposure Time (ms)	623±153	907±216	<0.001
Breast Volume (ml)	630±340	1079±549	<0.001
Compression Thickness (mm)	33.1±10.5	46.4±10.3	<0.001
Compression Pressure (kg)	14.8±3.0	15.0±3.2	0.126

*Mann-Whitney U test, SD: Standart Deviation

Table 7. Logistic regression analysis results of factors affecting radiation dose with and without technical parameters

	RR (%95 CI)	p value	RR (%95 CI)	p value
Voltage (kV)	3.35 (1.16-9.66)	0.025	-	-
Tube Current (mAs)	1.71 (1.48-1.97)	<0.001	-	-
Exposure Time (ms)	0.95 (0.94-0.97)	<0.001	-	-
Compression Pressure (kg)	1.01 (0.89-1.15)	0.870	-	-
Breast Volume (ml)	1.001 (0.999-1.002)	0.336	1.002 (1.001-1.002)	<0.001
Age (years)	0.99 (0.92-1.05)	0.680	0.95 (0.92-0.99)	0.006
Compression Thickness (mm)	0.89 (0.76-1.04)	0.135	1.30 (1.25-1.36)	<0.001
Nondense/Dense Breast	0.59 (0.21-1.70)	0.332	0.09 (0.05-0.15)	<0.001

RR: Relative risk, CI: confidence interval

DISCUSSION

In this study, we performed mean dose calculations for our mammography examinations and investigated the factors affecting radiation dose. According to our study, in addition to the technical parameters of the mammography device, factors such as patient age, breast tissue density, breast volume, and compression thickness affect the radiation dose. The most significant finding of our study is that among the factors other than the technical parameters influencing the radiation dose in mammography, compression thickness has the highest impact, followed by breast volume, age, and finally breast density.

In the literature, the American College of Radiology Imaging Network (ACRIN) Digital Mammographic Imaging Screening Trial (DMIST) study reported a radiation dose range of 1.7-2.5 mGy for a single projection.[24] According to our results, the average radiation dose for a single mammographic projection was found to be 2.04 mGy, the average dose per breast for two projections was 4.08 mGy, and the average total radiation dose for a mammography examination was 8.17 mGy. Baek et al.[25], in their study conducted on the Korean population, found an average dose of 1.81 mGy for a single projection. In a study conducted in the USA, Hendrick et al.[26] reported an average glandular dose of 1.86 mGy for a single projection. In a study conducted in Turkey, Soylu et al.[27] reported a dose of 2.18 mGy. Taking into account the dose weighting factor (0.12) of the International Commission on Radiological Protection (ICRP), the average dose received by a breast in our study was calculated to be 0.49 mSv.[12] According to the recommendations of the ICRP and FDA, the mammographic dose should not exceed 3 mGy for a single projection. In Europe, a dose limit of 2.5 mGy has been specified. When compared to the information and published doses in the literature, it can be said that the mammographic radiation dose rate in our hospital is within the allowed average values.

In our study, we found that higher breast density in patients resulted in increased radiation dose. Similarly, in the literature, it has been noted that higher breast density leads to higher dose exposure.[28,29] When compared to Europe and America, Asian women have been found to have denser breast tissue and consequently higher dose exposure.[25] Additionally, Nguyen et al.[14] stated that while breast density contributes to dose exposure, its impact is not as significant when evaluated in conjunction with other factors. They mentioned that only 10% of the dose increase is attributable to breast density. Similarly, in our study, through regression analysis, we found that among factors other than technical parameters, breast density ranked fourth in terms of its impact, following compression thickness, breast volume, and age.

Considering our findings and the literature, it is important to emphasize that patients with denser breast tissue, who are generally younger and may have concerns about increased radiation exposure due to lifelong mammographic screening programs, should not postpone their mammographic screenings. Furthermore, it has been stated in the literature that breast cancer screening programs, particularly through screening mammography, have resulted in a decrease in breast cancer-related mortality in the United States, and the benefit obtained from early detection of breast cancer outweighs the risk of radiation exposure associated with it. Therefore, it is crucial for patients with dense breast tissue to prioritize regular mammographic screenings without undue concern about radiation exposure.[14-16]

According to our study, there was a negative correlation between patient age and radiation dose. Our findings of higher radiation exposure in younger patients are consistent with similar studies in the literatüre.[25,27] These results suggest that younger women, who generally have denser breasts, require higher doses for optimal imaging. In a study by Raed et al.[30] where they modeled the cancer risk associated with mammography screening, they noted that the most important parameters influencing the overall effective risk from screening were the age at which screening begins and the number of screenings, as tissue radiosensitivity decreases with age. There is an ongoing debate regarding initiating screenings at earlier ages, particularly for women at higher risk due to genetic factors or family history. Additionally, Hendrick et al.[26] reported that even at the same mammographic radiation doses, young women have a higher risk of developing breast cancer. Considering the relatively higher level of radiation exposure and these findings, it indicates the added importance of dose management for women undergoing mammography at a younger age. Individualized dosing may be beneficial, particularly for young patients with a high familial risk factor.

In our study, the average breast tissue volume was calculated to be 595 mL. In the literature, breast volume in Western women has been reported to range from 552 to 774 mL on average.[31,32] Our results fall within this range. Baek et al.[25] reported a smaller average breast volume (380-466 mL) in the Korean population. Breast density, on the other hand, has been found to be approximately 37-51% in Western women and 62-86% in Korean women. In our study, the proportion of dense breast tissue was measured as 36%, which is significantly lower compared to Korean women but closer to the lower limit of Western women.[31,33,34] Furthermore, a study conducted in Turkey also reported a dense breast ratio of 36%, which is in line with our findings.[27]

In a study conducted on approximately 25,000 women, it has been reported that women with larger breasts are exposed to 1.7 times higher radiation dose.[20] In our study, we also found that patients with larger breast volumes were exposed to higher radiation doses. There was a strong positive correlation between breast volume and radiation dose, and we identified breast volume as the second factor influencing radiation dose after compressed breast thickness. Additionally, it can be stated that breast volume also affects the factor identified as the most influential, which is compressed breast thickness. In patients with larger breast volumes, it is inevitable for the compressed breast thickness to be higher as well.

In our study, we found that compressed breast thickness was the factor that most significantly influenced radiation dose exposure, apart from technical parameters. This finding is consistent with the literature, where Nguyen et al.[14] reported that an increase in compressed breast thickness accounted for 80% of the increase in breast radiation dose. Applying compression to the breast not only improves image quality but also reduces radiation exposure. When compression is not applied properly, when the breast volume is large leading to increased compressed breast thickness, or when axillary and surrounding tissues enter the field in MLO images, the thickness of the compressed tissue increases. This necessitates the use of higher kVp or mAs with the automatic exposure feature of the device to overcome the increased tissue thickness.[14]

According to our results, tube voltage, tube current, and exposure time were the most significant technical parameters affecting radiation dose. These parameters are expected to directly influence dose increase since they are dependent on the radiation dose emitted by the device. However, comparing these data with other studies in the literature is useful for dose optimization and determining dose reduction strategies. In our study, we obtained an average of 27.1 kV and 94.8 mAs values in a single projection. These values are similar or close to those reported in many other studies in the literatüre.[17,18,25]

Our study has several limitations. Firstly, the relatively small sample size, single-center design, and local nature of the study limit its generalizability and ethnic diversity. Additionally, all images in our study were obtained from a single device. While this allows for a more homogeneous group, it hinders the comparison of different devices. Another limitation was the subjective decision-making involved in classifying breast density according to the BIRADS category. We did not have access to computer programs that automatically measure breast density and provide numerical results. Furthermore, some factors affecting radiation dose were closely related or even inseparable, making it difficult to separate their effects in statistical analyses, especially in regression analyses. Additionally, due to the retrospective design of the study, we could not analyze parameters such as body mass index that may influence breast density and volume. Finally, we did not seek additional dose measurement support to measure radiation dose. We conducted our study using the dose values provided by the device. While many studies in the literature use this parameter, obtaining more accurate results may be possible with the integration of dose measurement devices. Although our results show similarities with studies conducted in populations with different demographic characteristics, they cannot be globally generalized. In the future, prospective longitudinal studies evaluating the dose differences in repeated control mammography scans of the same patients may provide additional benefit to investigate the factors affecting the dose.

CONCLUSION

Based on the local data obtained in our study, the radiation doses of our mammographic screening procedure fall within internationally acceptable dose values. The most important factors influencing radiation dose are technical parameters such as tube voltage, current and exposure time. However, apart from technical parameters, compressive breast thickness is the most influential factor, followed by breast volume, age, and least of all, breast density, in affecting radiation dose. Multicenter and multinational prospective studies are needed to obtain generalizable results.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kastamonu University Clinical Researches Ethics Committee (Date: 05.07.2023, Decision No: 2023-KAEK-79).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The impact of hypothyroidism and levothyroxine treatment on preeclampsia risk: unraveling the connection for improved maternal and neonatal outcomes

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ABSTRACT

Aims: Preeclampsia, a pregnancy-related complication, may develop in women with hypothyroidism. Possible risk factors may include comorbidities, predisposition to diabetes, obesity, advanced maternal age, and prior infertility treatments. The study aims to investigate the relationship between hypothyroidism and the risk of preeclampsia in pregnant women receiving levothyroxine by examining its application period.

Methods: This is a retrospective cohort included pregnant women who gave birth between December 2022-April 2023. Women with 110 preeclampsia and those without preeclampsia (152 controls) were identified and compared in terms of hypothyroidism status, type of hypothyroidism, and levothyroxine treatment.

Results: The results showed a significant association between the severity of the preeclampsia and its onset that early onset cases were more likely to be severe, while late onset cases were predominantly mild ($p<0.001$). The results showed no association between the onset of the preeclampsia and the starting treatment period ($p=0.372$). In the binary logistic regression, only one variable, "Apgar 5th minute" was significant in the logistic regression analysis ($p=0.032$). The coefficient indicates that as the "Apgar 5th minute" score increases, "during pregnancy" decrease by a factor of 0.603 (ODDs ratio; ranged from 0.380 to 0.957).

Conclusion: There was a difference in the distribution of mild and severe preeclampsia between

the euthyroid and hypothyroidism. Early threating hypothyroid with Levothyroxine might affect the Apgar score.

Keywords: Hypothyroidism, levothyroxine, preeclampsia, Apgar

INTRODUCTION

Thyroid disorders, particularly hypothyroidism, are common endocrine disturbances in women of reproductive age and pregnant women.[1] Overt hypothyroidism, subclinical hypothyroidism, isolated maternal hypothyroxinemia, and the presence of antithyroid antibodies in euthyroid patients are various manifestations of thyroid dysfunction.[2,3] Subclinical hypothyroidism is observed in 3-5% of pregnant women, whereas hypothyroidism occurs in 0.3-1% of the population.[4,5]

Hypothyroidism during pregnancy is associated with an increased risk of early and late obstetric complications, such as anemia, spontaneous abortion, congestive heart failure, preeclampsia, postpartum hemorrhage, placental abnormalities, and gestational hypertension in the mother.[6-8] Adverse neonatal outcomes, including low birth weight, premature birth, stillbirth, and neonatal respiratory distress, may also occur.9 The effects of subclinical hypothyroidism on pregnancy are not as well-defined as those of overt hypothyroidism Nonetheless, treatment of subclinical hypothyroidism during pregnancy is recommended to minimize obstetric complications.[10]

Preeclampsia, a pregnancy-related complication, may develop in women with hypothyroidism.[10,11] Severe preeclampsia are often associated with significant organ damage, as well as increased neonatal morbidity and mortality.[12] Despite previous studies indicating a higher risk of preeclampsia in pregnant women with thyroid disorders (mainly hypothyroidism), little is known about the specific risk factors that contribute to this increased risk.[13] Possible risk factors may include comorbidities, predisposition to diabetes, obesity, advanced maternal age, and prior infertility treatments.[14,15]

In this study, we aim to investigate the relationship between hypothyroidism and the risk of preeclampsia

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in pregnant women receiving levothyroxine. We will examine the presence and application period of levothyroxine treatment in women with preeclampsia and those without control group in a retrospective cohort.

METHODS

Ethical Statement

The study was carried out with the permission of Ankara Etlik City Hospital No:1 Clinical Researches Ethics Committee (Date: 13.06.2023, Decision No: AEŞH-EK1-2023-246). The study was conducted in accordance with the principles of the Declaration of Helsinki and Clinical Practice guidelines. All eligible participants were provided with an information sheet explaining the study's objectives, procedures, potential risks, and benefits. Oral informed consent was obtained from each participant before enrolling them in the study.

Study Design

This was a retrospective cohort conducted at the population that included pregnant women who gave birth between December 2022-April 2023. Women with 110 preeclampsia and those without preeclampsia (152 controls) were identified and compared in terms of hypothyroidism status, type of hypothyroidism, and levothyroxine treatment.

Enrolled Participants

The control group consists of 152 euthyroid preeclampsia patients who were monitored at our clinic due to preeclampsia and subsequently gave birth at similar gestational weeks. The case group, on the other hand, is comprised of preeclampsia patients who were treated with levothyroxine due to subclinical and overt hypothyroidism, and who were also monitored and gave birth at our clinic due to preeclampsia. Based on a comprehensive literature review, we established specific inclusion and exclusion criteria for this study to ensure a well-defined study population and minimize potential confounding factors. Pregnant women who gave birth at the Hospital between January 2016-December 2020 were included if they had singleton pregnancies and complete medical records, including data on key variables. Additionally, they needed to have a confirmed diagnosis of preeclampsia or no preeclampsia (controls) according to the American College of Obstetricians and Gynecologists criteria. Exclusion criteria were designed to eliminate potential biases and confounders. We excluded women with multiple pregnancies, a history of thyroidectomy or radioiodine, other known thyroid disorders, or pregnancies complicated by fetal anomalies or chromosomal abnormalities. Women with incomplete medical records or missing data on key variables, as well as those with a history of chronic hypertension or renal disease, were also excluded from the study.

Definitions of Preterm Delivery

Births between >34 and <37 weeks were classified as late preterm birth, which constitute approximately 90% of such cases. Births before 34 weeks were termed early preterm birth, comprising about 10% of cases. These early preterm births pose an increased risk due to the presence of significant maternal and/or perinatal morbidity or the relatively early gestational age at birth. The most commonly observed subtypes are early-onset (<34 weeks of gestation) and late-onset (≥34 weeks of gestation) preeclampsia.

Diagnostic Criteria of Thyroid & Data Collection

Hypothyroidism was defined as a TSH level >4.2 mU/l and a free T4 level <11 μmol/l, while subclinical hypothyroidism was defined as a TSH level >2.5 mU/l in the first trimester and a TSH level >M mU/l in the second and third trimesters with normal free T4 levels. Data were collected from the electronic medical records of the hospital. The following information was extracted for each participant: maternal age, body mass index (BMI) before pregnancy, parity, gestational age at delivery, hypothyroidism status, type of hypothyroidism (overt or subclinical), levothyroxine treatment status, and levothyroxine dosage. Additional data on comorbidities, such as diabetes, hypertension, and medical conditions were collected.

Laboratory Method

Blood samples were collected from each participant during routine prenatal visits in the first, second, and third trimesters. Samples were collected in serum separator tubes and centrifuged at 3000 rpm for 10 minutes. The serum was then aliquoted and stored at -80°C until analysis. Serum levels of thyroid-stimulating hormone (TSH), free thyroxine (fT4), and total thyroxine (TT4) were measured using an automated chemiluminescent immunoassay system according to the manufacturer's instructions. The reference ranges for TSH, fT4, and TT4 were established based on the guidelines provided by the American Thyroid Association. Internal and external quality control measures were implemented throughout the laboratory analysis process. These included the use of assay-specific quality control materials and participation in an external quality assessment scheme.

Statistical Analysis

The collected data were analyzed using IBM-SPSSv24. Descriptive statistics, including means, medians, and percentages, were calculated for the population. The relationship between hypothyroidism status, type of hypothyroidism, levothyroxine treatment, and

preeclampsia risk was assessed using logistic regression models. To determine the association between severity and onset, and to determine the association between the onset of the condition and the starting treatment period, we performed Chi-Square tests. we analyzed efficiency of early and late treatment of hypothyroid by levothyroxine by binary logistic regression. Adjustments were made for potential confounding factors such as maternal age, Abgar score, onset/late delivery, BMI, parity, and comorbidities. The statistical significance was set at $p<0.05$.

RESULTS

Demographics

As given in Table, our results showed no significant differences in age, with euthyroid women having a mean age of 30.6±6.6 years and hypothyroid women having a mean age of 31.9±6.3 years (p=0.108). Similarly, the BMI did not show any significant difference, with euthyroid women having a mean BMI of 32.8±6.3 kg/m² and hypothyroid women having a mean BMI of 33.2±5.5 kg/m² (p=0.523). Gravida, parity, abortions, living children, birth week, weight, and Apgar scores at the 1st and 5th minutes did not show any significant differences between euthyroid and hypothyroid pregnant women. For instance, the mean gravida for both groups were 3, with a range of 1-7 for euthyroid women and 1-6 for hypothyroid women (p=0.416). Parity also did not differ significantly, with a median of 1 (range: 0-5) in euthyroid women and 1 (range: 0-4) in hypothyroid women (p=0.127).

Table. All data comparison of the Euthyroid and Hypothyroidia groups

Variables	Control (n:152)	Hypothyroid (n:110)	P value
Age (years)	30.6±6.6	31.9±6.3	0.108
BMI (kg/m²)	32.8±6.3	33.2±5.5	0.523
FT3 (pg/ml)	4.98±0.74	4.52±0.83	0.001
FT4 (ng/dl)	14.28±2.56	13.34±2.87	0.006
TSH (µIU/ml)	1.57±0.65	4.17±1.91	0.001
Gravida	3 (1-7)	3 (1-6)	0.416
Parity	1 (0-5)	1 (0-4)	0.127
Abortions	1 (0-4)	0 (0-4)	0.550
Living	1 (0-5)	1 (0-4)	0.138
Birth week	35±4	35±3	0.749
Weight (g)	2405±957	2544±901	0.235
Apgar (1st minute)	8.1±1.4	8.2±1.4	0.752
Apgar (5th minute)	9.3±1.2	9.4±1.1	0.402

Abbreviations. BMI: Body Mass Index, FT3: Free Triiodothyronine, FT4: Free Thyroxine, and TSH: Thyroid Stimulating Hormone. The table presents the comparison of the Euthyroid and Hypothyroid Group with their respective means and standard deviations (Mean±SD) or Median (Min-Max). The p-values are provided for each variable to assess the statistical significance of the differences between the two groups.

Crosstab Analysis

As given in Figure 1, the control had 93 individuals (61.2%) with mild preeclampsia and 59 individuals (38.8%) with severe preeclampsia. On the other hand, the hypothyroidism had 76 individuals (69.1%) with mild preeclampsia and 34 individuals (30.9%) with severe preeclampsia that the asymptotic significance (2-sided) was 0.187. Based on this test, we cannot conclude that there is a significant difference in the distribution of mild and severe preeclampsia between the control and hypothyroidism groups. The results showed a significant association between the severity of the preeclampsia and its onset that Early onset cases were more likely to be severe, while late onset cases were predominantly mild ($p<0.001$). The results showed no significant association between the onset of the preeclampsia and the starting treatment period (p=0.372). The distribution of early and late onset cases appears to be independent of when the treatment was initiated (Figure 2 and 3).

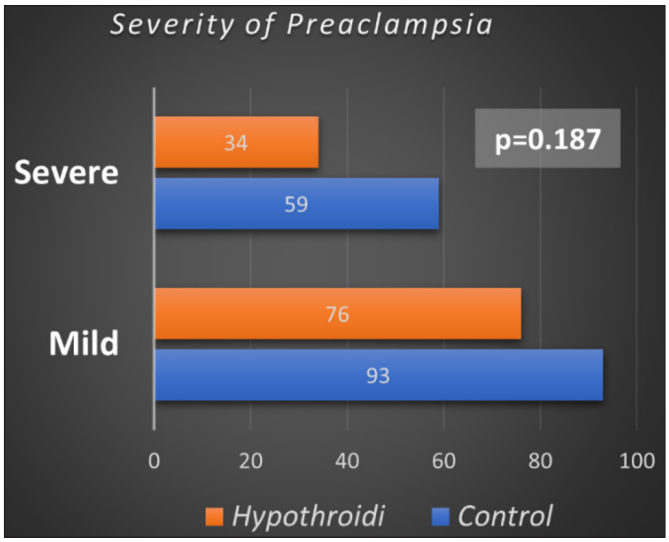

Figure 1. Severity of preeclampsia for hypothyroid or control groups

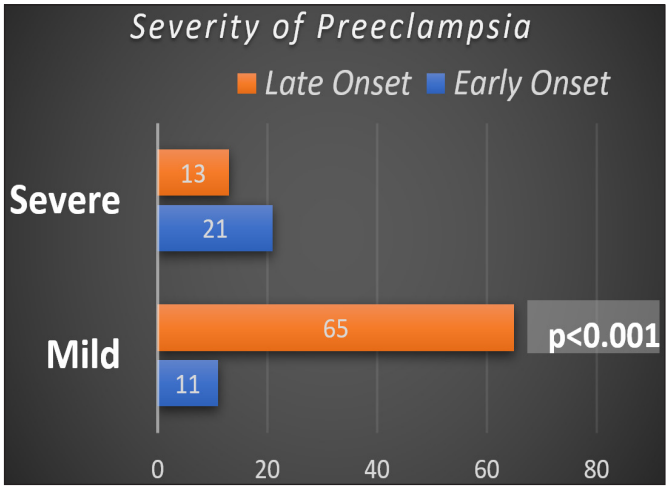

Figure 2. Severity of preeclampsia for early or late onset

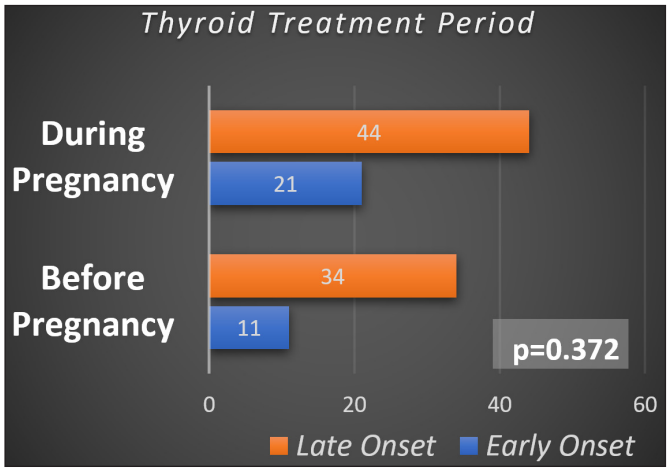

Figure 3. Thyroid Treatment Period of preeclampsia for early or late onset

Logistic Analysis

In the study, we analyzed efficiency of early and late treatment of hypothyroid by levothyroxine by binary logistic regression. The overall model was statistically significant, with an accuracy of 59.1%. Only one variable, "Apgar 5th minute" was significant in the logistic regression analysis (p=0.032). The coefficient indicates that as the "Apgar 5th minute" score increases, the odds of the pregnancy outcome being classified as "during pregnancy" decrease by a factor of 0.603 (ODDs ratio; ranged from 0.380 to 0.957). Several other variables were tested for their association with pregnancy outcomes, but none were significant. These variables included abortus (p=0.584), kilo (p=0.403), Apgar 1st minute (p=0.691), delivery type (p=0.81), gender of baby (p=0.311), delivery time (p=0.366), and severity of preeclampsia (p=0.208).

DISCUSSION

In this study, we investigated the relationship between initialing the hypothyroid treatment period in pregnant women with and without preeclampsia. The distribution of early and late onset cases appears to be independent of when the treatment was initiated. Our findings contribute to the growing body of evidence on the role of thyroid function in the development of obstetric complications, particularly in the context of levothyroxine therapy.

Hypothyroidism during pregnancy is associated with an increased risk of early and late obstetric complications, such preeclampsia and placental abnormalities. A recent meta-analysis by Li et al.[16] reported an increased risk of preeclampsia in pregnant women with hypothyroidism, while also highlighting that levothyroxine treatment may reduce this risk. Likewise, Zhang et al.[17] demonstrated that levothyroxine therapy in pregnant women with subclinical hypothyroidism was associated with a lower incidence of preeclampsia and other adverse pregnancy outcomes. Further supporting our findings, Gietka-Czernel et al.[18] reported that pregnant women with subclinical hypothyroidism who received levothyroxine treatment had a lower risk of preeclampsia compared to those who did not receive treatment. Moreover, two different studies demonstrated that appropriate levothyroxine dosage adjustment during pregnancy could significantly reduce the risk of obstetric complications, including preeclampsia.[19,20] According to our result, we cannot conclude that there is a significant difference in the distribution of mild and severe preeclampsia between the control and hypothyroidism groups. In the study, we analyzed efficiency of early and late treatment of hypothyroid by levothyroxine by binary logistic regression that the model was significant, with an accuracy of 59.1%. The coefficient indicates that as the "Apgar 5th minute" score increases, the odds of the pregnancy outcome being classified as "during pregnancy" decrease.

In a comparison with prior studies, the research conducted by Su et al.[21] demonstrated that hypothyroxinemia is only associated with a risk of preeclampsia-eclampsia, particularly escalating the risk in women with persistent hypothyroxinemia in the first half of pregnancy. Even though our study did not find a significant association with the severity of preeclampsia, these results support the relationship between thyroid dysfunction and preeclampsia risk. The study by Wang et al.[22] showcased the significance of thyroid function tests before and during pregnancy, indicating their role in the early diagnosis of hypothyroidism and in reducing the risk of preeclampsia. These findings are consistent with our study's results that early-onset cases are more severe, underscoring the importance of early diagnosis. The research by Maraka et al.[23] proposed that levothyroxine treatment could potentially reduce the risk of preeclampsia in pregnant women with subclinical hypothyroidism and isolated hypothyroxinemia, though this benefit may be limited. Even though our study did not find a significant relationship between the initiation of treatment and the onset of preeclampsia, it suggests that levothyroxine treatment could play an essential role in managing the risk of preeclampsia. In another study, Jiao and co-authors [24] evaluated the impact of thyroid dysfunction on the development of preeclampsia and found a significant association between subclinical hypothyroidism and the risk of preeclampsia. Despite the lack of a significant relationship with the severity of preeclampsia in our study, this research supports the connection between thyroid dysfunction and preeclampsia risk.

In the context of these recent findings, our study adds valuable insights into the complex interplay between thyroid function, levothyroxine treatment, and preeclampsia. However, it is important to acknowledge some limitations in our study. The sample size of the study may not be large enough to achieve sufficient power in statistical analyses, which can limit the generalizability of the study results and make it difficult to detect certain relationships. Measurement errors in continuous variables, such as thyroid hormone levels and other clinical measurements, can affect the accuracy of the analyses and the interpretability of the results. The study may not control for all potential confounding factors, for instance, genetic factors, nutrition, and lifestyle factors can have effects on thyroid dysfunction and preeclampsia risk and may not be fully addressed in this study. Prospective, long-term studies can help to better understand the causal relationships between thyroid dysfunction and preeclampsia.

CONCLUSION

Our study highlights the potential influence of thyroid function and levothyroxine treatment on the risk of abnormal invasive placentation in pregnant women with and without preeclampsia. According to our result, we cannot conclude that there is a difference in the distribution of mild and severe preeclampsia between the control and hypothyroidism. However, early threating hypothyroid with Levothyroxine might affect the Abgar score. Future research should involve larger, prospective studies to further explore the impact of levothyroxine treatment on the risk of preeclampsia.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Etlik City Hospital No:1 Clinical Researches Ethics Committee (Date: 13.06.2023, Decision No: AEŞH-EK1-2023-246).

Informed consent: Written consent was obtained from the patient participating in the study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Assessment of vitamin D deficiency and hyperparathyroidism in metabolically healthy and unhealthy obese patients

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ABSTRACT

Aims: This study aimed to compare the levels of vitamin D in metabolically healthy (MHO) and metabolically unhealthy obese (MUO) individuals and determine if there are differences between these two groups concerning vitamin D deficiency and hyperparathyroidism.

Methods: A total of 263 obese female patients were included in the study and divided into two groups based on metabolic syndrome diagnostic criteria. Biochemical and anthropometric data obtained after a 12-hour fasting period were analyzed.

Results: Among the patients, the average 25-OH vitamin D level was 10.9±6.5 ng/ml. A total of 242 patients (92%) had vitamin D deficiency, and 132 patients (50.2%) were diagnosed with hyperparathyroidism. Significant differences were found in vitamin D ($p=0.003$) and uric acid ($p<0.001$) levels between the MHO and MUO groups. Additionally, the groups with vitamin D deficiency showed significantly different glucose ($p=0.026$) and homeostatic model assessment for insulin resistance ($p=0.042$) values. Patients with hyperparathyroidism had higher waist circumference ($p<0.001$), waist-to-hip ratio ($p=0.018$), BMI ($p=0.006$), and systolic ($p=0.001$) and diastolic ($p<0.001$) blood pressure values compared to those with normal parathyroid hormone levels.

Conclusion: The study emphasizes the importance of monitoring vitamin D deficiency and hyperparathyroidism in obese patients, as these conditions are more prevalent in this population and might be associated with metabolic syndrome parameters, increasing cardiometabolic risk.

Keywords: Vitamin D deficiency, obesity, hyperparathyroidism, metabolic syndrome, cardiovascular disease

INTRODUCTION

Obesity, a chronic condition, has experienced a rise in prevalence in both developed and developing nations. Recent global assessments indicate that nearly 108 million children (approximately 5% prevalence) and 604 million adults (around 12% prevalence) are affected by obesity.[1] Generally, obesity rates are higher among women compared to men, and the risk tends to increase with age after 14 years.[1,2] In recent decades, there have been findings indicating that certain obese individuals have a notably reduced risk of developing cardio metabolic diseases. This has resulted in the introduction of the term "metabolically healthy obesity" (MHO) in scientific literature.[3] While the exact definition of MHO remains unclear, it is suggested that it can be identified by the absence of metabolic syndrome (MS) criteria.[4] Moreover, individuals with MHO are reported to exhibit lower levels of visceral fat, higher insulin sensitivity, and better-preserved beta cell reserve.[3,4]

Vitamin D deficiency is recognized as a significant global public health problem, impacting various bodily systems beyond musculoskeletal health.[5,6] Vitamin D deficiency in obese individuals is a well-documented observation; however, a definitive cause-and-effect relationship has not been established.[7,8] Recently, studies have suggested that vitamin D deficiency may be linked to the pathogenesis of MS components, including obesity, insulin sensitivity, hypertension and carbohydrate metabolism disorders.[5,7,9,10] Moreover, there are reports suggesting that secondary hyperparathyroidism, arising from vitamin D deficiency, is linked to obesity and its associated comorbidities.[11-13] It has even been suggested that secondary hyperparathyroidism may ameliorate with obesity treatment.[13]

Based on all the available data, the objective of this study is to examine whether there exists a disparity in

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vitamin D levels, secondary hyperparathyroidism and the incidence of vitamin D deficiency between patients with MHO and those with "metabolically unhealthy obesity" (MUO).

METHODS

Ethical Approval

The study was carried out with the permission of Erzincan Binali Yıldırım University Clinical Researches Ethics Committee (Date: 11.05.2023, Decision No: 2023-10/2). All procedures adhered to ethical guidelines and the principles outlined in the Declaration of Helsinki. Additionally, all participants provided informed written consent.

Study Population

Our study included 263 obese (body mass index, (BMI) ≥30 kg/m²) female patients, aged 18-65, who newly applied for follow-up at our hospital's obesity center. Patients who were pregnant or suspected to be pregnant, patients with chronic kidney insufficiency, those who had received vitamin D replacement therapy within the last 6 months, and patients using agents that could alter MS parameters were excluded from the study after screening.

Anthropometric Measures and Laboratory Analyses

Biochemical data and anthropometric measurement data obtained after 12 hours of fasting were obtained using the files of the obesity center. The recorded parameters included age (in years), waist and hip circumference (in cm), systolic and diastolic blood pressure (in mmHg), C-Reactive Protein (CRP), total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glucose, calcium, phosphate, creatinine, and uric acid levels (in mg/dl), HbA1c (in %), thyroid-stimulating hormone level (in U/ml), parathyroid hormone level (in pg/ml), and 25-OH-vitamin D level (in ng/ml).

Based on the baseline data provided above, BMI (in kg/m²), waist-to-hip ratio, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) values were calculated appropriately using the relevant formulas.

The presence of MS was assessed using the revised NCEP-ATP ATP III MS criteria.[14] According to this, the diagnosis of MS includes having three or more of the following criteria: elevated fasting blood sugar (≥100 mg/dl) or a diagnosis of type 2 diabetes (T2DM), high blood pressure (≥130/85 mm Hg) or a diagnosis of hypertension, low HDL cholesterol levels (less than 50 mg/dl for women), elevated triglyceride levels (≥150 mg/dl), and increased waist circumference specific to the population (≥90 cm as recommended by national guidelines for women in our country).[15]

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences 15.0 (SPSS Inc., Chicago, IL, USA) for Windows. Normality was tested by Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± standard deviation. Inter Quartile ranges were used for variables without a normal distribution. Data with a normal distribution were compared by Student's t test and comparisons of continuous variables with an asymmetric distribution were made by using the Mann-Whitney U test. Statistically significant differences between the groups were determined by the chi-square test for categorical variables. A p-value less than 0.05 was considered significant.

RESULTS

We included 263 obese female patients with an average age of 42.9±10.4 years and an average BMI of 37.5±4.5 kg/m² in our study. Among the patients, 49 were diagnosed with T2DM. Additionally, 5 new cases of T2DM were identified based on HbA1c and fasting glucose levels. During the assessment, 18 patients met all 5 criteria for MS, 40 patients met 4 MS criteria, and 85 patients had 3 positive MS criteria, totaling 143 cases of MS and MUO patients. Moreover, 85 patients had 2 MS criteria, 32 had 1 criterion, and 3 had no MS criteria, making a total of 120 MHO patients.

The average 25-OH vitamin D level among the patients was 10.9±6.5 ng/ml, while the average PTH (parathyroid hormone) level was 92.5±42.4 pg/ml. Out of the patients, 242 (92%) had a deficiency in 25-OH vitamin D, while 21 (8%) had a vitamin D level ≥20 ng/ml. Furthermore, 131 patients (49.8%) had PTH values within the normal range, and 132 patients (50.2%) were diagnosed with hyperparathyroidism.

As seen in **Table 1**, between the MHO and MUO patient groups, in addition to the MS parameters used for the definition and differentiation of the groups, 25-OH-D vitamin (p=0.003) and uric acid (p<0.001) were found to be statistically different. Among the groups with and without vitamin D deficiency, glucose (p=0.026) and HOMA-IR (p=0.042) values were found to be statistically different (**Table 2**). Moreover, in the patient group with hyperparathyroidism, waist circumference (p<0.001), waist-to-hip ratio (p=0.018), BMI (p=0.006), systolic (p=0.001), and diastolic blood pressure (p<0.001) values were found to be significantly higher compared to the group with normal parathyroid hormone levels (**Table 3**).

Table 1. Demographic and clinical parameters of metabolically healthy and unhealthy obese patients

Parameters	Metabolically healthy obese patients n=120	Metabolically unhealthy obese patients n=143	p value
Age (years)	42±10	44±11	0.098
Waist circumference (cm)*	102±10	109±8	<0.001
Hip circumference (cm)	125 (120-133)	127 (122-135)	0.484
Waist to hip ratio	0.81±0.1	0.86±0.1	<0.001
Body mass index (kg/m²)	36.3 (32.9-39.6)	37.8 (35.3-40.4)	0.075
Systolic blood tension (mmHg)*	110 (110-120)	130 (120-140)	<0.001
Diastolic blood tension (mmHg)*	70 (60-80)	80 (70-90)	<0.001
C-reactive protein (mg/L)	4.6 (3.3-8.4)	4.45 (3.2-8.6)	0.943
Total cholesterol (mg/dl)	198±41	203±39	0.298
HDL- cholesterol (mg/dl)*	53 (45-61)	47 (42-52)	<0.001
LDL- cholesterol (mg/dl)	129±30	134±28	0.182
Triglyceride (mg/dl)*	110 (83-135)	168 (125-226)	<0.001
Glucose (mg/dl)*	91 (85-95)	101 (91-114)	<0.001
HbA1c (%)*	5.5 (5.3-5.7)	5.7 (5.5-6.3)	<0.001
Calcium (mg/dl)	9.4 (9.2-9.5)	9.3 (9.1-9.6)	0.830
Phosphate (mg/dl)	3.6 (3.2-3.9)	3.7 (3.3-4)	0.093
Creatinine (mg/dl)	0.76 (0.71-0.82)	0.75 (0.7-0.83)	0.167
Uric acid (mg/dl)	4.6±1.1	5.2±1.2	<0.001
TSH (U/ml)	2.4 (1.5-3.4)	2.3 (1.4-3.9)	0.860
PTH (pg/ml)	77.7 (60.4-110.6)	87.5 (69.5-115.6)	0.051
25- OH-Vit-D (ng/ml)	11.3 (7.7-16.7)	9.3 (5.6-13.9)	0.003
25- OH-Vit D deficiency [n (%)]	14 (11.4%)	7 (4.9%)	0.044
HOMA-IR	2.33 (1.47-3.58)	3.99 (2.6-6.27)	<0.001

HDL; High-Density Lipoprotein, LDL; Low-Density Lipoprotein, HbA1c; Glycolyzed Hemoglobin, TSH; Thyroid Stimulating Hormone, PTH; Parathormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. Parameters with normal distribution are presented as the Mean±Standard deviation (Student t test). Parameters with abnormal distribution presented as median and interquartile range (Mann-Whitney U test). Chi-square test was used to compare categorical data. * Parameters used in the diagnosis of metabolic syndrome.

Table 2. Demographic and clinical parameters in patients with and without vitamin D deficiency

Parameters	Patients with a vitamin D value <20 ng/ml n=242	Patients with a vitamin D value ≥20 ng/ml n=21	p value
Age (years)	44 (36-50)	42 (37 -50)	0.625
Waist circumference (cm)*	106 100-113)	99 (95-109)	0.007
Hip circumference (cm)	126 (121-135)	124 (116.75-128)	0.053
Waist to hip ratio	0.83 (0.8-0.88)	0.82 (0.79-0.87)	0.269
Body mass index (kg/m²)	37.4 (35.1-40.1)	35.8 (32.6-39.3)	0.095
Systolic blood tension (mmHg)*	120 (110-133)	120 (110-130)	0.809
Diastolic blood tension (mmHg)*	80 (70-80)	70 (63-80)	0.470
C-reactive protein (mg/L)	4.6 (3.3-8.8)	3.6 (2.9-6.6)	0.073
Total cholesterol (mg/dl)	197 (174-224)	209 (183-236)	0.251
HDL- cholesterol (mg/dl)*	49 (43-59)	50 (46-55)	0.994
LDL- cholesterol (mg/dl)	131 (114-151)	135 (120-157)	0.207
Triglyceride (mg/dl)*	131 (97-189)	135 (79-174)	0.190
Glucose (mg/dl)*	95 (88-105)	92 (83-95)	0.026
HbA1c (%)*	5.6 (5.4-6)	5.6 (5.2-5.8)	0.076
Calcium (mg/dl)	9.3 (9.1-9.6)	9.4 (9.1-9.5)	0.911
Phosphate (mg/dl)	3.6 (3.3-3.9)	3.8 (3.3-3.9)	0.570
Creatinine (mg/dl)	0.75 (0.7-0.82)	0.76 (0.71-0.85)	0.727
Uric acid (mg/dl)	4.9 (4-5.7)	4.9 (4.5-5.9)	0.926
TSH (U/ml)	2.4 (1.5-3.6)	2.1 (1.02-3.1)	0.136
PTH (pg/ml)	87.3 (65.5-113.8)	64.3 (41.6-95.1)	0.006
25- OH-Vit-D (ng/ml)	9.5 (6.1-13.5)	24.7 (21.3-26.3)	<0.001
HOMA-IR	3.32 (1.98-4.99)	2.33 (1.21-3.30)	0.042

HDL; High-Density Lipoprotein, LDL; Low-Density Lipoprotein, HbA1c; Glycolyzed Hemoglobin, TSH; Thyroid Stimulating Hormone, PTH; Parathormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. Parameters with normal distribution are presented as the Mean±Standard deviation (Student t test). Parameters with abnormal distribution presented as median and interquartile range (Mann-Whitney U test). * Parameters used in the diagnosis of metabolic syndrome.

Table 3. Demographic and clinical parameters in obese patients with and without hyperparathyroidism

Parameters	Patients with Normal PTH value PTH<85 pg/ml n=131	Patients With Hyperparathyroidism PTH≥85 pg/ml n=132	p value
Age (years)	42.5 (36-48)	44 (35-52)	0.234
Waist circumference (cm)*	104±10	108±9	<0.001
Hip circumference (cm)	125 (119-134)	127 (122-135.75)	0.051
Waist to hip ratio	0.82 (0.79-0.86)	0.84 (0.81-0.88)	0.018
Body mass index (kg/m²)	36.8±4.8	38.3±4.1	0.006
Systolic blood tension (mmHg)*	120 (110-130)	120 (110-140)	0.001
Diastolic blood tension (mmHg)*	70 (60-80)	80 (70-90)	<0.001
C-reactive protein (mg/L)	4.6 (3.2-8.7)	4.3 (3.2-8.3)	0.584
Total cholesterol (mg/dl)	194 (172-227)	200 (176-223)	0.826
HDL- cholesterol (mg/dl)*	49 (43-57)	50 (43-58)	0.676
LDL- cholesterol (mg/dl)	132±31	131±27	0.712
Triglyceride (mg/dl)*	137 (100-192)	129 (93-184)	0.756
Glucose (mg/dl)*	95 (88-103)	95 (88-107)	0.644
HbA1c (%)*	5.6 (5.3-5.9)	5.6 (5.3-6)	0.955
Calcium (mg/dl)	9.4 (9.1-9.5)	9.3 (9.1-9.6)	0.928
Phosphate (mg/dl)	3.7 (3.3-4)	3.5 (3.3-3.9)	0.128
Creatinine (mg/dl)	0.76 (0.71-0.83)	0.75 (0.70-0.81)	0.837
Uric acid (mg/dl)	4.9±1.2	5±1.2	0.281
TSH (U/ml)	2.1 (1.4-3.1)	2.7 (1.7-3.9)	0.074
PTH (pg/ml)	64.3 (55.5-73.5)	112.5 (97.1-139.9)	<0.001
25- OH-Vit-D (ng/ml)	11.8 (8.65-17.1)	8.7 (5.1-11.8)	<0.001
HOMA-IR	3.15 (1.69-4.87)	3.30 (2.26-4.85)	0.089

HDL; High-Density Lipoprotein, LDL; Low-Density Lipoprotein, HbA1c; Glycolyzed Hemoglobin, TSH; Thyroid Stimulating Hormone, PTH; Parathormone, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. Parameters with normal distribution are presented as the Mean±Standard deviation (Student t test). Parameters with abnormal distribution presented as median and interquartile range (Mann-Whitney U test). * Parameters used in the diagnosis of metabolic syndrome.

DISCUSSION

The present study has several important findings. Firstly, the prevalence of vitamin D deficiency is 92% among obese patients. Secondly, lower levels of vitamin D and vitamin D sufficiency rates have been observed in patients with MUO. Lastly, vitamin D deficiency is associated with higher levels of glucose and insulin resistance, while elevated PTH levels are correlated with increased waist circumference, BMI, and blood pressure.

In our country, where patients receiving vitamin D replacement are not excluded, the rate of vitamin D deficiency can reach up to 71.5% in patient groups reflecting the general population.[16] Moreover, during winter months, it has been reported that the rate of vitamin D deficiency may increase up to 83.9% in individuals whose vitamin D use is excluded.[17] Additionally, higher rates of vitamin D deficiency have been reported in female patients.[18] In studies conducted outside our region, the rates of vitamin D deficiency in obese patients vary between 40% and 80%.[19] Therefore, our data from the known obese female patient group with higher rates of vitamin D deficiency have been appropriately evaluated according to the literature.

Multiple pathophysiological mechanisms have been proposed to explain the relationship between vitamin D and MS. One of these mechanisms suggests that vitamin D deficiency affects the capacity of beta cells to convert pro-insulin to insulin, leading to decreased insulin secretion and sensitivity.[7,19] Another mechanism points to increased visceral fat mass in MS patients, which could lead to an increase in the distribution volume of vitamin D and subsequently lower serum vitamin D levels.[7,8,19] Additionally, it is claimed that individuals with both MS and obesity may have low vitamin D intake due to their dietary habits.[7,19] Other possible mechanisms include reduced exposure to sunlight, variations in gene expression of enzymes involved in vitamin D metabolism, and impaired hepatic 25-hydroxylation.[7,8,19,20] Furthermore, Osmancevic and colleagues[21] discovered that there was a negative correlation between BMI and the rise in serum vitamin D3 following UVB exposure, even after considering other variables. Lastly, another significant mechanism is the suppressor effect that active vitamin D may have on its precursor, 25-OH-D levels. Studies have shown that obese individuals tend to have higher levels of active vitamin D compared to non-obese individuals.[22] It has been suggested that the inhibitory effect of this active metabolite on the liver's synthesis of its precursor could be a contributing factor to the lower vitamin D levels observed in obesity[8]. These mechanisms are among the potential factors that may play a role in the relationship between vitamin D and MS.

Many studies that did not include all obese patients have provided inspiration for these potential pathophysiological mechanisms concerning the relationship between MS and vitamin D deficiency. In a study conducted on postmenopausal women, the rate of MS was found to be 57.8% in patients with hypovitaminosis D, whereas it was 39.8% in patients without hypovitaminosis D.[23] Another study revealed that in individuals meeting the criteria for obese MS, vitamin D deficiency was significantly higher compared to those without vitamin D deficiency (60.9% vs. 33.3% respectively).[24] Similarly, Lee et al.[25] identified that low 25(OH)D levels were associated with an increased risk of MS. Researchers, by categorizing patients into quartiles based on their vitamin D levels, demonstrated a relationship between vitamin D levels and the prevalence of MS. The lower the vitamin D levels, the higher the prevalence of increased waist circumference, hypertriglyceridemia, and elevated low-density lipoprotein cholesterol (LDL) concentrations.[25] Zhu and Heil[26] reported an association between 25(OH)D concentrations and glucose and blood lipid concentrations, indicating that low vitamin D levels were related to the presence of MS. The authors of this study revealed that every 1 ng/ml increase in vitamin D was associated with a 54% decrease in MS risk.[26]

While the relationship between vitamin D and MS syndrome has been primarily emphasized, many studies have also given importance to the association between MS and PTH, stating that individuals with MS have higher PTH levels or a higher rate of secondary hyperparathyroidism.[11-13] However, in some studies, hyperparathyroidism in obese individuals has been attributed to factors other than vitamin D deficiency, such as high body weight and obesity.[12,27-29] This is supported by findings where normal vitamin D levels or even vitamin D supplementation did not result in a significant decrease in PTH levels.[30,31] Moreover, a recent article reported that the serum vitamin D level needed to suppress PTH levels was lower in patients before and after bariatric surgery compared to non-obese patients.[32] Therefore, it can be hypothesized that the inverse relationship between PTH and vitamin D in obesity is not causal, but both biochemical abnormalities may directly result from obesity itself. In our study, while there were only statistical differences in terms of glucose and HOMA-IR between the groups with and without vitamin D deficiency, significant differences were observed in waist circumference, waist-to-hip ratio, BMI, systolic and diastolic blood pressure between the groups with and without hyperparathyroidism. These findings suggest that hyperparathyroidism may be more closely related to the criteria of MS and may be closely associated with MS and obesity in addition to vitamin D deficiency.

Our study has certain limitations. Firstly, it is a cross-sectional study, and therefore, causal relationships cannot be established. Additionally, the study only included female patients and was conducted at a single center, which may limit its generalizability to the entire population. Furthermore, the relatively small size of the population without vitamin D deficiency in our study could be considered a weakness of the study.

CONCLUSION

High rates of vitamin D deficiency were found in obese patients. In people with MUO, vitamin D levels are lower and the prevalence of vitamin D deficiency is higher. Vitamin D deficiency is associated with glucose and insulin resistance, while hyperparathyroidism is associated with a greater number of parameters associated with the MS, such as waist circumference, waist-to-hip ratio, BMI, and systolic and diastolic blood pressure. Clinicians should be alert to the presence of vitamin D deficiency and hyperparathyroidism in obese patients, as these are more common in this population. In addition, clinicians should be aware that obese patients with vitamin D deficiency and hyperparathyroidism may be at increased cardiometabolic risk due to the association of vitamin D deficiency and hyperparathyroidism with MS parameters.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erzincan Binali Yıldırım University Clinical Researches Ethics Committee (Date: 11.05.2023, Decision No: 2023-10/2).

Informed consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The effects of behavioral therapy given to men with premature ejaculation on symptoms and their partners' sexual functioning and sexual quality of life

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ABSTRACT

Aims: This research aimed to evaluate the efficacy of behavioral therapy administered to men diagnosed with premature ejaculation (PE) and its consequent effects on their partners' sexual function and overall sexual quality of life.

Methods: Using a quasi-experimental pre-test post-test study design, men diagnosed with premature ejaculation and their partners from a urology outpatient clinic underwent behavioral therapy. The therapy incorporated the "stop-start technique" over six bi-weekly sessions, each lasting 45 minutes. Post-therapy evaluations were conducted after the sixth session.

Results: Post-treatment results showed a significant decrease in men's PEDT scores from 15.53±2.09 to 7.65±3.05 (p=0.012). Concurrently, their partners experienced an increase in FSFI scores from 13.90±11.1 to 21.70±7.86 (p=0.001) and SQOL-F scores from 37.82±8.50 to 84.01±9.68 (p=0.001). Significant improvements were also recorded in the FSFI subscales for desire (2.2±1.7 to 4.2±1.14), arousal (2.3±3.7 to 4.6±3.04), lubrication (2.5±2.7 to 3.7±1.7), orgasm (2.6±2.0 to 3.5±1.3), and satisfaction (2.0±2.2 to 4.4±1.8) for the female partners post-treatment, all with $p<0.05$. A notable decrease was observed in the pain subscale (2.3±2 to 1.3±0.9, $p<0.05$).

Conclusion: Behavioral therapy directed towards men with PE not only significantly alleviates their condition but also enhances their partners' sexual functionality and quality of life, emphasizing the therapy's comprehensive advantages.

Keywords: Premature ejaculation, behavioral therapy, PEDT, FSFI, SQOL-F, sexual function

INTRODUCTION

Premature ejaculation (PE) is a significant sexual dysfunction that impacts many men, as highlighted by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5).[1] While several definitions exist, the DSM-5 characterizes PE as an ejaculation pattern that consistently occurs within one minute of vaginal penetration, often against the individual's desires.[2] Two primary subtypes of this condition have been identified by the International Society for Sexual Medicine (ISSM) in 2014: Lifelong premature ejaculation (L-PE) and acquired premature ejaculation (A-PE). The former always or almost always results in ejaculation within one minute of vaginal penetration, coupled with emotional distress and frustration, leading to avoidance of sexual activities.[3]

Numerous studies have documented the psychological toll of PE, which can manifest as diminished self-worth, performance anxiety, and interpersonal tension.[4-6] Partners of those with PE may also face sexual difficulties, including issues with lubrication, achieving orgasm, and overall sexual satisfaction. Current treatments encompass a range of systemic drugs like SSRIs, tricyclic antidepressants, and PDE5 inhibitors, as well as local anesthetic applications.[7] Concurrently, psychological and behavioral therapeutic strategies have shown promise in particular, showcasing efficacy in addressing various sexual dysfunctions in both genders.[2]

Despite the body of research on the influence of behavioral therapy on men with PE, there is a gap concerning its effects on their partners' sexual function and overall quality of sexual life. This study aims to bridge that gap, exploring whether behavioral therapy directed towards men with PE can simultaneously alleviate their symptoms and enhance the sexual experiences of their partners.

METHODS

The study was carried out with the permission of İstinye University Human Researches Ethics Committee (Date: 29.06.2021, Decision No: 21-60). All procedures were

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carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

This study was conducted as a quasi-experimental pre-test post-test study to determine the effects of behavioral therapy given to men with premature ejaculation on the symptoms and their partners' sexual functions and sexual quality of life. The study was conducted on the men and their partners who applied to the urology outpatient clinic of the Hospital.

Patient Selection

The study sample consisted of men and their partners diagnosed with premature ejaculation who applied to the Hospital's urology outpatient clinic. The sample size was calculated using the G Power program. The expected confidence intervals were determined, and the confidence interval was calculated as 84 patients with α=0.05, test power (1-β) 0.95, and effect size d=1.14. The study reached 91 men and their partners, but 7 participants were excluded because they refused to participate (Figure 1). Since all the men included in the study were married, and the treatment with the couple would increase success, all the spouses were invited to the study.

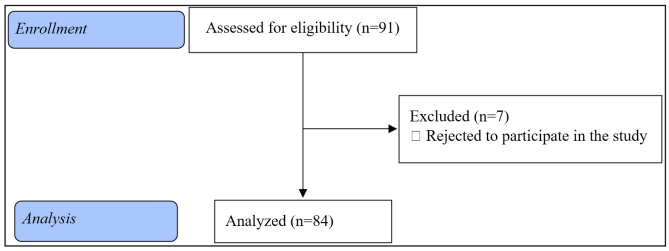

Figure 1. Workflow Diagram of the Men and their Partners Participating in the study

Study Procedures

The researchers interviewed the men and their partners who applied to the urology outpatient clinic and were diagnosed with premature ejaculation according to the DSM-5 criteria; they explained the purpose of the study and obtained informed consent forms from those who agreed to participate in the study. Pre-tests were applied to the men (Personal Information Form and PEDT) and their spouses (Personal Information Form, FSFI, and SQOL-F) just before the behavioral treatment was applied to the men in the treatment group. In the first interview with the men, the researchers delivered information about behavioral therapy and set therapy days and hours. There was no erectile dysfunction (ED) observed in the men participating in the study, and no ED developed in any male during the treatment.

The diagnosis of premature ejaculation was made by the researcher-author who is a urologist. In the study, for some patients, the duration was below 10 minutes in the first one or two days of the two-week period. However, since it exceeded 10 minutes in the following days, it was considered successful. No erectile dysfunction was observed in any male during the sessions. Not all men ejaculated once a day that ejaculating once a day could prolong intravaginal duration. Repeating every day is an effective and necessary act for learning a new behavior. It was emphasized that they should definitely use lubricants during masturbation, and they were educated on how to masturbate correctly to avoid traumatic masturbation before the first session. The men attended the sessions with their wives, and in addition to the behavioral therapy given to them, their wives received sexual education, including the anatomy and physiology of the female reproductive system and the orgasm cycle. No precautions were taken for the spouses of the men before the treatment, and there was no change in the level of contact between the therapist and the woman during the treatment.

According to the DSM-5 diagnostic criteria, sexual dysfunction and other sexual health problems in women were screened by the physician 8. Structured interviews, consisting of 6 sessions, were held once every two weeks for men with premature ejaculation problems. Behavioral therapy took place once every two weeks for six 45-minute sessions. The "stop-start technique" was the therapy used. Behavioral therapy interviews were conducted at the urology outpatient clinic of the hospital. Post-tests were administered to men with premature ejaculation (PEDT) and their spouses (FSFI and SQOL-F) immediately after the sixth session. Behavioral treatment content was applied with a structured interview technique, and no other questions were asked of the participants apart from the data collection tools. The workflow diagram of the research shows the interventions (Figure 2). Participants eligible for this study had to meet several criteria: they had to express a willingness to participate and be between the ages of 18 and 45, with literacy skills and no communication barriers. While they shouldn't have any personal history of sexual dysfunctions or medical conditions, such as psychiatric illnesses, pelvic anatomical disorders, or post-menopausal symptoms, that might impact sexual function, they needed to be married to someone diagnosed with lifelong premature ejaculation as per DSM-5 and ISSM.

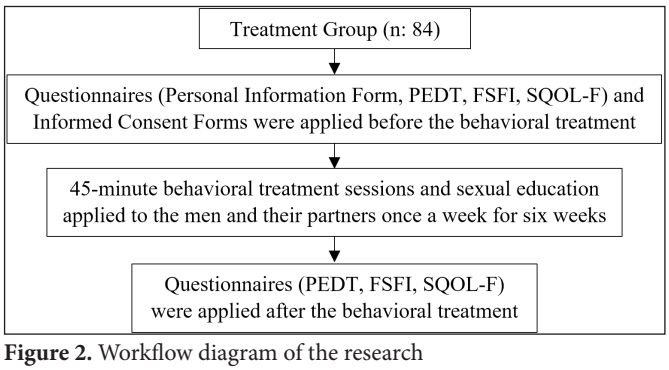

Figure 2. Workflow diagram of the research

Measures of the Structured Interviews' Guide

The first session: Sexual intercourse was prohibited. The stop-start technique was explained with videos and visuals. The patients were asked to perform masturbation exercises without sexual fantasy using the stop-start technique once a day for two weeks. In this technique, the penis was stimulated until the onset of the feeling of ejaculation, then the stimulation was stopped and then resumed. This cycle was repeated five times, and the patient was permitted to ejaculate the sixth time. A stopwatch measured elapsed time from the start to the point of ejaculation, with an ideal target time of 10-15 minutes. Corty and Guardiani (2008) studied normal and abnormal ejaculation times and how long vaginal intercourse should last. Accordingly, the interquartile range for the sex therapists' opinions regarding an "adequate" length for ejaculatory latency was from 3 to 7 minutes; "desirable" from 7 to 13 minutes; "too short" from 1 to 2 minutes; "too long" from 10 to 30 minutes. If the duration of the masturbation exercises remained under 10 min, the men repeated the first stage exercises for two weeks.

The second session: When the duration of the masturbation exercises reached over 10 minutes, the second stage began. In this stage, the men masturbated as before but now with sexual fantasies or watching pornographic movies. They did this once a day for two weeks. If the duration of the masturbation exercises remained under 10min, the men repeated the second-stage exercises for 2 weeks.

The third session: When the duration of the masturbation exercises reached over 10 minutes, the third stage began. In the third stage, the men's partners performed masturbation on them. This continued once a day for two weeks. If the duration of the masturbation exercises remained under 10 minutes, the men repeated the third-stage exercises for two weeks.

The fourth session: If the duration of the masturbation exercises was over 10 minutes, the fourth stage was started. In the fourth stage, the couple performed sexual intercourse using the woman on top rather than; in other words, the man was beneath the woman. The couple stopped intercourse with the onset of the feeling of ejaculation for a total of five cycles; then, the man was permitted to ejaculate in the sixth cycle. If the duration of these sexual intercourse exercises remained under 10 min, the couples repeated the fourth stage exercises for 2 weeks.

The fifth session: If the duration of sexual intercourse exercises was longer than 10 minutes, the couples continued to the fifth stage. In the fifth stage, sexual intercourse was performed in the missionary position, with the woman lying on her back and the man on top. This was repeated once every 2 days/2 weeks. If the duration of these sexual intercourse exercises remained under 10 min, the couples repeated the fifth stage exercises for two weeks.

The sixth session: If the duration of sexual intercourse exercises was longer than 10 minutes, the couples continued to the sixth stage. In the sixth stage, sexual intercourse was performed in different positions. With the onset of the feeling of ejaculation, the position was changed. Five positions were adopted, and the men ejaculated voluntarily in the sixth position. The couple performed sexual intercourse once every two days for two weeks and then came to the clinic for a check-up.

Premature Ejaculation Diagnostic Tool (PEDT)

It was developed by Symonds et al.[9] to better define premature ejaculation for use in clinical studies; this is a 5-point Likert-type scale consisting of 5 items. The scale was adapted to Turkish by Serefoglu et al.[10] The highest score that can be obtained from the scale is 20, and the lowest score is 0. Scores higher than 11 are defined as "PE," scores of 9-10 are defined as "possible PE," and scores of eight or less are defined as "no PE."

Female Sexual Function Index (FSFI)

Developed by Rosen et al.[11], the female sexual function index (FSFI) is a multidimensional scale consisting of six sections and 19 items evaluating female sexual function. The scale contains six sub-dimensions: desire, arousal, lubrication (wetting), orgasm, satisfaction, and pain. The highest score that can be obtained from the scale is 36.0, and the lowest is 2.0. As the score obtained from the scale increases, sexual function improves. The simple mathematical algorithm calculation is organized to determine the scoring of the subscales and the entire scale. Factor loads were determined as 0.6 for desire, 0.3 for arousal and lubrication, and 0.4 for orgasm, satisfaction, and pain. In the analysis performed for the internal consistency of the entire scale in this study, the Cronbach's alpha reliability coefficient was found to be 0.91 for the pre-test and 0.93 for the post-test. The pre-test Cronbach's alpha reliability coefficients for desire, arousal, lubrication, orgasm, satisfaction, and pain subscales were 0.89, 0.90, 0.87, 0.91, 0.88, and 0.94, and the post-test Cronbach's alpha reliability coefficients were 0.90, 0.91, 0.89, 0.95, 0.91, and 0.94, respectively.

Sexual Quality of Life-Female (SQOL-F)

It was developed by Symonds et al.[12] in 2005; the sexual quality of life-female (SQOL-F) is a six-point Likert-type questionnaire consisting of 18 items to evaluate women's sexual quality of life. Each item addresses sexual life over the preceding four weeks. The questionnaire uses

a 1-6 point system (1—agree, 2—strongly agree, 3—somewhat disagree, 5—strongly disagree, 6—totally disagree), and the range of points that can be obtained is between 18 and 108. As the score obtained from the questionnaire increases, the quality of sexual life increases. Before the total score is calculated, the scores of items 1, 5, 9, 13, and 18 are reversed. The total score obtained from the questionnaire is converted to 100 using the following formula: (taken raw score from the questionnaire-18)×100÷90.

Analytic Plan

The researcher coded and transferred the research data, then analyzed it using SPSS 26 version for Windows. In the present study, the application of behavioral therapy was the independent variable, and the "PEDT," "FSFI," and "SQOL-F" scores were the dependent variables. The Shapiro-Wilk test determined whether the scores of the men and their partners from the scales showed a normal distribution. One-factor ANOVA for dependent samples was used to determine the effect of behavioral treatment on PEDT, FSFI, and SQOL-F. Effect sizes were calculated with partial Eta squared (ηp2), and if they were calculated between .00 and .30, they were considered very low; if they were between .30 and .50, they were considered low; If they were between .50 and .70, they were considered moderate, if they were between .70 and .90, they were considered high; if they were between .90 and 1.00, they were considered very high. Paired Sample t-test was used for the pre-test and post-test comparison of the subscales of FSFI in the dependent group. The significance level was taken as $p<0.05$.

RESULTS

The mean age of the men participating in the study was 29.15±5.55, and the mean age of the women was 27.2±3.8. 53.5% of men and 41.7% of women were high school graduates, 94% of men and 58.3% of women were employed, 95.2% of couples had nuclear families, and 33.3% had been married for 2-5 years. 54.8% were diagnosed with premature ejaculation 1-5 years ago (Table 1).

To determine the effect of behavioral therapy given to men with premature ejaculation on PEDT, FSFI, and SQOL-F, a single-factor ANOVA test was performed to compare the pre-test and post-test results. Before behavioral treatment, men's pre-test PEDT mean score was 15.53±2.09, women's pre-test FSFI mean score was 13.90±11.1, pre-test SQOL-F mean score was 37.82±8.50; after behavioral therapy, men's post-test PEDT mean score was 7.65±3.05, women's post-test FSFI mean score was 21.70±7.86, and post-test SQOL-F mean score was 84.01±9.68. Accordingly, it was determined that the PEDT mean score of men decreased significantly in the post-test compared to the pre-test and had a moderate effect [F=14.15, p=0.012, ηp2=0.56]. It was determined that the FSFI mean score of women increased significantly in the post-test compared to the pre-test and had a moderate effect [F=32.824, p=0.001, ηp2=0.63]. It was determined that the SQOL-F mean score of women increased significantly in the post-test compared to the pre-test and had a moderate effect [F=11.302, p=0.001, ηp2=0.68] (Table 2).

Table 1. Sociodemographic characteristics of men and their partners

Characteristics	Men (n:84)	Women (n:84)
	n (%)	n (%)
Age		
18-25	23 (27.3)	33 (39.3)
26-35	36 (42.8)	30 (35.7)
36-45	25 (29.9)	21 (25.0)
Education Level		
Primary School	5 (5.9)	7 (8.3)
Secondary School	9 (10.7)	12 (14.3)
High school	45 (53.5)	35 (41.7)
University or Higher	25 (29.9)	30 (35.7)
Employment		
Employed	79 (94.0)	49 (58.3)
Unemployed	2 (2.3)	31 (36.9)
Student	3 (3.5)	4(4.8)
Family Type		
Nuclear family	80 (95.2)	80 (95.2)
Extended family	4 (4.8)	4 (4.8)
Marriage Duration		
0-1 year	20 (23.8)	20 (23.8)
2-5 years	28 (33.3)	28 (33.3)
6-10 years	21 (25.0)	21 (25.0)
11-15 years	15 (17.9)	15 (17.9)
The time when was diagnosed with premature ejaculation		
0-1 year ago	20 (23.8)	-
1-5 years ago	46 (54.8)	-
more than 5 years ago	18 (21.4)	-

Table 2. Means, SDs, effect sizes, and F-values for the PEDT, FSFI, and SQOL-F total mean scores

Scales	Group	Pre-test	Post-test	Effect size		
		M (SD)	M (SD)	F*	P	ηp2
PEDT	Treatment	15.03±2.07	7.55±3.04	14.15	.012	.56
FSFI	Treatment	13.90±11.1	21.70±7.86	32.824	.001**	.63
SQOL-F	Treatment	37.82±8.50	84.01±9.68	11.302	.001**	.68

*One-factor ANOVA test applied. **p<0.05

Women's pre-test mean score from the FSFI's subscale of desire was 2.2±1.7; the post-test mean score was 4.2±1.14; women's pre-test mean score from the FSFI's subscale of arousal was 2.3±3.7, the post-test mean score was 4.6±3.04; women's pre-test mean score from the FSFI's subscale of lubrication was 2.5 ±2.7, the post-test mean score was 3.7±1.7; women's pre-test mean score from the

FSFI's subscale of orgasm was 2.6±2.0, the post-test mean score was 3.5±1.3; women's pre-test mean score from the FSFI's subscale of satisfaction was 2.0±2.2, the post-test mean score was 4.4±1.8; women's pre-test mean score from the FSFI's subscale of pain was 2.3±2.0, the post-test mean score was 1.3±0.9. When the women's mean scores were examined in the FSFI's subscales of desire, arousal, lubrication, orgasm, and satisfaction, significant increases were found in the post-test compared to the pre-test ($p<0.05$). A significant decrease was found in the mean score in the subscale of pain in the post-test compared to the pre-test ($p<0.05$) (**Table 3**).

Table 3. Comparison of the total mean scores of the FSFI's subscales taken from the pre-test and post-test (n=84)

FSFI	Pre-test	Post-test	Test value and significance	
	X±Sd	X±Sd	t*	p
Desire	2.2±1.7	4.2±1.14	-23.955	0.001**
Arousal	2.3±3.7	4.6±3.04	-26.499	0.001**
Lubrication	2.5±2.7	3.7±1.7	-15.543	0.001**
Orgasm	2.6±2.0	3.5±1.3	-10.880	0.001**
Satisfaction	2.0±2.2	4.4±1.8	-39.039	0.001**
Pain	2.3±2.0	1.3±0.9	9.380	0.001**

* Paired sample t-test applied. **p<0.05

DISCUSSION

The study emphatically highlights the therapeutic potential of behavioral therapy in addressing PE among men. Beyond providing symptomatic alleviation for the afflicted men, the intervention concurrently amplifies the sexual function and overall quality of life for their partners. The observed enhancements across diverse facets of the female partners' sexual experiences, encompassing aspects from arousal to satisfaction, testify to the holistic advantages conferred by this therapeutic approach. Such compelling results necessitate a more intricate exploration of the inherent mechanisms and the wider ramifications of these interventions, especially in the context of PE's broader impact on conjugal relationships.

Couples enrolled in this study had been married for 2-5 years on average, and most men had been diagnosed with premature ejaculation 1-5 years before the study began. We observed that behavioral therapy decreased the PEDT scores compared to the pre-application. Some patients and their partners may have unrealistic preconceived notions about improving premature ejaculation symptoms and the success of therapy. At this point, it is essential to understand people's expectations before starting behavioral therapy and start the appropriate treatment in this direction.[13] Treatment should aim to increase the time spent in the vagina, control ejaculation, increase sexual satisfaction, and reduce stress.[14] The reason why behavioral therapy treats the symptoms of premature ejaculation may be that the increase in the time spent in the vagina increases the self-confidence and sexual satisfaction of the individuals, as well as the fact that the control over the ejaculation reflex has been learned better over time and that people have been relieved of psychological stress. Besides, the involvement of the partner in the therapeutic process is instrumental, as the establishment of greater involvement in the sexual relationship provides further stimulus for the patient in the recovery of their self-esteem, virility, and sense of adequacy of their sexuality.[12,15] When the studies in the literature are examined, there are studies on the effect of behavioral therapy on the PEDT score. In the study of Pavone et al.[16] the PEDT score decreased after the application. Mantovani et al.[17] found that the PEDT score decreased in the post-test compared to the pre-test. The results of the studies in the literature are similar to the results of this study. Studies presented that while these methods achieved impressive initial success, long-term follow-ups demonstrated significant relapses. In McCarty's study,[18] it was stated that re-sessions should be made at 6-month periods to prevent the relapses of behavioral treatments in premature ejaculation. In this study, relapse rates were not known because behavioral therapy was influential in the short term, but long-term follow-ups of the patients were not performed.

The behavioral therapy the study gave to men with premature ejaculation significantly increased their partners' post-treatment mean sexual function scores compared to pre-treatment scores.[19] The therapy may have improved the sexual functioning of the partners by prolonging the time the penis was inside the vagina and the duration of sexual intercourse, educating men about sex and the female orgasm, and improving the quality of sexual relations.[20] It has been presented in many studies that a period of at least 1 minute, which is taken as a criterion for premature ejaculation, is required for a woman to have an orgasm and that sexual intercourse times of 1 minute or longer are needed.[15,21] For this reason, it can be said that as the penis's duration of stay in the vagina increases, the sexual function and quality of the sexual life of the woman increases.[22] There is little premarital sex education or sexual experience in women raised in male-dominated cultures, so little sexual education significantly impacts female sexual functioning. In addition to the behavioral treatment given to the men, sex education was also given to their wives.[19] Sex education is when individuals acquire the necessary information and knowledge about sexuality and form their attitudes, beliefs, and values. This process contributes to interpersonal relationships, body image, and gender roles and maintains the mental health of individuals in the community. Lack of information or

misinformation about sexuality increases the risk of developing sexual dysfunctions.[23] Although many studies exist in the literature examining the effects of behavioral therapy on men with premature ejaculation, no study exists investigating the effects of this treatment on the sexual functioning of these patients' partners.[23-25]

For this reason, the results were discussed through studies examining the effect of behavioral therapy given only to women on women's sexual functions.[26,27] Mirzaae et al.[25] applied a behavioral therapy course for one and a half hours twice a week over a total of 8 sessions, and they observed that women's FSFI total mean score increased. Similarly, Omidi et al.[19] determined that cognitive behavioral therapy applied once a week for eight weeks improved women's sexual functioning. Additionally, Smith et al.[28] determined that psychoeducation given to 33 women between the ages of 28 and 70 increased the FSFI mean score. The studies in the literature support the current study.

Except for the FSFI mean score, our study observed increased total mean scores in the post-test compared to the pre-test in the subscales of desire, arousal, lubrication, orgasm, and satisfaction; a lower mean score was observed in the post-test compared to the pre-test in the subscale of pain.[29] Significant reductions in pain may be related to sexual intercourse with a partially or non-erect penis before the therapy and the insertion of a fully erect penis into the vagina after the therapy. These changes show that the behavioral therapy used to treat men with premature ejaculation benefits partners, reducing their reluctance to engage in sexual intercourse, increasing their sexual arousal and lubrication, enabling them to orgasm, and increasing their overall sexual satisfaction. A subsequent study conducted by Jalilian et al.[22] determined that sexual skills training, which was provided for women between the ages of 22 and 36 three times a week, enhanced sexual functioning in the subscales of sexual desire, arousal, lubrication, orgasm, and satisfaction. However, it had no effect in the subscale of pain.

The present study observed that behavioral therapy given to men with premature ejaculation significantly increased the post-treatment total mean score of the partners' sexual quality of life compared to pre-treatment. While women's sexual lives are affected by general living conditions, any changes/problems in their own or their spouse's sexual health and functioning affect their sexual quality of life. The behavioral approach addresses sexual education, sexual skills/techniques, relationship dynamics, power and control issues, and emotional and sexual intimacy. Alleviating premature ejaculation enhances interpersonal communication and satisfaction, positively affecting women's sexual quality of life. It can be argued that behavioral therapy and sex education improve couples' emotional bonds, relational intimacy, and quality of sexual life by learning to control unpleasant thoughts. Patrick et al.[30] found that almost half the partners of men with premature ejaculation reported that they had sexual difficulties, and Burri et al.[31] determined that the partners of men with premature ejaculation experienced high levels of sexual distress.[5,32] Before the present research, no study has examined how behavioral therapy given to men with premature ejaculation affects their partners' sexual functioning and sexual quality of life. The present study distinguishes from other studies because we conducted it for the first time in these terms, and we think this study would contribute to the clinical applications.

The study's strengths include its use of an up-to-date approach in treating premature ejaculation, pre-test/post-test measurements, and quasi-experimental design. Additionally, the dyadic approach was utilized in the treatment of PE. The sample was taken from only one center and was quantitatively small; the female partners of men with PE did not have sexual intercourse due to PE and had an artificially low sexual functioning score before treatment, the patients could not be followed up for a year or longer, and the absence of a control group can be considered as the limitations of the study. The relapse rate of premature ejaculation is not known because a 1-year follow-up could not be performed.

CONCLUSION

Premature ejaculation involves not only sexual problems but also relationship and communication difficulties, and behavioral approaches to treating premature ejaculation are promising. Couples' education is practical and easy to implement, and such measures as sexual education in behavioral treatment are expected to impact patients positively. Therefore, it is recommended to expand the application of behavioral therapy in treating premature ejaculation.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstinye University Human Researches Ethics Committee (Date: 29.06.2021, Decision No: 21-60).

Informed consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Assessment of the relationship between obstructive sleep apnea syndrome and sleep quality among dental students

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ABSTRACT

Aims: Dental students are under the risk of developing sleep disorders due to intensive curriculum and long study hours. Hence, we aimed to assess the prevalance of obstuctive sleep apnea syndrome and its relation with sleep quality among Turkish dental students.

Methods: A cross-sectional study was conducted among 314 dental students, who completed Epworth Sleepiness Scale (ESS) to identify the sleep quality (SQ) and excessive daytime sleepiness (EDS) and Berlin Questionnaire (BQ) for Obstructive Sleep Apnea Syndrome (OSAS). Demographic variables were also recorded. SPSS Version 23 (IBM Corporation, Armonk, NY, USA) was used for istatistical analysis. The statistical significance level was accepted as $p<0.05$.

Results: OSAS risk was found to be 16.8% in Turkish dental students. Higher hypertension, obesity and body mass index (BMI) values were found to be related with higher risk for sleep apnea. There was a statistically significant positive correlation between ESS and BQ assessments ($r=0.334$, $p<0.05$). Normal daytime sleepiness was observed in 46.6% of dental students.

Conclusion: OSAS risk was found to be high in dental students who will be the primary step in the diagnosis and treatment of sleep apnea. As the OSAS risk increases, sleep quality decreases. It should be emphasized that sleep and lifestyle habits may increase the risk of OSAS in dental students.

Keywords: Sleep disorders, obstructive sleep apnea syndrome, sleep quality, excessive daytime sleepiness, dental students

INTRODUCTION

Sleep, which is an important component in the growth and development of the body and in the formation of the circadian rhythm, is an indispensable part of our life. It is also known to be important for the maturation of cognitive functions and their optimal functioning. Poor sleep quality can cause a decrease in the individual's occupational performance at work or school,[1,2] impaired social functionality,[3] work accidents,[4] and poor physical health.[5] The negative impact that occurs in healthcare specialists such as physicians, nurses, medical and dental students, does not only affect the individual, but also affects the safety of patients.[6,7] New social and academic environment, unhealthy lifestyle, excessive use of social media cause sleep problems in undergraduate students.[8] In addition to these factors, dental students who are under high pressure, usually do not have good quality of life due to their high academic load, clinic practice responsibilities and long hours of night study. Thus, they face to lots of stress that causes poor sleep quality. Dental students are considered a population that is prone to sleep disorders.

Obstructive sleep apnea syndrome (OSAS) is a widespread disease that increases with age and is seen in approximately 2-4% of the adult population, characterized by recurrent apnea or hypopnea during sleep, episodes of upper airway obstruction, and decreased blood oxygen saturation. Although the diagnosis of the disease is only confirmed as a result of polysomnographic (PSG) evaluation, various screening tests in the form of surveys are also used to evaluate the individuals at risk.[9] It is thought that 80-90% of people with symptoms of the disease continue their lives without being diagnosed.[10] The Berlin Questionnaire (BQ) is the first test used to identify risk groups for OSAS.[11] Translation of BQ to Turkish[12] have been made and validated. Although there are various studies in the literature investigating sleep disorders in the healthcare professionals and medical students, there are few studies about sleep disorders among the dental students. Hence, in our study, we aimed to determine the prevalence of OSAS in Turkish dental students.

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METHODS

The study was carried out with the permission of İstinye University Human Researches Ethics Committee (Date: 21.07.2022, Decision No: 22-117). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The current cross-sectional study was carried out on 313 volunteer dental students (male: 118, female: 195) in İstanbul Beykent University School of Dentistry from August 2022 to November 2022. They provided written consent and participated on a voluntary and anonymous basis. All the participants completed a self-administered questionnaire. Sociodemographic characteristics (i.e. sex, age, height, weight, BMI) of the dental students were collected.

The BQ is a commonly used screening tool for OSAS and has been validated in primary care settings and other specific patient populations.[13,14] BQ contains 10 questions in three categories: The first category asks refers to snoring and witnessed apneas, the second category asks about sleepiness and fatigue and the last category asks about existence of hypertension and also body mass index (BMI) of >30 kg/m². Categories are evaluated separately from each other, 2 or more positive categories are considered as high risk of OSAS according to the Berlin questionnaire.

The Epsworth sleepiness scale (ESS) is a standard and validated questionnaire which is used to evaluate subjective excessive daytime sleepiness (EDS).[15] ESS consists of 8 questions for which the study participants are asked to rate on a 4-point scale from 0-3 (0=would never doze, 1=slight chance of dozing, 2=moderate chance of dozing, and 3=high chance of dozing), their chance of dozing off or falling asleep in 8 different conditions, or daily activities. According to this scale which range from 0-24, people who score above 10 are considered to be at high risk for excessive daytime sleepiness.

Statistical Analysis

Within the scope of the study, SPSS Version 23 (IBM Corporation, Armonk, NY, USA) was used for analysis. Mean and standard deviation values were used for open-ended data. One-way Anova and independent sample t-tests were used in the assessment based on gender and age. Correlation analysis was used to compare the participants' overall ESS scores and BQ scores. The statistical significance level was accepted as $p<0.05$. The sample size was calculated at a 95% confidence level using the G*Power programme (version 3.1.9.7; Heinrich Heine University, Dusseldorf, Germany). Based on the previous study comparing within the groups, considering an α of 0.05, a standardized effect size of 0.25, and a theoretical power of 95%, the minimum size for each group was estimated to be 57 and 285 in total.[16]

RESULTS

The characteristics of the study population was shown in Table 1. The total number of participants was 313 dental students, aged 18-40 years old (21.81±2.52), 62.3% of the participants were female and 37.7% of them were male. When Body Mass Indexes (BMI) were calculated, it was found that the mean was 22.27±3.60, the lowest BMI value was 15.70, and the highest BMI value was 36.7. 14.4% of the participants had a family history of OSAS.

Table 1. Main findings regarding age, height, weight and body mass index of participants

	N	Min	Max	Mean	SD
Age	313	18	40	21.81	2.52
Lenght	313	152	194	171.46	8.93
Weight	313	41	108	65.99	14.55
Body mass index	313	15.70	36.70	22.27	3.60

N: Number, Min: Minimum, Max: Maximum, SD: Standard deviation

As seen in Table 2, the presence of snoring was 23% (male: 36.4%, female: 14.4%) , frequency of snoring was 6.7% (male: 15.3%, female: 1.5%), snoring bothers other people was 3.8% (male: 9.3%, female: 0.5%) and it was determined that there was a statistically significant difference based on gender in terms of the presence and frequency of snoring and snoring bothers other people ($p<0.05$). The snoring severity was found 2.9% and respiratory arrest during sleep was 0.3% ($p>0.05$). While the general rate of participants who had a high frequency of feeling sluggish and tired upon waking from sleep is 39.9%, this rate is 35.6% for male participants and 42.6% for female participants, was found to be absent ($p>0.05$). However, while the general rate of participants who had a high frequency of feeling tired during the daytime was 42.2% (male: 33.9%, female: 47.2%, $p<0.05$). This rate was higher for female participants. There was a statistically important difference in term of fallen asleep while driving, the rate was found 16.1% for males, 4.6% for females ($p<0.05$). There was no significant gender-based finding in terms of feeling excessively sleepy while driving (male: 3.4%, female: 3.1%, $p<0.05$). Table 2 shows that male participants were more prone to hypertension and obesity than female ones (male: 6.8%, female: 1, $p<0.05$). The risk of OSAS was 27% in men and 10.8% in women. It was found to be 16.8% in the general population. When the Berlin risk assessment levels of the participants were compared according to the presence of hypertension or obesity, a statistically significant difference was found ($p<0.05$). The proportion of individuals in the high-risk group was higher in individuals with hypertension or obesity. Table 3 shows that there was a statistically significant difference between the OSAS risk in terms of BMI levels of the participants ($p<0.05$). The average BMI of the participants evaluated in the high-risk group was higher (Table 3).

Table 2. Comparison of Berlin Questionnaire Results by Gender

	Total (n=313)	Male (n=118)	Female (n=195)	p
Category 1				
Presence of snoring	72 (23%)	43 (36.4%)	29 (14.4%)	0.001
Snoring severity	9 (2.9%)	5 (4.2%)	4 (2.1%)	0.217
Frequency of snoring	21 (6.7%)	18 (15.3%)	3 (1.5%)	0.001
Snoring bothers other people	12 (3.8%)	11 (9.3%)	1 (0.5%)	0.001
Quit breathing during sleep	1 (0.3%)	0 (0.0%)	1 (0.5%)	0.623
Category 2				
Feel fatigue after waking up	125 (39.9%)	42 (35.6%)	83 (42.6%)	0.135
Daytime fatigue	132 (42.2%)	40 (33.9%)	92 (47.2%)	0.014
Fall asleep during driving	28 (8.9%)	19 (16.1%)	9 (4.6%)	0.001
Excessive sleepiness while driving	10 (3.2%)	4 (3.4%)	6 (3.1%)	0.560
Category 3				
Hypertension or obesity	10 (3.2%)	8 (6.8%)	2 (1.0%)	0.001

Table 3. Evaluation of Berlin Risk Categories According to Participants' BMI Levels

	N	Mean	SD	p
Low risk	258	21.78	3.23	0.001
High risk	52	24.81	4.29	

N: Number; SD: Standart deviation, BMI: Body mass index

According to the results of the BQ, Table 4 shows that 46.6% of low-risk participants and 19.2% of high-risk participants were in the normal ESS group, while 3.9% of low-risk participants had increased severe daytime sleepiness. In the high-risk group, 13.5% of the participants were in the increased severe daytime sleepiness group. Participants with high-risk status were more likely to experience increased moderate to severe daytime sleepiness by the ESS assessment (Table 4).

Table 4. Comparison of ESS and BQ results

	BQ		Total	p
	Low risk	High risk		
ESS				0.002
Normal daytime sleepiness	115 (46.6%)	10 (19.2%)	125 (40.3%)	
Normal but increased daytime sleepiness	90 (34.9%)	24 (46.2%)	114 (36.8%)	
Mild daytime sleepiness	28 (10.9%)	9 (17.3%)	37 (11.9%)	
Moderate daytime sleepiness	15 (5.8%)	2 (3.8%)	17 (5.5%)	
Excessive daytime sleepiness	10 (3.9%)	7 (13.5%)	17 (5.5%)	
Total	258 (100.0%)	52 (100.0%)	310 (100.0%)	

ESS: Epworth sleepiness scale, BQ: Berlin questionnaire

When the categories according to the general ESS evaluations of the participants and the risk levels according to the answers given to the BQ were compared; it was determined that there was a statistically significant low and strong positive correlation between ESS and Berlin assessments according to categories (r=0.334, p<0.05) (Table 5). There was no statistically significant relationship between ESS and Berlin questionnaire category 1 evaluation (r=0.081, p>0.05). There was a statistically significant low-level strong positive correlation between ESS and Berlin questionnaire category 2 evaluation (r=0.334, p<0.05). There was no statistically significant relationship between ESS and Berlin questionnaire category 3 evaluation (r=-0.042, p>0.05). However, there was a statistically significant low and strong positive correlation between ESS and BQ assessments (r=0.334, p<0.05) (Table 5).

Table 5. Evaluation of the Relationship between ESS and BQ Categories

	ESS	BQ Category 1	BQ Category 2	BQ Category 3	BQ Total
ESS	7.30±4.28	0.081	.395**	-0.042	.334**
BQ Category 1	0.37±0.74		0.07	.228**	.654**
BQ Category 2	0.94±0.97			0.049	.788**
BQ Category 3	0.03±0.18				.302**
BQ Total	1.34±1.30				

ESS: Epworth sleepiness scale, BQ: Berlin Questionnaire, **p<0.001, In the 1st category (questions 1 to 5) and 2nd category (questions 6 to 8), the patients considered "at risk" if they have answered positively to at least two questions. In the third category, the patient must have at least a positive response, or a BMI (body mass index) greater than 30. For a patient to be considered "high risk", they must have obtained a "positive" result in at least two categories of the Berlin questionnaire. "Low risk" subjects are those who will have at most one "positive" category.

DISCUSSION

An optimal sleep quality is an important part of healthy life.[2] When sleep quality deteriorates, it can cause different problems such as sleep disorders, psychiatric illnesses, etc. University students are a part of the group that is faced with stress due to getting used to a new order, being more responsible, being more free and having a heavy course load. Dental students also have to endure these pressures. However, dental students are bound to be under more stress due to their clinical responsibilities and practical lessons. Students should be taught methods of having optimal sleep and coping with stress. Various studies in the literature have investigated sleep quality in university students has been elaborated.[17-19] In the previous studies, the prevalence of sleep disorders were evaluated in university students.[16,20] However, few studies investigated the prevalence and risk factors of obstructive

apnea syndrome in dental students.[21,22] Therefore, in our study, our purpose was evaluating the prevalence of obstructive sleep apnea syndrome, which is one of the sleep disorders and can affect activities in daily life, and its relationship with sleep quality in Turkish dental students.

Since the polysomnography is the gold standard method in the OSAS diagnosis, we think that the value obtained in our study should be considered only as a risk assessment. In our study, 16.8% of people were found to be at high risk for the development of OSAS. In studies evaluating the prevalence of OSAS in the general population using the BQ, the risk of sleep apnea was reported to be 4.98% by Amra et al.[23] and 27.3% by Khazaei et al.[24] When studies of OSAS prevalence in healthcare workers using the BQ were examined, Geidr-Brown et al.[25] reported that 24% of nurses working 12-hour night shifts had a high risk of sleep apnea and Seyedmehdi et al.[26] found this risk to be 6.9% in health workers in their study. Similar to previous studies, Aydın Güçlü et al.[27] investigated the risk of OSAS in healthcare workers and reported that 15.2% of individuals had a high risk of sleep apnea. In the present study, the risk of OSAS was found to be 16.8%. However, OSAS risk in men was found to be statistically significantly higher than in women. Unlike our study results, Yavuz et al.[28] found no difference between gender and sleep apnea in medical students and 35.5% of students were found to be at risk for OSAS. Belingheri et al.[20] reported a huge prevalence of sleep disorders among medical and nursing students and an relationship between these symptoms and perceived stress. The difference in our results with the previous studies may be related to the difference in the scales used and diversity of the study population. However, there are also studies, argue that gender and OSAS risk are related in the literature.[27,28-30]

Studies have demonsrated that approximately 50% of OSAS patients have hypertension and approximately 30% of hypertensive patients have OSAS.[31,32] Obesity increases the risk of developing OSAS.[33] The risk of OSAS is 8-12 times higher in individuals with a BMI >29.[34] Similiar to Aydın Güçlü et al.[27] the present study also evaluated the association of sleep apnea with age, gender, BMI, hypertension, and obesity, and found that high sleep apnea risk was directly related to these factors. Similar to our study, dos Santos et al.[21] also examined OSAS in Brazilian dental students and found a relationship with BMI and OSAS.

If left untreated, OSAS can lead to impaired attention and concentration, decreased work performance, and academic failure. Aydin Güçlü et al.[27] in their study, 19.5% of healthcare professionals stated that they felt daytime fatigue and fatigue almost every day, while 14.1% of them fell asleep while driving. Similar to the previous study, Seyehmedhi et al.[26] reported that 22.6% of the participants felt tired almost every day during the day, while 15.4% felt excessive sleepiness and fell asleep while driving. However, in our study, 42.2% of the dental students stated that they felt tired almost every day, while the rate of those who fell asleep while driving was 10%. The fact that daytime fatigue is much higher than the previous studies, may be due to the fact that the dentistry curriculum is quite full of both clinical practice and theoretical course loads and the responsibility of the patients who have to be cared for during internships. It is important to identify the population at risk among healthcare workers. It is also very important to raise awareness of OSAS, especially since the dental students constitute the first step in diagnosis and treatment.

Sleep deprivation is a community health problem associated with obesity, morbidity, and mortality. The academic performance of dental students appears to be influenced by sleep.[35,36] In 2006, Curcio et al.[37] suggested that students' learning and academic performance were associated with sleep quality and the amount of sleep they received. Although relationship was found between the academic period of the student and sleep quality, gender and age were not linked to sleep quality or with excessive daytime sleepiness.[37] Ergin et al.[38] observed that, the best sleep quality was found in the Health Sciences students and the worst sleep quality was found in the dental students. Unlike to our study, the previous study results showed no statistically significant difference between gender and sleep quality.

Sleep disorders are a condition that can be triggered by people's lifestyles and habits, affecting the body and orofacial structures. For this reason, it is possible to detect respiratory disorders that occur during sleep as soon as possible and to inform individuals, and to prevent disease and improve health by clinical and/or surgical intervention. Problems such as sleep apnea can also cause sleep disturbance. Pasha et al.[22] evaluated sleep apnea risk factors in medical school students in Pakistan and stated that 27% of men and 12% of women had disturbing snoring. Similarly, in our study, 12% of dental students had the same discomfort. Also in our study, in the dental students at risk of OSAS, sleep quality also decreases and excessive daytime sleepiness increases. Hence we observed that OSAS and sleep quality were related according to our study results. Conditions such as lifestyle, attitudes towards events, academic load, social media and internet use of medical and dental students may be risk factors for sleep apnea. However, no study has been conducted to evaluate the prevalence and risk factors of sleep apnea in dental faculties in our country. In the literature, the prevalence of predictive factors for obstructive sleep apnea was investigated in dental and speech language hearing students in only one study.[21]

Our study has several limitations. The first one is that the study is a cross-sectional study. Only questionnaires were used to collect data in the study, no clinical examination was performed and imaging techniques were not used. The second limitation is the small number of the study population. The participation of all dental school students in Turkey may enable us to obtain more accurate results. Third, only two questionnaires were used in the study. In order to obtain clearer results, more scales from which we can obtain data such as stress, anxiety, life habits, etc., which may be risk factors for sleep apnea, should be used in future studies.

CONCLUSION

The risk of developing sleep apnea was found to be high in the dental students. Those with poor sleep quality are at greater risk. Since sleep disorders are also quite common among dental students, early diagnosis and treatment will reduce the risk of harm to students and patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstinye University Human Researches Ethics Committee (Date: 21.07.2022, Decision No: 22-117).

Informed consent: Written consent was obtained from the patient participating in the present study.

Referee Evaluation Process: Externally peer reviewed.

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

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Ultrasonic shear-wave elastography: a novel method for assessing the tumor grade in endometrial cancer: a prospective study

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ABSTRACT

Aims: To evaluate the diagnostic performance of the real time shear-wave elastography in patients with endometrial cancer in terms of tumor grade and myometrial invasion depth preoperatively.

Methods: In this prospective observational study, forty-eight women who were diagnosed with endometrioid type endometrium cancer in our gynecologic oncology clinic of a tertiary hospital between September 2020-January 2021 in Turkey. All patients underwent an ultrasonographic shear-wave measurements. Mean shear-wave values were measured from the tumor itself. Mean elasticity values were assessed in terms of tumor grade and myometrial invasion depth.

Results: The median [%25-%75] shearwave value of the participants was 29.45kPa (5.02-167.21). Shear-wave value for grade 3 endometrial cancer showed a statistically significant difference compared to grade 1 and 2 shear-wave values ($p<0.001$). To determine the myometrial invasion depth, lymph node involvement, lympho-vascular stromal invasion and cervical stromal invasion statues, shear-wave measurements did not show a significant result ($p>0.05$). ROC curve analysis showed significant results to determine the myometrial invasion depth and grade 3 endometrial cancer with the mean shear-wave cut-off values of 28.29 kPa and 57 kPa respectively ($p<0.001$).

Conclusion: Real-time shear-wave elastography is a promising tool to predict the grade 3 tumors and deep myometrial invasion in endometrial cancer patients.

Keywords: Endometrial cancer, Shear-wave elastography, shear-wave, tumor grade, grade 3 endometrial cancer

INTRODUCTION

Endometrial cancer (EC) is the most common gynecologic cancer of women in developed countries.[1] Although the majority of diagnosis are made in the postmenopausal period, nearly 15% of patients are diagnosed at premenopausal ages.[2] With the increase in life expectancy, endometrial cancer has become more frequent.[3] Because of this rising incidence, accurate diagnostic evaluation of patient is essential. Fortunately, EC has a favorable prognosis and early detection rates compared to other genital malignancies. Nevertheless some patients have worse prognosis due to many factors, such as; tumor grade, histological type, the depth of myometrial invasion, the tumor size, lympho-vascular invasion and lymph node status that can affect the course of the disease.[4]

Tumor grade is one of the important prognostic factors. As the tumor grade increases mortality and morbidity rates rise and extent of the surgery differentiates for the treatment. Endometrioid ECs are graded using the International Federation of Gynecology and Obstetrics (FIGO) classification system, which assesses the architectural pattern and nuclear grade; Grade 1-Less than 5 percent solid growth patterns Grade 2-6 to 50 percent solid growth patterns Grade 3-Greater than 50 percent solid growth

As seen in the histologic grading system, alterations in the growth pattern result in an increase in solid component of the cells, which may cause change in the tissue elasticity. Recently a novel method, elastography,

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has been used to assess the tissue stiffness quantitatively. Although it was firstly introduced in the late 1980s and early 1990s[5-8] clinical use of the application become more common in the recent years with the improvements in technology. Shear-wave ultrasonography (SWE) is a type of elastography which measures the tissue hardness without applying any external pressure to the tissue. In this method, transducer-generated acoustic force causes a displacement in the targeted tissue. As a result of this displacement shear waves arise from the tissue vertical to the acoustic force. Tissue-originated shear waves' velocity is then calculated quantitatively and converted to Young's modulus as Kilopascal (kPa).[9] The method's reproducible, and easy-to-learn nature makes it popular among the researchers. Several studies investigated the clinical use of SWE in the field of malignant-benign breast lesions, thyroid, superficial lymph nodes,[10-13] and rectum.[14] Also, cervix, myometrium and ectopic pregnancy were investigated by the gynecologist.[15,16]

However, to our knowledge, no study evaluated the SWE of EC in terms of histologic tumor grade, depth of myometrial invasion and lymph node status. Therefore, we aimed to investigate the relationship between SWE values and EC prognostic factors affecting the operation type, mortality and morbidity.

METHODS

The study was carried out with the permission of by Adana City Training and Research Hospital Clinical Researches Ethics Committee (Date: 26.08.2020, Decision No: 1043). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study population of this research, consisted of women who were diagnosed with endometroid EC in our gynecologic oncology clinic of a tertiary hospital between September 2020-January 2021. Preoperative endometrial sampling was performed to diagnose endometrial cancer, and final pathology confirmed our preoperative diagnosis. Total of 48 patients were enrolled for the study. All patients' SWE measurements were performed 24 hours before the surgery. Relationship between the final pathology and SWE values of the patients were analyzed. Inclusion criteria of the patient were: 1) having the pathological confirmation of endometrioid type EC, 2) a minimum 1cm. endometrial mass that could be seen on the ultrasound examination, 3) Body mass index (BMI) between 20-25 kg/m², 4) No other gynecologic benign and malign disease, 5) no other co-existing malignancy. Exclusion criteria were: 1) Patients with gynecologic benign or malign diseases such as, myoma uteri, adenomyosis or ovarian cancer etc. 2) patients with connective tissue disease, 3) tumor diameter 4 cm or greater 4) patients who were treated before the surgery. 5) BMI>25.

Ultrasound Examination

Examination of the participants were performed via a high-resolution ultrasound (US) device (Philips EPIQ 7), with a transabdominal 1-5 MHz convex probe (Philips Health Care, Bothell, WA, USA). Endometrial stiffness of the patient during the supine position was measured with ElastoPQ technique which is a point shear-wave (pSWE) elastography evaluation during the US examination, possible minimum compression was applied with the probe, which was maintained in a constant position. Elastography was performed with breath hold to minimize motion artifacts. After obtaining the conventional US images, target area was determined, and the measurements from region of interest (ROI) were taken. To achieve an optimum SWE measurement, grayscale mode was used. Our study's maximum ROI target distance was 8 cm, with a constant ROI box dimension of 1-0.5 cm. To evaluate the endometrial stiffness ten valid measurements were obtained from each patient's endometrial lesion and their average was calculated. If SWE measurement had low reliability due to operator (overpressure, not holding the probe fixed) or patient (cough or moving etc.) the measurement was ignored and to get a reliable measurement examination was repeated. The results were expressed as kPa. All the examinations were performed by a radiologist who is well-experienced in conventional US and SWE measurements. The specialist performs 500 SWE procedures annually with more than 5 years of experience. The average time to obtain a reliable measurement was about 25-30 minutes.

Statistical Analysis

IBM SPSS V.23 program was used for data analysis. The Shapiro-Wilk test was utilized to determine whether or not the continuous data followed a normal distribution. ANCOVA analysis was used to examine the effect of parameters on the mean shear-wave value by removing the effect of some variables. Roc Analysis was used to determine the cut-off value for the mean shear-wave value for the determination of grade 3 tumor and depth of myometrial Invasion. Analysis results were presented as frequency (percentage) for categorical variables and continuous variables were summarized as mean±standard deviation when it provided the assumption of normal distribution and as median [25%-75%] if it did not. A p-value of <0.05 was considered statistically significant.

RESULTS

A total of 48 patients were enrolled for this study. Table 1 summarizes the demographic features and tumor characteristics of the participants. The mean age (mean±SD) of the participants was 59.88±9.29 and the

median (%25-%75) tumor diameter of the patients was 2.75 cm. (1cm-3.7cm). Patients had a median (%25-%75) body mass index (BMI) of 22.66 kg/cm² (20.6-24.62) (Table 1). Main effect of tumor grade on mean shear-wave values was statistically significant (p<0.001). Shear-wave values of grade 1, 2 and 3 were 13.52 kPa, 33.24 kPa and 101.89 kPa respectively (Table 2). The shear-wave value for grade 3 EC showed a statistically significant difference compared to grade 1 and 2 shear-wave values (p<0.001) (Table 2). Main effect of myometrial invasion depth, lymph node involvement, lympho-vascular stromal invasion and cervical stromal invasion statues, on mean shear-wave values did not show a significant result. (p>0.05).

Table 1. The demographic features of patients and tumor characteristics

	n=48
Age (years) (mean ±SD)	59.88±9.29
Body mass index (kg/m²) (median [25%-75%])	22.6 [20.6-24.62]
Parity (median [25%-75%])	3 [0-12]
Tumor diameter (median [25%-75%])	2.75 [1-3.7]
Tumor grade (n, %)	
Grade 1	16 (33.3%)
Grade 2	17 (35.4%)
Grade 3	15 (31.3%)
Tumor stage (n, %)	
Stage I	25 (52.1%)
IA	20 (41.7%)
IB	5 (10.4%)
Stage II	9 (18.8%)
Stage III	12 (24.9%)
IIIA	5 (10.4%)
IIIB	1 (2.1%)
IIIC1	2 (4.2%)
IIIC2	4 (8.3%)
Stage IV	2 (4.2%)
IVA	1 (2.1%)
IVB	1 (2.1%)
Lymph node involvement (n, %)	
Yes	15 (31.3%)
No	33 (68.8%)
Myometrial invasion depth (n, %)	
<1/2	24 (50%)
>1/2	24 (50%)
Cervical stromal invasion (n, %)	
Yes	22 (45.8%)
No	26 (54.2%)
Lympho-vascular invasion (n, %)	
Yes	14 (29.2%)
No	34 (70.8%)

Table 2. Mean shearwave values of the prognostic factors

	Mean Shearwave Values	
	Mean±SD	p
Tumor grade		<0.05
Grade 1	13.52±7.2[b]	
Grade 2	33.24±18.48[b]	
Grade 3	101.89±33.13[a]	
Total	48.12±43.24	
Myometrial invasion depth		0.294
Invasion less than 1/2	23.2±23.66	
Invasion more than 1/2	73.04±44.33	
Total	48.12±43.24	
Lymph node involvement status		0.639
No	26.38±24.21	
Yes	95.96±37.04	
Total	48.12±43.24	
Cervical stromal invasion status		0.225
No	18.29±14.4	
Yes	83.37±39.18	
Total	48.12±43.24	
Lympho-vascular invasion status		0.639
No	29.79±27.62	
Yes	92.65±42.54	
Total	48.12±43.24	

a,b: There is no significant difference between the groups with same letter

A ROC curve was constructed to determine the tumor grade. The mean shear-wave value was used and the AUC was 0.994 (p<0.001). The mean shear-wave cut-off value of 57 kPa was used to diagnose grade 3 EC with 100% sensitivity and 96.97% specificity. Positive predictive value and negative predictive value were 93.75% and 100% respectively (Table 3) (Figure 1).

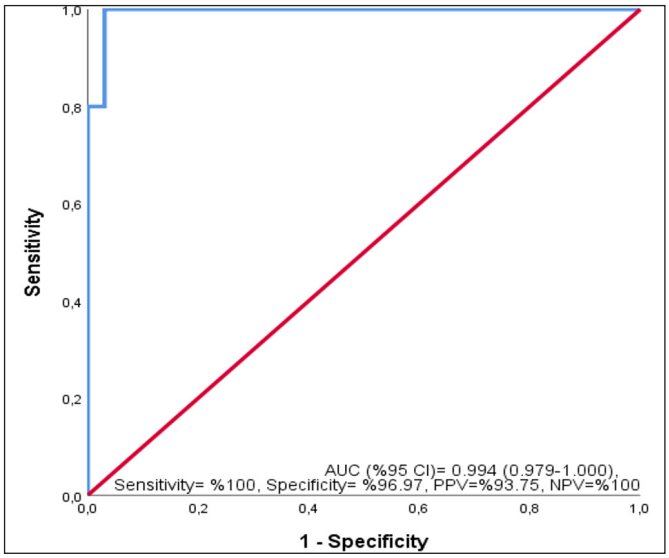

Figure 1. ROC curve to determine the grade 3 endometrial cancer

Table 3. Cut-off points analysis of the mean shearwave values to determine the grade 3 tumor and myometrial invasion depth

	Cut point	AUC	p	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Grade 3	≥57	0.994 (0.979-1.000)	<0.001	100%	96.97%	93.75%	100%
MID	≥28.29	0.853 (0.740-0.996)	<0.001	83.33%	79.17%	80%	82.61%

MID: Myometrial invasion depth

To determine the myometrial invasion depth another ROC analysis performed using mean shear-wave value. And AUC was 0.853 (p<0.001). The mean shear-wave cut-off value was 28.29 kPa with 83.33% sensitivity and 79.17% specificity. Positive predictive and negative predictive values were 80% and 82.61% respectively (Table 3) (Figure 2).

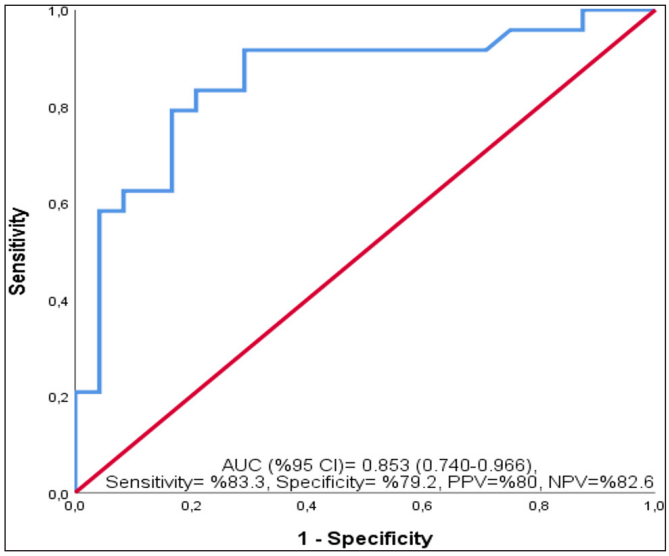

Figure 2. ROC curve to determine the myometrial invasion depth

DISCUSSION

So far, several researchers have investigated the diagnostic accuracy of shear-wave elastography in various malign and benign lesions of the organs including; breast, rectum, thyroid, liver and cervix.[13,14,17-19] Also a recent meta-analysis showed that SWE may have high diagnostic accuracy for the differential diagnosis of benign and malignant endometrial diseases.[20] In our study we also investigated the diagnostic performance of the shear-wave elastography in endometrial cancer patients in terms of the histologic grade of the tumor. In our study, we found that patients with grade 3 EC had higher shear-wave values compared to grade 1 and 2 patients 101.89 kPa, 13.52 kPa, 33.24 kPa, respectively (p<0.001). We think this difference may arise from the nature of the cancer cells. As far as we know, an increase in the tumor grade means an increase in solid component of the cells, which may cause a hardness in the tissue, and may result with a higher SWE value in high-grade lesion.[21] In addition, since the fibrous and vascular structure of the tissue is changed by the tumor, the tissue architecture is disrupted. Thus, cancerous tissue has more dens, fibrous structure with abnormal angiogenetic capillary vessels than normal tissue. These alterations may also contribute to the tissue stiffness.[22,23]

In 2020 European Society of Gynecological Oncology (ESGO), the European Society for Radiotherapy and Oncology (ESTRO), and the European Society of Pathology (ESP) updated the guidelines for the management of the patients with endometrial carcinoma. In this guideline the grade 3 EC patients were underlined as a high-risk group.[24] Grade 3 EC shows more aggressive behavior, with more local and distant relapse and poorer survival rates compared to low grade EC (Grade 1 and grade 2).[25-27] The National comprehensive cancer network (NCCN) also categorizes the grade 3 endometroid type tumor as a high risk. So even if it is an early-stage endometroid type endometrial cancer, NCCN recommends comprehensive surgery and adjuvant treatment for these patients.[28] In a study conducted by Gungorduk et al.[29] in 2021 they stated, comprehensive surgery with adequate lymphadenectomy with optimal cytoreductive approach is the key point for the grade 3 EC despite its poor prognosis. These results show us the importance of accurate evaluation of the patients preoperatively. Even though pathologist may define the grade of the tumor in EC patients, study of the M.-H. Baek et al.[30] found the preoperative pathological assessment is unreliable compared to final pathology in tumor grading. In 2017, a review also showed, preoperative sampling may be discordant with final pathology in tumor grade.[31] In this context, our study may contribute to the preoperative determination of the EC grade. This cheap and non-invasive method may be a helpful tool for clinicians' Current study revealed a 0.994 AUC value (p<0.001), while SWE measurement cut-off to determine the grade 3 EC was 57 kPa with 100% sensitivity and 96.97% specificity. Positive predictive value and negative predictive value were 93.75% and 100% respectively (Figure 1).

Myometrial invasion depth is another important prognostic factor that can change type of surgery in EC. Therefore, preoperative evaluation of myometrial depth is also an important issue. Magnetic resonance imaging (MRI) and transvaginal ultrasonography (TVS) are being used so far. In 2017 a meta-analysis that Alcazar et al.[32] studied, found an overall pooled 75% sensitivity and 86% specificity for detecting the deep myometrial invasion via TVS, while MRI revealed an 83% sensitivity and 82% specificity for deep myometrial invasion detection. In 2020 Zhao et al.[15] investigated the applicability of SWE in the preoperative diagnosis of deep myometrial invasion. Unlike our study, while they measured myometrial tissue stiffness with SWE, we measured the tumoral mass itself shear-wave value. They found a significant difference between patients with deep myometrial invasion and patients with superficial myometrial invasion. In that study, mean SWE cut off values were, to determine deep myometrial invasion of endometrial cancer, 28.17 kPa with a 92.9% sensitivity, 94.6% specificity and positive predictive value was 86.7%, negative predictive value was

97.2%. They attributed this difference to pathological changes caused by the tumoral cells invasion in myometrial tissue. We also found a 28.29 kPa cut-off value in the current study to determine the myometrial invasion depth (AUC= 0.853, p<0.001). This cut-off value has 83.33% sensitivity and 79.1% specificity with 80% positive predictive and 86.61% negative predictive value (**Figure 2**).

Study Strengths and Limitations

First of all, our study has a prospective design. For an accurate measurement of tissue elastography, operator needs to have certain training and operation experience.[33] In the study of Zhao et al.[15] the operator conducted 30 cases of training first to improve data repeatability. In our study SWE measurements were performed by an experienced radiologist with more than 5 years of experience in SWE studies. Conducting the study with a small sample size of the grade 3 EC group was a limitation of the study. However, grade 3 EC patients with BMI<25 are rare. In the literature many studies conducted with transvaginal probe.[20,34] In this study we used abdominal real-time shear-wave elastography. Abdominal wall fat has impact on measurement, so the impact of BMI on patients has not been studied, and it will be further explored in subsequent studies.[34,35]

CONCLUSION

Because of its poorer prognosis, grade 3 EC deserves extra attention for preoperative evaluation and optimal treatment strategy. World Health Organization and FIGO recommend identifying the risk groups for optimal treatment because of the prognostic relevance for tumor grade. Therefore, real-time shear-wave elastography may help the clinician with its high diagnostic accuracy regarding tumor grade and myometrial invasion depth. Hereby, SWE may be a valuable tool for deciding the treatment method and making a judgment about the future prognosis of the disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of by Adana City Training and Research Hospital Clinical Researches Ethics Committee (Date: 26.08.2020, Decision No: 1043).

Informed consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Examining the influence of sample rejection rates on the carbon footprint of clinical laboratories: a retrospective analysis

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ABSTRACT

Aims: Clinical laboratories play a vital role in healthcare, yet their operations contribute to resource consumption, waste generation, and greenhouse gas emissions. The need for sustainable practices in laboratories has led to guidelines for reducing their carbon footprint. This study aims to assess the impact of sample rejection rates (SRRs) on laboratory sustainability by calculating the carbon footprint and medical waste generated due to rejected samples.

Methods: This retrospective, single-center study obtained data from the Hospital Information Management System for two years (2021 and 2022). SRRs were calculated for different sample tube types. The carbon footprint caused by rejected samples was calculated using CO_2 emission (CO_2e) conversion factors. The weight of medical waste generated due to rejected samples was evaluated. Statistical analysis was performed using appropriate tests.

Results: In 2021 and 2022, SRRs for different sample tubes were calculated, with statistically significant differences observed. The total CO_2e value resulting from rejected samples over two years was 12.3 tons, and the medical waste generated was 3.7 tons. The highest SRR was observed in Blue top tubes, while yellow top tubes showed a significant reduction in SRR in 2022.

Conclusion: This study highlights the impact of SRRs on laboratory sustainability. The calculated CO_2e and medical waste values underscore the need to minimize sample rejections. While these values seem minor compared to global emissions, they reflect only a portion of the potential environmental impact. Reducing sample rejections not only improves patient safety and laboratory efficiency but also aligns with the larger goal of creating environmentally conscious and sustainable healthcare practices.

Keywords: Clinical laboratories, sustainability, environmental impact, waste reduction, preanalytical errors, sustainable healthcare

INTRODUCTION

Clinical laboratories play a significant role in disease management and medical decision-making.[1] However, their operations contribute to significant resource consumption, waste generation, and greenhouse gas emissions.[2] The urgency of addressing these environmental issues has led to the emergence of sustainable practices aimed at minimizing the ecological footprint of clinical laboratories. To this end, the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has published the "Green and Sustainable Laboratories guide" on the carbon footprint of laboratories.[2] This guide outlines the potential carbon footprint sources within laboratories and provides guidance on ways to mitigate it across four key areas: Chemicals, Energy, Waste, and Water (**Figure 1**).

When evaluating errors associated with waste management in laboratories, it becomes evident that the most common errors occur in the preanalytical phase (prior to analysis or reaching the laboratory).[3] One likely outcome of this situation is the rejection of samples due to preanalytical errors.[4] Preanalytical errors contribute significantly to sample rejections, unnecessary testing, and subsequent waste generation.[5] Therefore, initiatives aimed at reducing errors during sample collection and transportation can lead to substantial reductions in both environmental impact and costs.

With the growing global awareness of environmental issues, a new sustainability-oriented approach has emerged, based on the concept of ISO 14001 Environmental management systems - Requirements with guidance for use.[6] According to ISO 14001 and EFLM guidelines, each product has a life cycle, which includes all stages from production to disposal. In the case of laboratories, this cycle encompasses sample collection, delivery, analysis, and result reporting.[7] Evaluating sample rejections due to preanalytical errors, the most common cause of error throughout these processes, can provide insights into process control and improvements.

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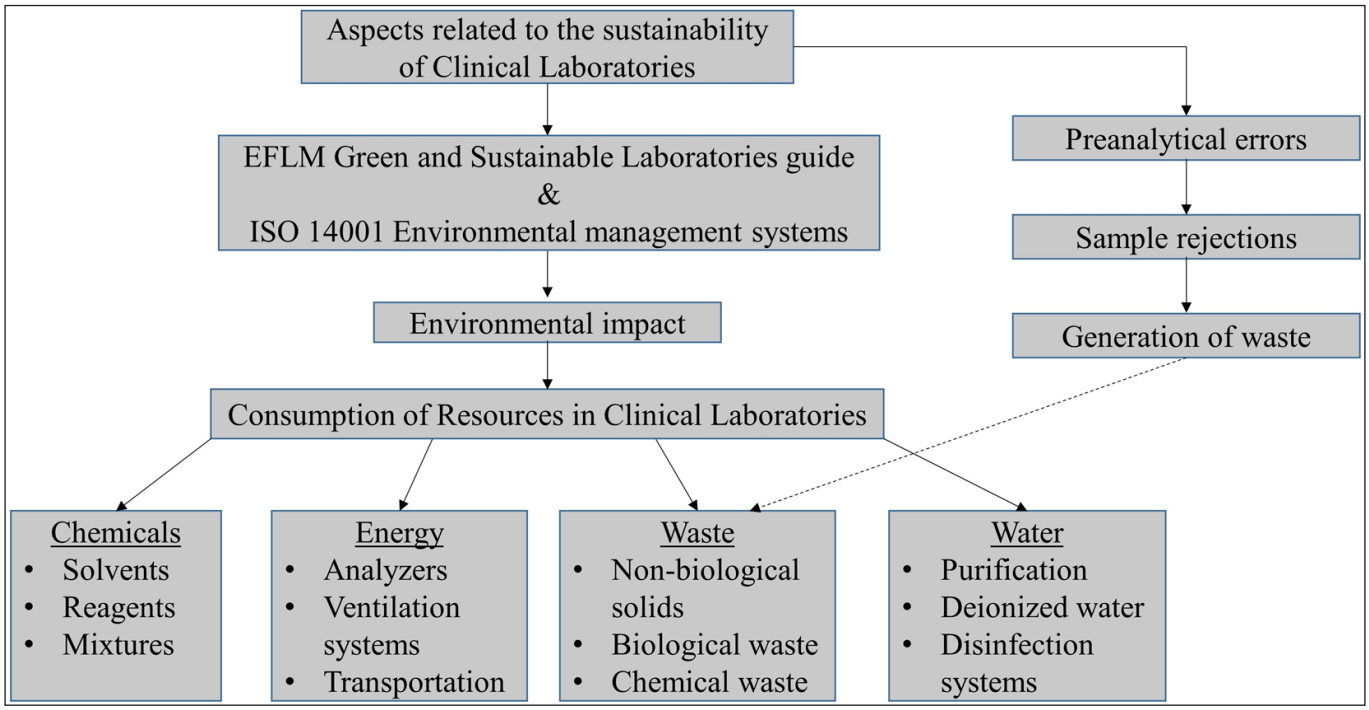

Figure 1. Potential carbon footprint sources of laboratories and their relation to sample rejections

While previous studies have explored the impact of sample rejections on patient safety[5,8,9] and even the CO_2 emission (CO_2e) levels related to the laboratories,[10,11] there appears to be a gap in directly assessing the CO_2e of rejected samples.

In this study, our aim was to calculate the sample rejection rates (SRRs) in our laboratory and to assess differences between years. Additionally, we sought to determine the potential additional CO_2e resulting from waste.

METHODS

The study was designed as a retrospective, observational, and single-center investigation. Approval was obtained from the İstanbul Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee (Date: 31.07.2023, Decision No: 324), and the study was conducted in accordance with the principles of the Declaration of Helsinki.

For the study, the number of sample tubes rejected by the laboratory over two years (2021 and 2022) and the total number of sample tubes received were retrieved from the Hospital Information Management System to calculate SRRs. Calculations were categorized into four groups based on the tube characteristics of the samples (yellow top/serum, blue top/coagulation, purple top/full blood count, urine container/urinalysis). The formula for calculating the SRR is as follows:

SRR (%)=(Rejected samples/Total received samples) *100

Furthermore, the carbon footprint resulting from the unnecessary usage of tubes due to sample rejections was quantified in terms of CO_2e, and the quantities of medical waste were assessed in kilograms. CO_2e conversion factors established by McAlister et al.[11] for the blood collection process, as well as the weights of materials used in blood collection such as gloves, cotton, blood collection needles, holders for vacuum tubes, blood collection tubes, and urine containers, were employed in the computation of medical waste. Calculations were conducted for each group. The formulas for calculating CO_2e and medical waste are presented below:

Gram CO_2e=Rejected samples * CO_2e conversion factors

The CO_2e conversion factors are determined as follows:

- 95 g CO_2e per serum tube with yellow top
- 79.6 g CO_2e per whole blood tube with purple top
- 84.3 g CO_2e per plasma tube with blue top
- 71.9 g CO_2e per urine container

Medical waste (kg)=Rejected samples * Weight of blood collection materials

The weight of tubes is established as follows:

- 30.84 g per serum tube with yellow top
- 23.71 g per whole blood tube with purple top
- 26.59 g per plasma tube with blue top
- 12.88 g per urine container.

To compare SRRs between the two years, SRRs were calculated separately for each year within the four groups. The Chi-square or Fisher's exact test was employed to assess differences in SRRs between the two years for these categorical groups. A p-value of less than 0.05 and a 95% Confidence Interval were considered statistically significant. Microsoft Office 365 (Microsoft Excel Software, Microsoft Corporation, US) and MedCalc® Statistical Software version 20.115 (MedCalc Software Ltd, Ostend, Belgium) were used for creating tables, generating graphs, and performing statistical analyses.

RESULTS

Out of the approximately 4 million samples accepted in 2022, approximately 77000 samples were rejected. A statistically significant reduction in the total SRRs was observed compared to 2021 (2021: 1.98%, 2022: 1.92%, p<0.001, Table 1). The SRRs of yellow top tubes significantly decreased in 2022 compared to 2021 (2021: 1.41%, 2022: 1.16%, p<0.001, Table 1). Conversely, the SRRs of purple top tubes showed a statistically significant increase in 2022 (2021: 1.41%, 2022: 1.67%, p<0.001, Table 1). The SRRs of blue top tubes did not exhibit a significant difference between 2021 and 2022 (2021: 6.59%, 2022: 6.52%, p=0.221, Table 1). The SRRs of urinalysis specimen containers significantly increased in 2022 (2021: 1.97%, 2022: 2.41%, p<0.001, Table 1). Figure 2 provides a visual representation of the SRRs.

The CO_2e value resulting from rejected samples was calculated as 12.3 tons CO_2e for the combined data of 2021 and 2022 (Table 1). The total weight of medical waste generated due to rejected samples amounted to 3.7 ton (with an average of 5.1 kg/day, Table 1). Detailed data including the total number of tubes accepted, rejected tubes, SRRs, kilogram (kg) CO_2e values, and medical waste (kg) values are presented in Table 1.

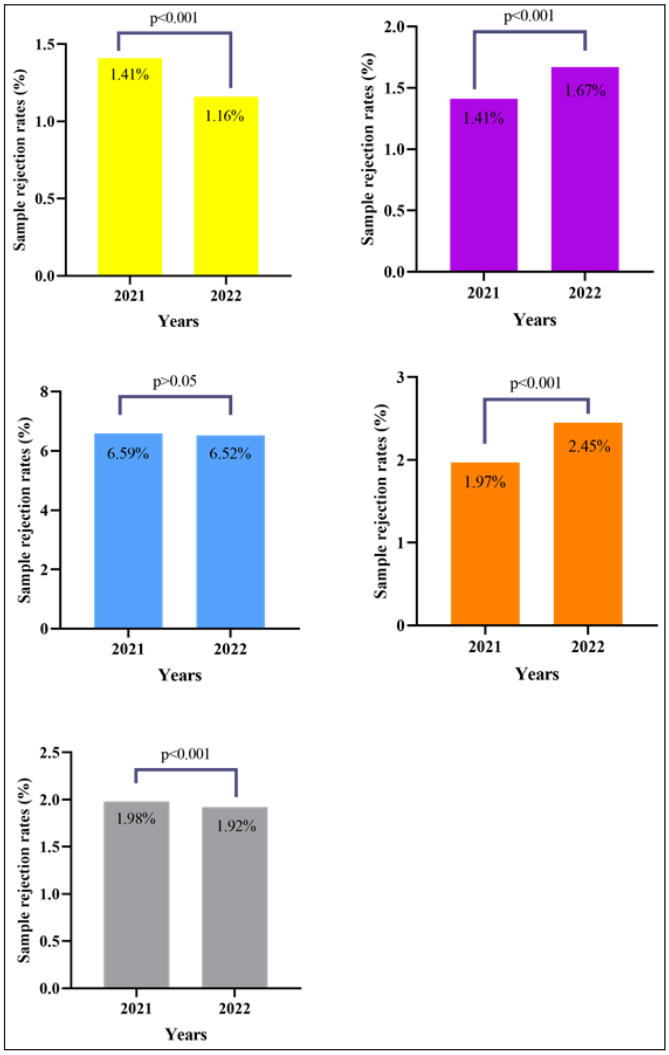

Figure 2. Sample rejection rates, categorized by year and tube types, in the study. Yellow: Yellow top tubes, Purple: Purple top tubes, Blue: Blue top tubes, Orange: Urinalysis container, Gray: Total rejection rates.

Table 1. Total number of tubes accepted by the laboratory and rejected tubes, rejection rates, CO_2e, and medical waste caused by rejected tubes, categorized by year and tube types.

Tube type	Year	Total tube number	Rejected tube number	Rejection rate	p value*	kg CO_2e**	Medical waste (kg)***
Yellow top serum tube	2021 2022	1726327 2123307	24303 24725	1.41% 1.16%	<0.001	2308 2348	750 763
Purple top CBC tube	2021 2022	1015974 1233229	14303 20635	1.41% 1.67%	<0.001	1138 1642	339 489
Blue top plasma tube	2021 2022	338358 379226	22305 24709	6.59% 6.52%	0.221	1880 2082	593 657
Urinalysis container	2021 2022	233048 290381	4592 7103	1.97% 2.45%	<0.001	330 510	59 91
Total	2021 2022 2021-2022	3313707 4026142 7339850	65503 77172 142674	1.98% 1.92% 1.94%	<0.001 NA	5657 6585 12242	1741 2000 3741

g: gram, kg: kilogram, NA: Not available. *p values were calculated by Chi-square test and statistical significance was determined as p<0.05 two-way. Statistically significant differences are indicated in bold. **According to the conversion table of McAllister et al.11 95 g CO_2e per serum tube with yellow top, 79.6 g CO_2e per whole blood tube with purple top, 84.3 g CO_2e per plasma tube with blue top and 71.9 g CO_2e per urine container were accepted. ***According to the medical waste amount tables determined by McAllister et al.11 for each tube, 30.84 g per serum tube with yellow top, 23.71 g per whole blood tube with purple top, 26.59 g per plasma tube with blue top and 12.88 g per urine container were considered to be generated.

DISCUSSION

In our study, we found SRRs of approximately 1.98% and 1.92% for 2021 and 2022, respectively, with the highest rejection rates observed in blue top tubes. The calculated total CO_2e resulting from these rejections was approximately 12.3 tons, and the medical waste amount was 3.7 tons (averaging 5.1 kg/day).

Turkey's total CO_2e value for 2021 is reported as 564.4 million tons, with a per capita CO_2e value of 6.7 tons.12 Globally, the European Commission Emissions Database for Global Atmospheric Research (EDGAR) group estimated the worldwide CO_2e value for 2021 at 37.9 gigatons.[13] While the calculated 12.3 tons from our data might appear relatively small in comparison, it is important to note that this value pertains solely to the production processes of blood collection devices. Moreover, CO_2e values related to waste disposal or extra transportation of waste were not incorporated due to the absence of calculable data. Rejected samples become wasted materials as they do not undergo desired testing, potentially leading to unfavorable outcomes. Furthermore, there exists a CO_2e value possibly generated by patients making unnecessary trips to health centers for results due to rejections.[14] Considering these factors, it is conceivable that our estimated CO_2e value might be an underestimate.

Apart from the generation of infectious waste in healthcare and laboratories, medical waste can give rise to financial and sustainability issues.[15] In their evaluation of 20 centers, Endris et al.[9] determined that laboratories generated an average of 4.9 kg/day of medical waste. Our study yielded a value of 5.1 kg/day, closely aligning with this finding. The overarching approach to mitigate laboratories' environmental impact can be summarized as "Reduce, Reuse, Recycle".[16] However, due to the risks posed by medical waste, the reuse and recycling stages for infectious materials are not feasible. Thus, reducing SRRs in laboratories is expected to help curtail medical waste and its adverse environmental effects.[17]

The impact of preanalytical errors on SRRs has been well-documented.[18] Previous research has highlighted that proper training in blood collection can significantly reduce preanalytical errors and subsequently lower SRRs.[8] Aykal et al.[19] for instance, demonstrated a reduction in SRRs from 2.35% to 1.56% following training. In our laboratory, we already implement a monthly SRR monitoring procedure and conduct blood collection training sessions. The observed decrease in SRRs during 2022, as revealed in our study, is likely attributed to the effective standardization of these rigorous monitoring and training processes.

Furthermore, our findings indicated that blue top tubes exhibited the highest SRR values in our laboratory (2021: 6.59%, 2022: 6.52%). This observation aligns with existing literature. Dikmen et al.[5] for instance, reported blue top tubes as the most frequently rejected samples in their study, with an SRR of 13.3%. Similarly, Atay et al.[8] found the highest SRR values in their laboratory to be associated with blue top tubes (SRR for blue top tubes: 2.28%). It is reasonable to attribute the proportional differences among these studies to variations in hospital and laboratory settings, as well as different working conditions.

While effective hazardous waste management is a subset of laboratories' journey toward becoming greener and more sustainable, it also encompasses chemical and solid waste. Shrank et al.[20] reported that a significant portion of the US healthcare system's expenditures (about a quarter) is allocated to waste management ($760 billion to $935 billion), a cost that notably increases when considering CO_2e equivalence. Moreover, assessments pertaining to Chemicals, Energy, and Water—factors that wield substantial impact on sustainability—are paramount for creating a "Green Lab".[2] These assessments incorporate Environmental, Social, and Economic criteria.[21] In addition to SRRs, a rational approach to test ordering can contribute to laboratory sustainability by reducing material and test chemical usage and the associated energy requirements.[22] Emphasizing the social and economic aspects of these efforts, and ensuring their persistence, can play pivotal roles in developing "Green and Sustainable Laboratories".[2,21] To achieve these aims, public awareness and support from patients, healthcare professionals, in-vitro diagnostics producers, and the general population are essential.[23] Laboratories will strengthen their standing within the healthcare sector and society as each sustainability milestone is achieved.

Our study, while providing valuable insights, does come with certain limitations. Specifically, we focused solely on calculating CO_2e values associated with the production and transportation stages of the tubes. However, as highlighted in the paper, there exists a noteworthy CO_2e related to the waste disposal phase. Unfortunately, due to the absence of relevant CO_2e values or conversion factors in the literature, we were unable to incorporate these aspects into our analysis. Furthermore, the repercussions of sample rejections extend beyond their immediate environmental impact. Such rejections can potentially lead to additional environmental harm through heightened transportation and consumption processes, be it patients returning unnecessarily for retests or the undue use of chemicals.

Another aspect to consider is that the conversion factors utilized in this study were sourced from a previously published work in the literature.[11] The author made this decision due to the obvious similarity between the blood collection devices (such as tubes, vacutainers, needles, and other consumables) employed in the referenced study and those utilized in our laboratory. Although there are valuable studies documenting and collating such data within our

country, accessing these specific values from a database proved challenging. Consequently, the decision was made to directly apply the established values to our laboratory, ensuring the utilization of dependable data. This study is believed to hold significance in raising awareness regarding the potential adverse environmental impacts of laboratories. It is believed that conducting a thorough and comprehensive study into these aspects would represent another crucial step towards achieving Green and Sustainable Laboratories.

CONCLUSION

In summary, our study reveals that SRRs in our laboratory averaged around 2% annually. The CO_2e value arising from sample rejections over two years totaled 12.3 tons, accompanied by a medical waste amount of 3.7 tons. Reducing sample rejections is anticipated to yield considerable gains, enhancing patient, clinician, and laboratory safety, in addition to mitigating the carbon footprint.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee (Date: 31.07.2023, Decision No: 324).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The transition from gel separatory serum tubes to lithium heparin gel tubes in the clinical laboratory

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ABSTRACT

Aims: To assess the viability of replacing serum samples with plasma samples in various clinical chemistry and immunoassay tests and to examine the implications of turnaround time (TAT) and sample quality during the transition process.

Methods: We compared the results of 27 paired clinical chemistry and 13 immunoassay tests from samples obtained using gel separator serum and gel separator lithium heparinized plasma (LIH) tubes. We used regression analysis, bias values, and Bland-Altman plots to compare the performance of serum and LIH tubes in various clinical chemistry and immunoassay tests. We collected and evaluated sample aspiration errors, hemolysis index values, and TAT data from the laboratory information system before and after switching to plasma in our study.

Results: Most tests showed no significant difference between the serum and LIH. However, for some analytes, total error (TE) values exceeded the total allowable error (TEa) limits derived from the biological variation database. Notably, insulin TE value did not exceed TEa, but it consumed near all its error budget. Consequently, we determined the alternative allowable error limits for some tests and found that plasma tubes could be used instead of serum tubes for most tests, except for lactate dehydrogenase (LDH). Plasma tubes improved the sample quality, reduced the incidence of aspiration errors, and decreased TAT in the emergency laboratory. We observed significant reductions in TAT after switching to plasma tubes.

Conclusion: Our study showed that LIH tubes can replace serum tubes in most clinical chemistry and immunoassay tests. Using LIH tubes in clinical laboratories can improve healthcare quality and reduce the workload of the laboratory staff.

Keywords: Plasma, serum, total error, turnaround time, sample quality

INTRODUCTION

Plasma and serum are the two most common types of samples used in medical laboratories. However, plasma has recently gained popularity because of its advantages over serum. These advantages include no need for clotting before centrifugation, a larger sample volume, and better sample quality with fewer clots and fibrin residues.[1] Turnaround time (TAT) is the time interval between when a lab receives a test sample, processes it, and sends the results to the physician or other medical provider who orders it. The literature measures TAT intervals as the time from request to report or acceptance to report.[2] All laboratory processes require good planning and evaluation to improve TAT targets.[3] One of these improvements is the use of plasma samples instead of serum, particularly in emergency laboratories.[4] Evidence also supports the use of plasma to prevent interference that may result from serum clotting. Plasma is recommended for most laboratory tests because its constituents represent a patient's pathological state more accurately than serum. Anticoagulants can stop the clotting process from having an interferant effect.[1] Our study aimed to assess the viability of replacing serum samples with plasma samples and to examine the implications of TAT and sample quality during the transition process.

METHODS

The study was carried out with the permission of Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee (Date: 22.03.2023, Decision No: 117). All procedures were carried out in accordance with the ethical rules and principles of the Declaration of Helsinki.

The study was conducted in April 2023 and included data from patients who were treated in the year 2022 in our retrospective analysis. The participants were 38 males and 38 females aged 25-65 years, who were randomly recruited from the emergency department of our hospital. A single experienced phlebotomist trained in preanalytical errors

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sampled the antecubital vein using a 21-gauge blood collection needle. Two tubes were randomly assigned for sampling, and each tube was used to collect samples from the contralateral arms. We applied the inclusion criteria specified in the Clinical and Laboratory Standards Institute (CLSI) GP34-A:2010 document.[5,6] We followed the recommendations of the tube manufacturers for tube inversion, clotting time, and centrifugation characteristics. We centrifuged the samples at 1800g for 10 minutes. After centrifugation, we visually evaluated sample quality indicators such as hemolysis, lipemia, icterus, fibrin, and clots in the serum/plasma. During the analysis, we also measured a semi-quantitative hemolysis index and excluded samples with evident hemolysis (>50 mg/dl free hemoglobin). We used the gel separator-clot activator tube VACUETTE® Greiner Bio-One (Kremsmuenster, Austria) 13*100 mm, catalog number 456073 for serum and gel separator plasma tubes with lithium heparin VACUETTE® 13*100 mm, catalog number 456087, Greiner Bio-One (Kremsmuenster, Austria). We used a pneumatic system to send all tubes to the laboratory after blood collection. Serum tubes were upright for 25-30 minutes before centrifugation. The plasma tubes were centrifuged immediately after receiving the tubes without delay. We compared the results of 27 paired clinical chemistry and 13 immunoassay tests from samples obtained using gel separator serum and gel separator lithium heparinized plasma (LIH) tubes. Measurements were performed randomly on a Roche COBAS 8000 clinical chemistry analyzer with c 701, e 602 and ion-selective electrodes (ISE) modules. (Roche Diagnostics, Basel, Switzerland); the measurement method applied for biochemical testing was spectrophotometry, whereas immunoassays utilized electrochemiluminescence, and electrolyte tests made use of ion-selective electrodes. Analyses were performed in duplicate within a single study cycle, using the averages of the calculated results. Precision and reproducibility studies were conducted using same-brand internal quality control materials, with twice-daily measurements.

Statistical Analysis

The study compared serum and LIH tubes using regression analysis, bias values, and Bland-Altman plots. The Kolmogorov-Smirnov test was used to check for normality, and the paired t-test or Wilcoxon test was used to determine statistical significance. The present study aimed to assess clinically significant differences using biological variation in TEa (Total allowable error) targets.[7,8] MedCalc Statistical Software 19.0.7 (Ostend, Belgium) was used for the statistical analysis.

$$Bias\% = [(compared\ tube\ LIH) - (control\ tube\ serum)/(control\ tube\ serum)]X100$$

$$Total\ error\%: |\%Bias| + 1.65X\%CV$$

CV (%) is the coefficient of variation for measuring between day imprecision.

We followed different sources for the TEa targets depending on the availability of the test parameters. We mainly used the biological variation database from the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) website but also referred to the combined performance specifications from Westgard's website for some parameters. For N-terminal pro B-type natriuretic peptide (PBNP) and Procalcitonin (PCT) tests, we relied on the targets from the literature.[7-9]

RESULTS

Paired statistical analyses performed on the test results obtained from serum and plasma tubes revealed significant differences in K, ALP, ALT, ALB, GGT, Na, CK, TP, AST, LDH, PHOS, Mg, UA, LIP, COL, TG, PBNP, TNT-hs, FER, TSH, CA125, CA15-3, CA19-9, and INS ($p<0.05$). The total error values for the Na, K, Cl, TP, and LDH tests exceeded the TEa limits for biological variation (BV). Some tests did not meet the performance criteria of BV models.[10] Therefore, the alternative TEa limits for Na, K, Cl, TP, and LDH were determined and evaluated. The total protein (TP) test was beyond these limits due to fibrinogen in the plasma, and further evaluation was not required.[4] Analytical performance specifications for BIL-D, CKMB, PBNP, HCG-BETA, PCT, and CA15.3 were obtained from other sources because they were not included in the BV database (**Table 1**).

Table 2 shows the slope and intercept values obtained from the Passing and Bablok regression analyses. The regression lines are shown in **Figure 1**. According to the regression analysis results, considering the 95% confidence interval for the slope, there is no proportional difference between the two groups if the confidence interval covers a value of 1. Except for the Ca and INS tests, the 95% confidence interval for the slope value included 1 for all tests. As is common knowledge, the intercept value is assessed as a measure of the systemic difference between two groups. If the 95% confidence interval of the intercept values from the Passing and Bablok regression analysis covers 0, then we assume that the two sample groups do not differ systematically. This assumption was valid for all tests except for Ca and GGT when we compared serum and plasma samples based on the intercept values obtained in our study. In our study, most test points were within the set limits, as shown in **Figure 2**. We also reported the mean biases (%) from the Bland-Altman analysis in **Table 1**. We collected alerts related to device aspiration errors from the laboratory information system to evaluate the effects of plasma use in our laboratory (**Table 3**).

Table 1. Comparison of the results of clinical chemistry tests using serum and plasma tubes

Test (Unit)	n	Serum (Gel separator tube)	Plasma (Lithium-heparinized plasma tube)	CV%	Mean bias (%)	TE (%)	TE[a] (%)
K (mmol/L)	76	4.44 (4.23-4.75)	4.24 (4.04-4.48)	1.88	-4.66	7.77	4.8
GLU (mg/dl)	75	100.5 (92.73-124.08)	101 (92.68-125.6)	1.32	0.51	2.69	6.5
CREA (mg/dl)	76	0.84 (0.61-0.94)	0.83 (0.61-0.95)	3.12	-0.45	5.60	7.4
Urea (mg/dl)	73	28 (22.13-36.54)	28 (22.04-36.79)	1.56	-0.39	2.97	17.8
ALP (IU/L)	76	74 (59.4-98)	71 (58.68-92.7)	2.1	-3.22	6.68	10.5
BIL-T (mg/dl)	76	0.39 (0.24-0.62)	0.4 (0.26-0.61)	3.9	7.03	13.46	24.8
ALT (IU/L)	76	19.95 (13.0-29.75)	17.95 (13.05-26.75)	1.6	-5.16	7.80	16.1
ALB (g/L)	75	45.4 (39.65-47.85)	44.8 (39.45-47.46)	1.25	-1.15	3.21	3.4
AMY (IU/L)	76	58.95 (46.3-76.9)	59.38 (46.3-77.23)	1.0	0.28	1.93	13.2
GGT (IU/L)	76	18 (12.95-29.95)	20.13 (14.15-30.88)	1.72	13.21	16.05	18.9
CRP (mg/L)	75	3.98 (1.28-20.35)	4.06 (1.25-20.29)	1.64	-1.48	4.19	50.7
Na (mmol/L)	76	139.05 (137.55-140.4)	138.63 (137.23-140.03)	1.3	-0.31	2.46	5.0
CK (IU/L)	71	85.85 (55.74-136.5)	85.2 (56.48-133.25)	1.0	-1.08	2.73	22.6
Cl (mmol/L)	75	102.45 (99.54-104.09)	102.8 (99.96-104.2)	1.59	0.25	2.88	5.0
TP (g/L)	71	71.58±5.6	73.58±5.7	1.9	2.83	5.96	8.0
BIL-D (mg/dl)	76	0.16 (0.11-0.26)	0.16 (0.12-0.25)	1.96	6.55	9.79	44.5
Ca (mg/dl)	76	9.27±0.593	9.26±0.54	1.28	-0.11	2.23	2.3
AST (IU/L)	76	21.55 (16.65-30.75)	22.78 (17.75-29.35)	1.95	4.82	8.04	13.6
LDH (IU/L)	75	181.5 (158.5-218.75)	205 (174.88-250.38)	1.0	13.95	15.60	15
CKMB (ng/ml)	64	1.61 (1.16-2.22)	1.65 (1.17-2.21)	2.44	1.68	5.71	25
PHOS (mg/dl)	74	3.26±0.65	3.11±0.65	1.28	-4.63	6.74	9.7
Mg (mg/dl)	74	2.03 (1.9-2.17)	2.06±0.29	1.89	0.47	3.59	4.0
UA (mg/dl)	74	4.95±1.84	4.7 (3.8-5.8)	1.31	0.46	2.62	12.8
LIP (IU/L)	75	30.7 (23.04-40.03)	30.5 (23.13-39.94)	1.71	0.22	3.04	14.2
CHOL (mg/dl)	70	187±47.49	184.2±46.94	2.6	-1.50	5.79	8.8
HDL (mg/dl)	69	42.7 (35.0-49.05)	44.23±13.33	2.9	0.09	4.87	10.9
TG (mg/dl)	57	121.7 (83.55-186.43)	118.4 (80.0-183.63)	1.9	-2.60	5.73	27
FT3 (pg/ml)	70	2.94 (2.55-3.24)	2.87±0.59	2.07	-0.17	3.59	6.5
PBNP (pg/ml)	66	50.93 (24.8-134)	50.33 (25.1-134)	3.37	-2.53	8.09	30
FT4 (ng/dl)	66	1.26±0.226	1.26±0.23	3.04	-0.06	5.07	6.3
TNT-hs (ng/L)	69	4.92 (3.0-11.33)	5.18 (3.0-11.73)	2.58	7.54	11.79	17.6
FER (ng/ml)	66	99.78 (41.1-184)	96.08 (40.65-180)	2.38	-0.70	4.63	13.8
HCG-BETA (mIU/ml)	66	0.2 (0.2-0.89)	0.2 (0.2-0.9)	3.12	3.62	8.77	18.0
TSH (uIU/ml)	66	1.52 (1.06-2.14)	1.52 (1.07-2.06)	2.76	-0.50	5.05	24.6
PCT (ng/ml)	66	0.05 (0.04-0.07)	0.05 (0.04-0.07)	3.87	2.03	8.41	20.3
CA125 (IU/ml)	64	10.25 (7.38-18.25)	9.99 (7.12-17.75)	2.14	-3.56	7.09	13.9
CA15-3 (IU/ml)	63	15.9 (12.63-21.93)	15.4 (12.28-20.68)	3.63	-3.38	9.37	20.8
CA19-9 (IU/ml)	63	8.04 (4.67-14.78)	8.01 (4.63-14.73)	4.65	-0.92	8.59	17.9
INS (uIU/ml)	66	13.6 (8.08-22.5)	8.73 (5.18-19.6)	4.23	-22.97	29.95	31.5
VITD (ng/ml)	69	12 (8.16-18.2)	13.1 (8.69-18.83)	2.76	4.98	9.53	12.4

[a]Total error targets derived from biological variation. [b]CLIA (Clinical Laboratory Improvement Amendments) [c]Westgard consolidated comparison of chemistry performance specifications, consolidated comparison of immunoassay performance specifications. *Values are presented as mean±standard deviation; percentiles (Q1-Q3) are given in parentheses. CV: Coefficient of variation. TE: Total error. TEa: Total Allowable Error. Alanine aminotransferase (ALT), albumin (ALB), alkaline phosphatase (ALP), amylase (AMY), aspartate aminotransferase (AST), bilirubin direct (BIL-D), bilirubin total (BIL-T), C-reactive protein (CRP), inorganic phosphorus (PHOS), gamma-glutamyl transferase (GGT), glucose (GLU), HDL cholesterol (HDL), calcium (Ca), creatine kinase (CK), creatine kinase-MB (activity) (CKMB), creatinine (CREA), lactate dehydrogenase (LDH), lipase (LIP), magnesium (Mg), total cholesterol (CHOL), total protein (TP), triglycerides (TG), uric acid (UA), chloride (Cl), potassium (K), and sodium (Na), free triiodothyronine (FT3), N-terminal pro-b-natriuretic peptide (PBNP), free thyroxine (FT4), troponin T (TNT-hs), ferritin (FER), chorionic gonadotropin (HCG-BETA), thyroid stimulating hormone (TSH), procalcitonin (PCT), cancer antigen 125 (CA125), cancer antigen 15-3 (CA15-3), cancer antigen 19-9 (CA19-9), insulin (INS), total 25-hydroxy vitamin D (VITD).

Table 2. Results of Passing-Bablok regression analysis comparing the values of 40 clinical chemistry tests in serum and plasma.

Test	Passing-Bablok slope (95% CI)	Passing and Bablok intercept (95% CI)	Measured concentration range
K	0.968 (0.847-1.101)	-0.085 (-0.670-0.456)	2.01-5.47
GLU	1.0 (0.976-1.017)	0.200 (-1.423-2.791)	60.3-562.6
CREA	1.007 (0.989-1.033)	-0.010 (-0.032-0.007)	0.459 -1.965
URE	1.003 (0.996-1.001)	-0.240 (-0.448-0.005)	9.1-103
ALP	0.955 (0.940-0.971)	0.568 (-0.738-1.964)	33-258
BIL-T	0.969 (0.934-1.000)	0.016 (0.000-0.029)	0.04-1.755
ALT	0.981 (0.954-1.011)	-0.127 (-0.731-0.535)	8.4-317.6
ALB	0.969 (0.937-1.000)	0.898 (-0.500-2.334)	29.2-51.2
AMY	1.000 (0.986-1.005)	0.000 (-0.254-0.799)	16-944
GGT	1.000 (0.966-1.023)	1.000 (0.592-1.858)	6-260
CRP	0.981 (0.976-0.988)	0.046 (0.031-0.071)	0.125-197.22
Na	1.038 (0.940-1.150)	-5.579 (-21.197-8.066)	129-143.3
CK	0.996 (0.985-1.002)	-0.530 (-1.200-0.581)	10-805.5
Cl	0.982 (0.922-1.041)	1.972 (-4.018-8.162)	87.5-107.9
TP	1.017 (0.951-1.080)	0.821 (-3.710-5.530)	55.6-83.9
BIL-D	1.000 (0.949-1.043)	0.001 (-0.005-0.010)	0.04-0.8
Ca	0.903 (0.838-0.971)	0.877 (0.224-1.489)	6.89-10.25
AST	1.000 (0.973-1.052)	0.700 (-0.368-1.445)	11.1-636.5
LDH	1.028 (0.938-1.150)	13.649 (-6.870-29.531)	97.6-520.5
CKMB	0.986 (0.964-1.004)	0.026 (0.004-0.052)	0.317-9.18
PHOS	1.042 (1.000-1.081)	0.029 (-0.095-0.170)	1.57-4.73
Mg	1.000 (0.950-1.063)	-0.020 (-0.145-0.079)	0.9-3.56
UA	1.000 (1.000-1.008)	0.005 (-0.024-0.005)	1.6-12.05
LIP	0.999 (0.986-1.007)	-0.251 (-0.528-0.202)	8.4-2435
CHOL	1.011 (0.992-1.031)	0.843 (-2.269-4.360)	75.5-307.3
HDL	1.000 (0.977-1.017)	0.000 (-0.730-0.874)	15.8-92.6
TG	1.001(0.994-1.023)	2.495 (0.897-4.369)	46.3-443.2
FT3	1.007 (0.978-1.042)	-0.023 (-0.126-0.059)	0.855-3.89
PBNP	0.982 (0.972-0.997)	-0.114 (-1.105-0.467)	8.765-10328
FT4	1.015 (0.977-1.058)	-0.023 (-0.076-0.026)	0.854-1.805
TNT-hs	0.966 (0.953-0.991)	0.103 (0.027-0.141)	3-126
FER	0.979 (0.970-0.989)	0.355 (-0.110-1.135)	2.48-2000
HCG-BETA	1.000 (1.000-1.035)	0.000 (-0.007-0.000)	0.2-10000
TSH	0.987 (0.977-0.999)	0.001 (-0.011-0.013)	0.171-14.1
PCT	0.949 (0.902-1.041)	0.002 (-0.002-0.005)	0.02-2.57
CA125	0.968 (0.947-0.983)	0.028 (-0.164-0.207)	2.33-400
CA15-3	0.967 (0.945-0.987)	-0.063 (-0.351-0.291)	3.65-46.9
CA19-9	0.981 (0.972-0.993)	0.037 (-0.006-0.059)	2-64.8
INS	1.179 (1.097-1.306)	0.683 (-0.192-1.384)	0.866-106
VITD	0.989 (0.946-1.027)	0.372 (-0.080-0.951)	3-72.4

CI=Confidence interval

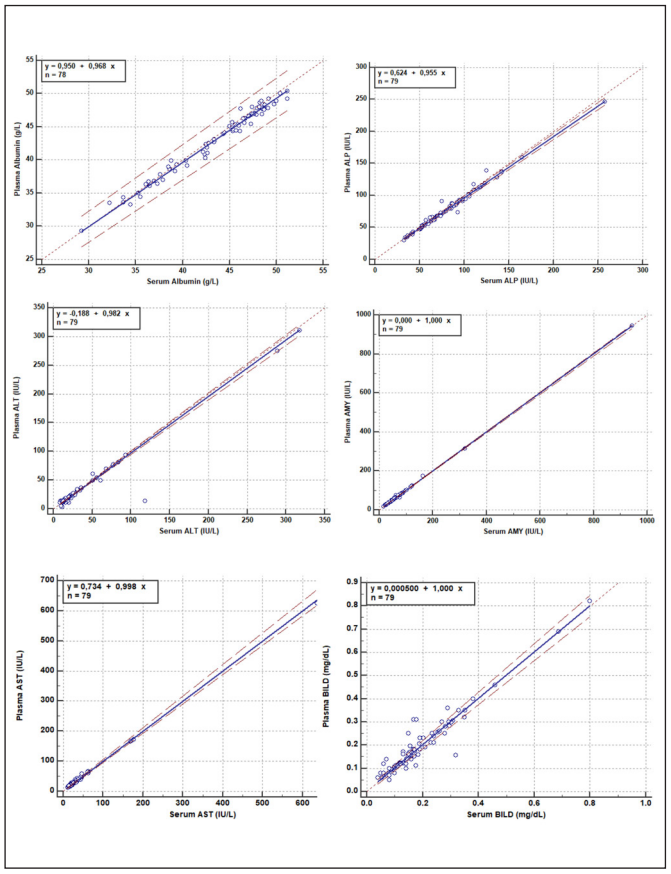

Figure 1A

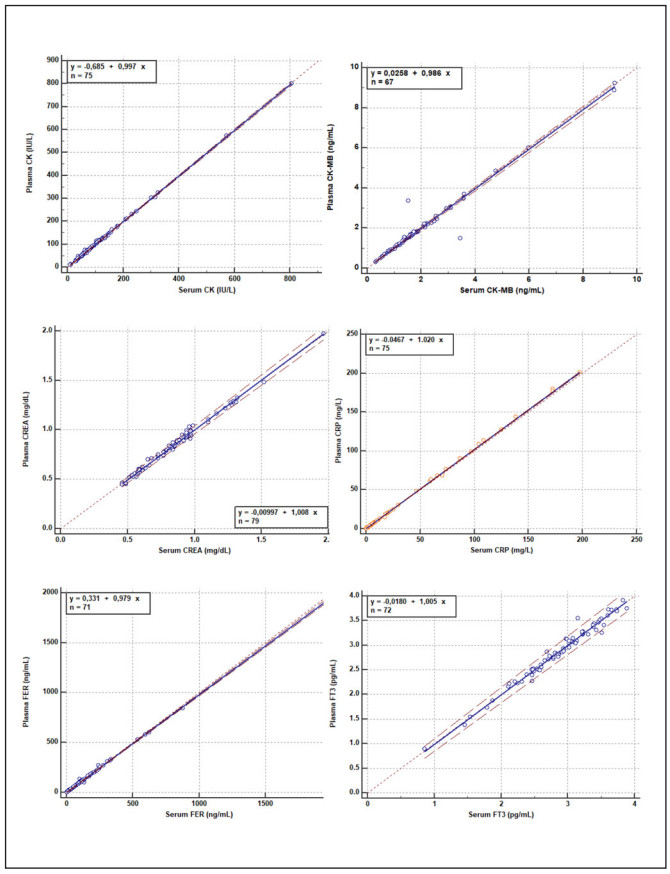

Figure 1C

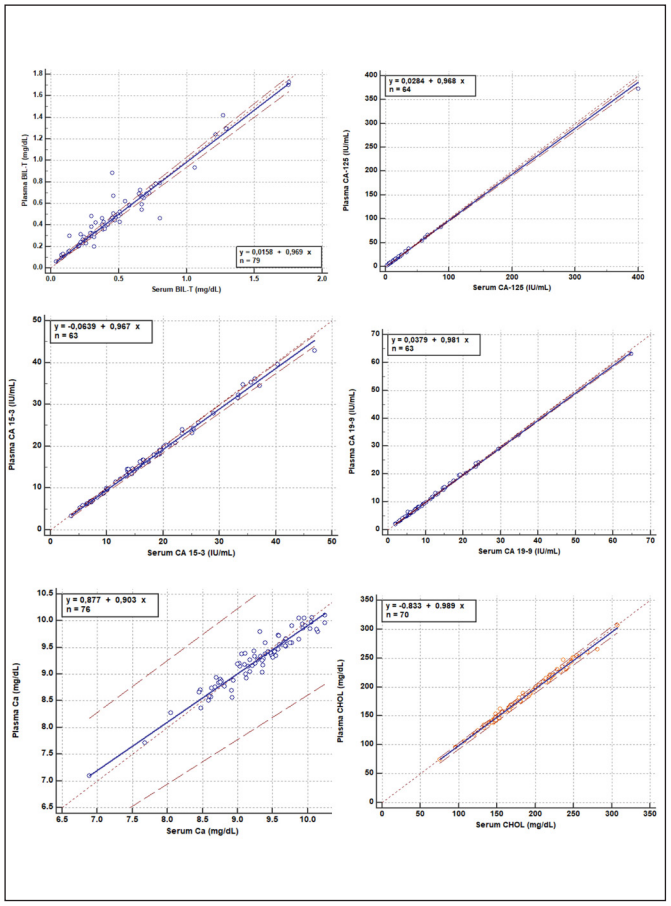

Figure 1B

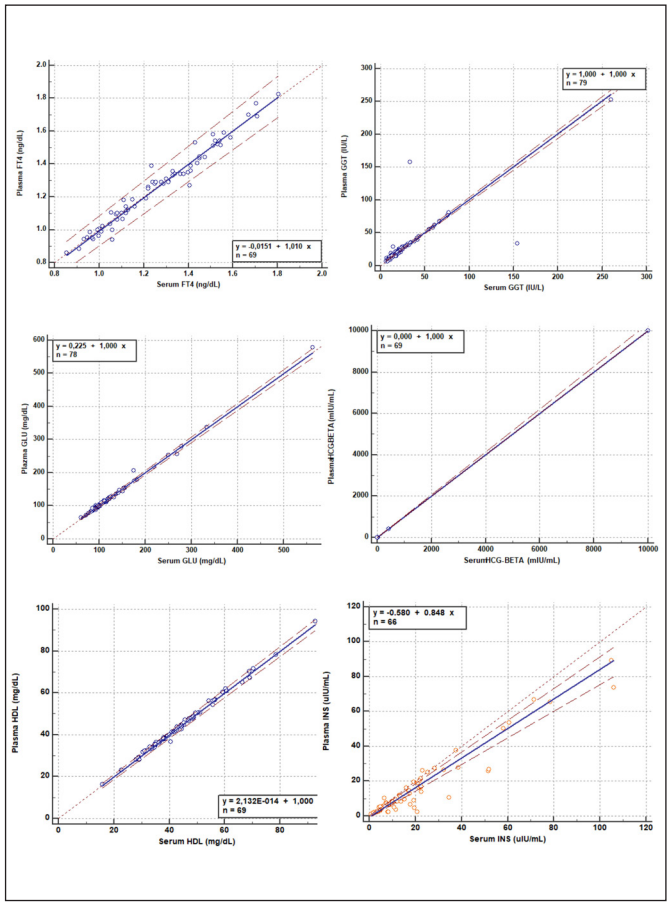

Figure 1D

Figure 1 (A - G): Serum and heparinized plasma test results shown on the Passing-Bablok regression analysis.

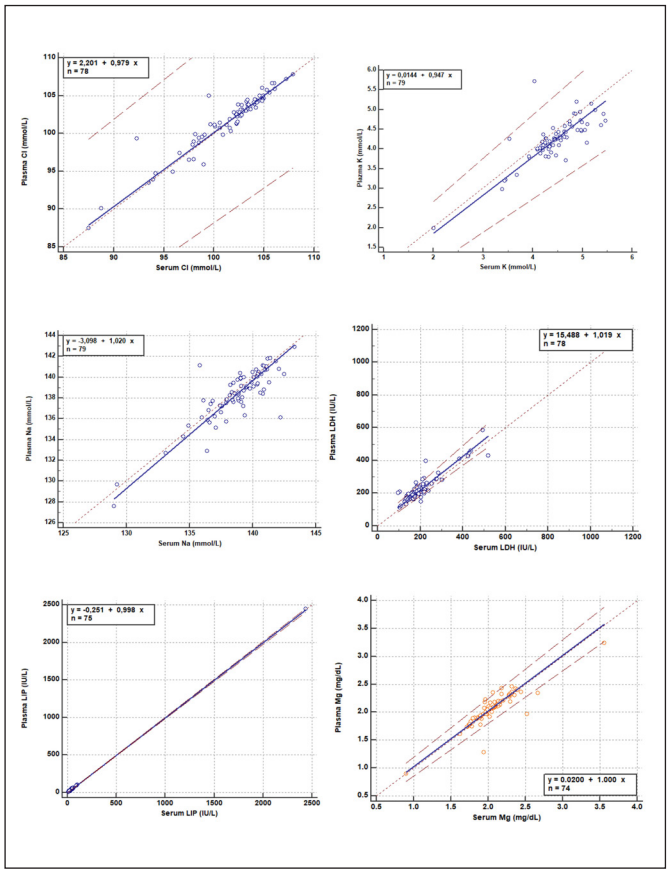

Figure 1E

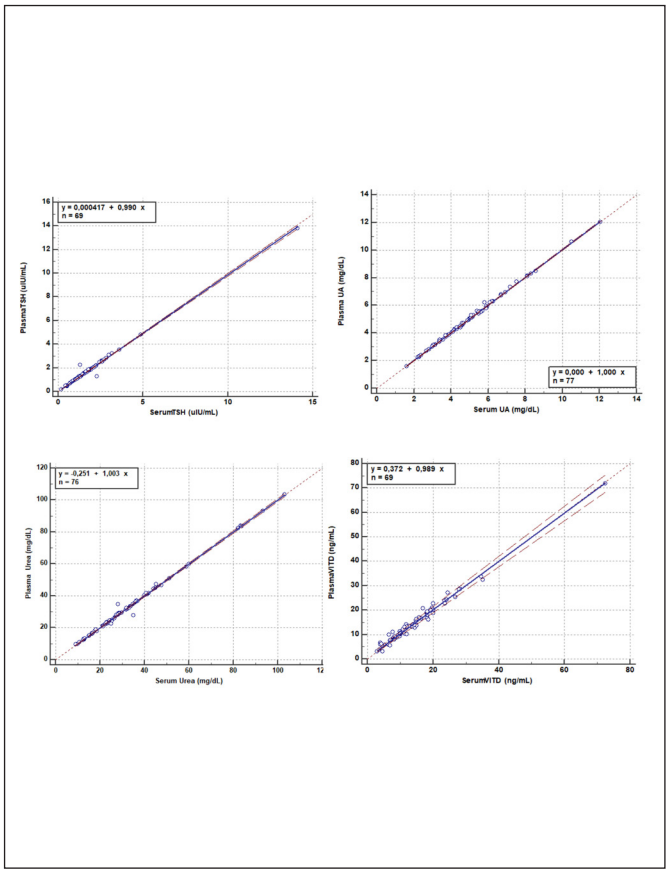

Figure 1G

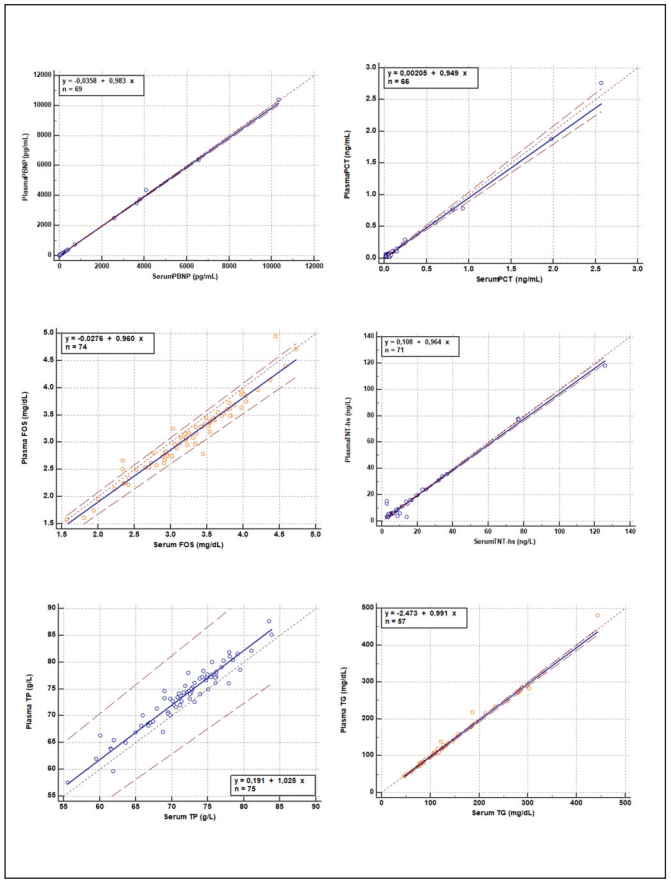

Figure 1F

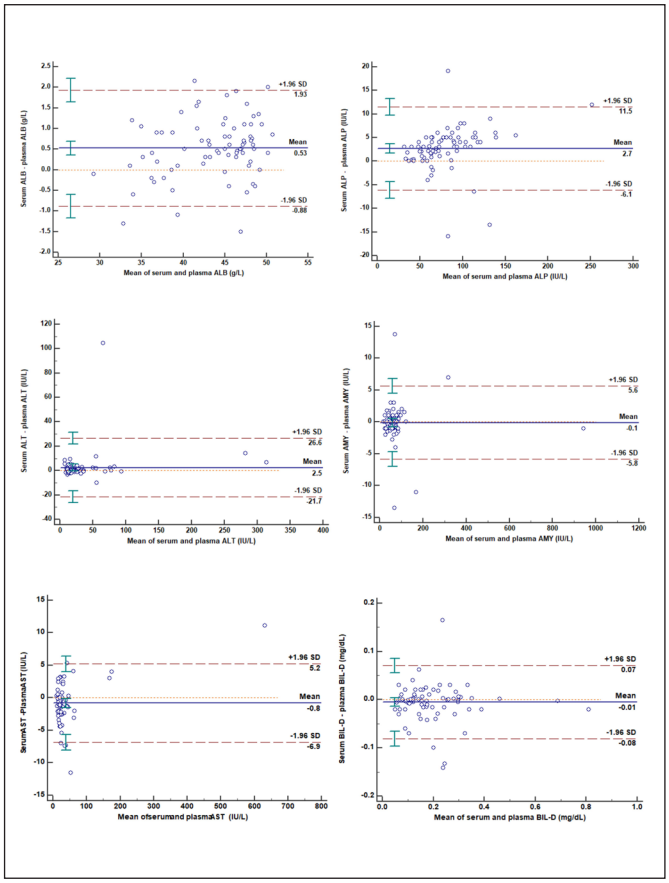

Figure 2A

Figure 1 (A - G): Serum and heparinized plasma test results shown on the Passing-Bablok regression analysis.

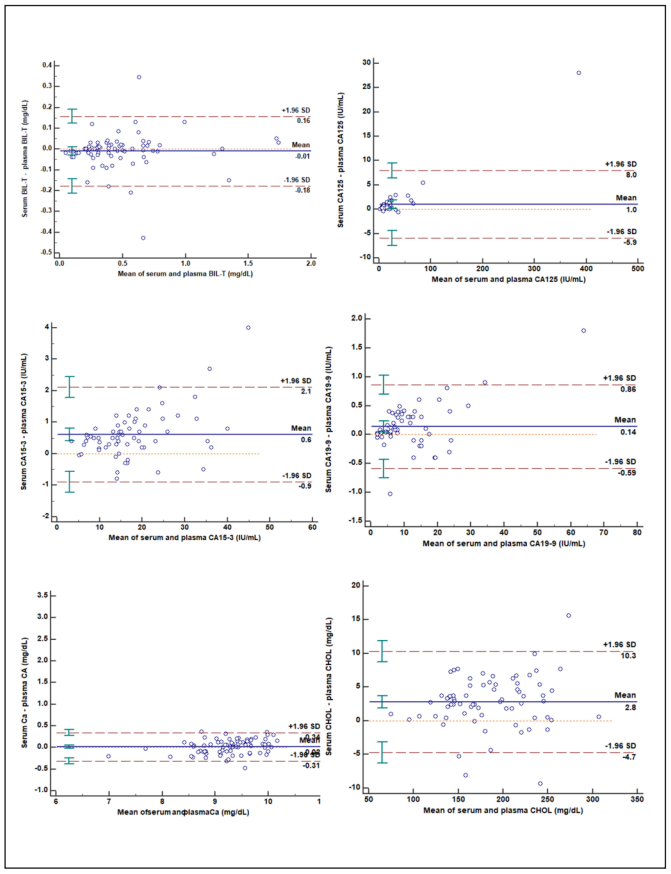

Figure 2B

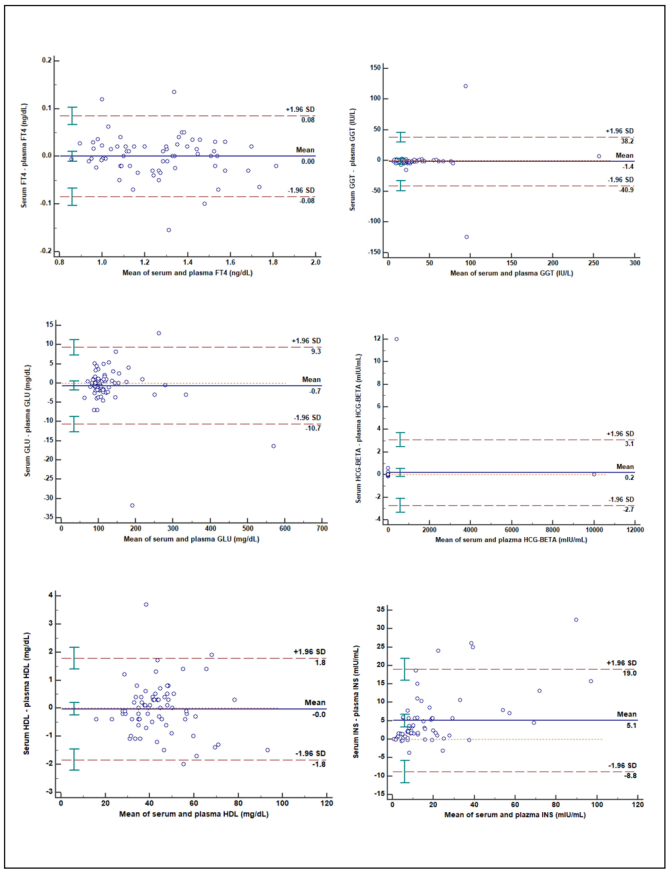

Figure 2D

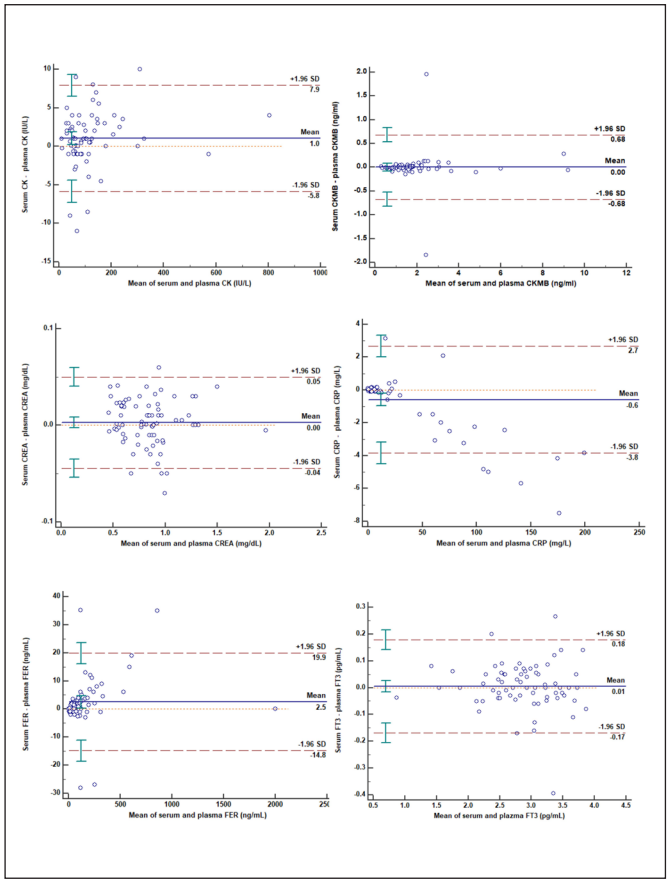

Figure 2C

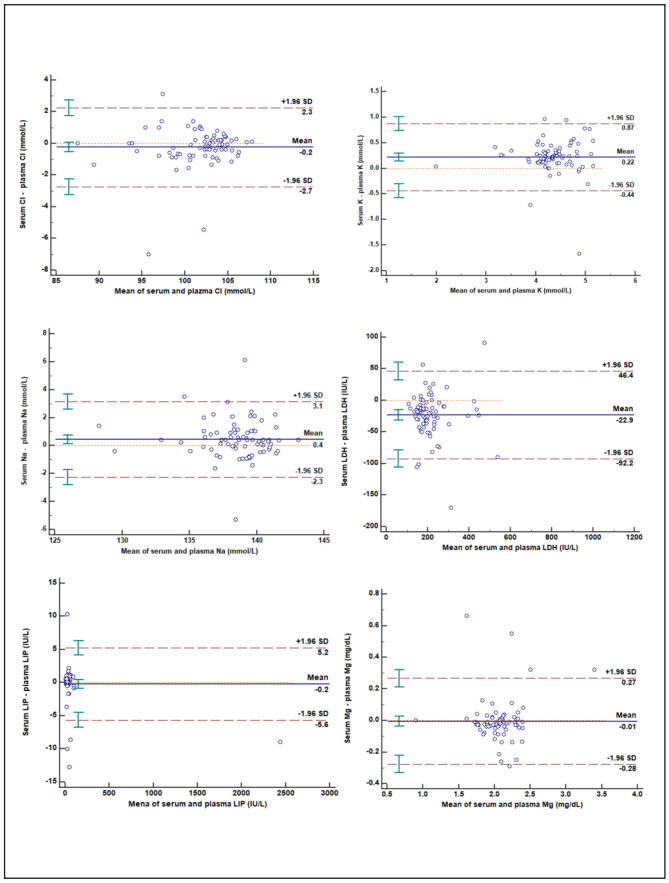

Figure 2E

Figure 2 (A - G): Bland-Altman plots for serum and plasma test results

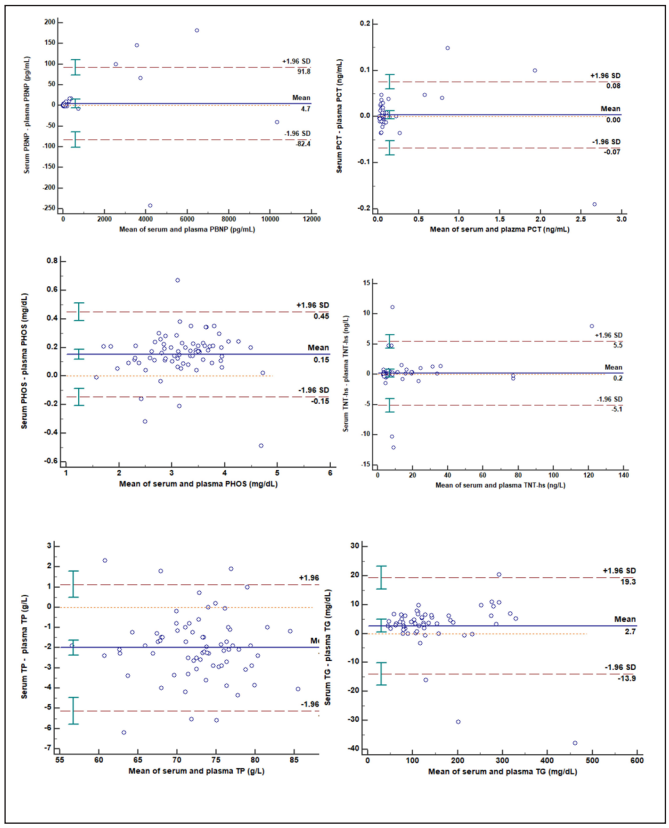

Figure 2F

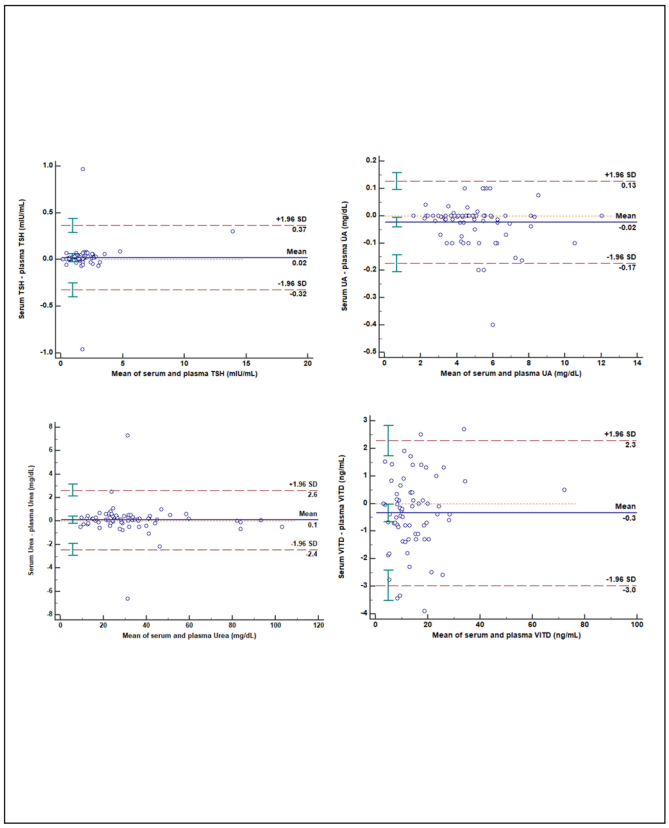

Figure 2G

Figure 2 (A - G): Bland-Altman plots for serum and plasma test results

Table 3. Sample probe errors and hemolysis index values

		Before the use of LIH	After the use of LIH
Total number of samples		15866	45059
Sample probe aspiration errors[a]		141 (0.88%)	200 (0.44)
Hemolysis[b]	Negative	12996 (81.9%)	35979 (79.8%)
	+	1558 (9.8%)	5084 (11.3%)
	++	1142 (7.2%)	3486 (7.7%)
	+++	118 (0.7%)	352 (0.8%)
	++++	52 (0.3%)	158 (0.4%)

[a]X^2=41.718, df=1, p<0.001[b]X^2=34.130, df=4, p<0.001. LIH: Lithium heparin tubes

Before the introduction of the LIH tube (February-March 2023), 141 aspiration errors appeared as instrument alerts in the system. The proportion of tubes with aspiration errors in the total number of samples was 0.88%. The proportional reduction in total aspiration errors and related device warnings was statistically significant two months after switching to plasma tubing. The proportion of specimens with aspiration errors to the total number of specimens was 0.44% between April and May 2022 (X 2=41.718, p<0.001). A medical laboratory technician takes approximately 15 minutes on average to deal with a tube with an aspiration failure warning. This involves identifying the specimen, locating it (removing it from the instrument), checking for clots visually, re-centrifuging, aliquoting, ensuring that the rerun is programmed in the instrument, and transferring the results to the LIS. These steps can divert the technician's attention from other tasks.[11] Therefore, aspiration errors can significantly impact TAT goals. We retrospectively assessed the specimen reception-device result output times of serum K and TNT-hs tests to measure the effect of switching to plasma use on TAT (March-April 2023). These were the two most common tests requested by the emergency department (Figure 3). LIH reduced the percentage of K orders that lasted more than 90 minutes between order-result time from 5.48% to 4.09% (X2=36.75, p<0.001).

Similarly, for TNT-hs orders, the percentage of time outliers decreased from 6.97% to 4.86% (X2=23.93, p<0.001). LIH successfully lowered the percentage of samples that exceeded the TAT out of the total number of samples. Using plasma reduced the order-result time for 95% of the emergency biochemistry and immunoassay samples from the emergency department. Before the intervention, 95% of 20957 samples took less than 110 minutes, while after the intervention, 95% of 21433 samples took less than 102 minutes (Figure 4).

We compared the median time from sample acceptance to device entry before and after plasma use (Figure 5). The median and interquartile values for this period decreased after plasma use in March and April 2023. Before plasma use (n=6838), the median and interquartile range were 21 (16-28) minutes, while after plasma use (n=12682), they were 17 (15.3-21.2) minutes (p<0.001).

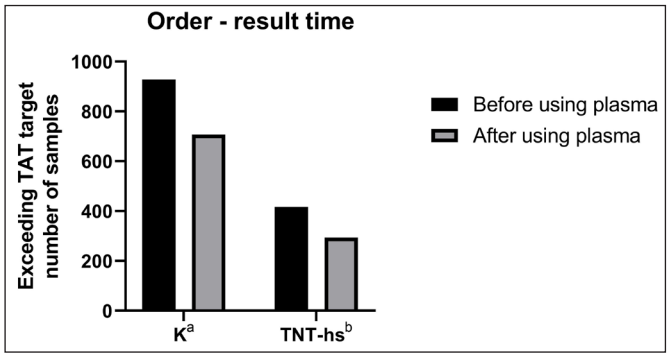

Figure 3. Order - result time analysis K and TNT-hs tests

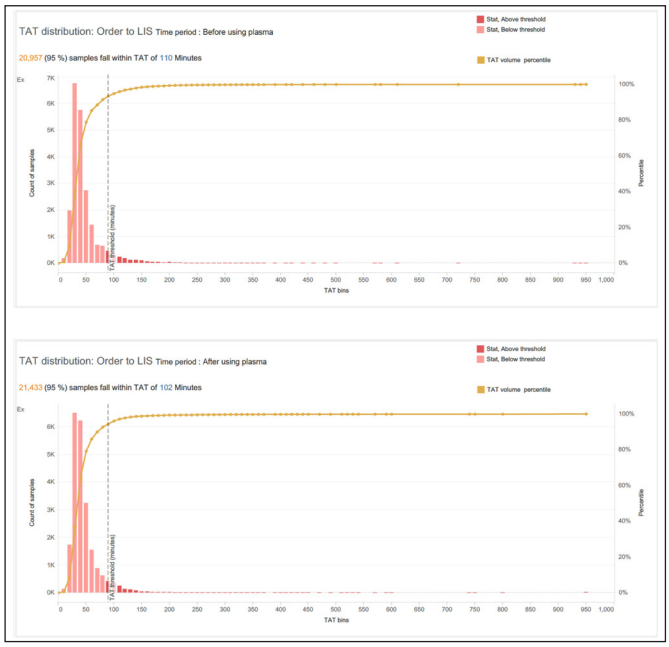

Figure 4. Sample Turnaround Time (TAT) distribution analysis.

DISCUSSION

Sample quality defects and clotting problems can cause errors in laboratory processes, especially with new-generation high-performance analyzers. Plasma is often a more suitable sample for laboratory tests than serum because it reduces TAT and avoids false results caused by fibrin remnants. Correct sample processing is essential for accurate and reliable test results, and the CLSI recommends validating plasma tubes locally before routine use.[1,6,12,13]

Our study found a mean bias of -4.66% for K and 5.96% for TP. These results are consistent with those of previous studies on these analytes. For example, using gel lithium heparin tubes, Ercan et al.[14] reported mean bias values of -2.63% and 2.37% for K and TP, respectively. The literature has previously documented a negative bias for K and a positive bias for TP in plasma tubes compared to serum tubes.[4,5,15] Consistent with the studies in the literature, our study found a positive bias of 13.95% for LDH compared to serum tubes.

Additionally, our study found no statistically significant differences between the hemolysis indices of plasma and serum tubes. In contrast to our findings, Arslan et al.[16] reported a negative bias for LDH. According to Hetu et al.[11] a positive bias for LDH in a Barricor mechanical separator heparinized plasma tube was consistent with our findings. The reason for this phenomenon is not fully understood. It is believed that LDH may be released from platelets and other cells into plasma during centrifugation.[17]

The observed total error (TE%) values for Na and Cl analytes exceeded the TEa% limits derived from the BV database. The mean bias (%) for Na and Cl analytes was -0.31% and 0.25%, respectively, while the calculated TE% values were 2.46% and 2.88%, respectively. Although these bias% values were consistent with the literature, the TE% values obtained by researchers in previous studies also exceeded the TEa% targets derived from BV. According to Oosterhuis et al.[10] analytes, such as Na and Cl, which are tightly regulated by homeostatic mechanisms, can be accurately measured. However, they recommend questioning the quality criteria set

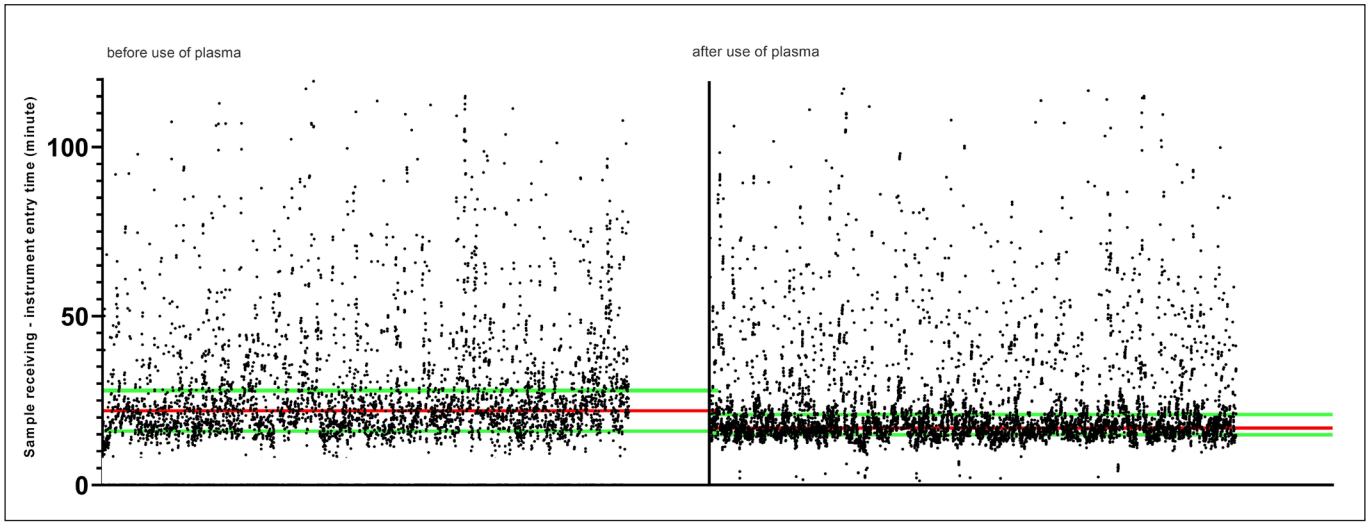

Figure 5. The median of the times for sample reception and instrument entry (Pre and post plasma period)

by BV and using alternative methods to determine the performance specifications for sodium.[10] Based on the limitations of BV targets, alternative targets for allowable bias were set for Na and K; the targets were±4 mmol/L and±3 mmol/L, respectively. For Cl TEa% was 5%, while for TP, the chosen limit was 8%.18 Table 4 summarizes the alternative targets for Na, K, Cl, TP, and LDH, according to Westgard's consolidated quality requirement targets. Upon re-evaluation using alternative targets, it was determined that a plasma sample could be used instead of a serum sample for these tests, except for LDH. Although our comparison results for LDH were close to the alternative error limit, the total error budget was used. Therefore, it is recommended that a reference interval transfer be performed if a plasma sample is used in the LDH assay.[6]

Table 4. Alternative TEa limits

Test	Mean bias (%)	TE (%)	TEa (%)-BV	TEa (%) -Westgard
Na	- 0.31 (0.4 mmol/L)*	2.46	0.7	± 4 mmol/L**
K	- 4.66 (0.2 mmol/L)*	7.77	4.8	± 3 mmol/L**
Cl	0.25	2.88	1.3	5
TP	2.83	5.96	3.5	8
LDH	13.95	15.60	15	15

*Mean bias (mmol/L),** Allowable absolute bias,TE: Total error, TEa: Total allowable error, BV: Biological variation

Many studies have compared serum and plasma samples using statistical and medical criteria. They used Bland-Altman differences plots and Passing-Bablok regression analyses to assess the statistical difference and agreement between the samples and TEa% targets from various sources to assess the medical significance of the differences. The regression analysis and the difference plot have different ways of interpreting the agreement and significance of the difference based on the confidence intervals of certain values.[4,14,19] Ercan et al.[14] used the Turkish Ministry of Health Total Error Guideline for TEa limits to compare with serum and gel plasma tubes. They found that the TE values for all the tests were below the TEa limits. They concluded that heparinized lithium plasma with a gel separator could replace the serum.[14] In a similar study, Arslan et al.[16] used BV TEa targets and obtained larger differences than the desired bias values for the K, LDH, and TP analytes in the plasma tube. However, when assessing this difference in the assays, a clinically significant difference was considered to be present when the difference between the mean concentration in one tube and the reference tube exceeded±2.8x (long-term standard deviation). Since they found clinically significant differences for K, LDH, and TP analytes, they reported that they could be used with reference value transfer.[4] Orhan et al.[20] observed that the K test in tubes with mechanical separators exceeded the TEa limit they set. Still, they suggested its use in the laboratory because of the benefits of using plasma. Hetu et al.[11] compared 65 different analytes and found that, except for K, TP, LDH, progesterone, and rheumatoid factor, the TE values obtained in plasma tubes with mechanical separators were within the BV TEa limits.[11]

Numerous research have been undertaken in the academic literature to investigate the disparities observed in the lactate dehydrogenase (LDH) test results when comparing plasma and serum samples, as well as to identify the underlying causes contributing to these discrepancies. There are several factors that contribute to this disparity, including variations in tube brands, plasma contamination with platelets, fragmentation of platelets, and fragmentation of erythrocytes. The literature has highlighted the notable issue of the impact of platelet residues and platelet lysis on plasma LDH levels, specifically in terms of their amplifying influence. These findings offer significant criteria that necessitate consideration when evaluating LDH levels. It has been noted that LDH measurements can be influenced by many processes that take place during serum preparation, including hemolysis and coagulation. In summary, it is important to consider the type of sample, preparation process, and tube selection when aiming to achieve accurate evaluation and interpretation of LDH measurement outcomes. The acquisition of this information holds significant value in enhancing the precision and dependability of outcomes in laboratory testing.[12,16,21-27]

In the Passing-Bablok regression analysis (Figure 1), the slope value for all tests, except for Ca and INS, had a 95% confidence interval that included 1. The slope value for Ca was 0.90 95% CI (0.84-0.97), and for INS, it was 0.84 95% CI (0.77-0.91). The intercept value confidence interval for all tests, except GGT and Ca, passed through 0. The intercept values and 95% CI for GGT and Ca were calculated as 1.00 (0.59-1.86) and 0.88 (0.22-1.49), respectively. The systematic error and proportional difference observed for the Ca, GGT, and INS analytes were not clinically significant, as all three tests remained within the BV %TEa limits (Table 1). Kösem et al.[23] similarly interpreted their findings on parathormone. According to Ercan et al.[14] the confidence interval for the CRP intercept did not contain a value of 0. However, they reported that this bias was not clinically significant. Our study, following that of Ferrari et al.[15] represents the second investigation examining insulin in various tubes. However, our findings concerning insulin did not correspond with the earlier report. We observed a negative bias compared to the serum tube. We believe future research should focus on evaluating insulin values at low, normal, and high levels for a more comprehensive understanding.

We collected and evaluated sample aspiration errors, hemolysis index values, and TAT data from the LIS before and after switching to plasma in our study. During the 2-month period we measured, the proportion of aspiration errors in all samples was 0.88% before the implementation phase, while this proportion dropped to 0.44% after switching to plasma (Table 3). This decrease was statistically significant and has been reported in similar studies. Ramakers et al.[28] reported a decrease in aspiration errors from 2.3% to 0.4% over six months. Hetu et al.[11] found that aspiration errors dropped from 2.01% before to 0.77% after the implementation. Our findings were consistent with those reported in the literature. However, the reduction rate of aspiration errors was low because of the relatively low number of aspiration errors and the short evaluation period. In our initial implementation phase, as shown in Table 3 with data sourced from actual patient samples, we did not observe a decrease in hemolysis rates. We assume that the utilization of plasma tubes did not influence our hemolysis rates. Such an outcome might be attributed to persistent preanalytical errors during blood collection.

Nevertheless, our reduction rate is significant for high-volume emergency laboratories. These data indicate that the sample quality improved in the plasma phase compared with the previous phase using serum samples. This resulted in a decrease in the time required by the laboratory staff for clotting time and corrective actions related to fibrin. As a result, laboratory staff can spend more time on other critical tasks and less time on non-value-added work.

Previous studies have shown the positive effects of plasma on TAT. Hetu et al.[11] studied TAT for the K test and found significant reductions in the average time between sample acceptance and result confirmation. Similarly, Ramakers et al.[28] reported a significant decrease in median TAT time using Barricor tubes. Badiou et al.[29] reported significant reductions in the median time to specimen acceptance and turnaround time in the first 15-day measurement period using Barricor tubes instead of gel LIH tubes. Their study reduced the time between sample collection from the emergency department and transfer of results to the LIS by 10 minutes for 95% of TAT results for some analytes such as creatinine, CRP, K, Na, and Na and Hs-cTnT measured for eight analytes.[11,28,29] In our laboratory, we performed three separate analyses to measure improvements in TAT after switching to plasma tubing. Using the K and TnT-Hs assays as examples, we observed a significant decrease in the number of samples exceeding the TAT target ($p<0.001$). As shown in Figure 3, the reductions in TAT time after the transition phase are consistent with those reported by Hetu et al.[11] Our second analysis examined sample-based TAT distributions before and after the transition phase. When we analyzed the time between ordering and receiving test results for all patient samples, we found that 95% of the patients received results in 102 minutes instead of 110 minutes when using serum, as shown in Figure 4. In our other sample-based analysis, when we analyzed the median values of the laboratory sample acceptance-device entry times, we decreased from 21 minutes to 17 minutes ($p<0.001$). We think that a partial reduction occurred here due to not having to wait before centrifugation Figure 5. According to our findings, these significant reductions in TAT times are in agreement with the literature. Our study encountered certain limitations concerning insulin. Given the conflicting results for plasma insulin, we propose that examining various plasma/serum insulin levels is essential to determine if these discrepancies are influenced by such variations.

CONCLUSION

In this study, we compared the performance of serum and LIH tubes in various clinical chemistry and immunoassay tests. According to the regression analysis and Bland-Altman plots, most tests had no significant difference between the serum and LIH. However, for some analytes, TE values exceeded the TEa limits derived from the BV database. Therefore, we determined the alternative allowable error limits for some tests and found that plasma tubes could be used instead of serum tubes for most tests, except for LDH. We also observed that the use of lithium heparinized plasma tubes improved the sample quality reduced the incidence of aspiration errors, and decreased TAT times in the emergency laboratory. Our study showed that LIH tubes can replace serum tubes in most clinical chemistry and immunoassay tests. Plasma not only improved the sample quality but also reduced TAT times and the incidence of aspiration errors. Therefore, using LIH tubes in clinical laboratories can improve healthcare quality and reduce the workload of the laboratory staff.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Başakşehir Çam and Sakura City Hospital Clinical Researches Ethics Committee (Date: 22.03.2023, Decision No: 117).

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

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Prognostic value of inflammatory markers for mortality in hemodialysis patients: a retrospective study with over 3-year follow-up

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ABSTRACT

Aims: In chronic kidney disease (CKD), chronic systemic inflammation contributes to premature ageing and morbidity; it is a predictor of overall mortality. In this study, we aimed to investigate prognostic value of inflamatory markers including systemic immune-inflammation index (SII), pan-immune-inflammation value (PIV), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) for mortality outcomes in hemodialysis patients.

Methods: In this retrospective study, CKD patients on maintenance hemodialysis between January 1, 2020 and January 31, 2020 were included. SII, PIV, NLR, PLR values of the patients were calculated. SII was calculated by (neutrophil count x platelet count)/lymphocyte count; PIV was calculated by (neutrophil count x platelet count x monocyte count)/lymphocyte count. Mortality rate of the study population during approximately 38-month follow-up period was calculated. The relationships of inflamatory markers and other variables with mortality were analysed.

Results: Of 162 patients, 53.1% were male and 46.9% were female (mean age: 61.6±13.5). During 38-month follow-up period, a total of 60 patients (37%) died. Compared with surviving group, NLR values, mean age and the rate of diabetes mellitus (DM) and coronary artery disease (CAD) comorbidities were higher (p=0.012, p <0.001, p=0.008, p <0.001 respectively) and albumin, uric acid and creatinin levels were lower (p <0.001, for each) in the nonsurvivor group. There was no difference between these two groups in terms of PIV and SII values and CRP levels. In Cox regression analysis, presence of CAD (Exp ß: 2.116; 95% CI, 1.222-3.648; p=0.007), age (Exp ß: 1.049; 95% CI, 1.022-1.077; p <0.001), serum uric acid (Exp ß: 0.721; 95% CI, 0.559-0.929; p=0.011) and albumin levels (Exp ß: 0.395;95% CI, 0.158-0.984, p=0.046), NLR (Exp ß: 1.345; 95% CI, 1.152-1.1570; p <0.001) and PLR (Exp ß: 0.993; 95% CI, 0.989-0.997; p=0.002) were found to be associated with mortality.

Conclusion: There was no difference between nonsurviving and surviving group in terms of PIV and SII values and CRP levels. Age, presence of CAD, serum uric acid and albumin levels and NLR and PLR values were associated with all-cause mortality, independently. Prospective studies with larger number of hemodialysis patients and serial measurements of inflammatory markers are needed.

Keywords: Hemodialysis, systemic immune-inflammation index, pan-immune-inflammation value, mortality, inflammatory markers

INTRODUCTION

During the course of chronic kidney disease (CKD) progression, there is a gradual decrease in kidney function, which may eventually lead to the necessity of renal replacement therapy (RRT). Hemodialysis is one of the most important RRT and despite the developments in hemodialysis technology, the mortality rate among hemodialysis patients is still high.[1,2] Some factors such as age, albumin levels, comorbid conditions, underlying kidney disease, cardiovascular and psychosocial status, residual renal function, dietary factors, etc, may affect mortality rates in hemodialysis patients.[2,3]

In CKD patients, chronic systemic inflammation has multifactorial etiology; it contributes to premature ageing and morbidity (especially cardiovascular disease, malnutrition and anemia) and it is a predictor of all-cause mortality.[4] In some previous studies in hemodialysis patients, it was reported that the increased systemic inflammation-based prognostic scores including Glasgow prognostic score and modified Glasgow prognostic score (inflammation-based scores based combination of albumin and CRP levels), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte

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ratio (PLR), prognostic index and prognostic nutritional index were associated with mortality during 42-month follow-up; and inflammation scores, which include NLR, monocyte-to-lymphocyte ratio and PLR, might be useful in predicting prognosis and all-cause mortality.[5,6]

The systemic immune-inflammation index (SII), calculated using neutrophil, platelet and lymphocyte counts has recently been introduced as a new and powerful prognostic marker in cancer patients.[7,8] In a study conducted in Turkey, it has been showed that high SII could predict mortality in cancer patients receiving palliative care.[9] In a recent study, it was reported that pan-immune-inflammation value (PIV) (a novel inflamatory marker calculated using neutrophil, platelet, monocyte and lymphocyte counts) was significantly associated with an increased risk of all-cause mortality in peritoneal dialysis patients.[10] In the literature, there is limited data about relation between SII and mortality outcomes of hemodialysis patients.[11,12] As far as we know, there is no data about prognostic value of PIV in hemodialysis patients.

In this retrospective study, we aimed to investigate prognostic value of inflamatory markers including SII, PIV, NLR and PLR for mortality outcomes and also to investigate other factors related with all-cause mortality in hemodialysis patients during 38-month follow-up period.

METHODS

The study was carried out with the permission of by KTO Karatay University Faculty of Medicine Non-medicine and Non-medical Device Researches Ethics Committee (Date: 27.04.2023, Decision No: 2023/018). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this retrospective study, CKD patients (aged >18 years) who were continuing on maintenance hemodialysis in dialysis center between January 1, 2020 and January 31, 2020 were included. The patients on hemodialysis less than 3 months; the patients who had acute or chronic infection, who had rheumatological or hematological disease; the patients under immunesuppressive or anti-inflammatory treatment and the patients with missing data were excluded from the study.

Demographical, clinic and laboratory data of patients, such as age and gender, primary cause of kidney disease, comorbid conditions, smoking status, hemoglobin level, neutrophil, lymphocyte, platelet count, Kt/V value, serum lipids, creatinine, sodium, potassium, parathormon, ferritin, phosphorus, calcium, uric acid, C-reactive protein (CRP) and albumin levels were obtained from the patients' file records. Blood samples for routine blood tests were taken before dialysis. In all patients in this study, hemodialysis was performed with heparin except two patients with high risk of bleeding. NLR, PLR, SII and PIV of the patients were calculated. SII was calculated by (neutrophil count x platelet count)/lymphocyte count; PIV was calculated by (neutrophil count x platelet count x monocyte count)/lymphocyte count. NLR ratios were also grouped as 'low' and 'high' with cut-off value of 3 (<3 and ≥3 respectively).[13]

For mortality outcomes, the patients' clinical status on April 1, 2023 were detected from the medical records and the mortality rate of the study population during this approximately 38- month follow-up period was calculated. The relationships of the inflamatory markers (SII, PIV, NLR and PLR) and also other variables with mortality were analysed.

Statistical Analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) for Windows version 22 program. Categorical variables were compared using Chi-square test. T-test and Mann Whitney U test were used for comparisons between groups. Backward Cox regression analysis was used to identify independent variables related with mortality. In Cox-regression analysis, parameters with $p < 0.1$ in paired comparison tests (T-test, Mann Whitney U test and Chi-square test) were included (age, CAD, DM, NLR, PLR, SII, serum creatinine, uric acid, albumin, phosphorus, lymphocyte, vascular access). P values <0.05 were accepted as statistically significant.

RESULTS

A total of 195 patients were included in the study at the beginning. But, during follow-up period, 27 patients were transferred to other hemodialysis centers, 3 patients underwent renal transplantation and hemodialysis was discontinued in two patients. Finally, data of remaining 162 patients were analysed. Mean age was 61.6±13.5; 76 patients (46.9%) were female and 86 (53.1%) were male. Median time on hemodialysis was 65.5 (3-324) months. Underlying etiologies for kidney failure were diabetes mellitus (DM) (52 patients, 32.1%), hypertension (37 patients, 22.8%), polycystic kidney disease (15 patients, 9.3%), urological causes (15 patients, 9.3%), glomerulonephritis (12 patients, 7.4%), other etiologies (12 patients, 7.4%) and unknown underlying etiologies (15 patients, 9.3%). As vascular access for hemodialyis, 123 patients (76.9%) had arteriovenous fistula (AVF) or arteriovenous graft (AVG); 39 patients (24.1%) had central venous catheter (CVC). Of the patients, 107 (66%) had hypertension; 69 (42.6%) had coronary artery disease (CAD); 66 (40.7%) had DM; 19 (11.7%) had chronic obstructive pulmonary disease as comorbid diseases; 39 patients (24.1%) had smoking history. Median PIV and

SII values of all study group were 431 (5-3570) and 835.5 (112.5-7843.7) respectively. Demografic, clinical and laboratory features of the study population were shown in Table 1. During 38-month follow-up period, a total of 60 patients (37%) died; remaining 102 patients (63%) were on hemodialysis.

When the group of patients who died and those surviving were compared, NLR values (as absolute value and as grouped with specific cut-off value of 3, p=0.012 and p=0.035 respectively), mean age (p <0.001) and the rate of DM (p=0.008) and CAD comorbidities (p <0.001) were higher and serum albumin, uric acid and creatinin levels were lower (p <0.001, for each) in the group of patients who died (Table 1). As vascular access for hemodialyis, 66.7% of patients had AVF/AVG and 33.3% had CVC in nonsurviving group; these numbers in surviving group were 82.2% and 17.8%, respectively (p=0.034) (Table 1). There was no differences between these two groups in terms of PIV, SII values, CRP, ferritin, serum lipids and the other parameters (Table 1).

Table 1. Baseline characteristics of the patients and comparison of survivor and nonsurvivor groups

Parameter	All patients(n=162)	Survivors(n=102)	Nonsurvivors(n=60)	P value
Age, year	61.6±13.5	57.513.5	68.7±10.5	<0.001
Gender,				0.961
Female, n(%)	76 (46.9%)	48 (47.1%)	28 (46.7%)	
Male, n(%)	86 (53.1%)	54 (52.9%)	32 (53.3%)	
Smoker, n(%)	39 (24.1%)	24 (23.5%)	15 (25%)	0.234
Presence of diabetes, n (%)	66 (59.3%)	33 (32.7%)	33 (55.0%)	0.008
Presence of hypertension, n (%)	107 (66.0%)	63 (62.4%)	44 (73.3%)	0.174
Presence of COPD, n (%)	19 (11.7%)	11 (10.9%)	8 (13.3%)	0.625
Presence of CAD, n (%)	68 (42.0%)	32 (30.7%)	37 (61.7%)	<0.001
Vascular access,				0.034
AVF/AVG	123 (76.0%)	83 (82.2%)	40 (66.7%)	
CVC, n(%)	39 (24.0%)	19 (17.8%)	20 (33.3%)	
Hemodialyis vintage, month	65.5 (3-324)	45.5 (3-226)	49.0 (3-324)	0.504
PIV value	431.1 (5-3570)	280.9 (5.6-3570.7)	322.3 (22.3-3423.9)	0.162
SII value	835.3 (112.5-7843.8)	607.8 (112.5-7540.8)	711.5 (139.4-7843.5)	0.062
NLR value	3.62 (0.9-43.8)	2.79 (0.90-18.1)	3.21 (1.2-43.8)	0.012
< 3	83 (51.2%)	59 (% 57.8)	24 (% 40)	0.035
≥ 3	77 (48.8%)	43 (% 42.2)	36 (% 60)	0.035
PLR value	168 (51-1737)	134.7 (53.9-1737.5)	155.9 (51.5-1627.3)	0.087
Hemoglobin, g/dl	12.7±1.6	12.8±1.6	12.7±1.6	0.649
Monocyte, 10^3/µl	0.50±0.25	0.51±0.26	0.50±0.23	0.853
RDW, %	14.1 (9.6-20.4)	14.0 (9.9-19.3)	13.8 (9.6-20.4)	0.931
MPV, µm^3	8.16 (5.6-14.8)	7.9 (5.9-13.5)	8.1 (5.6-14.8)	0.312
Neutrophil, 10^3/µl	4.9 (0.72-11.8)	4.5 (0.72-10.90)	4.79 (1.68-11.80)	0.459
Lymphocyte, 10^3/µl	1.69 (0.11-4.54)	1.68 (0.24-4.54)	1.47 (0.11-3.67)	0.075
Platelet, 10^3/µl	228.7 (74.5-454.0)	227.5 (91.1-454.0)	215 (74.5-443.0)	0.548
Serum albumin, g/dl	3.9 (2.1-4.8)	4.0 (2.6-4.8)	3.8 (2.1-4.5)	<0.001
Serum uric acid, mg/dl	5.7±1.0	6.0±1.0	5.4±1.0	<0.001
C-reactive protein, mg/L	12.2 (0.1-144)	138 (132-144)	138 (129-143)	0.784
Serum creatinine, mg/dl	7.8±2.1	8.4±2.2	7.0±1.7	<0.001
Calcium, mg/dl	8.7±0.8	8.6±0.8	8.8±0.7	0.107
Potassium, mmol/L	4.9±0.65	4.96±0.64	4.81±0.67	0.140
Phosophorus, mg/dl	4.8 (1.7-9.9)	5.0 (1.7-8.5)	4.6 (2.2-9.9)	0.079
Sodium, mmol/L	138.7 (129-143)	138 (132-144)	138 (129-143)	0.784
Triglyserides, mg/dl	189.0 (42.0-822.0)	164.0 (42.0-603.0)	162.5 (48.0-822)	0.820
HDL-cholesterol, mg/dl	37.5 (15.0-78.0)	37.0 (15.0-78.0)	36.0 (20.0-64.0)	0.628
LDL-cholesterol, mg/dl	89.7 (40.0-210.0)	85.0 (40.0-210.0)	87.5 (40.0-183.0)	0.864
Triglyserides/ HDL-cholesterol	5.87 (1.04-39.14)	4.75 (1.04-23.19)	4.6 (1.17-39.14)	0.880
ALT, U/L	15.3 (1.0-100.0)	12.0 (1.0-100.0)	12.0 (1.0-66.0)	0.824
Ferritin, µg/L	805.2 (11.9-1650)	603.2 (47.9-1650)	855.0 (11.9-1650)	0.213
Parathormon, ng/L	562.5 (0.1-2941)	495.4 (0.1-2941.9)	422.6 (12.5-1862.5)	0.600
HbA1c, %	7.0 (4.1-13.2)	6.6 (4.1-13.2)	6.9 (5.4-11.1)	0.247
Kt/V	1.6 (0.95-2.19)	1.6 (0.95-2.19)	1.61 (1.36-1.97)	0.295

Data are given as mean±SD or median (minimum-maximum). Abbreviations: COPD, chronic obstructive pulmonary disease; CAD, coronary artery disease; AVF/AVG, arteriovenous fistula/arteriovenous graft; CVC, central venous catheter; PIV, pan-immune-inflammation value; SII, systemic immune-inflammation index; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RDW, red blood cell distribution width; MPV, mean platelet volume; HDL, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; HbA1c, glycosylated hemoglobin.

In Backward Cox regression analysis, performed to determine the independent markers of mortality, the parameters with p <0.1 in paired comparison tests (age, CAD, DM, NLR, PLR, SII, uric acid, albumin, phosphorus, lymphocyte, serum creatinine, vascular access) were included and presence of CAD (Exp ß: 2.116; 95% CI, 1.222-3.648; p=0.007), age (Exp ß: 1.049; 95% CI, 1.022-1.077; p <0.001), serum uric acid (Exp ß: 0.721; 95% CI, 0.559-0.929; p=0.011) and albumin levels (Exp ß: 0.395;95% CI, 0.158-0.984, p=0.046), NLR (Exp ß: 1.345; 95% CI, 1.152-1.1570; p <0.001) and PLR (Exp ß: 0.993; 95% CI, 0.989-0.997; p=0.002) were found to be associated with mortality, independently (Table 2).

Table 2. Cox regression analysis (Backward) to determine independent variables

Variable	Model 1 Chi-square=66.008 P Exp(B) CI %95	Model 6 Chi-square=65.231 P Exp(B) CI%95
Presence of CAD	0.005 2.317 (1.284-4.182)	0.007 2.116 (1.228-3.648)
Age, year	0.001 1.047 (1.018-1.076)	<0.001 1.049 (1.022-1.077)
NLR value	0.004 1.328 (1.093-1.613)	< 0.001 1.345 (1.152-1.570)
PLR value	0.001 0.993 (0.988-0.997)	0.002 0.993 (0.989-0.997)
Lymphocyte, 10^3/µl	0.996 0.999 (0.636-1.569)	0.084 1.907 (0.916-3.969)
Serum uricacid, mg/dl	0.007 0.674 (0.505-0.899)	0.011 0.721 (0.559-0.929)
Serum albumin, g/dl	0.034 0.329 (0.118-0.918)	0.046 0.395 (0.158-0.984)
Presence of diabetes	0.886 0.958 (0.535-1.716)	
SII value	0.753 1.000 (0.999-1.001)	
Phosophorus, mg/dl	0.503 1.103 (0.828-1.469)	
Serum creatinine, mg/dl	0.748 1.029 (0.863-1.227)	
Vascular access	0.251 0.721 (0.413-1.260)	

Parameters with p <0.1 in paired comparison tests were included in the Cox regression analysis. Abbreviations: CAD, coronary artery disease; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index.

DISCUSSION

Findings of this study showed that all-cause mortality rate in our hemodialysis patients was 37% during 38-month-follow-up period and age, presence of CAD, serum uric acid and albumin levels and NLR and PLR values were associated with all-cause mortality.

In a previous study from Turkey, mortality rate in hemodialysis patients was reported to be 39.2% during 4-year follow-up.[2] In United States Renal Data System, the mortality rate in 2016 was 166 per 1,000 patient-years for hemodialysis patients.[1] In a study from Japan, all-cause mortality rate among hemodialysis patients was 29.1% during 42-month follow-up.[5]

In our study, in the group of patients who died during follow-up period, NLR values, age and the rate of DM and CAD as comorbidities were higher; whereas albumin, uric acid and creatinin levels were lower; there was no differences between nonsurviving and surviving group in terms of PIV and SII values and CRP levels. A recent study including a part of the MONitoring Dialysis Outcomes (MONDO) participants, nonsurviving patients were older, and they had lower albumin and creatinin levels, and higher levels of inflammatory markers (white blood cell count, neutrophil count, NLR, CRP) at baseline.[14] Findings of this study showed that these inflammatory markers which decreased after initiation of hemodialysis, increased approximately 6 months preceding death; whereas lymphocyte count, serum albumin, and hemoglobin levels, which increased after hemodialysis initiation, decreased in months before death.[14]

In a retrospective study, higher inflammation scoring including monocyte-to-lymphocyte ratio, NLR and PLR was found to be independently associated with all-cause mortality in hemodialysis patients, whereas CRP, a traditional marker of inflammation, was not associated with mortality.[6] Unlike our study, in one of limited number of the previous studies investigating the relationship between SII and prognostic outcomes in hemodialysis patients, 1-year cumulative survival rate was reported to be lower in SII high group.[12] These may be atributed to the different follow-up times, different sample size and methodology between studies. In a study in coronavirus disease-2019 (COVID-19) hemodialysis patients, it was concluded that SII could be used to predict the need for intensive care unit and mortality risk.[11] However, in another study in maintenance hemodialysis patients with COVID-19, SII was not found as an independent risk factor for mortality in the multivariate regression analysis; it was reported that NLR had favorable predictive value and SII did not contribute more than NLR in predicting mortality.[15] In the study of Kato et al.[5], it was shown that increased Glasgow prognostic score/modified Glasgow prognostic score (inflammation-based scores including combination of albumin and CRP levels), NLR and PLR values were associated with all-cause mortality in hemodialysis patients. Our Cox regression analysis revealed that, age, presence of CAD, serum uric acid and albumin levels and NLR and PLR values were associated with all-cause mortality, independently. Similarly, in the study of Alanlı et al.[2], older age, low albumin levels and low left ventricle ejection fraction values were among the factors related with increased mortality risk in hemodialysis patients. Albumin level is related with inflamation and nutritional status. Inflamation and malnutrition which are prevalent in hemodialysis patients, are interrelated creating a vicious cycle and closely linked to atherosclerosis-

related cardiovascular diseases: They all contribute to the increased risk of morbidity and mortality in hemodialysis patients.[16] Regarding uric acid level, our nonsurviving group had lower uric acid level and our Cox regression analysis showed that uric acid level associated with all-cause mortality, in accordance with the studies of Li et al.[16] and Murea et al.[17] Underlying mechanism of this effect in hemodialysis patients is speculative.[18] In the study of Li et al.[16] uric acid levels were found to be correlated with nutritional and inflammatory status.

We could not found any data about prognostic value of PIV in hemodialysis patients. Unlike our study, in a previous study in peritoneal dialysis patients, PIV value was significantly associated with an increased risk of all-cause mortality.[10] In the study of Kazan et al.[18] it was reported that PIV and SII values might be useful in predicting remission in low-moderate risk idiopathic membranous nephropathy patients.

There are some limitations of our study: First, it is a single center and retrospective study with relatively small sample size and it would be better to perform aditional analysis in subgroups that match each other regarding parameters that affect mortality such as age, CAD, etc. Second, according to our study design, we performed analyzes on a single measurement in patients undergoing dialysis within a specified time period. It might be more appropriate to investigate the prognostic value of inflammation markers, including SII, PIV, NLR and PLR, for mortality by performing 6-month serial measurements starting from the initiation of hemodialysis and even just before hemodialysis. These may be considered in future studies.

CONCLUSION

Our findings showed that, there was no difference between the nonsurviving group and the surviving group in terms of PIV and SII values and CRP levels. In the group of patients who died during follow-up period, NLR values were higher; age, presence of CAD, serum uric acid and albumin levels and NLR and PLR values were associated with all-cause mortality, independently. To determine prognostic role of inflammatory markers for mortality in hemodialysis patients, prospective studies with larger number of patients and serial measurements of inflammatory markers are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of by KTO Karatay University Faculty of Medicine Non-medicine and Non-medical Device Researches Ethics Committee (Date: 27.04.2023, Decision No: 2023/018).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Impact of Ramadan fasting on eGFR in patients with late stage chronic kidney disease

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ABSTRACT

Aims: Ramadan fasting is a significant religious practice observed annually by approximately 1.9 billion adult Muslims worldwide. However, its potential impact on kidney health in individuals with chronic kidney disease (CKD) remains a subject of concern. This study aimed to investigate the effects of Ramadan fasting on renal function in patients with stage 3-5 CKD and to identify any associated risk factors.

Methods: A single-center, self-controlled longitudinal observational study was conducted on 192 stable patients with stage 3-5 CKD who observed Ramadan fasting. The fasting period was about 14-15 hours per day for one month Various clinical parameters, including eGFR, blood urea nitrogen (BUN), sodium (Na^+), potassium (K^+), calcium (Ca^{++}), phosphorus, parathyroid hormone (PTH), albumin, uric acid, fasting glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and hemoglobin (Hgb), were measured before and after Ramadan fasting. $P<0.05$ was assumed significant.

Results: The study results demonstrated no significant deterioration in eGFR during Ramadan fasting (pre-Ramadan: 43.54±11.04 vs post-Ramadan: 44.28±11.51, $p=0.063$). Additionally, traditional risk factors for CKD progression, such as diabetes mellitus (DM), hypertension (HT), cardiovascular disease CVD, and age, did not show a significant association with eGFR changes during fasting ($p>0.05$). Furthermore, the use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARB) did not impact eGFR (0.084). Notably, Ramadan fasting led to improvements in metabolic parameters, such as fasting glucose and lipid profile, except for triglyceride levels ($p<0.001$).

Conclusion: The study results indicate that Ramadan fasting does not appear to have adverse effects on kidney function in individuals with CKD, although certain metabolic changes were observed.

Keywords: Ramadan fasting, chronic kidney disease, glomerular filtration rate, metabolic parameters, kidney function

INTRODUCTION

Ramadan fasting is a fundamental pillar of Islam and involves abstinence from food and drink from dawn to sunset for approximately 30 days. This annual period of fasting poses potential physiological challenges to the human body, including the renal system. Existing literature is controversial regarding the impact of Ramadan fasting on kidney function in individuals with chronic kidney disease. This controversy may vary depending on factors such as the study design, duration of fasting, presence of comorbidities, and guidance provided by healthcare providers.[1-4] Additionally, previous studies have primarily focused on acute renal hemodynamic alterations during the Ramadan period, and the long-term impact of Ramadan fasting on renal function remains unknown.[1,2]

Understanding the effects of Ramadan fasting on kidney health is of great importance for healthcare professionals to ensure appropriate guidance and management for individuals observing fasting during this holy month. It allows for the identification of potential risks and the implementation of preventive measures to safeguard renal function. Furthermore, it assists in addressing the concerns of individuals with pre-existing kidney diseases who wish to observe the fast.

The Muslim calendar follows a lunar cycle spanning 355 days per year. Consequently, the month of Ramadan can occur during any period of the Gregorian calendar, completing its full cycle approximately every 33 years. This leads to Ramadan fasting taking place across different seasons, resulting in varying durations of

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fasting. In equatorial regions, the average fasting period ranges from 12 to 14 hours, while in certain geographical locations, it can extend up to 22 hours.[5]

This study aimed to investigate renal function longitudinally for six months in patients with stage 3-5 chronic kidney disease (CKD) who underwent approximately 14-15 hours of Ramadan fasting for one month.

METHODS

The study was carried out with the permission of by Zonguldak Bülent Ecevit University Non-interventional Clinical Researches Ethics Committee (Date: 10.06.2022, Decision No: 2022/14). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This is a single-center, self-controlled longitudinal, and observational study conducted at our state hospital's nephrology outpatient clinic between March 2021 and July 2022. The study included 195 patients with stable stage 3-5 chronic kidney disease (CKD) who were regularly monitored in our nephrology outpatient clinic and observed fasting during the month of Ramadan (Figure 1). Participants were followed up for a duration of six months. Demographic and clinical characteristics of the participants were recorded, and informed consent was obtained. We relied on the measurements that were performed in our central laboratory.

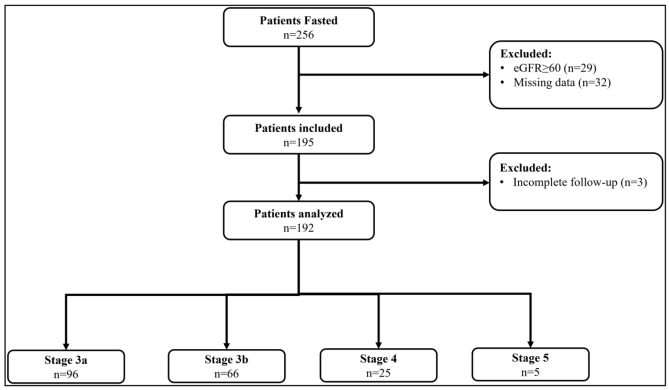

Figure 1. Flowchart of the study population, Abbreviations: eGFR: estimated glomerular filtration rate

Inclusion criteria consisted of participants aged above 18 years, those who observed fasting during Ramadan and had an estimated glomerular filtration rate (eGFR) of <60, as well as patients with a recent blood sample analysis before the study commencement.

Exclusion criteria encompassed patients under 18 years of age, those with active malignancy, acute kidney injury on CKD, a history of hospitalization or missing data.

Measurements: The study collected data on a range of variables, including age, diabetes mellitus (DM), body mass index (BMI), hypertension (HT), gender, cardiovascular disease (CVD), cerebrovascular event (CVE), lung disease, smoking, use of oral antidiabetic medication (OAD) and insulin, utilization of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARB), beta blockers, and alpha blockers, as well as parameters such as proteinuria, physician consultations, chronic kidney disease (CKD) etiology, CKD stages, estimated glomerular filtration rate (eGFR), blood urea nitrogen (BUN), sodium (Na^+), potassium (K^+), calcium (Ca^{++}), phosphorus, parathyroid hormone (PTH), albumin, uric acid, fasting glucose, total cholesterol (T. cholesterol), triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and hemoglobin (Hgb). These measurements were taken before Ramadan (pre-Ramadan), after Ramadan (post-Ramadan month-6), and, in some cases, during the 6-month follow-up period. The study aimed to assess changes in these laboratory parameters during and after Ramadan fasting in patients with chronic kidney disease.

BMI calculation: BMI = weight (in kilograms) / (height [in meters])^2.

Physician consultations: A group of patients with CKD (but not patients with eGFR <30 ml/min/1.73 m²) applied to their physician for Ramadan fasting counseling and received advice relating to drug and diet adjustment during Ramadan fasting.

Data source: Data were collected from the hospital software and nephrology clinic documents.

Statistical Analysis

The data analysis was conducted using SPSS version 15.0 for Windows. Descriptive statistics, including mean (± standard deviation), median (range or interquartile range), and proportions (n, %), were employed to summarize and describe the demographic characteristics and baseline measurements of the study population. Paired t-tests or Wilcoxon signed-rank tests were utilized to compare paired measurements within the same group, such as pre-Ramadan versus post-Ramadan month-6 data. Chi-square tests and Fisher's exact tests were employed to assess the association between categorical variables, such as gender and comorbidities, and outcomes of interest, including cardiovascular disease, lung disease, and smoking status. Regression analysis was conducted to explore the impact of independent variables on dependent variables, potentially revealing any relationships between different factors. A significance level of $p < 0.05$ was considered statistically significant, indicating that results with a p-value below this threshold were considered noteworthy and unlikely to occur due to chance alone.

RESULTS

A total of 195 participants were initially evaluated for the study. However, three patients (two from stage 4 and 1 from stage 5) had to undergo hemodialysis treatment due to worsening kidney functions during the follow-up and were subsequently excluded from the analysis. These individuals had an eGFR of less than 20 ml/min/1.73 m² and had participated in Ramadan fasting without prior counseling. The vast majority of the remaining patients (nearly all) successfully completed a full month of Ramadan fasting, adhering to a fasting duration of 14-15 hours.

For an additional subset of the study population, 30 CKD patients with an eGFR below 30 ml/min/1.73 m² observed Ramadan fasting without receiving any prior counseling. Their experiences were recorded retrospectively based on their statements. Table 1 presents a comprehensive analysis of the demographic characteristics, comorbidities, and clinical parameters of the study participants who had stage 3-5 CKD and participated in Ramadan fasting.

Table 1. The clinical and labarotory features of the participants

Age, years, mean±SD	62.64±12.59
BMI, kg/m², mean±SD	29.17±4.93
Gender, male, n (%)	107 (55.7)
eGFR (CKD-EPI 2021), mean±SD	42.03±11.79
Mean Ramadan days, mean±SD	29.74±1.18
Smoking, n (%)	11 (5.7)
ACEi/ARB, n (%)	72 (37.5)
Beta blocker, n (%)	38 (19.7)
Alpha-blocker, n (%)	15 (7.8)
Physician consultation pre-Ramadan, n (%)	50 (26.04)
CKD etiology, n (%)	
DM	54 (28.1)
HT	110 (57.3)
PCKD	5 (2.6)
GN	8 (4.2)
Other	15 (7.8)
DM, n (%)	51 (25.5)
HT, n (%)	180 (93.7)
CVD, n (%)	33 (17.2)
CVE, n (%)	0 (0.0)
Lung disease, n (%)	6 (3.1)
OAD, n (%)	51 (26)
Insulin, n (%)	20 (10.4)
Polypharmacy, n (%)	27 (14.06)
Proteinuria, mg/day †	556 (0-5123)
CKD stage, n (%)	
Stage 3a	96 (50.0)
Stage 3b	66 (34.4)
Stage 4	25 (13.0)
Stage 5	5 (2.6)

Abbreviations: ACEi: angiotensin converting enzyme inhibitor, ARB: aldosteron receptor antagonist, BMI: body mass index, CVD: cardiovascular disease, CVE: cereberovascular event, DM: diabetes mellitus, eGFR: estimated glomerular filtration rate, GN: golmerulonephritis, HT: hypertension, PCKD: polyscystic kidney disease,, OAD: oral antidiabetic drug, †Expressed as range (minimum -maximum)

Out of the total 195 CKD patients, 50 received a consultancy just before Ramadan and were provided with relevant advice.

The study's results revealed a slight increase in estimated glomerular filtration rate (eGFR) from pre-Ramadan (43.54±11.04) to post-Ramadan month-6 (44.28±11.51), as illustrated in Figure 2 and Table 3. However, this difference was not found to be statistically significant (p=0.063) (Table 2). Additionally, Ramadan fasting demonstrated variable effects on different laboratory parameters, with significant changes observed in potassium (p=0.003), calcium (p=0.023), fasting glucose (p<0.001), total cholesterol (p=0.001), HDL cholesterol (p<0.001), and LDL cholesterol levels (p<0.001). On the other hand, other parameters did not display significant differences between the pre-Ramadan and post-Ramadan month-6 measurements (p>0.05) (Table 2).

Table 2. The comparison of the pre and post-Ramadan labarotory results

Parameter*	Pre-Ramadan	Post-Ramadan Month-6	P value
eGFR**	43.54±11.04	44.28±11.51	0.063
BUN (mg/dl)	54.42±24.12	56.34±25.17	0.876
Na^{+} (mmol/L)	139.98±2.84	139.66±9.63	0.673
K^{+} (mmol/L)	4.83±0.48	4.98±0.55	0.003
Ca^{++} (mg/dl)	9.19±0.53	9.26±0.48	0.023
Phosphorus (mg/dl)	3.79±0.60	3.79±0.70	0.997
PTH (ng/L)	102.57±61.54	102.18±74.10	0.921
Albumin (g/dl)	4.29±0.32	4.31±0.26	0.283
Uric acid (mg/dl)	6.60±1.79	6.62±1.73	0.726
Fasting Glucose (mg/dl)	119.48±39.90	111.14±25.98	<0.001
T. cholesterol (mg/dl)	171.93±42.35	162.39±41.50	0.001
Tryglyceride (mg/dl)	156.41±46.14	143.15±101.68	0.191
HDL cholesterol (mg/dl)	44.96±10.22	50.16±14.01	<0.001
LDL cholesterol (mg/dl)	116.48±28.91	106.74±28.94	<0.001
Hgb (g/dl)	12.50±1.53	12.52±1.63	0.816

*Expressed as mean±SD, **eGFR (CKD-EPI 2021 Formula) is given as ml/min/1.73 m², Abbreviations: BUN: eGFR: estimated glomerular filtration rate, HDL: high density lipoprotein, Hgb: hemoglobin, LDL: low density lipoprotein, PTH: Parathyroid hormone

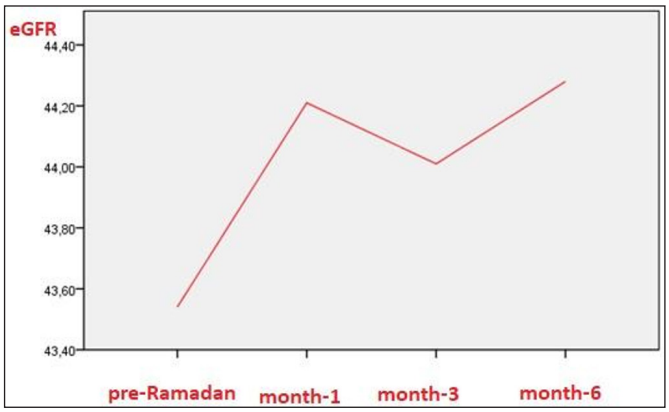

Figure 2. eGFR changes during 6-month, following Ramadan fasting, eGFR; estimated glomerular filtration rate (pre-Ramadan vs post-Ramadan month-6, p=0.063), eGFR (CKD-EPI 2021 Formula) is given as ml/min/1.73 m²

The impact of ACEi/ARB on eGFR change was analyzed, revealing no statistically significant effect (p=0.084). Initially, participants not taking ACEi/ARB had lower pre-Ramadan eGFR levels compared to ACEi/ARB users (eGFR; 41.64±12.28 vs. 45.29±10.50, p=0.030). However, after Ramadan, an improvement was observed among ACEi/ARB-free individuals, leading to the disappearance of the difference (eGFR; 43.62±12.30 vs. 45.38±10.50, p=0.310). Age, HT, DM, CVD, polypharmacy (p=0.581), and BMI were found to have no significant impact on eGFR change (p=0.780, p=0.389, p=0.780, p=0.836, p=0.829, and p=0.182, respectively). Surprisingly, the pre-Ramadan evaluation by a physician also had no impact on eGFR change (Delta eGFR [pre-Ramadan eGFR - post-Ramadan eGFR / pre-Ramadan eGFR]; -0.02±0.11 vs. -0.02±0.13, p=0.966). **Table 3** illustrates the changes in eGFR according to the stages of CKD, showing that there was no significant alteration in eGFR, even among patients with advanced-stage CKD. Pre-Ramadan proteinuria had no impact on post-Ramadan eGFR change (p=0.208 and r^2=0.08). However, the impact of Ramadan fasting on proteinuria progression could not be investigated due to lack of the data.

Table 3. eGFR changes following Ramadan fasting according to the CKD stages.

eGFR*	Pre-Ramadan	Post-Ramadan	P value
Stage 3a, n=96	50.23±6.83	50.88±7.68	0.963
Stage 3b, n=66	41.15±6.28	41.68±6.89	0.910
Stage 4, n=25	26.80±9.38	28.48±11.42	0.366
Stage 5, n=5	10.00±1.22	11.00±1.41	0.463

*eGFR (CKD-EPI 2021 Formula) is given as ml/min/1.73 m^2, Abbreviations: eGFR: estimated golmerular filtration rate.

DISCUSSION

The results of this study indicate that Ramadan fasting did not significantly affect the eGFR, even among patients in advanced stages of CKD. Importantly, the use of ACEi/ARB did not lead to worse outcomes in this context. Additionally, certain laboratory parameters related to metabolic features, including fasting glucose, total cholesterol, HDL cholesterol, and LDL cholesterol, exhibited significant improvement during the fasting period. This is the largest sample-sized longitudinal study in the literature and the only study including a small group of patients with non-dialysis stage 5 CKD. These findings will support future research to gain deeper insights into the effects of Ramadan fasting on renal function and to identify effective strategies for managing CKD in individuals who observe fasting practices.

As of the year 2020, the population adhering to the Islamic faith comprises approximately one-fourth of the global population.[6] Muslims annually observe a one-month fast from the time of dawn to sunset, with the total fasting duration varying based on the specific timing and geographic location of the observance, in accordance with the Islamic lunar calendar. Despite the widespread and extensive participation in this religious practice, its health-related consequences remain insufficiently understood. The lack of knowledge on the subject is further compounded by the absence of similar long-term mass religious practices in Western culture.[7]

The extended periods of fasting during Ramadan, which often last around 18 hours per day, have raised concerns regarding the potential deterioration of kidney function. Unfortunately, there is no single guideline published for CKD and Ramadan fasting. The International Diabetes Federation and Diabetes and Ramadan (IDF-DAR) Practical Guidelines for 2021 represent the sole acknowledged data concerning the prevention of fasting-related complications in patients with diabetes and CKD.[8] In this cohort, DM and HTN were the leading underlying causes of CKD, as previously reported in other Muslim countries.[9-12] The mean fasting duration in our study was 14-15 hours per day, which is similar to previous studies, and all patients fasted for almost one month.[11]

There are several small studies that have addressed the impact of fasting on the glomerular filtration rate (GFR).[1,2,13] However, the results of these studies are controversial. For instance, Wakil et al.[1] reported a non-significant reduction in GFR and an increase in urine N-acetyl-B-D-glucosaminidase levels, indicating potential renal tubular cell injury at the end of Ramadan fasting. In contrast, Mbarki et al.[2] found a higher risk of acute kidney injury (AKI) associated with Ramadan fasting, especially for patients with pre-Ramadan creatinine clearance of <60 mL/min/1.73 m^2. Nasrallah et al.[14] reported a link between elevated serum creatinine and Ramadan fasting, with a higher risk of creatinine elevation observed among those receiving renin-angiotensin-aldosterone system antagonists (RR=2). However, serum creatinine levels generally returned to baseline by the end of the fasting month. This study also demonstrated a higher occurrence of major adverse cardiovascular events among fasting CKD patients with pre-existing cardiovascular disease.

Numerous studies have reported that Ramadan fasting was not associated with an increased risk of declining renal function in patients with stages 2-4 CKD, even among kidney recipients.[4,13,15-17] However, the authors of these studies noted that elderly individuals might still be at a higher risk, and the amount of pre-fasting water consumption may play a significant role as a predictor of renal function. This study represents the largest sample size in the literature, and the study cohort was older compared to previous studies, yet GFR did not decrease

following Ramadan at the 6-month follow-up, consistent with previous studies.[11,17,18] Nevertheless, it is essential to acknowledge that this study was not designed to demonstrate the acute changes in GFR within the holy month of Ramadan.

Evidence-based risk factors for major complications during Ramadan fasting in individuals with diabetes indicate that age is a significant risk factor for hospitalization.[19] Abdullah et al.[19] suggested that a small subgroup of individuals with preexisting comorbidities may experience fasting-related kidney function issues. Kara et al.[13] also emphasized age as a risk factor for the deterioration of kidney function. However, in this current study, regression analysis demonstrated that age had no impact on the change in glomerular filtration rate (GFR) following Ramadan fasting.

The available literature on risk factors for GFR deterioration due to Ramadan fasting in CKD patients is limited. Paradoxically, the perception that prolonged fasting and thirst pose a risk for the deterioration of CKD in CKD patients is not supported by the evidence. This study indicates that traditional risk factors for CKD progression, such as DM, HT, CVD, and age, do not show any significant association with GFR changes in fasting patients. Furthermore, ACEi/ARB use was not found to impact GFR. It is possible that the acute hemodynamic changes experienced during Ramadan fasting may reverse after the conclusion of the holy month, at least in this particular cohort. Another explanation could be that the study participants had well-controlled or mild comorbidities that were not affected by fasting.

Data regarding patients with CKD stage 5 and Ramadan fasting is lacking as previous studies have often excluded this patient group.[4,20,21] In this study, a small group of patients with CKD stage 5 did not exhibit a significant change in GFR following Ramadan fasting.

Previous studies suggest that seeking consultation before Ramadan may lead to improved outcomes and better disease monitoring. However, this study did not find a significant impact of pre-Ramadan consultation on GFR changes. Since this was not a controlled study, participants may have received consultancy from different physicians or obtained information from various media sources.

Ramadan fasting has been associated with beneficial effects on metabolic parameters, such as blood glucose and lipid profile, as demonstrated in previous studies.[22,23] Consistent with these findings, our study also revealed similar improvements in lipid profile, with the exception of triglyceride levels. It is worth noting that fasting during Ramadan induces an acute increase in osmolarity and serum electrolytes while reducing body water composition.[24] In our study, we observed significant increases in potassium and calcium values during Ramadan fasting; however, the final values remained within the normal physiological range. These observations suggest that the changes in electrolyte levels during fasting were not of clinical concern and did not pose any adverse health effects.

Limitations of the Study

Sample size: The study's sample size may be relatively small, limiting the generalizability of the findings to a broader population of individuals with CKD observing Ramadan fasting.

Lack of control group: The absence of a control group of non-fasting individuals with CKD hinders the ability to compare the fasting group's outcomes to those not participating in Ramadan fasting, making it challenging to attribute specific effects solely to fasting.

Retrospective data: The study's retrospective nature may introduce inherent biases and limitations in data collection and analysis, potentially affecting the accuracy and completeness of the information obtained.

Single-center study: Conducting the study at a single center may limit the diversity of the study population and restrict the representation of individuals with CKD from different geographic regions or healthcare settings.

Short-term follow-up: The six-month follow-up period may not be sufficient to capture long-term changes or complications related to Ramadan fasting in individuals with CKD. A longer follow-up duration would provide more comprehensive insights into the effects of fasting on renal function over time.

Variability in fasting practices: Ramadan fasting practices can vary among individuals, with some adherents following strict fasting while others may modify their fasting patterns due to health conditions or personal preferences. The heterogeneity in fasting practices within the study population might introduce variability in the results.

Lack of dietary and fluid intake data: Detailed dietary and fluid intake information during Ramadan fasting was not recorded, which could potentially influence kidney function and confound the results.

Missing data: Incomplete or missing data on certain variables may limit the study's ability to analyze specific factors' impact on the outcomes.

External factors: Other external factors, such as climate, physical activity, or medication adherence, were not fully accounted for in the analysis and may have influenced the study results.

CONCLUSION

These findings suggest that, in our study cohort, Ramadan fasting does not appear to have adverse effects on kidney function. However, it is essential to consider individual variability in fasting practices and the potential influence of factors such as age, comorbidities, and medication use. Further research is warranted to explore the acute changes in kidney function within the holy month of Ramadan and to examine the effects of fasting on specific subgroups, such as elderly individuals and those with advanced CKD.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of by Zonguldak Bülent Ecevit University Non-interventional Clinical Researches Ethics Committee (Date: 10.06.2022, Decision No: 2022/14).

Informed consent: All patients included in the study signed informed consent.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The role of disease activity as a determinant of body awareness and central sensitization in patients with axial spondyloarthritis: a cross-sectional study

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ABSTRACT

Aims: The aim of this study was to investigate the effects of disease activity on body awareness and central sensitization in patients with axial spondyloarthritis (axSpA).

Methods: This cross-sectional study included patients diagnosed with axSpA. Disease activity was evaluated using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and a score of four or higher was considered high disease activity. Patients were divided into two groups according to BASDAI: high disease activity (BASDAI ≥ 4) group (HG) and the low disease activity (BASDAI<4) group (LG). Body awareness levels were assessed with the Body Awareness Questionnaire (BAQ). The Central Sensitization Inventory (CSI) was used for central sensitivity. Simple linear regression analyses were performed to investigate which of the independent variables could explain the disease activity.

Results: Sixty-two patients with a mean disease duration of 10.8 years were included. The mean BASDAI, CSI-A, and BAQ were 4.2, 38.5, and 73 points, respectively. Demographics and clinical characteristics were comparable between the groups (p>0.05). The CSI-A score was higher in HG compared to LG (44 (31-54) vs. 31 (21-41), p=0.008). The HG had poorer BAQ scores than the LG (61 (52-85) vs. 85 (64-96), p=0.017). BASDAI was moderately associated with CSI-A (r=0.145, R2=0.172, p=0.001). No significant correlation was found between BASDAI and BAQ (p=0.167). The results of the simple linear regression analysis suggested that CSI-A explained 17.2% of the disease activity. BASDAI (β = 0.415, p=0.001) significantly predicted central sensitization. BASDAI was strongly correlated with VAS (r=0.665, R2= 0.442, p<0.001). The VAS explained 44.2% of the disease activity, and BASDAI (r=0.665, p<0.001) significantly predicted pain severity.

Conclusion: High disease activity adversely affects central sensitization and body awareness in patients with axSpA. Physicians should also consider a multimodal biopsychosocial perspective in the management of patients with high disease activity.

Keywords: Central sensitization, spondyloarthritis, awareness, pain

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease characterized by sacroiliitis, enthesopathy, and spondyloarthropathy, with variable peripheral joint involvement.[1] Chronic pain, stiffness, fatigue, sleep disturbance, and functional impairment are common symptoms of axSpA, and these symptoms limit activity and worsen quality of life during the active phase of the disease.[2] At present, the most widely used patient-based outcome measure to assess disease activity in patients with axSpA is the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). This scale assesses parameters such as fatigue, peripheral joint pain, spinal pain, attachment point inflammation and duration and severity of morning stiffness.[3] The assessment of disease activity in axSpA is crucial in the management of the disease, follow-up of patients, and prediction of prognosis.

Different medical approaches utilized in controlling the disease and disease activity provide significant gains to the patient in patients with axSpA, however, sometimes they may not adequately treat disease-related pain, fatigue, sleep disturbance, anxiety, and depression.[4] Chronic inflammation in axSpA may trigger both peripheral and central modifications of pain pathways, and lead to central sensitization (CS). CS is described as an increased sensitivity of nociceptive neurons in the central nervous system to usual or subthreshold peripheral stimuli,[5] and the high prevalence of CS in patients with axSpA despite biologic therapy suggests that inflammation may not be the sole cause of pain and that additional pain mechanisms, such as

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perception and interpretation of pain, may play a role in the persistence of pain.[6,7] Recently, CS has been recognized as a possible underlying pathophysiological mechanism of the chronic pain associated with axSpA. In addition, previous studies suggested chronic musculoskeletal pain, cognitive impairment and emotional state related to body awareness.[8,9] However, little is known about body awareness in patients with axSpA and its association with disease activity.

Body awareness is defined as the ability to focus attentively on and be aware of internal body sensations or to recognize nuances in body cues.[10,11] Body awareness occurs through the integration of many sensory inputs such as interoceptive, proprioceptive, exteroceptive, vestibular, and it is involved sensory, physical and physiological mechanisms.[12] Many cortical areas are interconnected to help the perception of body position, the relationship between body segments and the body itself, and body awareness, which is the ability to recognize one's own body, is formed.[13] Previous studies have suggested a negative relationship between body awareness, pain and emotional mood in healthy individuals[14] and that cognitive impairment and chronic musculoskeletal pain reduce body awareness in older adults.[8] Shifting the focus of attention from pain sensations to different mental tasks may be beneficial in reducing pain, indeed, previous studies on experimental pain or other models of acute pain show such benefits.[15,16] Preliminary evidence suggests that body awareness may have significant benefits in the management of chronic painful diseases and improving health-related quality of life.[17,18] Our knowledge about body awareness in axSpA characterized by chronic musculoskeletal pain, fatigue, systemic inflammation, and other complaints are limited. Furthermore, disease activity, which is commonly assessed in the clinical setting, may affect both body perception and central sensitivity. The aim of this study was investigating the effects of disease activity on body awareness and CS in patients with axSpA. The primary hypothesis suggests that high disease activity is associated with impaired body awareness. The secondary hypothesis suggests that disease activity is a determinant factor of the body awareness and CS.

METHODS

Study Design

The study was carried out with the permission of Ankara Medipol University Non-interventional Clinical Researches Ethics Committee (Date: 11.04.2023, Decision No: 44). Informed consent was obtained from all patients, and all procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The data was obtained in the Ankara Etlik City Hospital Department of Rheumatology between April 2023 and July 2023.

Participants

Inclusion criteria were age ranged from 18 to 65 years, diagnosed with axSpA by criteria of Assessment of Spondyloarthritis International Society classification criteria for axial spondyloarthritis[19] for at least 1 year, had chronic musculoskeletal pain for more than 3 months. Patients with any cardiovascular disease (history of acute myocardial infarction, heart failure, etc.), neurological diseases (Parkinson, multiple sclerosis, stroke, dementia), the presence of active malignancy, neuropsychiatric medical treatment, locomotor disorders (fractures and prostheses) and/or osteoporosis, history of lower extremity and spine surgery, visual and hearing problems, chronic addiction to alcohol, and pregnancy were excluded from the study. All patients included in the study were divided into two groups according to BASDAI: high disease activity (BASDAI ≥ 4) group (HG) and the low disease activity (BASDAI<4) group (LG).

Instruments

Information about disease duration, comorbidities and medications were recorded. Smoking history and regular exercise habits were questioned. Regular exercise habits were considered to be at least 2-3 days a week for at least 30 minutes. Routine laboratory findings such as erythrocyte sedimentation rate (ESR) (mm/h), C-reactive protein (CRP) (mg/L), creatine kinase (U/L), vitamin D3 (nmol/L), folic acid (ng/ml), vitamin B12 (ng/L), ferritin (µg/L), and TSH (mIU/L) were obtained on the day of the assessment.

Pain Severity

The severity of the pain was assessed with the Visual Analogue Scale (VAS). The 10-cm horizontal line was defined as 0 "no pain" and 10 "very severe pain", and the patients were asked to mark a line indicating their musculoskeletal pain, and then the line was measured in centimeters.[20]

Disease Activity

Disease activity was determined using BASDAI, which is a patient reported outcome measure. BASDAI includes six questions related to fatigue, spinal pain, peripheral joint pain, attachment point inflammation, and duration and severity of morning stiffness. A total score ranges from 0 to 10, and a higher score indicates more severe disease activity. A BASDAI score ≥4 is a threshold, which indicate considered as active of disease.[3]

Central Sensitization

Central Sensitization Inventory (CSI) is used to assess the presence and severity of CS. The CSI is a self-reported tool and is consists of two parts: the first part (CSI-A) contains 25 items investigating emotional and somatic disorders associated with CS. Each response is scored

between 0 and 4, and the total score is obtained as 0-100. High scores indicate CS symptoms of increasing severity. The second part of the scale (CSI-B) investigates disorders diagnosed by a physician that may be related to CS, such as restless leg syndrome, chronic fatigue syndrome, fibromyalgia syndrome, temporomandibular joint problems, migraine, irritable bowel syndrome, anxiety, and depression.[21] The cut-off point of the CSI is 40 points. The Turkish validity and reliability study of the scale was conducted by Düzce et al.[22]

Body Awareness

The Body Awareness Questionnaire (BAQ) is determined whether the body's level of sensitivity is normal or abnormal. The BAQ includes 18 items. Each item is rated by the patients on a scale of 1 to 7 (1=not true for me at all, 7 = completely true for me). The questionnaire uses the total score for the rating. The maximum total score that can be obtained from the questionnaire is 126 and the minimum score is 18 points. Higher scores indicate a better level of body awareness.[23] The Turkish validity and reliability study of the scale was conducted by Karaca et al.[23]

Statistical Analysis

A priori sample size analysis was performed with G Power software (Version 3.1.9.2, Franz Faul, University of Kiel, Kiel, Germany). The sample size of at least 58 individuals was found to have a power of 0.80, an effect size of 0.31 (medium effect d≥0.3), correlation test r^2= 0.10, and an alpha value of 0.05 (one-tailed). BM SPSS (Statistical Package for the Social Sciences, ver. 22.0) was used for statistical analyses. Descriptive data were given as mean (standard deviation) or median (IQR) and minimum to maximum for numerical data, and number (n) and percentage (%) values were calculated for non-numerical data. Kolmogorov-Smirnov tests were performed to determine whether variables were normally distributed. The relationship among disease activity, body awareness, and CS was evaluated with Pearson's correlation analysis due to parametric conditions. The size of the correlation coefficient was considered to be very high (0.90 to 1.00), high (0.70 to 0.89), moderate (0.50 to 0.69), low (0.30 to 0.49), and negligible (0 to 0.29).[24] Simple linear regression analyses were performed to investigate which of the independent variables (VAS, CS) could explain the dependent variables (BASDAI) in presence of the significant correlations. Each analysis is performed only one independent variable. p value <0.05 was considered for statistical significance.

RESULTS

Sixty-two patients (38 male, 24 female, mean age: 43.9 (9.7) years, mean disease duration: 10.8 (4.8) years) were included. The demographic and clinical features of patients with axSpA are shown in Table 1. The groups had similar characteristics in terms of clinical and demographic characteristics (p>0.05). CRP is higher in HG compared to LG (p<0.001). The mean BASDAI score of all patients was 4.2 (1.4) points. The CS is positive in 48.4% of all patients. CS presence is more common in HG than LG (20 (60.6) vs 10 (34.5), p=0.036).

A comparison of CS and body awareness between the groups is presented in Table 2. There were significant among-group differences in the CSI-A scores (p=0.008), BAQ scores (p=0.017), and VAS (p<0.001). HG had higher CSI-A (44 (31-54) vs 31 (21-41)), poorer BAQ (61 (52-85) vs 85 (64-96)), and more severity pain (7 (6-8) vs 5 (4-6)) compared to LG.

BASDAI is moderately correlated with the CSI-A scores (r=0.415, R2= 0.172, p=0.001) and is highly correlated with VAS (r=0.665, R2= 0.442, p<0.001). No significant correlation was found between BASDAI and BAQ (p=0.167). The results of the simple linear regression analysis suggested that disease activity explained 17.2% of the variance. BASDAI (r= 0.415, p=0.001) significantly predicted CS. The VAS score is explained 44.2% of the variance and BASDAI (r=0.665, p<0.001) significantly predicted pain severity (Figure 1). The relationship between the disease activity, CS and pain severity of axSpA patients are shown in Table 3.

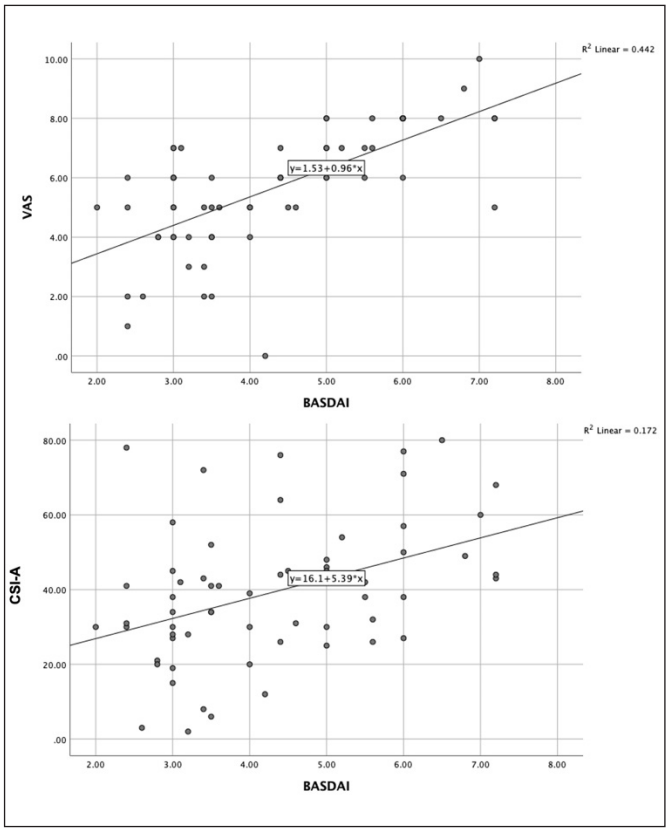

Figure 1. Scatter plots of disease activity, central sensitization, and pain severity

Tablo 1. The demographic and clinical features of patients with axial spondyloarthritis

	AxSpA (All patients) (n=62)	HG (BASDAI ≥ 4) (n=33)	LG (BASDAI<4) (n=29)	p value*
Age (year), mean (SD), min-max	43.9 (9.7) (25-63)	44 (10.2)	43.8 (9.3)	0.947
Male, n (%)	38 (61.3)	24 (72.7)	14 (48.3)	0.049
BMI (kg/m²), mean (SD)	28.0 (3.46)	27.8 (3.2)	28.1 (3.7)	0.778
Disease duration (year), mean (SD), min-max	10.8 (4.8) (2-20)	10.5 (5.2)	11.4 (4)	0.632
History of smoking, n (%)				
None	45 (72.6)	24 (72.7)	21 (72.4)	
Active	17 (27.4)	9 (27.3)	8 (27.6)	0.978
Pack-year, median (IQR)	10 (7.5-16)	10 (10-16)	8 (5-15)	0.340
Regular exercise habits n (%)	13 (21)	6 (18.2)	7 (24.1)	0.565
Comorbidity n (%)				
Diabetes mellitus	6 (9.7)	5 (15.2)	1 (3.4)	0.201
Hypertension	8 (12.9)	5 (15.2)	3 (10.3)	0.430
Coronary artery disease	2 (3.2)	1 (3)	1 (3.4)	0.721
Thyroid disorder	2 (3.2)	0 (0)	2 (6.9)	0.215
Chronic renal failure	1 (1.6)	1 (3)	0 (0)	0.541
COPD-asthma	7 (11.3)	4 (12.1)	3 (10.3)	0.574
Depression	1 (1.6)	0 (0)	1 (3.4)	0.468
Laboratory findings, median (IQR)				
ESR (mm/h)	18 (9-27)	18 (12-26)	15 (9-30)	0.553
CRP (mg/L)	7.8 (4-20)	14 (7-21.5)	5 (1-8)	<0.001
Creatine kinase (U/L)	69.5 (59.5-103)	92 (66.5-117)	63.5 (58.5-77)	0.094
Vitamin D3 (nmol/L)	39 (27-78)	39 (33-66.5)	30 (17-78)	0.635
Folic acid (ng/ml)	7 (6-10)	6.6 (6.1-10.5)	8.4 (6-10)	0.831
Vitamin B12 (ng/L)	360 (275-447)	403 (317-463)	360 (235-432)	0.472
Ferritin (µg/L)	57.5 (30-88)	71.5 (30-95)	54 (31.5-74)	0.241
TSH (mIU/L)	1.86 (1.14- 2.45)	1.98 (1.5-2.3)	1.76 (1.08 2.80)	0.910
Active medications, n (%)				
Methotrexate	3 (4.8)	2 (6.1)	1 (3.4)	0.549
Sulfasalazine	20 (32.3)	13 (39.4)	7 (24.1)	0.200
Biological DMARDs	34 (54.8)	18 (54.5)	16 (55.2)	0.961
Anti-TNF alpha drug	33 (53.2)	19 (57.6)	14 (48.3)	0.633
NSAIDs	21 (33.9)	12 (36.4)	9 (31)	0.658
BASDAI, mean (SD)	4.2 (1.4)	5.4 (0.9)	3.02 (0.4)	-
CS positive, n (%)	30 (48.4)	20 (60.6)	10 (34.5)	0.036

AxSpA, axial spondyloarthritis; HG, high disease activity group; LG, low disease activity group; TNF, Tumor Necrosis Factor; DMARDs, disease-modifying antirheumatic drugs; BMI, Body mass index; COPD, chronic obstructive pulmonary disease; IQR, interquartile range, ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; TSH, thyroid stimulating hormone; NSAIDs, non-steroidal anti-inflammatory drugs; CS, central sensitization. * p value indicates comparison of HG and LG. *x^2 test or Fisher exact test **Independent t test or Mann-Whitney U test.

Table 2. Comparison of central sensitization and body awareness between the groups

	AxSpA (All patients) (n=62)	HG (BASDAI ≥ 4) (n=33)	LG (BASDAI<4) (n=29)	z	p value
CSI-A (point), median (IQR)	38.5 (28-48)	44 (31-54)	31 (21-41)	-2.640	0.008
CSI-B (point), median (IQR)	0 (0-1)	0 (0-0)	0 (0-1)	-0.649	0.517
BAQ (point), median (IQR)	73 (53-90)	61 (52-85)	85 (64-96)	-2.379	0.017
VAS (cm), median (IQR)	6 (4-7)	7 (6-8)	5 (4-6)	-4.659	<0.001

AxSpA, axial spondyloarthritis; HG, high disease activity group; LG, low disease activity group; CSI-A, Central Sensitization Inventory Part A; CSI-B, Central Sensitization Inventory Part B; BAQ, Body Awareness Questionnaire; VAS, Visual Analogue Scale. * p value indicates comparison of HG and LG. * Mann-Whitney U test.

Table 3. Relationship between the disease activity, central sensitization and pain severity of axial spondyloarthritis patients

					CSI-A				
	r	R^2	P	F	B coefficient	Std. error	β	t	p
Constant					-3.035	0.391	-	7.753	<0.001
Disease activity	0.415	0.172	0.001	12.480	0.032	0.009	0.415	3.533	.001
					VAS				
Constant					1.688	0.401	-	4.211	<0.001
Disease activity	0.665	0.442	<0.001	47.501	0.462	0.067	0.665	6.892	<0.001

CSI-A, Central Sensitization Inventory Part A; VAS, Visual Analogue Scale. * Linear regression analysis.

DISCUSSION

The present study aimed to investigate the effects of disease activity on body awareness and CS in patients with axSpA. This study's results showed that high disease activity adversely affected body awareness and CS. As well as, disease activity is an important determinant of CS in patients with axSpA. As the disease activity increased, CS and pain severity deteriorated.

A study involving healthy individuals reported an inverse association between body awareness and pain intensity and emotional state.[14] In older adults, chronic musculoskeletal pain and cognitive impairment are reduced body awareness.[8] Hider et al.[25] reported that body totality was associated with disease acceptance in patients with ankylosing spondylitis and that body image, including body totality and body self-consciousness, was inversely related to depression. Another study examining the relationship of physical activity level with body awareness and balance showed that physical activity level is associated with body awareness in patients with ankylosing spondylitis.[26] About 50% of ankylosing spondylitis patients reported discomfort about their appearance by Ward et al.[27] study. A brief report showed high disease activity negatively affected catastrophic thoughts, body awareness, and kinesiophobia in patients with ankylosing spondylitis.[28] The present study showed that patients with high disease activity have poorer body awareness compared to low disease activity, which is consistent with Karaca et al.[28] study. In addition, in present study, CRP level of HG is higher compared to LG. The BASDAI scores ≥ 4 with high CRP levels may indicate poor body awareness. In the few studies conducted on patients with axSpA related to body awareness, the present study would improve our knowledge of body awareness in treatment and management of AS.

Previous studies in which the prevalence of CS for axSpA was reported ranged from 45% to %57.[4,6,29-32] Another study reported that CS rates were 45.1% for axSpA and that the frequency of severe forms of CS was higher in patients with axSpA than in healthy individuals.[30] In a study including different rheumatic diseases, authors reported that CS syndromes were present in almost half the patients: 45% of axSpA, 41% of rheumatoid arthritis, 62% of osteoarthritis, and 94% of fibromyalgia patients.[29] The present study detected clinical CS in 48.4% of patients with axSpA, which is consistent with the literature. In addition, the study determined that patients having high disease activity (also high CRP levels) showed two times more common clinical CS compared to low disease activity. Clinical CS is a common condition in patients with axSpA and should be considered in treatment management and patient follow-up.

Kieskamp et al.[6] showed that CS, specific illness perceptions and obesity were all independently associated with BASDAI. Another study reported that CS adversely affects disease activity in axSpA.[30] Sariyildiz et al.[31] confirmed that worse disease activity, more enthesal involvement, and anxiety independently predict the development of CS in axSpA. Unlike these studies, Guler et al.[29] did not find a relationship between CSI score and disease activity in patients with axSpA. The present study found patients with axSpA with high disease activity have higher CS scores than those with low disease activity. In addition, the results of the regression analysis performed in the present study showed that CS and disease activity were moderately associated. The disease activity may predict the presence and severity of CS in patients with axSpA, which is consistent with the previous studies. As the disease activity increases, the severity of CS is also increased.

Study Limitations

The study has some limitations. Firstly, patients' psychological states weren't evaluated in this study, but body awareness and CS may be affected by psychological factors. Secondly, we did not evaluate neuropathic pain profiles of patients, which may be involve the CS. An important strength of our study is that body awareness is assessed in detail and to investigate relationship of disease activity with body awareness and CS in patients with axSpA. Future studies may assess the effects of body awareness therapies on disease activity, health-related quality of life, psychological and emotional status, and CS.

CONCLUCION

Our study provides comprehensive evidence of the relationship between disease activity, body awareness, and CS in patients with axSpA. This study is focused on body awareness and disease activity in patients with axSpA, which are missing in the literature. Early detection of impaired body awareness and CS would help in planning treatment and management of axSpA. In addition to pharmacological treatments, nonpharmacologic treatments such as exercises therapy, pain-coping strategies, and cognitive behavioral therapies, body awareness therapy may reduce pain and CS, and increase the body awareness. Physicians should also consider a multimodal biopsychosocial perspective in patients with high disease activity.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara Medipol University Non-interventional Clinical Researches Ethics Committee (Date: 11.04.2023, Decision No: 44).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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"Structured with contemporary education methods" regarding anaesthesiology and reanimation internship education evaluation of student feedback

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ABSTRACT

Aims: This study aims to evaluate our training program, which is carried out with contemporary education methods in anaesthesia internship, for the students of our faculty, with student feedback.

Methods: In order to evaluate the comments of the students about the internship content, 1051 feedback forms filled out since 2008 were examined. There were 16 questions in the form, 15 of which were closed-ended and 1 open-ended.

Results: In the general evaluation of the internship program, 48.4% (n=509) of the students evaluated it as very productive and 46.1% (n=484) as productive. The percentage of respondents who said they were proficient in the "skill of vascular access," one of the abilities taught on models during clinical skills training and later used on patients, was 95.1% (n=999). 29.7% (n=312) of the students admitted that their "intubation practice" was inadequate.

Conclusion: It is crucial that the anaesthetic internship, which teaches one of the most fundamental medical skills—airway safety—be developed in accordance with the feedback received from the students. Standardised applied training programs increase the successful outcome of anaesthesia internship education.

Keywords: Anaesthesiology, educational methods, medical education

INTRODUCTION

In recent years, the increase in the knowledge and skills desired by the physician who graduated from the medical faculty necessitates the use of "learning to access information" in the education process and "learning by doing" techniques in skill education.[1] One of the critical steps of training good physicians is that these techniques, whose validity has been accepted by the medical faculties, are made effective by the education units.[2]

Few would contest the importance of providing medical undergraduates with clinical training in anaesthesia and intensive care medicine, even though these are postgraduate fields.[3] The literature discussed changes to undergraduate medical school curricula as well as the special advantages anaesthesiologists working in operating rooms, intensive care units, and pain clinics may offer medical students at all levels of their training.[4]

Renewal and standardisation studies in the medical education curriculum, which started with the contributions of the Health Sciences Education Council in our country in 2000, also pioneered the implementation of new educational techniques. This program was implemented in medical faculties as the National Core Education Program (NCEP) in the 2003-2004 academic year.[5]

The World Federation of Medical Education published the universal standards for the improvement of quality in medical education in 2007. This standardisation was translated into our language by the Turkish Medical Association in 2010 and a recommendation was made to apply it to the medical faculties of our country.[6] In our faculty, these standardisations and the structuring of our education and examination system were carried out in 2008.

In the educational process, it is crucial to understand the learning preferences of medical faculty students. Education should follow the concepts of adult education.[7] In 2005, our clinic started to work on the use of modern education methods in our internship. The content of the NCEP was examined in terms of the knowledge and

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skills to be acquired during the anaesthesia internship. In addition, the subjects that may be related to the internship content were also examined. For each subject, the aims and learning objectives were determined by the faculty members and the appropriate educational techniques were reviewed. At the same time, measurement and evaluation techniques were restructured.

This study aims to evaluate our training program, which is carried out with contemporary education methods in anaesthesia internship, for the students of our faculty, with student feedback.

METHODS

The study was carried out with the permission of Mersin University Clinical Researches Ethics Committee (Date: 12.02.2015, Decision No: 2015-40). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. In order to evaluate the comments of the students about the internship content, 1051 feedback forms filled out since 2008 were examined.

Internship Education Program

Settings: Students take 20 hours of practical and 16 hours of theoretical lessons in a two-week period until 2018. Since 2019, this period has been updated as 3 weeks. Three internship groups were formed, each undergoing rotating training in pain clinics and intensive care units for three days each, and anaesthesia for seven days. After the students practice their skills training on models, working one-on-one with the lecturer, they perform these skills on the patient in anaesthesia, algology, and intensive care departments.

Assessment and Evaluation

It is performed with the objective structured exam technique (OSCE). It consists of three phases: skill practices, multiple choice questions and verbal (structured).

Filling the Form

The feedback forms are distributed to the students by the internship group representative (student) after each final exam, and the forms filled in by the student are sent to the training officer (lecturer) by the group representative. There are no student names or any identifying marks on the forms. There were 16 questions in the form, 15 of which were closed-ended and 1 open-ended. In its content, questions are asked about the general evaluation of the internship, the duration of the internship, the course hours, the course environment, the proficiency in patient practices and the evaluation of the exam. There are also areas to write their own comments on them freely.

Survey questions were as follows:

- What is your general evaluation of the anaesthesia internship?
- What is your general opinion about the internship period?
- Were the lecture hours sufficient?
- Was the environment conducive to learning?
- Did you contact enough patients during the internship?
- Who provided you the necessary practical skills and inspection techniques?
- Do you believe that enough time is focused on teaching clinical skills?
- What practical skills did you acquire at the end of this internship?
- What practical abilities do you believe will be lacking after this internship?
- The courses that I thought I benefited the most during my internship were as follows;
- The courses that I thought I did not benefit the most during my internship were as follows;
- In the exam; The questions were consistent with the topics covered.
- In the exam; the questions serve to measure my real knowledge.
- Everyone is evaluated equally in the exam.
- I think my performance during the internship is taken into consideration during the exam
- Your thoughts and recommendations on internship.

Statistical Analysis

The Statistical Package for Social Sciences version 24 (SPSS v.24) program was used to enter the data for the statistical analysis. Additionally, calculations were performed using the E-PICOS tool in accordance with the MedicReS Good Biostatistical Practice guidelines. For categorical data, descriptive statistics were used, and percentages were used to describe frequency calculations. Cross-comparison tables used the chi-squared test. To compare the mean values, independent-group and dependent-group t-tests were run. Statistical significance was defined as a p-value of 0.05.

RESULTS

All 1051 forms filled out by students were evaluated. The number of internship groups to which the questionnaire was applied was 66. The number of students in the groups was at least 12 and at most 20.

In the general evaluation of the internship program, only 0.9% (n=9) of the participants found the internship unproductive. Other assessments are detailed in **Table 1**. Those who did not find the course environment

suitable were students belonging to a single internship group. In this internship group, it was determined that the applications were made in the operating room environment. Students' comments; it was that they were distracted in the operating room. Students who stated that the internship period was short stated they could not find enough time, especially for skills. Those who used the expression insufficient in response to the question of whether the course hours are sufficient also stated that the time for skill training should be increased.

Table 1. Evaluation of the internship program

Evaluation	% (n)*
General evaluation	
Productive	48.4 (509)
Agreeable	46.1 (484)
Might be improved	4.3 (45)
Unproductive	0.9 (9)
Duration of internship	
Adequate	71.2 (748)
Insufficient	22.7 (239)
Long	5.1 (54)
Lecture hour adequacy	
Adequate	89 (935)
Inadequate	12 (31)
Is the classroom appropriate?	
Appropriate	83.6 (879)
Inappropriate	16.1 (169)

n*: the number of respondents

The clinic practices were given enough time, according to 72.2% (n=748) of the students. In contrast to the 77.9% (n=819) who said they could contact to the patient, 20.8% (n=219) said they were unable to. The proportions of those that assisted the student during the clinical practice are displayed in Table 2. Of the 239 participants who believed the internship period was too short, 64.4% (n=154) indicated they had experienced enough patients, while 35.6% (n=85) said they had not been able to contact enough patients ($p<0.001$).

Table 2. The proportions of instructors who assisted the student with applications on the patient

	% (n)*
Lecturer	32.5 (342)
Research assistant	12.5 (131)
Allied health personnel	19.2 (202)
More than one group	35.5 (373)

n*: the number of respondents

The percentage of respondents who said they were proficient in the "skill of vascular access," one of the abilities taught on models during clinical skills training and later used on patients, was 95.1% (n=999). 29.7% (n=312) of the students admitted that their "intubation practice" was inadequate. Table 3 lists further outcomes.

Table 3: Clinical skill competence assessment

Lecture	Adequate % (n) *	Inadequate % (n)*
Vascular access	95.1 (999)	4.9 (52)
Adult advanced life support	92.8 (975)	7.1 (75)
Intubation	82.6 (868)	17.4 (183)
Bag-mask ventilation	92.3 (970)	7.7 (81)
Serum set preparation	91.2 (958)	8.8 (92)
Laryngeal mask airway	88.5 (930)	11.4 (120)
Medication (adrenaline) preparation	74.0 (778)	26.0 (273)

n*: the number of respondents

The rates related to the answers to the survey questions about measurement and evaluation are given in Table 4. Comments written concerning the evaluation; It was stated that the oral exam was stressful, had three stages, and was tiring.

Table 4. The responses to the questions about the measurement-evaluation at the end of the internship.

	% (n)*
Exam questions were consistent with the internship content	89.1 (936)
Equally evaluated	87.3 (917)
The knowledge and skills taught were measured in detail	86.4 (908)
Exam results were impacted by internship performance	70.5 (741)

n*: the number of respondents

DISCUSSION

In our country, the process of medical education is undergoing significant changes. The number of medical education institutions and units is growing daily and is becoming more academically robust. The practice of learning clinical skills and attitudes is improving medical education.[8] Nkabinde et al.[9] stated that the lack of structured internship training in anaesthesia and other branches in their country caused the students to lack skills. In the general evaluation of the internship program, only 0.9% (n=9) of the participants found the internship unproductive. We believe that our structured educational system is related to this pleasure.

Education is fast moving toward a student-centred structure, increasing the student's influence on the curriculum, and emphasising the evaluation of student feedback.[10] Karabilgin et al.[11] published an article on the use of student feedback in the evaluation of educational effectiveness. They claimed that student feedback is a part of the educational process in formative assessment. It was stated that the feedback forms should be created and evaluated and that the groups should not be less than ten. Structured feedback forms have been applied and evaluated in our clinic for 15 years. Since our internship groups consist of at least 12 people, students can openly share their thoughts.

Due to their extensive knowledge and workload, students focus solely on the course or courses that will be evaluated. As a result, the examination should also be educational, and students should fill in what is missing in their knowledge and abilities based on test results. In terms of convenience, evaluation is the best technique to review and impart the curriculum.[12]

In the study of 187 research assistants conducted by Hacıbeyoğlu et al.[13], aimed to measure the contribution of Anaesthesiology and Reanimation rotation to airway management. Ninety-five per cent of respondents said they had received training on airway equipment and its usage; 82% said they had received their first instruction on its use during their anaesthetic rotation. 56.7% of the participants stated that they performed the first endotracheal intubation during their anaesthesia rotation, and 59.2% stated that they used the airway device first time during their anaesthesia rotation. 34.2% stated that they used a laryngeal mask airway before, and 50% said they performed it for the first time during anaesthesia rotation. With 1051 participants, our study has one of the largest sample sizes in the literature for this subject. In our study, 82% of participants said that they performed intubation, and 88% claimed that they performed laryngeal mask adequately in their anaesthesia internship. When we look at all these, it can be predicted that research assistants who do not receive appropriate airway training in anaesthesia internship continue working careers with inadequate airway management. However, we believe that our students' success in practice is higher as we have been applying the standards of the World Medical Education Federation since 2008.

In the study Budakoglu et al.[14] conducted in different medical faculties, it was stated that anaesthesia internship was 5 days. The duration of our faculty's anaesthesia internship was 2 weeks until 2018. With the feedback received from the students, this period has been updated as 3 weeks since 2019. Although 22.7% of our participants found the anaesthesia internship period to be short, it is pleasing that 64.4% of them stated that they contacted a sufficient number of patients.

Muray[15] indicated that simulation training programs have been shown to increase the skill of anaesthesiology physicians. Hauben et al.[16] indicated that one of the most significant advances aimed at developing effective residency programs is the portfolio. The importance of cooperation in anaesthetic procedures and instruction has previously been demonstrated in the literature.[17,18] In our clinic, the operating room is shared by faculty, senior assistants, and allied health professionals. Professors and senior research assistants train students. Yet anaesthetic technicians can also help students with practical abilities. In our study, 19% of participants said they only received assistance from allied health professionals. Despite the anaesthetic technician's assistance, this situation leads us to assume that the operating room physician was forgotten about while responding the question.

According to the literature, while the tests are being designed as part of the medical faculty's end-of-internship evaluation, comprehensive, fair, and related questions should be asked regarding the internship's subject matter.[19] Additionally, Dökmeci et al.[20] suggested that student report cards be used to evaluate internship performance. Ninety per cent of the students agreed that the tests were equally evaluated, measured in detail and relevant to the course content. However, 70% of the participants claimed that the exam considered how well they performed during their internship.

CONCLUSION

It concluded that learning by doing is a successful strategy for clinical skills training to improve the student's sense of satisfaction and demonstrate the power of hands-on training. It is crucial that the anaesthetic internship, which teaches one of the most fundamental medical skills—airway safety—be developed in accordance with the feedback received from the students. We believe that standardising anaesthesiology internship programs and adopting contemporary teaching techniques improves medical education.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Mersin University Clinical Researches Ethics Committee (Date: 12.02.2015, Decision No: 2015-40).

Informed Consent: All participants signed and free and informed consent form.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Does ramelteon have an ameliorative effect in MTX-induced testicular injury?

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ABSTRACT

Aims: The aim of this study was to investigate the potential protective effect of Ramelteon (RMT), which exhibits antioxidant, anti-inflammatory and antiapoptotic properties, against testicular damage induced by Methotrexate (MTX), which is widely used in the treatment of various diseases, including chemotherapy.

Methods: 32 Wistar albino rats were equally divided into four groups: Control (group I), MTX (group II), MTX+RMT (group III) and RMT (group IV). Histologic evaluation was performed using Hematoxylin and Eosin (H&E) staining, immunohistochemical analysis using TNF-alpha and Cas-3, and biochemical evaluation using TAS, TOS and OSI.

Results: Histologic analysis using H&E staining revealed a significant difference between group I and groups II and III ($p<0.05$), while no significant difference was observed between group IV and the other groups ($p>0.05$). While normal histologic structures were observed in groups I and IV, histopathologic findings were noted in groups II and III. Immunohistochemical evaluation of TNF-alpha and Cas-3 showed a significant difference between group I and groups II and III ($p<0.05$), while no significant difference was observed between group IV and other groups ($p>0.05$). The highest immunostaining intensity was observed in group II. Biochemical evaluation revealed statistically significant differences in TAS, TOS and OSI parameters reflecting oxidative stress differences between the groups ($p<0.05$).

Conclusion: Anti-inflammatory, antioxidant and antiapoptotic properties of RMT demonstrated its protective effect against MTX-induced testicular injury in rats. Histological, immunohistochemical and biochemical analyses underline the potential role of RMT as a protective agent in MTX-induced testicular injury. This research aims to contribute to the understanding of potential applications to reduce the adverse effects of MTX therapy on reproductive health.

Keywords: Methotrexate, ramelteon, rat, testicular injury

INTRODUCTION

Methotrexate (MTX, 4-amino-10-methylfolic acid), an antimetabolite and cytotoxic agent, has been widely used in the clinical treatment of malignant and non-malignant diseases since the 1950s.[1-5]

MTX, a folate antagonist, antimetabolite, and dihydrofolate reductase inhibitor possess antitumoral, antiproliferative, anti-inflammatory, antimicrobial, immunosuppressive and immunomodulatory effects.[3,6-8]

MTX exerts its antiproliferative effect by inducing cytotoxicity during active phases of cell proliferation. Therefore, it has been reported to have toxic effects not only on cancer cells but also on highly proliferative cells such as bone marrow, gastrointestinal mucosa, and spermatogenic cells.[8]

Studies have demonstrated its toxic effects on systems such as gastrointestinal, hematologic, and central nervous systems, as well as its impact on infertility due to negative effects on oogenesis and spermatogenesis.[2] Furthermore, it has been reported to cause permanent azoospermia and infertility by inducing testicular toxicity.[1,6,9]

Oxidative stress, inflammation, and apoptosis are known to play roles in the tissue toxic effects of MTX.[3] Excessive production of reactive oxygen species (ROS) can weaken enzymatic and non-enzymatic antioxidant defence systems that protect cells from various damage mechanisms.[3,4]

Previous studies have reported that MTX increases ROS production in testicular tissue, leading to toxic effects.[2] The testicular toxicity caused by MTX has been associated with structural damage (disorganization and vacuolization) in the seminiferous tubules of the testes, rapid increase in abnormal sperm morphology, reduction in sperm

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count and density, and DNA damage in sperm.[1,2,6,8,10] In addition to these, it has been reported that MTX leads to a decrease in testicular weight and hormone levels such as FSH, LH, and testosterone.[8] Numerous studies have also demonstrated apoptotic cell death as a result of testicular damage associated with MTX.[5]

Oxidative stress is a significant pathogenic factor in MTX-induced testicular damage. Excessive production of reactive oxygen species (ROS) can lead to oxidative damage, alteration of membrane structure and function, and lipid peroxidation. It is well known that testes are highly sensitive to MTX toxicity, and studies on testicular toxicity models have shown that free radical scavengers, antioxidants, anti-inflammatory cytokines, and other drugs can mitigate testicular injury. Induction of oxidative stress and inflammatory factors are recognized as important mechanisms of MTX-related testicular toxicity.[7]

Considering all these mechanisms, there is a need for new agents with antioxidant properties to mitigate oxidative stress-induced organ damage and oxidative stress-induced inflammation caused by cancer cells and chemotherapeutic agents.[2,8,11]

RMT (RMT, (S)-N-[2-(1,6,7,8-tetrahydro-2H-indeno-[5,4-b]furan-8-yl)ethyl] propionamide, $C_{16}H_{21}NO_2$) is an orally administered hypnotic agent approved by the U.S. Food and Drug Administration (FDA) in 2005 for the treatment of chronic insomnia.[3,12,13] As a high-affinity melatonin receptor agonist, RMT is a potent and highly selective agonist for melatonin MT1/MT2 receptors believed to mediate the circadian rhythm in mammals. It also has antioxidant and anti-inflammatory effects.[3,14,15]

The aim of this study is to determine whether RMT, which has antioxidant and anti-inflammatory effects against testicular toxicity caused by acute MTX, has a protective effect through histochemical, immunohistochemical, and biochemical analyses.

METHODS

Animals

The experimental design was approved by the Süleyman Demirel University Animal Experiments Local Ethics Committee (Date: 11.09.2020, Decision No: 06/12). The study adhered to the animal research guidelines of the National Institute of Health.

Experimental Groups

A total of thirty-two male Wistar albino rats weighing 200-300 grams were included in the study. All rats were housed in standard conditions (temperature: 22-23°C, humidity: 60 ± 5%, 12-hour light/dark cycle) with access to standard laboratory chow and tap water ad libitum. The rats were randomly divided into four groups (n=8 each):

- **Control (Group I):** Received 0.1 ml saline orally for 7 days and a single intraperitoneally (IP) dose of saline on day 2.
- **MTX (Group II):** Received a single IP dose of MTX (20 mg/kg) on the second day (MTX 50 mg/ml, flk, Kocak, Turkey), and 0.1 ml saline was given orally for 7 days.[16]
- **MTX+RMT (Group III):** Received a single IP dose of MTX (20 mg/kg) on the second day and 0.1 ml RMT (10 mg/kg) orally for 7 days.[17]
- **RMT (Group IV):** Received a single IP dose of saline (0.1 ml) on the second day and 0.1 ml RMT (10 mg/kg) orally for 7 days.

Twenty-four hours after the final drug administration, rats were euthanized via intraperitoneal injection of a ketamine (90 mg/kg, Alfamine, Alfasan IBV) and xylazine (10 mg/kg, Alfazin, Alfasan IBV) combination. The testis was removed, with half stored at -20°C for subsequent analysis and the other half fixed in 10% neutral buffered formalin for histopathological and immunohistochemical evaluations of Cas-3 and TNF-alpha protein expression and biochemically, TAS, TOS and OSI values have been assessed.

Histological Analysis

Histopathological analysis: Testis tissues were fixed in a 10% buffered formaldehyde solution, dehydrated, cleared, and embedded in paraffin. Sections of 4-5 µm thickness were cut by microtome (Leica SM2000R, Germany), stained with hematoxylin and eosin (H&E), and the prepared slides were examined and imagined with a camera-equipped light microscobe with scale X20, X40 (DM500, Leica, Germany) microscopically. Seminiferous tubules, Leydig cells, and germ cells were evaluated and scored based on modified semi-quantitative criteria.

Immunohistochemical analysis: Immunohistochemical detection of Cas-3 and TNF-alpha was performed. Sections were deparaffinized, dehydrated, treated with hydrogen peroxide, blocked with Ultra-V Block, incubated with primary and secondary antibodies, followed by streptavidin peroxidase. DAB staining and hematoxylin counterstaining were carried out. Immunoreactivity was scored using a modified semi-quantitative scale.

Biochemical Analysis

Oxidative stress parameter measurement: Testis tissue samples were homogenized, TAS and TOS were measured spectrophotometrically. The OSI was calculated as TOS/TAS.

TAS analysis involved the reaction of Fe^{2+}-o-dianisidine with hydrogen peroxide to form OH radicals, with subsequent reaction with o-dianisidine producing dianisidyl radicals. Antioxidants suppress oxidation,

preventing colour formation. TOS analysis involved oxidation of the ferrous ion-o'dianisidine complex to ferric ion, forming a coloured complex with xylenol orange in an acidic medium. Colour intensity correlated with oxidant levels and was measured spectrophotometrically.

Statistical Analysis

Statistical evaluations of histological studies were performed using SPSS 29.0 and for semi-quantitative assessment, the Kruskal-Wallis test was applied. The non-parametric Mann-Whitney U test was used for pairwise group comparisons. For biochemical analyses, Levene's homogeneity test was conducted. Since the data did not exhibit homogeneity according to Levene's test, the non-parametric Kruskal-Wallis test was continued, followed by pairwise comparisons using the Mann-Whitney U test to determine the source of the difference between groups. Variables were presented as mean±standard deviation (SD), and p-values below 0.05 were considered statistically significant ($p<0.05$).

RESULTS

Histological results

Histological results of testis tissue sections from the control group and experimental groups were evaluated based on the grading system established by Refaiy et al.[18] In the H&E-stained testis tissue sections, significant differences were observed between the control (group I) and the experimental groups (group II - group III) ($p<0.05$), while no significant difference was found between the RMT (group IV) and the control group ($p>0.05$). When comparing the experimental groups (group II - group III - group IV) among themselves, the highest histopathological findings were observed in group II. In group III, these findings significantly decreased, while in group IV, these findings were not present. The observed histopathological findings included the following changes in seminiferous tubules: large-nucleus, vacuolization, enlargement, and degeneration of germ cells; granulomatous cells; dilatations; seminiferous tubules with reduced sperm content in their lumens; increased interstitial connective tissue between seminiferous tubules; and degeneration in Leydig cells (Figure 1, Table 1).

Table 1. Evaluation of histopathological findings between all groups

Histopathological Findings	Control Group I	MTX Group II	MTX+RMT Group III	RMT Group IV
Degeneration in Leydig cells	-	++/+++	+	-
Interstitial connective tissue	-/+	++	++/+++	-/+
Degeneration and dilatation of seminiferous tubules	-	+++	+++	-/+
Large-nucleus in germ cells	-	++	++	-
Enlargement in germ cells	-	+++	+/++	-
Degeneration in germ cells	-	+++	++/+++	-/+
Vacuolization in germ cells	-	+++	+++	-/+
Amount of sperm in the lumen	-	++	+/++	-

(-) (negative score): No structural changes, (+) (1 positive score): Light structural changes, (++) (2 positive score): Middle structural changes, (+++) (3 positive score): Serious structural changes. -:($p>0.05$), -/+:($p>0.05$), +:($p<0.05$), +/++:($p<0.05$), ++:($p<0.05$), ++/+++:($p<0.05$), +++:($p<0.05$).

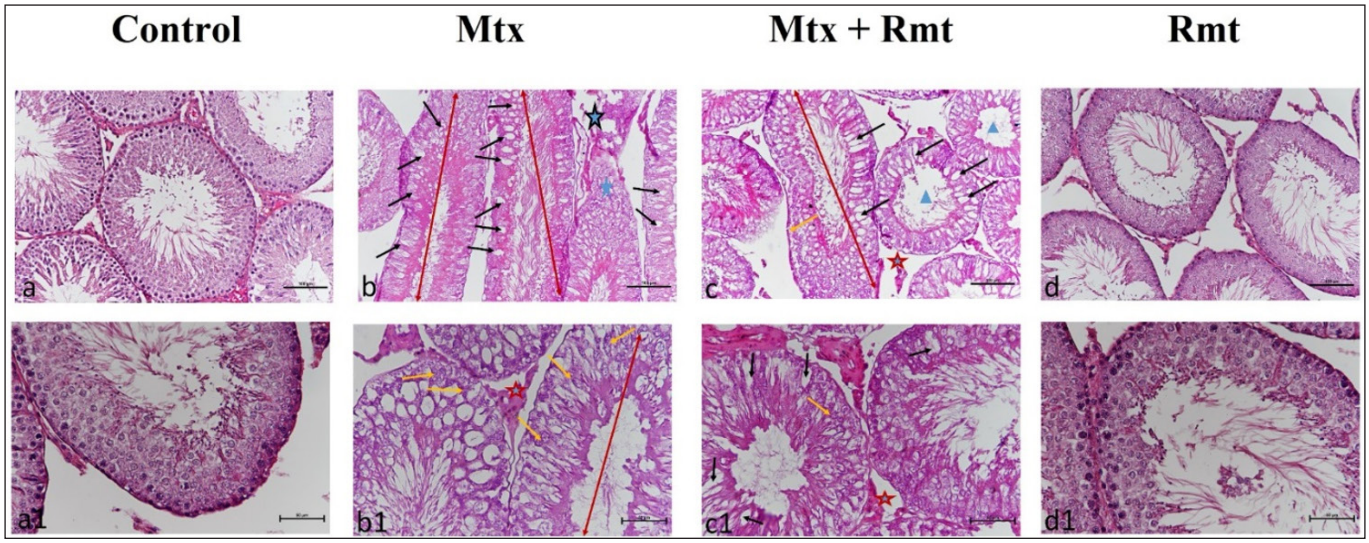

Figure 1: Histopathological findings in testis tissues belonging to control and experimental groups: a - a1, control group; normal histological structure of seminiferous tubules, Leydig cell, and interstitial area. b - b1, MTX group; lots of histopathological findings; degeneration and dilatation of seminiferous tubules (red arrows), vacuolization in germ cells (black arrows), increase of interstitial area (black star), granulomatous cells in seminiferous tubules (blue star), degeneration of Leydig cells (red star), degeneration of germ cells (yellow arrows), a few of sperm in the lumen (blue triangles) c - c1, MTX+RMT group; mild histopathological findings, d - d1, RMT group; normal histological structure. (a, b, c, d, H- E, x20 - a1, b1, c1, d1, H-E, x40).

Immunohistochemical Results

Immunohistochemical staining of testis tissue sections showed significant differences between the control group (group I) and the experimental groups (group II - group III) ($p<0.05$), while no significant difference was found between the RMT group (group IV) and the control group ($p>0.05$). When comparing the experimental groups (group II - group III - group IV) among themselves, the highest immunostaining was observed in group II. In group III, immunostaining decreased significantly compared to group II, while the lowest immunostaining was observed in group IV, which was equally comparable to the control group (Figure 2, Table 2). No significant differences were observed in the degrees of staining among TNF-alpha and Cas-3 antibodies.

Table 2. Degrees Staining of TNF-Alpha, Cas-3

Staining Levels			
Control Group I	MTX Group II	MTX+RMT Group III	RMT Group IV
+/-	+++	++	+/-

Biochemical Results

Oxidative stress parameters in the testicular tissue were compared among all groups using the Kruskall-Wallis test, and statistically significant differences were observed among groups in terms of TAS, TOS, and OSI parameters ($p=0.006$, $p=0.019$, $p=0.005$, respectively) (Figure 3). To understand from which group the difference originated, pairwise comparisons were conducted using the Mann-Whitney U test (Figure 4). When comparing the Control and MTX groups, there were significant differences in all three parameters (TAS, TOS, and OSI) ($p=0.001$, $p=0.007$, $p=0.002$, respectively). These data, in accordance with the materials and methods, reveal that the systemic toxicity induced by MTX leads to a depletion of TAS and an elevation of TOS levels in testicular tissue. There were no significant differences in all three parameters between the Control and RMT groups ($p=0.105$, $p=0.574$, $p=0.798$, respectively). This indicates that the investigated RMT preparation alone did not have a significant impact on the oxidative and antioxidative systems, either positively or negatively. Similarly, no statistically significant difference was found between the Control and MTX + RMT groups ($p=0.382$, $p=0.505$, $p=0.083$, respectively). These data reflect the positive effects of RMT for all three parameters. When administered alone, MTX-induced increases in TOS and decreases in TAS levels were brought back to control levels upon co-administration with RMT. Statistically significant differences were observed in all three parameters between the MTX and MTX + RMT groups ($p=0.010$, $p=0.003$, $p=0.005$, respectively). In the MTX-treated group, the increased oxidative stress and depleted TAS levels displayed an opposing trend in the group that received RMT alone, similar to the control group. When comparing the MTX and MTX + RMT groups, a significant difference was only observed in TAS ($p=0.021$). Although there was a decrease in TOS in the MTX + RMT group in terms of other parameters, this difference was not statistically significant ($p=0.065$). There were no significant differences in all three parameters between the RMT and MTX + RMT groups. This suggests that RMT exhibits a protective effect, resulting in a restorative effect that brings the MTX + RMT group closer to control levels.

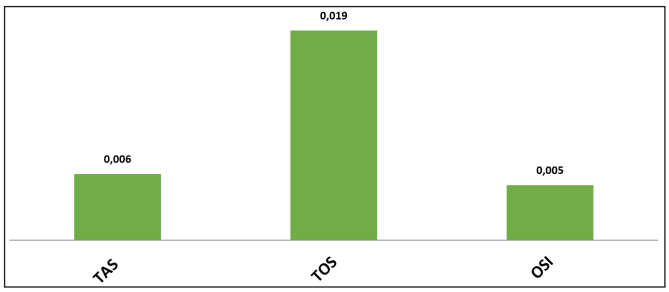

Figure 3. Levels of difference between groups in TAS, TOS, and OSI parameters as determined by Kruskal-Wallis Test

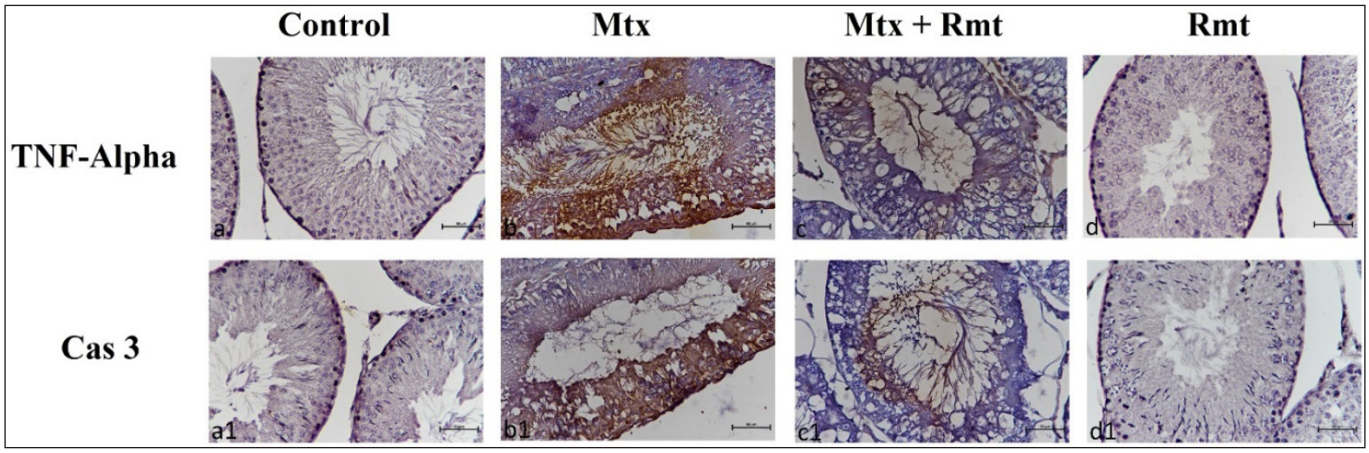

Figure 2. TNF-alpha, and Cas-3 immune stainings in testis tissues belonging to control and experimental groups. a- a1, control group; no positive staining, b - b1, MTX group; positive staining, c - c1, MTX+RMT group; mild positive stainings, d - d1, RMT group; no positive staining, immun staining, x40

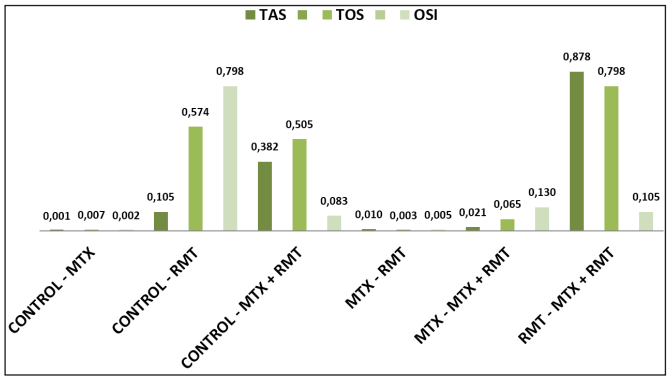

Figure 4. Levels of difference between pairwise groups in TAS, TOS, and OSI parameters as determined by Mann-Whitney U Test

DISCUSSION

MTX, extensively used in tumour chemotherapy,[7] structurally resembling folic acid and being able to inhibit folate-dependent enzymes, suppresses the synthesis of tetrahydrofolate required for the formation of purines and pyrimidines, crucial for cell replication, by binding to dihydrofolate reductase. Suppression of purine and pyrimidine synthesis results in DNA damage and subsequently leads to apoptosis.[4]

As a folate antagonist, MTX effectively suppresses cellular growth. Hence, it finds applications in various clinical conditions including autoimmune diseases like psoriasis and rheumatoid arthritis, non-neoplastic and neoplastic diseases like graft-versus-host disease, multiple sclerosis, keratoacanthomas, psoriatic arthritis, systemic lupus erythematosus, acute lymphoblastic leukaemia, non-Hodgkin lymphoma, breast cancer, head and neck malignancies and dermatomyositis, as well as ectopic pregnancy.[1-4,10,11,19,20]

Studies have shown that chemotherapy drugs can lead to side effects in various organs, including the reproductive system, causing conditions such as azoospermia, testicular damage, sex hormone dysfunction, and infertility in both human and animal models due to their antiproliferative properties.[19]

Previous studies on MTX have demonstrated negative effects on the male reproductive system, causing irregularities and vacuolization in the seminiferous epithelium, reduced sperm counts, sperm DNA damage, decreased testicular weight, and diminished seminal vesicle and prostate gland sizes.[19] Our study also exhibited notable histopathological changes, especially in the group treated with MTX. Various histopathological findings were observed, including large-nucleus germ cells with vacuolated appearance, enlargement, and degeneration; granulomatous cells; dilations; seminiferous tubules with low sperm content in their lumens; increased interstitial connective tissue; and Leydig cell degeneration. Conversely, these findings were alleviated in the MTX + RMT group, suggesting the positive impact of RMT on the damage caused by MTX.

Oxidative stress and inflammation play a significant role in tissue damage due to various causes, including drug toxicities. An imbalance between the production of high levels of reactive free radicals and the antioxidant defence systems results in oxidative stress. Consequently, the body experiences elevated oxidant radicals and reduced antioxidant molecules. To assess the overall oxidant and antioxidant status, markers such as TOS, TAS, and OSI have been developed.[4]

MTX's adverse effects have been associated with increased reactive oxygen species (ROS) in the testes. Furthermore, MTX induces testicular germ cell apoptosis, leading to increased apoptotic cell counts and oxidative stress due to enhanced apoptotic indices, and mRNA expression of caspase-3, caspase-8, and caspase-9.[19]

In our study, all groups were compared, and statistically significant differences were found among groups in terms of TAS, TOS, and OSI parameters. These findings indicate that systemic toxicity induced by MTX increases TOS levels in testicular tissue. When MTX was administered alone, the increased TOS and decreased TAS levels were restored to control levels in the MTX+RMT group. This outcome highlights the positive effects of RMT.

CONCLUSION

This study demonstrates the potential protective effect of RMT on the damage induced by MTX, owing to RMT's anti-inflammatory, antioxidant, and antiapoptotic properties. As a high-affinity melatonin receptor agonist, RMT is used in insomnia treatment and has potential applications in anticancer therapy. The study could serve as a reference for future research, investigating the protective effects of RMT on chemotherapeutic agent-induced damage.

ETHICAL DECLARATIONS

Ethics Committee Approval: The experimental design was approved by the Süleyman Demirel University Animal Experiments Local Ethics Committee (Date: 11.09.2020, Decision No: 06/12). The study adhered to the animal research guidelines of the National Institute of Health.

Informed consent: This project is an animal experiment, so informed consent has not required.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Histopathological evaluation of the effects of sildenafil on organ damage in a diabetic rat model

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ABSTRACT

Aims: In this study, it was aimed to show the effects of sildenafil on heart, liver and kidney tissues histopathologically by creating an experimental diabetes model with streptozocin.

Methods: Male adult Sprague dawley rat (48) was used in the study. The rats were first divided into three groups as control group, the diabetes group and the diabetes+sildenafil group. Each group was divided into two groups within itself. Streptozotocin 40 mg/kg was administered intraperitoneally to the rats in the groups that would develop diabetes and diabetes+sildenafil diabetes. Rats with blood glucose levels of 250 mg/dl and above after 48 hours were considered diabetic. Sildenafil citrate 10mg/kg/day was given by gavage to the diabetes+sildenafil group. At the end of the experiment heart, liver and kidney tissues were placed in formaldehyde solution. Hematoxylin-Eosin staining was applied to the sections taken. Histological changes in the stained sections were evaluated by a histologist. Histological evaluation was performed semi-quantitatively in heart, liver and kidney tissue. In the assessment, the findings of the tissues were scored and statistical analysis was performed.

Results: Histological findings of heart, liver and kidney tissues were examined. It was determined that less organ damage was seen in the diabetes+sildenafil group compared to the Diabetes group.

Conclusion: In our study, it has been demonstrated histologically that sildenafil can be a drug that has an antioxidant effect in tissue by helping to protect cell structure and architecture against heart, liver and kidney tissue damage caused by diabetes. It should not be overlooked that it is important to determine the appropriate dose and frequency of use of sildenafil in revealing these effects.

Keywords: Sildenafil citrate, diabetes, streptozocin, organ damage

INTRODUCTION

Diabetes is a complex chronic disease in which the number of patients gradually increases with the changing lifestyle, decrease in physical activity and increase in obesity. Reducing the harmful effects of this disease by keeping it under control has been and continues to be the focus of many past studies. Retinopathy, nephropathy, neuropathy, myocardial infarction due to diabetes, and atherosclerosis of the vessels may occur, especially in diabetic patients, due to failure to control hyperglycemia.[1] The underlying problem of these diseases is the increased concentration of free radicals caused by hyperglycemia. Oxidative stress due to free radicals is a known pathogenetic mechanism in diabetic complications.[2] Oxidative/antioxidant balance disrupted by oxidative stress causes macro and microvascular complications of diabetes.[3,4]

Sildenafil, known as a phosphodiesterase type 5 (PDE 5, the enzyme responsible for cGMP hydrolysis) inhibitor, is a therapeutic drug of choice in neurodegenerative diseases,[5] including age-related macular degeneration, used in the treatment of erectile dysfunction and pulmonary arterial hypertension.[6] It is known that sildenafil regulates vascular superoxide release by affecting nitric oxide (NO) release and reduces oxidative stress by causing a vascular antioxidant effect in insulin-resistant rats.[7] In addition, sildenafil is known to regulate oxidative stress markers such as malondialdehyde (MDA),8 reduced glutathione (GSH) and proinflammatory cytokines such as interleukin-4 (IL-4).[9]

These positive effects of sildenafil on oxidative stress are too important to ignore. The multiple effects of diabetes on the organs cause the whole organism to be affected and

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pave the way for the emergence of secondary diseases. The most affected of these organs are the heart, liver and kidney. It will be important to reveal the relationship of sildenafil with diabetes simultaneously through the histological changes in these organs. In this study, it was aimed to show the effects of sildenafil on heart, liver and kidney tissues histopathologically by creating an experimental diabetes model with streptozocin (STZ).

METHODS

The study was carried out with the permission of Erciyes University Animal Experiments Local Ethics Committee (Date: 03.03.2021, Decision No: 21-56). The study adhered to the animal research guidelines of the National Institute of Health.

Experimental Design

48 male adult Sprague Dawley rats weighing 250-300gr were used in the study. The rats were first divided into three groups: The Control group, the Diabetes group, and the Diabetes+Sildenafil group. Each group was divided into two groups within itself (Control 1 group, Control 2 group, Diabetes 1 group, Diabetes 2 group, and Diabetes+Sildenafil 1 group, Diabetes+Sildenafil 2 group). Streptozocin 40 mg/kg was administered intraperitoneally (IP) to the rats in groups Diabetes and Diabetes+Sildenafil that would develop diabetes (dissolved in citrate buffer). Intraperitenoal 1 ml of saline was administered to the control group. Two days after the procedure, blood glucose was measured from the tail vein of the rats, and the rats with a blood glucose level of 250 mg/dl and above were considered diabetic.[10,11] Sildenafil citrate was administered to the Diabetes+Sildenafil group by gavage at 10 mg/kg/day. 1st groups were sacrificed on the 4th day, and the 2nd groups were sacrificed on the 7th day. The reason for dividing the groups within themselves is to show the possible effect of sildenafil as it changes within days. The rats were sacrificed by the decapitation method. The rats were fed as standard in this process and cared for under standard conditions.

Histological Procedure

At the end of the experiment, heart, liver and kidney tissues of rats were fixed in 4% formaldehyde. Then histological tissue follow-up was applied. The tissue was first passed through a graded alcohol series (50%, 70%, 80%, 96%, 3 x Absolute alcohol). Then, it was passed through xylene (x3) and the melt was taken to paraffin. Hematoxylin Eozin (HE) staining was performed to determine histological changes by taking 5 µm sections from the tissues fixed on paraffin blocks. Histological changes in the stained sections were evaluated by a histologist.[12] Histological evaluation was performed semi-quantitatively in heart, liver and kidney tissue. In the assessment, the findings of the tissues were scored and statistical analysis was performed. A total of ten areas in each group were evaluated from tissue sections of each animal, scoring 0: no, 1: slight, 2: moderate, and 3: severe.[13]

Hematoxylin-Eosin Staining

After the preparations of the heart, liver and kidney tissue sections were kept in the oven, the paraffin was removed by taking xylene. Tissues were rehydrated by passing through a series of alcohol (3x Absolute alcohol, 96%, 80%, 70%, 50%). Afterward, the sections were washed with water and kept in a Hematoxylin solution. Sections washed with water again were taken in Eosin solution. The re-washed sections were first examined by passing through the alcohol series, then through xylene and closed with Entellan.[14]

Statistical Analysis

All statistical analyses were carried out by using GraphPad Prism version 7.00 for Mac, GraphPad Software, La Jolla, California, USA. D'Agostino Pearson's omnibus test was used to identify the normal distribution of the data. In the case of normal distribution, quantitative variables were compared using one-way ANOVA analysis of variance and Tukey's posthoc test. The data were expressed as the mean of normalized data standard deviation of the mean. $p<0.05$ was considered statistically significant.

RESULTS

Heart Tissue Histological Findings

Cardiac muscle fibres in the heart tissue of the control 1 group had a normal histological appearance in terms of their regular arrangement, nucleus and cytoplasm. The heart tissue sections of the Control 2 group were also similar to the Control 1 group. Myofibril loss was observed in cardiomyocyte cytoplasm in some areas of the heart tissue belonging to the diabetes 1 group. Disorganization of the cardiomyocyte bundles and loss of myofibrils in the cell cytoplasm were observed in the heart tissue of the diabetes 2 group. Cardiomyocytes, which can be distinguished by their dense eosinophilic cytoplasm, were found in some areas. Cardiomyocyte arrangement in the heart tissue sections of the Diabetes+Sildenafil 1 group was similar to the control groups in terms of cytoplasm and nucleus. In the Diabetes+Sildenafil 2 group, cardiomyocyte bundle disorganization was partially improved compared to the diabetes group. Cardiomyocytes with dense eosinophilic cytoplasm were also less common in this group. Statistical analysis results of heart tissue myofibril loss, cardiomyocyte disorganization and eosinophilic

cardiomyocyte findings are shown in Table 1 and Table 2. The histological findings of all experimental groups are shown in Figure 1.

Table 1. Statistical analysis results of heart tissue myofibril loss, cardiomyocyte disorganization and eosinophilic cardiomyocyte findings in Control 1, Diabetes 1 and Diabetes+Sildenafil 1 groups.

	Control 1	Diabetes 1	Diabetes+ Sildenafil 1	p
Myofibril loss	0.3±0.4[a]	1±0.8[b]	0.5±0.5[ab]	0.0517
Cardiomyocyte disorganization	0.3±0.4	0.8±0.6	0.5±0.5	0.1438
Eosinophilic cardiomyocytes	0.2±0.4[a]	1.1±0.9[b]	0.4±0.5[ab]	0.0181

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Table 2. Statistical analysis results of heart tissue myofibril loss, cardiomyocyte disorganization and eosinophilic cardiomyocyte findings in Control 2, Diabetes 2 and Diabetes+Sildenafil 2 groups.

	Control 2	Diabetes 2	Diabetes+ Sildenafil 2	p
Myofibril loss	0.3±0.4[a]	1.1±0.7[b]	0.6±0.5[ab]	0.0178
Cardiomyocyte disorganization	0.3±0.4	0.8±0.6	0.3±0.4	0.0732
Eosinophilic cardiomyocytes	0.2±0.4[a]	1.1±0.8[b]	0.5±0.5[ab]	0.0127

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Liver Tissue Histological Findings

The liver sections of the Control 1 and Control 2 groups had a regular structure with the vena centralis in the middle forming the classical lobule structure and hepatocyte cell cords and sinusoids radially located towards the periphery. Portal areas located in the periphery of the classical lobule also preserved their regular histological structure. Deterioration was observed in the hepatocyte cords located close to the vena centralis in the classical lobule structure of the liver belonging to the diabetes 1 group. It was observed that some hepatocytes lost their euchromatic nuclei and transformed into cells with denser eosinophilic cytoplasm. In the liver sections of the diabetes 2 group, disruption of radial hepatocyte cords was observed. In addition, cells with altered nuclei and intense eosinophilic staining were observed more frequently in the Diabetes 2 group. While the liver tissue of the Diabetes+Sildenafil 1 group had a generally good appearance, there were almost no cells with an intense eosinophilic appearance. While the sections had a regular appearance in the Diabetes+Sildenafil 2 group, cells with a changed structure and dense eosinophilic cytoplasm were found very rarely. Statistical analysis results of liver tissue hepatocyte cords disorganization and eosinophilic hepatocytes findings are shown in Table 3 and Table 4. The histological findings of all experimental groups are shown in Figure 2.

Table 3. Statistical analysis results of liver tissue hepatocyte cords disorganization and eosinophilic hepatocytes findings in Control 1, Diabetes 1 and Diabetes+Sildenafil 1 groups.

	Control 1	Diabetes 1	Diabetes+ Sildenafil 1	p
Hepatocyte cords disorganization	0.2±0.4	0.7±0.6	0.6±0.6	0.1733
Eosinophilic hepatocytes	0.1±0.3[a]	1.2±0.6b	0.3±0.4[a]	0.0001

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

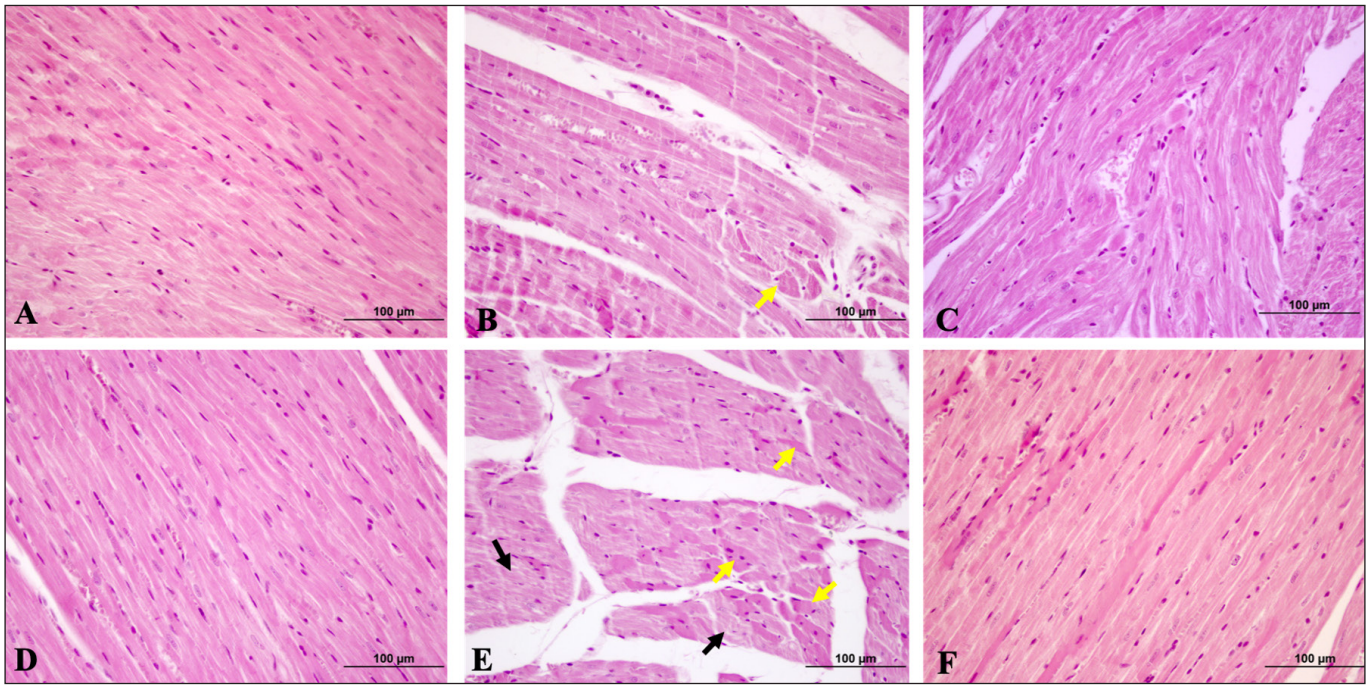

Figure 1. HE staining of heart tissue belonging to the experimental groups. yellow arrow; eosinophilic stained cardiomyocytes, black arrow; shows the loss of myofibrils in cardiomyocytes. A) Control 1 group, B) Diabetes 1 group, C) Diabetes+Sildenafil 1 group, D) Control 2 group, E) Diabetes 2 group, F) Diabetes+Sildenafil 2 group. Scale bar 100 µm.

Table 4. Statistical analysis results of liver tissue hepatocyte cords disorganization and eosinophilic hepatocytes findings in Control 2, Diabetes 2 and Diabetes+Sildenafil 2 groups.

	Control 2	Diabetes 2	Diabetes+ Sildenafil 2	p
Hepatocyte cords disorganization	0.1±0.3[a]	1.2±0.6[b]	0.7±0.4[b]	0.0002
Eosinophilic hepatocytes	0.1±0.3[a]	1.7±0.8[b]	0.2±0.4[a]	0.0001

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Kidney Tissue Histological Findings

In the kidney sections of Control 1 and Control 2 groups, glomeruli, proximal and distal tubule structures forming the nephron, and collecting tubules were evaluated. In both groups, glomerular structure, Bowman's space and parietal leaf were distinguished by their normal appearance. The proximal tubule and distal tubule epithelium had a regular appearance with their cytoplasm and lumens. In general, all cortex and medulla connective tissue and collecting tubules were regular. Histological changes were found mostly in the cortex layer in the diabetes groups. In the diabetes 1 group, disruption of the cytoplasm of the proximal tubule epithelium, scattering in its apical parts, and shed epithelial cells in some tubule lumen was observed. No significant histological changes were observed in the nephron structure and distal tubules. In the diabetes 2 group, epithelial damage in the proximal tubule and cells spilt into the lumen were observed more intensely. Disorganization was also observed in the distal tubule epithelium. In the Diabetes+Sildenafil 1 group, nephron structures had a regular appearance. The number of proximal tubules with epithelial spilt lumen was much lower than in the diabetes groups. Both proximal and distal tubule epithelial cytoplasm also had a more regular appearance. Nephron structures were regular and some disorganization was observed in the proximal tubule epithelium in the Diabetes+Sildenafil 2 group. Shedding was less in some proximal and distal tubule epithelium of the kidney tissue compared to the diabetes group. Statistical analysis results of kidney tissue proximal tubule injury and distal tubule injury findings are shown in Table 5 and Table 6. The histological findings of all experimental groups are shown in Figure 3.

Table 5. Statistical analysis results of kidney tissue proximal tubule injury and distal tubule injury findings in Control 1, Diabetes 1 and Diabetes+Sildenafil 1 groups.

	Control 1	Diabetes 1	Diabetes+ Sildenafil 1	p
Proximal tubule injury	0.2±0.4[a]	1.7±0.6[b]	0.9±0.7[c]	0.0001
Distal tubule injury	0.4±0.5[a]	1.9±0.7[b]	1.1±0.8[ab]	0.0004

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

Table 6. Statistical analysis results of kidney tissue proximal tubule injury and distal tubule injury findings in Control 2, Diabetes 2 and Diabetes+Sildenafil 2 groups.

	Control 2	Diabetes 2	Diabetes+ Sildenafil 2	p
Proximal tubule injury	0.4±0.5[a]	1.8±0.7[b]	0.8±0.7[a]	0.0005
Distal tubule injury	0.4±0.5[a]	1.9±0.7[b]	0.9±0.8[a]	0.0003

Data are shown as ± standard deviation. p <0.05 was considered significant. There was no significant difference between the groups containing the same letter (a, b and c).

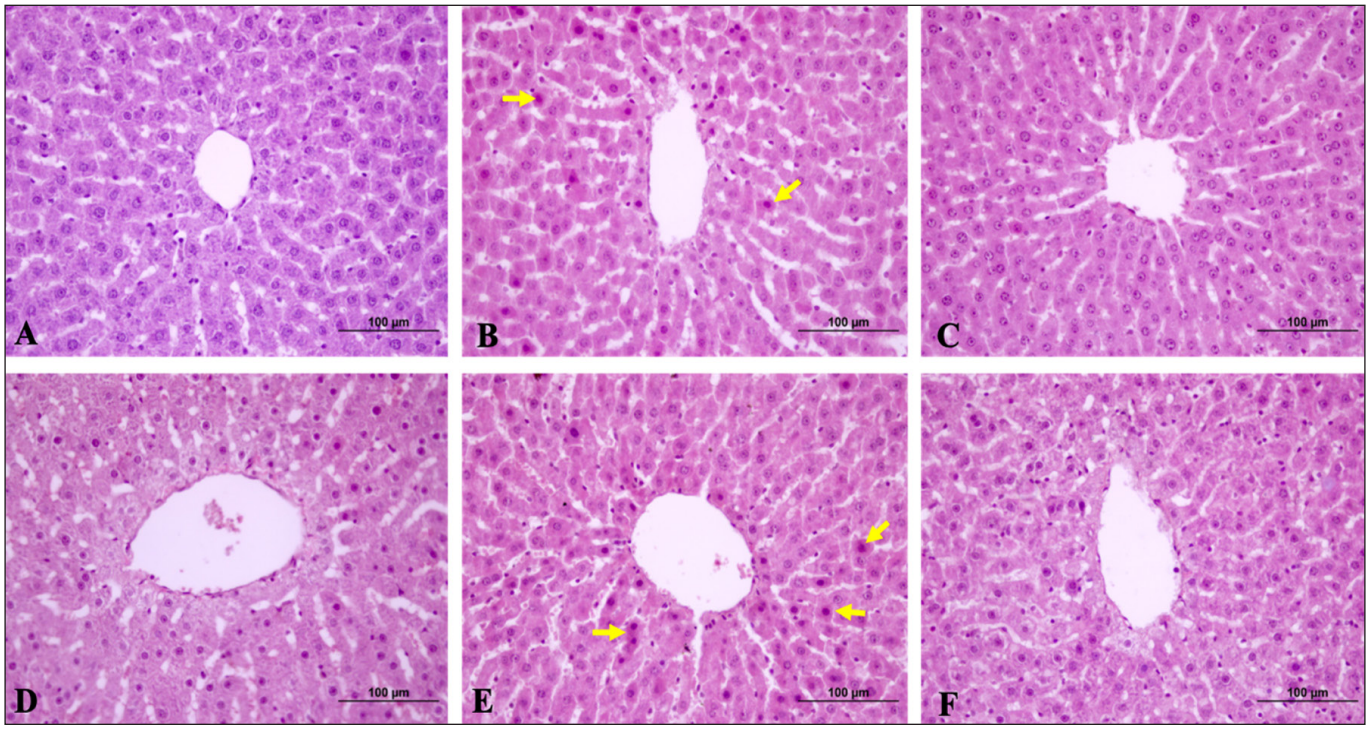

Figure 2. HE staining of liver tissue of the experimental groups. yellow arrow; eosinophilic stained hepatocytes A) Control 1 group, B) Diabetes 1 group, C) Diabetes+Sildenafil 1 group, D) Control 2 group, E) Diabetes 2 group, F) Diabetes+Sildenafil 2 group. Scale bar 100 µm.

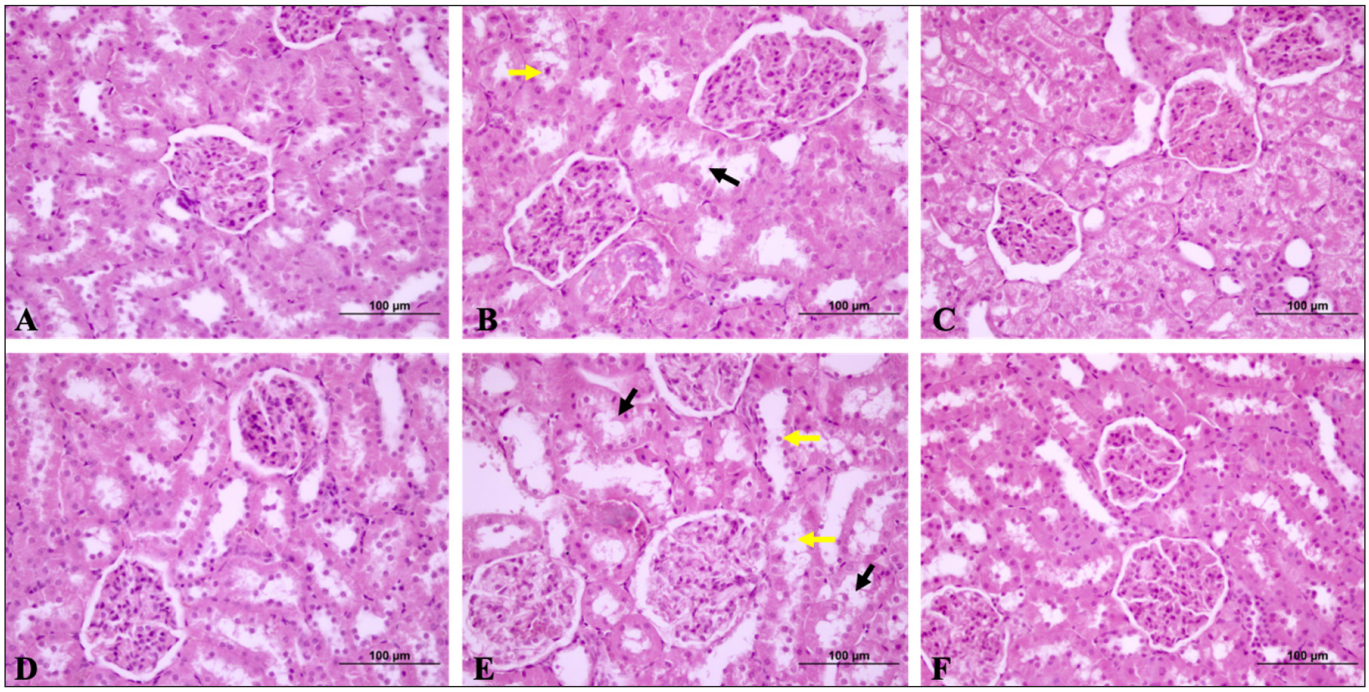

Figure 3. HE staining of kidney tissue of the experimental groups. yellow arrow; epithelial cell spilling into the lumen, black arrow; shows disorganized tubules. A) Control 1 group, B) Diabetes 1 group, C) Diabetes+Sildenafil 1 group, D) Control 2 group, E) Diabetes 2 group, F) Diabetes+Sildenafil 2 group. Scale bar 100 µm.

DISCUSSION

Hyperglycemia from diabetes is a widely recognized cause of increased free radical concentration, whereas the involvement of oxidative stress in glycemic regulation is still debated. Glucose transport is a sequence of events that begins with the interaction of insulin with its receptor on the plasma membrane and ends with intracellular glucose metabolism. Every step plays an important role in this complex sequence of events and can be prevented by reducing oxidative stress.[2] Because elevated blood glucose level stimulates the production of proinflammatory cytokines, and activates lipid peroxidation and apoptotic process, causing various diabetes complications. It has been found that oxidative stress plays a central role in lipid peroxidation, protein oxidation, DNA damage and neuronal death in humans and experimental animals.[15] This study, it was aimed to show the effects of sildenafil on diabetes-induced damage in heart, liver and kidney tissue.

It is known that diabetic rats have a decrease in the activity of antioxidant enzymes and an increase in oxidant enzyme activity, as well as an increase in lipid peroxidation, protein carbonylation, free oxygen radical production, and proinflammatory cytokines. The role of inflammation in the pathogenesis and progression of myocardial damage in chronic diabetes is well established. The detrimental effect of chronic diabetes on the myocardium may result from inflammatory signalling or dysregulation of anti-inflammatory signalling systems.[16] The presence of cytokines due to increased oxidative stress caused by diabetes damages the tissue. In our study, it was concluded that myofibril loss can be prevented and the oxidative and inflammatory response in the tissue can be improved by preserving the cardiomyocyte arrangement, cytoplasm, and nucleus in the heart tissue sections belonging to the Diabetes+Sildenafil group. Considering the properties of sildenafil, it should not be forgotten that its effects on the heart should be revealed not only by histopathological examination but also by methods such as biochemical analyses and gene expression. The appearance of intense eosinophilic cells, especially in the heart and liver tissue, can be considered as an indicator of changes in the cell cytoplasm due to impaired function loss in the tissue. Sildenafil, which is widely used in the treatment of erectile dysfunction, has been reported to reduce the level of IL-8 associated with diabetic cardiomyopathy by suppressing inflammatory processes through cyclic guanine monophosphate (cGMP), which belongs to the drug class that inhibits phosphodiesterase type 5.[17] Sildenafil is known to have strong cardioprotective effects in addition to its antioxidant activity and can reduce apoptosis and necrosis in heart tissues after ischemia-reperfusion injury.[18] Sildenafil also has a relaxing effect on smooth muscle cells of arterioles via cGMP, enhancing its effects through NO production and affecting the liver. NO affects hemodynamic parameters by increasing hepatic microvascular blood flow without increasing pressure due to the relaxation of both presinusoidal stellate cells and portal vein smooth muscle and terminal arterioles, but it is also known that excess NO accumulation is toxic to hepatocytes.[19] It has been reported that liver tissue damage is reduced and hepatocyte cytoarchitecture is regulated

by regulated NO level and antioxidant balance due to sildenafil.[20] In our study, sildenafil was found to be effective in maintaining tissue integrity by regulating the impaired hepatocyte structure and sequence due to diabetes. In the pathogenesis of diabetic nephropathy, it is seen that increased angiotensin II levels cause advanced glycation end products, cytokine increase due to induction of oxidative stress and then kidney tissue damage, especially glomerular damage.[21] Therefore, it can be said that the regulation of NO level in the kidney tissue as in the liver tissue improves the tissue by regulating the abnormal NO and cGMP levels of sildenafil in the damage caused by diabetes. In our study, it was observed that sildenafil was effective in reducing diabetes-related renal tissue tubule damage and protecting the glomerular structure. The absence of biochemical parameters in our study is considered a deficiency, but the histological demonstration that sildenafil produces positive results in tissue in diabetes-induced damage should not be overlooked. In addition to these positive effects, it should be noted that frequent use and high doses of sildenafil may cause damage to liver and kidney tissues.[22]

In our study, a dose of 10 mg/kg/day Sildenafil was applied, and the effect of different doses was not examined. In the studies of Ala et al.[23] it was shown that oxidative stress and antioxidant effects of Sildenafil at different doses (5, 10 and 40 mg/kg/day) may be different, 10 and 40 mg/kg/day doses of Sildenafil were shown to be more effective on oxidative stress and inflammatory cytokines compared to 5 mg/kg/day. Similarly, in the studies of Hafez et al.[24] it was shown that the oxidative stress and antioxidant effects of Sildenafil at different doses (5 and 10 mg/kg/day) may vary. These dose-dependent changes could not be demonstrated in our study because a single dose (10 mg/kg/day) of Sildenafil was administered.

In the study of Jeong et al.[25] it was shown histologically that sildenafil prevented kidney damage in streptozocin-induced diabetic rats. It has been shown histologically that sildenafil prevents kidney damage in models of kidney injury created by different mechanisms. In the study of Jorge et al.[26] it was shown histologically that sildenafil prevented kidney damage in the kidney injury induced by bothrops alternatus snake venom model. These results are similar to our study.

CONCLUSION

In our study, it was revealed that sildenafil may be a drug with antioxidant effects in tissue by helping to preserve cell structure and architecture at the histological level against heart, liver and kidney tissue damage caused by diabetes. It should not be overlooked that determining the appropriate dose and frequency of use of sildenafil is important in revealing these effects.

CONCLUSION

Treatment of ARDS associated with COVID-19 requires a multidisciplinary approach. In this patient group with high mortality and cost, the use of ECMO by considering prognostic factors and guidelines is seen as factors that increase the chance of success. The patients in our study were treated with ECMO in accordance with established guidelines. However, given the high mortality recorded in the present study, we believe that studies on the effectiveness of additional supportive treatments that can reduce ECMO-related complications are needed. As mortality in patients with ARDS due to COVID-19 is higher than that in patients with ARDS unrelated to COVID-19, potential risk factors for mortality other than ARDS need to be reviewed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Animal Experiments Local Ethics Committee (Date: 03.03.2021, Decision No: 21-56).

Informed Consent: This study was designed as an experimental animal study.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Role of hemogram parameters as predictive markers for propofol injection pain in reproductive and postmenopausal women: a prospective study

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ABSTRACT

Aims: In clinical practice, propofol injection pain (PIP) is a frequent condition that clinicians could face every day. The aim of this prospective study was to investigate the correlation between PIP and hemogram parameters in reproductive and postmenopausal women.

Methods: In this prospective study, 40 reproductive and 40 postmenopausal female patients who underwent elective surgery were enrolled. Baseline data including age, weight, height, hemogram parameters, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and systemic immune inflammatory score (SII) were recorded preoperatively. The pain was classified as 0: no pain, 1: mild pain, 2: moderate pain, 3: severe pain. Patients were previously informed about the questioning of pain scores during propofol.

Results: The proportion of patients experiencing PIP in postmenopausal (n=16;40%) group was significantly higher than those in the reproductive group (n=6;15%) (p=0.009). When reproductive and postmenopausal groups were compared according to the presence or absence of pain, no difference was found between the groups in terms of hemogram ratios and platelet indices (p>0.05). The NLR, PLR and SII ratios were not significant in predicting the presence of PIP.

Conclusion: The findings of our study showed that the occurrence of PIP in postmenopausal women was higher compared to women in reproductive age. Hemogram values, NLR platelet, PLR and SII ratios were not significantly associated with the presence of PIP.

Keywords: Propofol, pain, neutrophil, hemogram, postmenopausal

INTRODUCTION

Propofol is one of the most used hypnotic agents for the induction and maintenance of general anesthesia, procedural sedation, and sedation in intensive care units.[1,2] While the risk of severe complications related to propofol injection, such as propofol-related infusion syndrome is rare, milder side effects are more prevalent. Amongst these side effects, the preeminent and commonly encountered adverse circumstance for clinicians is the manifestation of propofol injection pain (PIP).[3]

The pain experienced during propofol injection is considered to result from various factors, including damage to the inner lining of blood vessels, differences in osmolality, abnormal pH levels, and stimulation of pain receptors and nerve endings in veins. These factors are considered to contribute to the pain experienced during injection, although the underlying cause is not well-established.[3,4] The incidence of PIP ranges from 28% to 90% and is known to create a negative memory of the anesthesia experience for individuals.[3-5] The majority of research conducted on the topic of PIP has primarily concentrated on mitigating discomfort through the administration of agents like lidocaine, ephedrine, ondansetron and ketamine prior to the injection.[5-8] Nevertheless, in recent years, a limited number of studies have also been undertaken to explore the predictive factors associated with PIP in various patient populations.[9,10]

The neutrophil- lymphocyte ratio has been shown to have a relationship with the perception of postoperative pain and pain in chronic diseases. Therefore, aimed to determine if there was a link between pain on propofol injection (POPI) and various hemogram parameters

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and ratios. This prospective study aimed to investigate the potential correlation between PIP and hemogram parameters and derived ratios, including the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and systemic immune inflammation score (SII), in a cohort of reproductive and postmenopausal women undergoing elective surgery. The objective was to assess whether hematological markers could serve as an indicator of pain in this patient population.

METHODS

This prospective study was carried out with the permission of Doctor Burhan Nalbantoglu State Hospital Ethics Committee (Date: 18.07.2023, Decision No: 1.01-25/23). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In this prospective study, a cohort comprising 40 reproductive and 40 postmenopausal female patients who underwent elective surgery at a tertiary hospital in July and August 2023 was planned to enroll. All participants provided informed consent. The study specifically focused on patients scheduled under general anesthesia for elective procedures in the fields of orthopedics, abdominal surgery, urology, otolaryngology, or plastic surgery, and classified as American Society of Anesthesiology (ASA) physiological score I or II. Exclusion criteria included hematological, oncological, or endocrinological diseases, a history of chronic drug or steroid use within the last three months, upper-lower respiratory tract infection within the last three weeks, and regular alcohol consumption, as these factors could potentially impact their surgical outcomes. Patients with a history of psychiatric illness and those requiring tranquilizers due to severe anxiety prior to surgery were also excluded from the study, as these factors may influence their pain perception.

Prior to the surgical procedure, relevant information was gathered from the patients, including age, weight, length, and various indicators from their hemogram. These indicators included white blood cell count, neutrophil count, lymphocyte count, platelet count, as well as platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). Additionally, the levels of hemoglobin and hematocrit were measured. All hemograms were performed using the Sysmex XT 1800i device manufactured by Sysmex Corporation in Kobe, Hyogo, Japan. Using this data, calculations were made to determine the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and systemic immune inflammation score (SII). The SII was derived using the formula SII = Platelet (P) x Neutrophil (N) / Lymphocyte (L) counts. The primary outcome was the association between presence of the PIP and hemogram parameters. Secondary outcomes included hemogram derivated parameters and the comparison of the presence of the PIP between two study groups. The study design and process are depicted in the flow diagram (**Figure 1**).

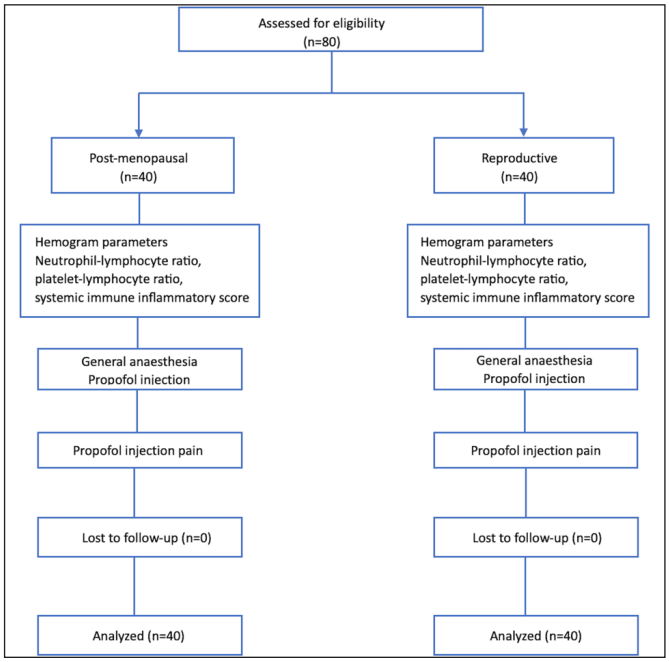

Figure 1: Flow diagram of the study

Patients underwent routine monitoring (including electrocardiography, pulse oximetry, and noninvasive blood pressure). Intravenous access was performed on the dorsum of the hand with a 20 gauge cannula. A face mask was used to deliver 6 lt/min of fresh oxygen and fraction of inspired oxygen (FiO2) for three minutes before induction. Prior to propofol injection, patients were informed about the possibility of experiencing pain. The anesthesiologist utilized McCririck and Hunter's verbal rating score (VRS) to evaluate the intensity of pain.11 The pain was categorized into four groups: 0 (no pain), 1 (mild pain), 2 (moderate pain), and 3 (severe pain). A solution containing 200 mg/20 mL of 1% propofol and 2 mL of 2% lidocaine (40 mg) was prepared in a 50 mL syringe. The patients' baseline heart rate and blood pressure were measured, and the infusion of propofol was initiated at a rate of 18.3 mL/min until a dosage of 2.5 mg/kg was achieved. The anesthesiologist regularly assessed the patients' pain levels every five seconds until they became unconscious. Additionally, the patients' heart rate and blood pressure were recorded after the induction of anesthesia.

In this study, a blinded researcher conducted a comparison between hemogram parameters and pain perception scores. To analyze the data, the statistical software SPSS 16.0 was employed. The hemogram parameters were assessed using an independent sample

t-test if they followed a normal distribution. However, if the distribution was not normal, the Mann-Whitney U test was used instead. For categorical evaluations, the Chi-square test with Yates correction was applied. The cut-off levels for parameters were determined through the analysis of the receiver operating characteristic curve. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

In this study, a total of 80 consecutive patients, 40 in each group (reproductive and post-menopausal), were evaluated. While the mean age was 36.87±7.56 years in the reproductive group, it was 60.55±3.50 years in the postmenopausal group. The descriptive data of the groups such as age, weight and height are presented in Table 1.

Table 1. Descriptive characteristics of patients

	Reproductive (n:40)	Post-Menopause (n:40)	p
Age (years)	36.87±7.56	60.55±3.50	NA
ASA I/II	28/12	11/29	0.0001
Length (cm)	165.75±5.28	161.20±4.93	0.0001
Weight (kg)	68.72±8.39	78.5±6.48	<0.0001
BMI (kg/cm²)	25.03±2.99	30.28±3.04	<0.0001
Type of surgery			
General surgery	17 (42.5%)	19 (47.5%)	
Orthopedics	9 (22.5%)	12 (30%)	
Neurosurgery	5 (12.5%)	4 (10%)	0.689
Otolaryngology	7 (17.5%)	3 (7.5%)	
Plastic surgery	2 (5%)	2 (5%)	

American Society of Anesthesiology class: ASA, Body Mass Index: BMI

When the groups were compared in terms of complete blood count results and obtained rates, there was no statistical difference between the groups (Table 2). The distribution of pain densities according to age groups is presented in Table 3 and this distribution was found to be statistically different ($p<0.05$). When the groups were classified as pain (+) and no pain, a statistical difference was found after Yates' correction ($p=0.024$) and this significance was due to the number of patients with pain in the postmenopausal group. When reproductive and postmenopausal patients are grouped according to the presence or absence of pain, no difference was found between the groups in terms of hemogram ratios and platelet indices ($p>0.05$) (Table 4).

In both groups, the NLR, PLR, and SII ratios were not significant in predicting PIP. Area under the curve for NLR, PLR, and SII related to the PIP, and cut-off values of each predictor with sensitivity and specificity values for PIP was shown in Table 5. However, no significant result was present for cut-off values in predicting PIP.

Table 2. Laboratory results of patients

	Reproductive (n:40)	Post-Menopause (n:40)	p
WBC	8.97±4.45	9.16±3.78	0.837
HGB	12.56±2.29	12.20±1.76	0.432
HCT	37.44±6.15	36.14±2.02	0.207
PLT	308.4±73.07	302.95±127.95	0.815
PDW	12.03±2.10	12.27±2.06	0.607
MPV	10.30±0.97	10.52±0.89	0.293
PCT	0.31±0.07	0.31±0.11	0.999
NEUT#	6.08±4.43	6.05±3.85	0.974
LYMPH#	2.18±0.93	2.37±1.52	0.502
NLR	3.67±4.32	3.31±3.25	0.674
PLR	173.23±102.03	166.41±152.22	0.814
SIII	1320.02±2188.56	1255.28±2506.63	0.901

White Blood Cell: WBC; Hemoglobin: HGB; Hematocrit: HCT; Platelet: PLT; Platelet Distribution Width: PDW; Mean Platelet Volume: MPV; Plateletcrit: PCT; Neutrophil Count: NEUT#; Lymphocyte Count: LYMPH#; Neutrophil Lymphocyte Ratio: NLR; Platelet Lymphocyte Ratio: PLR; Standard Derivation: SD; Systemic Immune Inflammation Score: SII; Number of Individuals:

Table 3. Pain density levels according to groups

	VRS-0 (n)	VRS-1 (n)	VRS-2 (n)	VRS-3 (n)	p
Reproductive	16	15	7	2	0.009
Post-Menopause	6	11	16	7	

McCririck and Hunter's Verbal Rating Score: VRS

Table 4. Evaluation of hemogram ratios and platelet indices when reproductive and postmenopausal patients are grouped according to the presence or absence of pain

Reproductive	No-Pain (n:16)	Pain (+) (n:24)	p
NLR	2.89±2.35	4.03±5.24	0.356
PLR	156.76±87.20	177.39±109.02	0.522
SIII	817.58±644.79	1617.47±2757.71	0.183
PLT	287.53±51.76	323.20±82.84	0.126
PDW	12.12±2.07	12.09±2.13	0.964
MPV	10.23±1.18	10.37±0.85	0.664
PCT	0.29±0.05	0.33±0.08	0.083
Post-Menopause	**No-Pain (n:6)**	**Pain (+) (n:34)**	**p**
NLR	4.06±2.72	3.25±3.36	0.580
PLR	193.31±124.44	165.41±158.54	0.673
SIII	1161.05±825.47	1288±2702.82	0.826
PLT	292.42±46.85	304.02±137.73	0.689
PDW	11.07±1.35	12.42±2.15	0.147
MPV	10.15±0.34	10.57±0.95	0.062
PCT	0.29±0.05	0.31±0.12	0.459

Neutrophil Lymphocyte Ratio: NLR; Platelet Lymphocyte Ratio: PLR; Standard Derivation: SD; Systemic Immune Inflammation Score: SII; Platelet: PLT; Platelet Distribution Width: PDW; Mean Platelet Volume: MPV; Plateletcrit: PCT; Number of Individuals: n; No-Pain: VRS-0, Pain (+): VRS-1-2-3

Table 5. ROC analysis for NLR, PLR and SII parameters

Factor	AUC (95% CI)	Cut-off	p	Sensitivity (%)	Specificity (%)
Reproductive					
NLR	0.497 (0.309-0.606)	2.73	0.978	66.7	50
PLR	0.487 (0.299-0.675)	124.50	0.890	46.8	68.8
SII	0.482 (0.298-0.666)	747.46	0.847	58.3	50
Post-Menopause					
NLR	0.537 (0.223-0.850)	4.40	0.776	88.2	50
PLR	0.525 (0.225-0.824)	156.74	0.850	70.6	50
SII	0.529 (0.230-0.849)	1131.90	0.762	85.3	50

Neutrophil Lymphocyte Ratio: NLR; Platelet Lymphocyte Ratio: PLR; Confidence Interval: CI ; Systemic Immune Inflammation Score: SII; Area under the curve: AUC

DISCUSSION

The findings from our study revealed a higher occurrence of PIP in postmenopausal women compared to women in reproductive age. However, it is important to note that the presence of this pain cannot be accurately predicted by analyzing hemogram parameters and the derived indices obtained from the hemogram.

Several variables, including vein diameter at the site of propofol injection, injection rate, lipid composition of the commercial formulation, and other factors, may influence the occurrence of PIP. On the other hand, chronic inflammation in the patient was also associated with the pain perception.[9,12] In our study, we also investigated the relationship between propofol injection-related pain and hemogram-derived parameters, which increase in acute or chronic inflammation, but could not determine it.

We conducted a study that was methodologically quite similar to this study, but only included male patients.[9] In that study, we detected a linear correlation between PIP and NLR,PLR and SII. In our study, however, we could not determine a correlation between these parameters and the presence of PIP, in which case one of the effective factors may be gender per se. Hanci et al.[13] found a relationship between the phase of the menstrual cycle of women and the presence of PIP in their study. It has been previously revealed that lymphocyte count increases in the premenstrual period compared to other periods of the menstrual cycle.[14] In our study, we did not standardize our patients in the reproductive period in terms of menstrual phase, and therefore we could not achieve homogenization in our data. We also know that there are serious differences in leukocyte composition in the postmenopausal period compared to the reproductive period.[15] Given the variations observed in hemogram data and the influence of multiple factors, it is plausible that the examined relationship could not be conclusively determined. This discrepancy can be attributed to the inherent differences in hemogram profiles between individuals of reproductive age and those in the post-menopausal stage. It is possible that a definitive association between PIP and pain perception could be established by conducting investigations within more homogenous groups.

Moreover, the current literature does not provide clear evidence regarding the association between hemogram parameters and the perception of acute or chronic pain. Some studies have identified a linear correlation between parameters such as NLR and pain perception, while others have observed a reverse correlation or no correlation at all.[11,16,17] Moreover, it is important to note that several articles investigated these parameters as predictors of treatment outcomes and mortality rates.[18-20]

In our study, we clearly found that the perception of pain associated with propofol injection was more pronounced in postmenopausal women. In a study comparing extracorporeal shock wave lithotripsy-related pain in postmenopausal and reproductive women, it was reported that postmenopausal women felt less pain.[21] However, in a study investigating musculoskeletal pain and the reproductive life stage, no relationship was found between pain and reproductive life stage.[22] When examining the relationship between pain perception and the reproductive life stage of women, it is crucial to consider the specific type, mechanism, and source of pain as distinct factors. Currently, the mechanism underlying PIP remains unknown, and it is possible that conducting studies with larger sample sizes may yield more conclusive findings. Thus, it is imperative to approach the investigation of PIP with a comprehensive understanding of its various dimensions in order to obtain meaningful and reliable results.

Our study has several limitations, some of which have been discussed earlier. One significant limitation is the lack of evaluation of women of reproductive age based on the specific phase of the menstrual cycle. Additionally, postmenopausal cases were not standardized, presenting another limitation. Furthermore, the absence of a randomized controlled trial introduces potential bias. Moreover, the classification of patients based on verbal statements rather than utilizing techniques such as hormone measurements to determine reproductive life stage is another limitation. Implementing such techniques would provide more precise and reliable data. Demographic data was different between the groups, different results can be obtained with more similar descriptors with matching to the propensity score. Lastly, no previous studies investigated the association between PIP and hemogram parameters, therefore, we could

not calculate power analysis according to the previous studies. However, this study could provide data for a sample size calculation for future trials.

CONCLUSION

In the present prospective study, our objective was to investigate the relationship between pain experienced during propofol injection and the reproductive life stage of women. Our findings indicate that postmenopausal women exhibit a higher incidence of pain compared to women of reproductive age. Furthermore, our analysis suggests that the use of hemogram and hemogram parameters alone is not sufficient to accurately predict the occurrence of pain in this context. These results highlight the need for further research to identify more reliable predictors and develop effective interventions for pain management during propofol injection in postmenopausal women.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Doctor Burhan Nalbantoglu State Hospital Ethics Committee (Date: 18.07.2023, Decision No: 1.01-25/23).

Informed Consent: Written informed consent was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The change of antibiotic resistance rates over the years in *Enterococcus* spp. isolated from clinical specimens

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ABSTRACT

Aims: The aim of this study was to retrospectively evaluate the change in antibiotic resistance rates of *Enterococcus* species isolated from various clinical samples of outpatients and inpatients in our hospital over the years.

Methods: Between January 2018 and December 2021, various clinical samples sent to Tekirdağ Namık Kemal University Hospital Microbiology Laboratory from outpatients, inpatients and intensive care patients were retrospectively examined. The samples were inoculated on 5% sheep blood agar, chocolate agar and EMB agar media according to their types and incubated at 37°C. The blood cultures were performed by BACTEC 1280 System (Becton Dickinson, MA, USA). Bacterial identification and antimicrobial sensitivity tests were made using conventional methods and automated systems.

Results: A total of 417 *Enterococcus* strains were isolated in our laboratory at four years and included in the study. Of the 417 isolates, 204 (48.9%) were isolated from male patients and 213 (51.1%) from female patients. The mean age of the patients was 57.79±22.9 years (0-96 years). It was determined that 36.9% of the isolates belonged to outpatients, 33.4% to inpatients and 29.7% to intensive care unit patients. Of the 122 enterococci isolates identified as species, 49.2% were typed as *Enterococcus faecalis* (*E. faecalis*) and 40.2% as *Enterococcus faecium* (*E. faecium*). Of the 417 isolates, 60.4% were isolated from urine samples, 24.2% from blood samples, and 8.9% from wound samples. Considering the total antibiotic resistance rates; ampicillin was 34.9%, ciprofloxacin was 46.4%, vancomycin was 8.4%, tigecline was 3.2%, high-level gentamicin was 49.0%. Linezolid and nitrofurantoin resistance were not detected. Ampicillin and vancomycin resistance rates were determined to have a statistically significant increase within four years. Ampicillin, ciprofloxacin, vancomycin and high-level gentamicin resistance rates were found to be significantly higher in isolates obtained from inpatients and intensive care patients compared to enterococcal isolates obtained from outpatients.

Conclusion: In our study, it was determined that antibiotic resistance in enterococcal isolates, which are the causative agents of infection in our hospital, increased over the years. In this way, determining the change in antibiotic resistance rates is beneficial in determining appropriate antibiotic use policies. It is thought that conducting surveillance studies on antibiotic resistance periodically and taking new measures according to changing antibiotic resistance rates will be beneficial in terms of treatment.

Keywords: *Enterococcus*, antibiotic resistance, vancomycin, vancomycin resistant enterococci (VRE)

INTRODUCTION

Enterococci are gram-positive cocci that are found as single, pairs or short chains as one of the main elements of the microbiota in the mouth and vagina, especially in the gastrointestinal tract of humans and all other land animals.[1,2]

Enterococci cause various community and hospital-acquired infections such as urinary tract infection, bacteremia, neonatal sepsis, endocarditis, intra-abdominal and pelvic infections, wound and tissue infections, meningitis, hospital-acquired pneumonia. The ability of enterococci to transfer resistance and virulence genes, the colonization ability of the bacteria, widespread or incorrect antibiotic use, invasive applications like catheters, serious diseases of the patients or long-term hospital stays cause an increase in the incidence of hospital-acquired enterococcal infections. Enterococci have increased the importance of infecting patients with impaired host defenses, increasing resistance to antibiotics used in treatment, and causing serious and high-mortality infections. In recent years, the clinical importance of enterococci has increased with the increase in resistance to antibiotics, including vancomycin.[3-7]

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Antibiotic resistance is one of the most important health problems of today, and it is very important for each hospital to regularly monitor their own antibiotic resistance surveillance and to determine antibiotic restriction programs accordingly.[8]

In this study, we aimed to analyze retrospectively the change in antibiotic resistance rates of *Enterococcus* species isolated from various clinical samples of outpatients and inpatients in our hospital over the years.

METHODS

The study was carried out with the permission of Tekirdağ Namık Kemal University Non-interventional Clinical Researches Ethics Committee (Date: 26.04.2022, Decision No: 2022.51.04.01). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Bacteria Isolation

Between January 2018 and December 2021, various clinical specimens (urine, blood, wound, respiratory, sterile body fluids, catheter, vaginal secretion samples) sent to the microbiology laboratory of Tekirdağ Namık Kemal University Hospital from outpatients, inpatients and intensive care patients were retrospectively analyzed. Clinical specimens were inoculated on 5% sheep blood agar (Oxoid, UK), chocolate agar (Oxoid, UK) and EMB agar (Oxoid, UK) media. Peritoneal and wound samples were incubated for 72 hours, CSF samples for 120 hours and other samples for 24-48 hours at 37°C. Blood cultures were followed in the BACTEC (Becton Dickinson, USA) automated blood culture system. Isolates were isolated by conventional methods (colony morphology, Gram stain, catalase, PYR) and automated bacterial identification system (VITEK2, bioMérieux, France/2018-2019 ve BD Phoenix 100, Becton Dickinson, USA/2020-2021). The first isolate was evaluated in repeated samples from the same patient.

Antibiotic Susceptibility Test

Enterococcal strains isolated from clinical samples were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion and automated system (VITEK2, bioMérieux, France/2018-2019 and BD Phoenix 100, Becton Dickinson, USA/2020-2021) using European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards.[9] Ampicillin, nitrofurantoin and ciprofloxacin were reported for uncomplicated UTIs only. Also nitrofurantoin was evaluated only in *E. faecalis* strains according to EUCAST criteria. Isolates with vancomycin resistance in both methods were confirmed by gradient test (Liofilchem, Italy). Internal quality control for disk diffusion method is made once in a month and external quality control three periods in a year (*E. faecalis* ATCC 29212 strain used).

Statistical Analysis

IBM SPSS Statistics for Windows Version 22.0 (Statistical Package for the Social Sciences, IBM Corp, Armonk, NY, USA) package program was used for statistical analysis of the obtained data. Quantitative and categorical results were expressed as numbers and percentages. Chi-square test was used to compare and evaluate different groups of variables, and a p value of 0.05 or less was considered statistically significant.

RESULTS

A total of 417 enterococci strains were isolated in our laboratory in a four-year period and included in the study. Of the 417 isolates, 204 (48.9%) were isolated from male patients and 213 (51.1%) from female patients. The mean age of the patients was 57.79±22.9 years (0-96 years).

The distribution of enterococci isolates according to the clinics (outpatient, inpatient and intensive care unit) and years of isolation is given in **Table 1**. It was determined that 36.9% of the isolates belonged to outpatients, 33.4% to inpatients and 29.7% to intensive care unit patients.

Species identification was made for only 122 isolates out of 417 isolates included in the study. Of the isolates identified to species, 49.2% were *Enterococcus faecalis* (60/122), 40.2% were *Enterococcus faecium* (49/122), 3.3% were *Enterococcus durans* (4/122), 2.5% were *Enterococcus casseliflavus* (3/122), 1.6% were *Enterococcus raffinosus* (2/122), 2.5% were *Enterococcus gallinarum* (3/122) and 0.8% was *Enterococcus avium* (1/122). *E. faecalis* was the most frequently isolated one, followed by *E. faecium*.

Table 1. Distribution of Enterococci isolates by isolated clinics and years (n/%)

Clinics	Years								Total	
	2018		2019		2020		2021			
	n	%	n	%	n	%	n	%	n	%
Outpatient clinics	42	44.7	29	38.7	27	28.1	56	36.8	154	36.9
Inpatient clinics	33	35.1	22	29.3	34	35.4	50	32.9	139	33.4
Intensive care unit	19	20.2	24	32.0	35	36.5	46	30.3	124	29.7
Total	94	100	75	100	96	100	152	100	417	100

417 isolates were isolated from patients treated in 44 different clinics. Isolates were mostly obtained from intensive care unit patients with 29.5% and urology clinic patients with 24.5% (nephrology 7.4%, pediatrics 6.0%, oncology 5.0%, surgery 3.8%, infectious diseases 3.8%, orthopedics 3.4%, hematology 3.1%, internal medicine 2.2% and others clinics 11.2%). When the distribution of *E. faecalis* and *E. faecium* isolated from clinics was analyzed, both species were isolated mostly from intensive care unit patients with a rate of 51.7% and 49%, respectively.

Isolates were mostly isolated from urine, blood and wound samples, respectively. Of 417 isolates, 253 (60.4%) were isolated from urine samples, 101 (24.2%) from blood samples and 37 (8.9%) from wound samples. The remaining 26 isolates were isolated from sputum, cerebrospinal fluid (CSF), throat, drainage, catheter, peritoneal fluid, respiratory secretion and vaginal samples (Table 2). Of the species identified isolates, both *E. faecalis* and *E. faecium* were highest in blood, urine and wound samples, respectively.

In our study all isolates were analyzed for resistance rate without species discrimination and the highest resistance rates were high-level gentamicin resistance with 49.0% (39.5%in *E. faecalis*, 89.5% in *E. faecium*) and ciprofloxacin resistance with 46.4% (42.9% in *E. faecalis*, 93.3% in *E. faecium*). Ampicillin resistance rate was 34.9% (12.5% in *E. faecalis*, 93.3% in *E. faecium*) , vancomycin was 8.4% (3.4% in *E. faecalis*, 30.4% in *E. faecium*). Vancomycin resistance was detected in 2 of 59 *E. faecalis* (intensive care unit) isolates and 14 of 46 *E. faecium* (8 inpatient clinics, 6 intensive care unit) isolates and confirmed by gradient test. While resistance was detected against tigecycline at the rate of 3.2% in all 417 isolates, no resistance was detected in *E. faecium* and *E. faecalis* isolates. Linezolid resistance (0%) was not detected in the isolates (Table 3).

Table 3: Antibiotic resistance rates of Enterococci isolates (%)

Antibiotics	*Enterococcus* spp. (n=417)	*E. faecalis* (n=60)	*E. faecium* (n=49)
AMP	34.9	12.5	93.3
CIP	46.4	42.9	93.3
VAN	8.4	3.4	30.4
LZD	0.0	0.0	0.0
TIGE	3.2	0.0	0.0
GEN	49.0	39.5	89.5
NIT	-	0.0	-

AMP: Ampicillin, CIP: Ciprofloxacin, VAN: Vancomycin, LZD: Linezolid, TIGE: Tigecycline, GEN: High-level gentamicin, NIT: Nitrofurantoin

The distribution of antibiotic resistance rates among outpatient clinic, inpatient clinic and intensive care unit patients from whom enterococcal isolates were isolated was analyzed and ampicillin, ciprofloxacin, vancomycin and high-level gentamicin resistance rates of isolates obtained from inpatients and intensive care unit patients were statistically significantly higher than enterococcal isolates obtained from outpatients (p<0,05) (Table 4).

Table 4. Antibiotic resistance rates according to the clinic where Enterococci isolates are isolated (%)

Antibiotics	Total	Outpatient clinics	Inpatient clinics	Intensive care	p
AMP	34.9	14.1	66.7	47.5	0.000*
CIP	46.4	30.1	69.1	67.6	0.000*
VAN	8.4	2.0	11.9	12.5	0.002*
LZD	0.0	0.0	0.0	0.0	-
TIGE	3.2	0.0	3.0	4.0	0.759
GEN	49.0	9.1	54.5	50.0	0.020*
NIT	0.0	0.0	0.0	0.0	-

AMP: Ampicillin, CIP: Ciprofloxacin, VAN: Vancomycin, LZD: Linezolid, TIGE: Tigecycline, GEN: High-level gentamicin, NIT: Nitrofurantoin (only for *E. faecalis*)
*p<0,05

Table 2. Distribution of Enterococci isolates according to the samples and years of isolation (n/%)

Samples	Years								Total	
	2018		2019		2020		2021			
	n	%	n	%	n	%	n	%	n	%
Urine	61	64.9	49	65.3	53	55.2	90	59.3	253	60.4
Blood	17	18.1	24	32.0	25	26.0	35	23.0	101	24.2
Wound	10	10.6	2	2.7	11	11.5	14	9.2	37	8.9
Peritoneal fluid	1	1.1	0	0.0	0	0.0	6	3.9	7	1.7
Sputum	2	2.1	0	0.0	1	1.0	2	1.3	5	1.2
Respiratory secretion	2	2.1	0	0.0	2	2.1	1	0.7	5	1.2
Vaginal secretion	1	1.1	0	0.0	0	0.0	4	2.6	5	1.2
CSF	0	0.0	0	0.0	1	1.0	0	0.0	1	0.2
Throat	0	0.0	0	0.0	1	1.0	0	0.0	1	0.2
Drainer	0	0.0	0	0.0	1	1.0	0	0.0	1	0.2
Catheter	0	0.0	0	0.0	1	1.0	0	0.0	1	0.2
Total	94	100	75	100	96	100	152	100	417	100

*CSF: Cerebrospinal fluid

The change in antibiotic resistance rates of 417 isolates over the years was analyzed and it was observed that the resistance rates to ampicillin, ciprofloxacin, vancomycin and high-level gentamicin detected in 2018 decreased in 2019 and increased again in 2020 and 2021. The increase in resistance to ampicillin and vancomycin continued in 2021 and was found to be statistically significant ($p<0.05$) (Table 5).

Table 5. Antibiotic resistance rates of Enterococci isolates over the years (%)

Antibiotics	Year				p
	2018	2019	2020	2021	
AMP	26.2	24.5	49.1	38.2	0.004*
CIP	41.0	39.6	48.1	53.5	0.244
VAN	11.0	2.7	11.9	12.5	0.002*
LZD	0.0	0.0	0.0	0.0	-
TIGE	0.0	0.0	2.5	8.2	0.111
GEN	65.2	36.4	46.3	49.3	0.268
NIT	0.0	0.0	0.0	0.0	-

AMP: Ampicillin, CIP: Ciprofloxacin, VAN: Vancomycin, LZD: Linezolid, TIGE: Tigecycline, GEN: High-level gentamicin, NIT: Nitrofurantoin (only for *E. faecalis*)
*p<0,05

DISCUSSION

Enterococci, which are among the human flora elements, cause many clinical manifestations in both outpatients and inpatients, especially urinary system infections. They are considered to be one of the most common causes of nosocomial infections, especially due to their colonization ability and their ability to develop resistance to antibiotics used in treatment. In the literature, they are also reported as common pathogens in wound and bloodstream infections.[10-15] Mataj et al.[16] investigated the prevalence of the microorganisms isolated from blood cultures for a ten-year period and found that *Enterococcus* spp. isolation rates has increased between the first and the last five-year period. In our study, it was determined that 63.1% of the isolates belonged to inpatients. Also, most of the isolates were isolated from urine samples with a rate of 60.4%, followed by blood samples with 24.2% and wound samples with 8.9%, and this order did not change over the years. This result suggests that *Enterococcus* species may be an important cause of bacteremia in addition to urinary tract infections in our hospital.

It is reported that 80-90% of enterococcal infections are caused by *E. faecalis* and 5-10% by *E. faecium*, but in recent years, the incidence of *E. faecium* strains has increased in relation to its ability to resist more than one antibiotic, especially in inpatients.[10,17] In two studies conducted from samples of inpatients in Brazil and Italy, 82.2-87% of isolates were reported to be *E. faecalis* and 10.8-17.8% were reported to be *E. faecium* (18,19). In our country, Agus et al.[20] identified 77% of the isolates as *E. faecalis* and 23% as *E. faecium* in outpatients and inpatients. Celik et al.[21] identified 60.8% of the isolates as *E. faecalis* and 38.2% as *E. faecium* only in inpatients. In our study, only 122 isolates were identified at the species, 49.2% of them were typed as *E. faecalis* and 40.2% as *E. faecium*. Species identification of *Enterococcus* in our hospital is frequently made in samples of inpatients, and we think that this may cause the frequency of *E. faecium* to be detected a little higher than in other studies.

Studies on ampicillin, the first-line antimicrobial agent used in the treatment of enterococcal infections, have shown that ampicillin resistance rates have increased in recent years. Sig et al.[22] determined ampicillin resistance as 28.9% in *Enterococcus* spp., 2.4% in *E. faecalis* and 85.6% in *E. faecium*. Odemis et al.[17] found ampicillin resistance in 50% of *E. faecalis* and 94% of *E. faecium* in samples from inpatients in 2010-2015. Bilgin et al.[23] determined ampicillin resistance as 8.1% in *E. faecalis* and 95% in *E. faecium* in outpatient and inpatient samples in 2018. Simsek[24] reported ampicillin resistance as 31.9% in *Enterococcus* spp., 10.6% in *E. faecalis*, and 83.9% in *E. faecium* in samples from outpatients and inpatients in 2019. In our study, it was observed that the ampicillin resistance rates were consistent with the studies and showed a statistically significant increase over the years.

Ciprofloxacin is used in the treatment of urinary tract infections due to enterococci.[24] Etiz et al.[25] found ciprofloxacin resistance rate as 66.6% in *Enterococcus* spp., 42.5% in *E. faecalis* and 94.3% in *E. faecium* in a study including only enterococci isolates from urine samples. In our study, the ciprofloxacin resistance rate of 417 enterococcal isolates was 46.4%. It was determined that ciprofloxacin was the second antibiotic with the highest resistance rate in the study and that the resistance increased over the years, although not statistically significant. With these results, we believe that ciprofloxacin is not a suitable option for empirical treatment of enterococcal UTIs in our hospital.

Nitrofurantoin is an antibiotic that can be used in the treatment of urinary tract infections due to *E. faecalis* in accordance with EUCAST recommendations.[26] Yenisehirli et al.[27] reported nitrofurantoin resistance as 4.3% in *E. faecalis* isolates obtained from urine samples between 2011 and 2014. In our study, nitrofurantoin resistance was evaluated only in *E. faecalis* strains that isolated in urine samples, and no resistance was observed.

In different studies, high-level gentamicin resistance has been reported in the range of 39.4-52% in *Enterococcus* spp., 27-57.2% in *E. faecalis*, and 39.7-70% in *E. faecium*.[10,28-30] In our study, high-level gentamicin resistance was determined as 49% in total, 39.5% in *E. faecalis* and 89.5% in *E. faecium*. It can be said that the total

high-level gentamicin resistance rate and the resistance rate of *E. faecalis* isolates in our study are compatible with other studies, but the resistance rate of *E. faecium* isolates was found to be higher than other studies. In the evaluation of resistance rates, the clinic from which the sample was isolated (outpatient or inpatient) is a factor affecting the result. In our study, high-level gentamicin resistance was statistically significantly higher in inpatients compared to outpatients. The high resistance observed in *E. faecium* may be related to this.

Glycopeptides are among the most effective antibiotics in enterococcal infections. Vancomycin-resistant enterococci (VRE) were identified in 1986 and enterococcal strains resistant to these antibiotics have been observed in many countries.[31] In the reports of the European Antimicrobial Resistance Surveillance System published in 2019, it was reported that there was a significant increase in VRE rates in Europe and the vancomycin resistance rate of *E. faecium* isolates was 18.3%.[32] In the summary report of the National Healthcare Associated Infections Surveillance Network conducted in our country in 2019, the vancomycin resistance rate in *E. faecalis* was reported as 3.8% and 18.9% in *E. faecium*.[33] In the 2019 annual report published by the National Antimicrobial Resistance Surveillance System (UAMDSS), the vancomycin resistance rate was reported as 1% and 13% for *E. faecalis* and *E. faecium* isolates, respectively.[34] In recent studies conducted in our country, vancomycin resistance was detected between 4.5-16% in *Enterococcus* spp., 0-4% in *E. faecalis* and 8.2-23% in *E. faecium*.[10,29,35,36] In our study, vancomycin resistance was found to be 8.4% in all isolates, 3.4% in *E. faecalis* and 30.4% in *E. faecium* isolates. The resistance rates we found are higher than other studies. There could be several reasons for this. First of all, since species identification could not be made all of the *Enterococcus* spp., isolates innate resistant to vancomycin could not be excluded. In addition, the low number of *E. faecalis* and *E. faecium* isolates included in the study may be the reason for high resistance rates. Also, it is observed that the number of resistant isolates increased in 2020 and 2021. Therefore, we think that some precautions should be taken in terms of VRE infections in our hospital.

Linezolid is an important antimicrobial used for the treatment of VRE.[37] In the 2016 report published by the National Antimicrobial Resistance Surveillance System, it was reported that the linezolid resistance rate was 1% for *E. faecium* isolates, while no resistance was detected for *E. faecalis*.[30] In a study by Dadashi et al.[38] analyzing 114 studies conducted between 2000 and 2020, linezolid resistance was reported as 2.8%, 2.1% and 0.7% for *E. faecalis* and 0.9%, 1.8% and 3.4% for *E. faecium* in Asia, Europe and the Americas, respectively. In a 15-year meta-analysis from Turkey, the mean resistance rates to vancomycin and linezolid was reported to 1.0±2.2% and 1.9±2.6% in *E. faecalis* and 10.3±11.3% and 2.4±0% in *E. faecium*, respectively.[39] In our study, no linezolid-resistant isolates were detected. This is a good result for our hospital.

Tigecycline is a broad spectrum antibiotic with antimicrobial activity in gram positive and gram negative bacteria and is used in VRE infections.[38] In different studies, tigecycline resistance rate in *Enterococcus* spp. has been reported to be around 1%.[24,38] In our study, the rate of tigecycline resistance in enterococcal isolates was determined as 3.2%. The tigecycline resistance rate we obtained in our study was higher than other studies. This may be because more than 60% of the samples included in our study belonged to inpatients and intensive care unit patients. In addition, tigecycline resistance could not be studied with the reference method, the broth microdilution method, in our study. This may have caused our results to be higher than other studies.[40]

In our study, only 122 of the 417 isolates we isolated were identified as species, and 295 isolates were *Enterococcus* spp. was typed. This is the main limitation of our study. The retrospective design of the study and the inability to study tigecycline resistance with the broth microdilution method in line with EUCAST recommendations are other limitations.

CONCLUSION

In our study it was determined that enterococci can be an important infectious agent in our hospital, resistance rates have increased over a four-year period, and this increase is statistically significant for antibiotics that are important in the treatment of enterococcal infections such as ampicillin and vancomycin. Antibiotic resistance is one of the most important health problems of today, and isolates resistant to last-line antibiotics such as vancomycin pose a serious risk for public health. In this way, determining the change in antibiotic resistance rates is useful in determining the appropriate antibiotic use policies. It is thought that periodic surveillance studies on the basis of country, province, district and hospital regarding antibiotic resistances and taking new measures according to changing antibiotic resistance rates will be beneficial in terms of treatment selection and success.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Tekirdağ Namık Kemal University Non-interventional Clinical Researches Ethics Committee (Date: 26.04.2022, Decision No: 2022.51.04.01).

Informed Consent: Written informed consent was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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Extra corporeal membrane oxygenation therapy in acute respiratory distress syndrome due to Coronavirus-2019 (COVID-19): a retrospective study

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ABSTRACT

Aims: Extra corporeal membrane oxygenation (ECMO) has been used as a supportive treatment in ARDS due to COVID-19. Although different results have been reported in the literature regarding its efficacy, ECMO is recommended as a salvage therapy for severe forms of the disease after standard therapy fails. In our study,we aimed to evaluate the survival outcomes of patients supported with ECMO for COVID-19.

Methods: Our study was conducted by scanning the data of consecutive adult patients hospitalized in our intensive care unit due to COVID-19. The ECMO process was planned according to the Extracorporeal Life Support Organization (ELSO) and Berlin criteria.

Results: 51 patients hospitalized for acute respiratory failure due to COVID-19 were taken to ECMO. Demographic data of patients; 39 (76.5%) men and 12 (23.5%) women. 46 (90.2%) of the patients died. The mean intubation time before ECMO is 3.9 days, and the mean time for non-invasive mechanical ventilation is 5.8 days. The mean PaO_2 value before ECMO was79.09 mmHg, the mean PCO_2 value was 63.62 mmHg and the mean PaO_2/FiO_2 ratio was 82.80.

Conclusion: The use of ECMO by considering prognostic factors and guidelines is seen as factors that increase the chance of success.Despite the fact that the patients were admitted to ECMO in accordance with the guidelines in our study, the high mortality rate suggests that there is a need for investigation of other supportive treatments and studies to reduce ECMO complications.

Keywords: COVID-19, extracorporeal membrane oxygenations, respiratory distress syndrome, mortality, prognosis

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory distress syndrome has resulted in high economic costs for healthcare systems worldwide and placed a substantial burden on healthcare staff. During this pandemic, where mortality has been high globally, face masks, isolation, hand hygiene, and vaccines have been used as protective measures. As treatments, drugs, such as interleukin-1 antagonists, interleukin-6 antagonists, and remdesivir, have been tried. However, these drugs have not proved efficacious in COVID-19 patients with severe hypoxemia, where extracorporeal membrane oxygenation (ECMO) has been necessary as supportive therapy. The purpose of ECMO is to eliminate hypoxemia and to maintain tissue perfusion while allowing the patient to recover. Although different results have been reported in the literature regarding the efficacy of ECMO, it is recommended as a rescue treatment for severe forms of COVID-19 disease after standard treatment fails.[1] Early administration is recommended, especially in young patients before multiple organ dysfunction syndrome or severe ventilator-related lung injury occurs.[2,3] According to a report by the Extracorporeal Life Support Organization (ELSO) in 2018, the survival rate of patients receiving respiratory support provided by ECMO was 58.7-73.2%, whereas that of patients on circulatory support was 42.7-52.6%.[4] Despite continuous developments in technology, the incidence of ECMO-related complications and mortality remains high.[5] ECMO is a high-cost, extremely complex type of life support system, which is available only in specialized and experienced centers trained in its use. Thus, the use of ECMO is limited, which has led to insufficient studies and experience.[6]

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Our primary outcome is to evaluate the success of using ECMO by considering prognostic factors and guidelines in this patient group with high mortality and cost, and the secondary outcome is to evaluate the survival results of ECMO supported COVID-19 patients, and to share our clinical experiences and data.

METHODS

The study was carried out with the permission of İstanbul Medipol University Non-interventional Clinical Researches Ethics Committee (Date: 06.01.2022, Decision No: 07). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In the present study, data were collected from the hospital records of consecutive adult patients hospitalized due to COVID-19 between 01.01.2021 and 01.11.2021 in the General Intensive Care Unit (ICU) of İstanbul Medipol University Faculty of Medicine Hospital.

All patients had SARS-CoV-2 infection, as documented by nasopharyngeal swabs or lower respiratory tract aspiration and real-time polymerase chain reaction (PCR) results (LightCycler 96; Roche). Demographic data of the patients, hemodynamic parameters, blood gas and laboratory values, drugs administered, ventilator and ECMO parameters, and complications during ECMO were evaluated. The relationship of these parameters with mortality and survival was calculated. Patients with severe liver disease, hypoxic encephalopathy, patients with vascular disease, metastatic cancer patients, patients with immune deficiency and patients over the age of 70 were not taken to ECMO.

All the patients, except those with comorbidities, were placed in the prone position prior to ECMO. Neuromuscular blocking agents were administered to patients without contrindications before ECMO. ECMO cannulation was performed by a team of anesthesiologists, cardiovascular surgeons, perfusionists, and intensive care specialists under ultrasound guidance and chest X-ray control. ECMO follow-up was undertaken by the intensive care team. Femoral vein cannulation was performed using a 23-27 Fr internal jugular cannula and a 17-21 Fr cannula.

In cases of acute respiratory distress syndrome (ARDS), the ELSO recommends initiating veno-venous (VV)-ECMO prior to conventional therapies. 4 In our study, despite ventilator optimization in ARDS cases, VV-ECMO was planned for patients with the following clinical conditions:

1. PaO_2/FiO_2<150 mmHg
2. Any of the following: i) PaO_2/FiO_2<60 mmHg for>6 hours, ii) PaO_2/FiO_2<50 mmHg for>3 hours, or iii) pH<7.20 and $PaCO_2$>80 mmHg for>6 hours
3. ECMO if no contraindications or $PaO_2/FiO_2 \geq 150$ mmHg; pH <7.20, with $PaCO_2$>80 mmHg for>6 hours; or no contraindications for ECMO

The activated clotting time was used for monitoring VV-ECMO anticoagulation with unfractionated heparin, with a target time of 150-250 seconds. The hemoglobin threshold was determined as 7-8 g/dl for red cell transfusions and (< 50 thousand/ml) for platelet transfusions.

For data analysis, SPSS 16.0 was used. <0.05 p value was conserved as statistically significant.

RESULTS

In total, 51 patients hospitalized in our ICU with acute respiratory failure due to COVID-19 received ECMO. In terms of demographic data, there were 39 (76.5%) males and 12 (23.5%) females (Table 1). Only 3 (5.9%) of our patients had received the COVID-19 vaccine (Sinovac vaccine was available in all three patients). Before ECMO, 40 (78.4%) patients were placed in the prone position, and 27 (52.9%) patients received a neuromuscular blocker. There were 28 (54.9%) patients with comorbidities and 3 (5.9%) pregnant patients. All our patients received VV-ECMO. ECMO support was terminated in 46 (90.2%) patients due to death. Of the surviving patients, 4 (7.8%) were discharged home, and one died in the hospital, with the death unrelated to COVID-19.

Table 1. Variables

	n= 51 (%)
Age (years)	51 (27-77)
Gender (male)	39 (76.5)
BMI (kg/m^2)	30 (23-42)
Comorbidities	
Obesity	2 (3.9)
Diabetes mellitus	6 (11.7)
Hypertension	9 (17.6)
Asthma	3 (5.8)
Coronary artery disease	4 (7.8)
Postpartum	3 (5.8)
PCR Positive	48 (94.1)
Vaccinated patients	3 (5.8)

Values are expressed as mean (interquartile range) or number (%)

Steroids (hydrocortisone, dexamethasone, or methylprednisolone) were administered to all patients before and during ECMO. Twenty-six (51%) patients

were taking vasopressors before ECMO. Before or during ECMO, drugs, including colchicine (n=32, 62.7%), remdesevir (n=3, 5.9%), IVIG (intravenous immunoglobulin) (n=2, 3.9%), an IL-6 antagonist (n=28, 54.9%), or an IL-1 antagonist (n=6, 11.8%), were administered. Patients also received immune plasma therapy (n=3, 5.9%), a sepsis filter (n=19, 37.3%), or continuous VV-hemodiafiltration (CVV-HDF) (n=32, 62.7%) (Table 2).

Table 2. Additional medications and supportive treatments

Additional medications and supportive treatments	Patients (N)	Percentage (%)
Steroid	51	100
Remdesevir	3	5,9
Colchisine	32	62,7
IVIG	2	3,9
IL-6 antagonist	28	54,9
IL-1 antagonist	6	11,8
Sepsis filter	19	37,3
CVV-HDF	32	62,7
Immune plasma	3	5,9

IVIG (intravenous immunoglobulin), IL-6 ((interleukin-6), IL-1 (interleukin-1), (continuous veno-venous hemodiafiltration), CVV-HDF (continuous veno-venous hemodiafiltration)

Bacterial growth occurred in blood (n=25, 49%) and endotracheal aspiration (n=35, 68.6%) cultures. Methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* and methicillin-sensitive *S. aureus* (MSSA) and *Stenotrophomonas maltophilia* constituted the majority of bacteria in both cultures. Complications (bleeding, thrombosis, thrombocytopenia, pneumothorax, and circulatory disorders) occurred in 35 (68.6%) of the ECMO patients. Only 7 (13.7%) patients did not receive blood or blood product replacement. In 19 (37.3%) patients with thrombocytopenia or other blood disorders, acetyl salicylic acid, dipyridamole, or clopidogrel was used rather than heparin.

Before ECMO, the average number of orotracheal intubation days and noninvasive mechanical ventilation days was 3.90 and 5.80, respectively. The average number of days on ECMO support was 12.73. The average number of ECMO devices sets used was 1.45. Table 3 shows the average driving pressure, PaO_2, PCO_2, and P/F ratio values prior to ECMO. Although these values were marginally improved on the first, third, and seventh days of ECMO, parameters for removal of ECMO support were reached in only five of the 51 patients. Compared to pre-ECMO levels, only IL-6 (1015-131) and ferritin (1212-709) levels were significantly decreased in patients who could be weaned. There was no change in D-dimer, lymphocyte, thrombocyte, and hemoglobin levels between pre- versus post ECMO levels.

Table 3. Pre ECMO data

	n= 51
Pre ECMO days	12 (2-42)
Intubation time	3.9 (1-16)
NIMV time	6.08 (1-20)
Blood gas	
pH	7.27 (6.80-7.50)
$PaCO_2$ (mmHg)	63.62 (37-113)
PaO_2/FIO_2	82 (49-131)
Ventilation parameters	
Respiratory rate (/min)	34 (18-40)
Tidal volüme (ml/kg)	350 (200-580)
PEEP (cmH_2O)	14 (10-18)
FIO_2 (%)	96 (60-100)
PIP (cmH_2O)	16 (10-32)
Neuromuscular blocker	27 (52.9)
Prone position	40 (78.4)
APACHE II	20 (9-32)
Driving pressure (cmH_2O)	20,66 (12-42)

Values are expressed as mean (interquartile range) or number (%), NIMV: non-invasive mechanical ventilation time

When we examined the data on the survivors, these patients had received only standard steroid therapy (i.e., no other drugs or procedures). One patient was given remdesevir and an IL-6 antagonist, and one patient was treated with a sepsis filter and CVV-HDF due to ETA and *S. maltophilia* growth in blood culture. Another patient was treated with an IL-6 antagonist, sepsis filter and CVV-HDF due to the growth of *S. maltophilia* and MSSA in blood culture. One patient was given remdesevir and an IL-6 antagonist.

The most common complications were bleeding (from drain sites), thrombocytopenia, thrombosis, and circulatory disorders. Pneumothorax was observed in only one patient (Table 4). The mean APACHE II score of the patients was 20.49.

Table 4. Clinical results

	n= 51
Decannulation (n)	5 (9.8)
Discharge from ICU (n)	5 (9.8)
Discharge from hospital (n)	4 (7.8)
ECMO time (days) median, IQR [Q1-Q3]	8 [5-18]
Complications	35 (68,6)
Blood products transfusion	44 (86.2)
Pneumothorax	1 (1.9)
Thrombosis	12 (23.5)
Positive blood cultures	25 (49)
Positive tracheal cultures	35 (68.6)

Datas were expressed as median IQR [Q1-Q3] or mean±SD

DISCUSSION

In our study, although the mean driving pressure, PaO_2, PCO_2, and P/F ratio values before ECMO improved marginally on the first, third, and seventh days of ECMO, the parameters for removal of ECMO support were achieved in only five of 51 patients. Compared with pre-ECMO levels, only IL-6 (1015-131) and ferritin (1212-709) levels were significantly reduced in weaning patients. There was no change in D-dimer, lymphocyte, platelet and hemoglobin levels between pre- and post-ECMO values.

During the COVID-19 pandemic, hospitals, especially ICUs, had to cope with a heavy patient load. Standard patient admission procedures were either not implemented or delayed. In our study, the Berlin criteria were used in the diagnosis and treatment of ARDS, and the criteria of the ELSO were used in in deciding whether to initiate ECMO. All of the 51 patients, the majority of whom were diagnosed with severe ARDS (mean P/F: 82.80), received ECMO. The P/F ratio in our study was higher than that reported in studies by Barbaro et al.[7] (P/F ratio: 72), Combes et al.[8] (P/F ratio: 51), and Schmidt et al.[9] (P/F ratio: 60). In our study, 78.4% of the patients were placed in the prone position before ECMO. In the study by Schmidt et al.[10] 94% of patients were placed in the prone position prior to ECMO,[8] whereas 56% and 26% of patients were placed in the prone position in the EOLIA study. As it has been see in literature, there is significant differences among studies in relation to the placement of the patient in the prone position before ECMO.

In our study, neuromuscular blockers were used in 52.9 % of patients before ECMO. The recruitment maneuver was applied in these patients. During ECMO, 56.8% of patients were placed in a prone position. In the study by Barbaro et al.[7] mortality 90 days after the start of ECMO was 37.4%. In the study of Combes et al.[8] 60-day mortality was 35% in the ECMO group and 46% in a conventional treatment group. In our study, mortality rate was 90.2%. The mean duration of invasive and noninvasive mechanical ventilation in our study was 3.8 and 5.8 days, respectively. We attribute the higher mortality rate in our study as compared with that in the literature to the late transition to ECMO, despite more prone positioning and a higher P/F ratio compared to that in the other studies. Henry et al.[11] stated that initiation of ECMO 10 days after invasive ventilator use in patients with COVID-19 reduced the probability of successful treatment. They also recommended close monitoring of IL-6 and lymphocyte levels. Previous studies reported a significant difference in lymphocyte counts and IL-6 concentrations of COVID-19 survivors and nonsurvivors.[12,13] Similarly, in our laboratory follow-ups, we observed a significant decrease in post-ECMO IL-6 and ferritin levels compared to pre-ECMO levels. However there was no difference between D-dimer, lymphocyte, thrombocyte, and hemoglobin levels pre- and post-ECMO. In our study, the mean driving pressure value before ECMO was 20.66 cmH_2O, and the mean value on the first day was 16.98 cmH_2O. Similar driving pressures (20±7 vs. 14±4 cm H_2O) were observed by Schmidt et al.[10]

In a previous study, Jacobs et al.[14] presented findings on 32 consecutive COVID-19 patients admitted to nine different hospitals who received ECMO for 24 days. In their study, 17 patients were still receiving ECMO at the time of writing, 10 died before or shortly after decannulation, five were successfully extubated after ECMO, and one of these five patients was discharged. The mortality rates in their study were similar to those in our study based on the literature, mortality rates were high in the early pandemic period and in centers where ECMO experience was insufficient.

Other than the timing of ECMO and associated treatment options, we think that the location of the cannula and the characteristics of the catheter are important factors affecting mortality. Among COVID-19 patients who receive ECMO, there is a strong positive correlation between mortality and high cytokine levels, the most important being IL-6.[15] Ruan et al.[16] noted that IL-6 concentrations differed significantly between COVID-19 survivors and nonsurvivors. Mehta et al.[17] found up to 1.7 times higher IL-6 levels in nonsurvivors as compared to those of survivors. In our study, IL-6 and ferritin levels of the survivors were lower than the baseline values, and a sepsis filter was applied to two of these patients. In addition, the surviving patients were given antibody treatments, such as IL-1 or IL-6 antagonists, remdesevir, and antivirals. In the European Union, an extracorporeal cytokine adsorbent approved to reduce toxic levels of cytokines can be used with ECMO to treat the cytokine storm associated with COVID-19 pneumonia. Additional research is needed on each of these treatment options.

In a meta-analysis by Tran et al.[18] which aimed to determine the relationship between pre-ECMO prognostic factors and mortality risk, patient-related factors, such as advanced age and male gender, were among the factors associated with increased mortality, with medium or high precision. Other factors, such as chronic lung disease, longer symptom duration, and longer duration of invasive mechanical ventilation, in addition to precannulation factors, such as higher arterial CO_2 partial pressure, higher driving pressure, and less ECMO experience, were also noted. In the study of Uysal et al.[19] it was stated that the mortality rate in COVID-19 patients with chronic renal failure hospitalized in intensive care is high, and as the severity of the disease increases, the rate of patients being connected to mechanical ventilation

and death increases.In our study, prognostic factors for high mortality included comorbidities, male gender, long symptom duration, and mechanical ventilation duration.

Study Limitations

One limitation was that this was a retrospective study. Another limitation was the lack of a randomized controlled study, making it impossible to draw definitive conclusions.

CONCLUSION

Treatment of ARDS associated with COVID-19 requires a multidisciplinary approach. In this patient group with high mortality and cost, the use of ECMO by considering prognostic factors and guidelines is seen as factors that increase the chance of success. The patients in our study were treated with ECMO in accordance with established guidelines. However, given the high mortality recorded in the present study, we believe that studies on the effectiveness of additional supportive treatments that can reduce ECMO-related complications are needed. As mortality in patients with ARDS due to COVID-19 is higher than that in patients with ARDS unrelated to COVID-19, potential risk factors for mortality other than ARDS need to be reviewed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul Medipol University Non-interventional Clinical Researches Ethics Committee (Date: 06.01.2022, Decision No: 07).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Can hematologic parameters predict isolated oligohydramnios and isolated polyhydramnios?

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ABSTRACT

Aims: We fulfilled this study to anticipate the diagnosis of isolated oligohydramnios (IO) and isolated polyhydramnios (IP) by using the first trimester value of hematologic parameters.

Methods: We conducted a retrospective research 32 and 42 weeks of gestation women with IO and IP between in a single tertiary center in Turkey. In this cohort research three groups are composed of 65 IO patients and 56 IP patients and normal 97 patients that had normal volume of amniotic fluid.

Results: While PLR were significantly increased in the IO pregnants ($p < 0.05$) the distinction was not displayed in the IP pregnants. In addition, there was no notable variation in hematologic parameters; in terms of NLR, WBC, Hgb, MCV, PLT, PDW between patients and control patients ($p > 0.05$).

Conslusion: PLR values were independently associated with isolated oligohydramnios but not to isolated polyhydramnios. Hematologic parameters can be helpful in predicting isolated oligohydramnios.

Keywords: First trimester, NLR, PLR, isolated oligohydramnios, isolated polyhydramnios

INTRODUCTION

Oligohydramnios refers to the amniotic fluid volume (AFV) that is less than the minimum expected for gestational age and can be related with deseases like membranes' premature rupture, placental insufficiency and chromosomal or structural anomalies. Diagnosed by ultrasound examination, preferably based on an objective measurement such as amniotic fluid volume (AFV) ≤5 cm or one deepest pocket at least (SDP) <2 cm, but a subjective assessment of decreased AFV is also acceptable.[1] Isolated oligohydramnios (IO) is described that oligohydramnios while absence of fetal structural and chromosomal abnormalities, fetal growth restriction, intrauterine infection, and maternal disease. The percentage of IO ranges from 0.5 to 5% accordingly the difference in definition.[2] Even IO is noticed at >37 weeks of pregnancy to prevent sudden death labor induction is recommended, this pregnancies were at increased risk for meconium aspiration syndrome, cesarean birth for an fetal heart rate abnormalities, increased risk of follow-up in neonatal intensive care unit.[3,4] These harmful perinatal consequences are related with utero-placental insufficiency between uterus and placenta, and/or amniotic fluid with meconium because of umbilical cord compression.[5] Although the reason is unknown, yet it has been thought an indicator of placental insufficiency in the manner of fetal growth retardation (FGR) and preeclampsia.[6]

Polyhydramnios, is a pathologic excess of AFV in pregnancy ranges from 0.2 to 1.6% of the whole of pregnant women and is related with perinatal side effects due to a higher incidence of intrauterine fetal demise, prematüre birth, early breaking of membranes, cord prolapse, fetal macrosomia, breech presentation, cesarean delivery, and postpartum hemorrhage.[7,8] Idiopathic causes and various diseases can be reasoned with polyhydramnios. Approximately 20% are due to a congenital anomaly and 60% to 70% are idiopathic with no identified underlying cause. Fetal anomalies (often associated with an underlying genetic abnormality or syndrome) are the most common conditions associated with severe polyhydramnios, while maternal diabetes, multiple gestation, and idiopathic factors are more often associated with milder cases.[9] Defectivenes which improve the swallowing reflex such as esophageal atresia, duodenal atresia and neuromuscular disorders such as myotonic dystrophy.[10] The diagnosis of polyhydramnios

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is based upon sonographic visualization of increased AFV and single deepest pocket (SDP) ≥8 cm or amniotic fluid volume (AFV) ≥24 cm.[11]

Pregnancy is a chronic mild inflammatory condition that may directly or indirectly affect certain hematological parameters observed in blood tests.[12] Some of these changes may vary in pathological conditions. It has been detected to increase in preeclampsia both neutrophil-lymphocyte ratio (NLR) and Red blood cells Distribution Width (RDW).[13] Studies of the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) have shown a significant association with cancer and many diseases with inflammation; these parameters are beginning to be considered as possible inflammation markers.[14] This research purposes to appraise the first trimester hematological values in complete blood count for predicting IO and PO.

METHODS

This retrospective comparative study was carried out at Department of Obstetric and Gynecology in Kayseri City Training and Research Hospital, a tertiary referral center. Kayseri City Hospital Clinical Researches Ethics Committee (Date: 01.04.2021, Decision No: 376). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. We consider for diagnose to the oligohydramnios and polyhydramnios the table according to weeks in the study describing Rinat Gabbay-Benziv's 3rd TM AMS index published in JMFNM in 2020. We retrospectively reviewed 1572 obstetric us reports in Department of Radiology, Kayseri City Hospital, between January 1, 2020 and December 31, 2021. Among these pregnants, singleton pregnancies between 32-42 weeks of gestation without fetal anomalies and fetal neuromuscular disorders, without IUGG, without PROM, without a diagnosis of a certain systemic disease such as DM or HT were screened to determine the IO and IP. Pregnant women aged 18-40 ages ≥28 weeks with IO and IP current first trimester complete blood count results were collected for the study. The patients with normal amniotic fluid created from low-risk term pregnancies with a normal amount of amniotic fluid (AFI 5-25 cm) 387 healthy pregnant women. A total of 65 women that fit these criteria with IO and 56 woman with IP were detected. We evaluated of all groups first trimester complete blood count parameters. These parameters were white blood cell count (WBC), hemoglobin (Hgb), mean corpuscular volume (MCV), neutrophil and lymphocyte count, neutrophil lymphocyte ratio (NLR), platelet count, platelet lymphocyte ratio (PLR), red cell distribution width (RDW), platelet distribution with (PDW) and mean platelet volume (MPV). In addition, the records of 97 healty women who gave birth at the same hospital and time were accepted as a control group. The information that are contained maternal age, parity, gestational age at delivery is saved.

Statistical Analysis

The data were investigated with the SPSS 21.0 program. Whether the continuous variables were suitable for normal distribution according to the groups was analyzed with the Kolmogorov-Smirnov test. A Mann-Whitney U test was used to compare variables that did not conform to a normal distribution. One-way analysis of variance (ANOVA) was used to compare normally distributed data according to three or more groups, and the Kruskal-Wallis test was used to compare non-normally distributed data. The Chi-square test or Fisher's exact probability test was used to determine whether the frequency distributions of categorical variables were homogeneously distributed among groups.

RESULTS

A sum of 218 patients were included. Of these, 65 pregnant women were with isolated oligohydramnios (study group), 56 pregnant women were with isolated polyhydramnios and 97 pregnant women with a normal amount of amniotic fluid (control group). The demographic features of the patient groups are demonstrated in Table 1 and Table 2. Statistically significant distinction was not displayed between groups concerning the age, obstetric history (gravida, parity, number of abortion), history of previous caesarean delivery. Mean birth week was 38.34±1.14 for the oligohydramnios group, 38.58±1.31 in the polyhydramnios group and 38.73±0.88 healthy control group, and this finding was not statistically significant.

Table 1. Features of patients with and without oligohydramnios

Parameters	Oligohydramnios (n=65)	Healthy pregnant (n=97)	p value
Age [year]	31.68±4.68	31.02±4.492	0.74
Birth weeks	38.34±1.14	38.73±0.88	0.120
Gravidity	2.79±1.46	2.81±1.276	0.89
Parity	1.20±1.46	1.23±0.51	0.46

Table 2. Features of patients with and without polyhydramnios

Parameters	Polyhydramnios (n=56)	Healthy pregnant (n=97)	p value
Age [year]	32.12±2.96	31.02±4.492	0..81
Birth weeks	38.58±1.31	38.73±0.88	0.77
Gravidity	2.59±1.86	2.81±1.276	0.86
Parity	1.02±1.85	1.23±0.51	0.38

In terms of hematological parameters investigated in the first trimester, there was no statistically significant distinction between the groups' WBC, hemoglobin,

neutrophil, or lymphocyte counts (p > 0.05). İmportant distinction was not displayed between the groups with regards to platelet counts, RDW, MPV, NLR values in the first trimester (p >0.05). PLR values were significantly higher in the oligohydramnios group in comparison with the healthy control group (p < 0.05) and not in the polyhydramnios group (Table 3, 4).

Table 3. Comparison of the hematological test results measured in the first trimester of patients with and without oligohydramnios between the groups

Parameters	Oligohydramnios (n=65)	Healthy pregnant (n=97)	p value
WBC (×10³/L)	9.81±0.12	9.79±0.55	0.62
Hemoglobin, g/L	11.07±0.19	10.61±0.12	0,85
Platelets (×10³/L)	281.967+7.44	261.508±3.62	0.01
Neutrophils (×10³/L)	6.37+0.28	6.47±1.08	0.67
Lymphocytes (×10³/L)	3.05±0.06	2.81±0.11	0.84
RDW	12.4+0.24	11.91±0.13	0.70
MPV	9.7±0.2	9.3±0.21	0.24
NLR	2.032±0.11	2.12±0.04	0.72
PLR	118.6±3.15	112.13±3.62	0.03

Abbreviations: WBC:white blood cells; RDW=:red blood cell distribution width; MPV=mean platelet volume (MPV); NLR=Neutrophil to lymphocyte ratio; PLR= Platelet to lymphocyte ratio

Table 4. Comparison of the hematological test results measured in the first trimester of patients with and without Polyhydramnios between the groups

Parameters	Polyhydramnios (n=56)	Healthy pregnant (n=97)	p value
WBC (×10³/L)	8.98±0.19	9.79±0.55	0.44
Hemoglobin, g/L	11.22±0.12	10.61±0.12	0,63
Platelets (×10³/L)	270.895+8.04	261.508±3.62	0.43
Neutrophils (×10³/L)	6.22+0.25	6.47±1.08	0.83
Lymphocytes (×10³/L)	2.23±0.22	2.81±0.11	0.66
RDW	11.89+0.22	11.91±0.13	0.59
MPV	9.14±0.18	9.3±0.21	0.31
NLR	3.10±0.26	2.12±0.04	0.1
PLR	114.08±4.28	112.13±3.62	0.35

DISCUSSION

The study aimed to be helpful the early prediction of IO and IP pregnancy thanks to the complete blood count values which are routinely used in many obstetrics clinics.

We evaluated maternal serum levels of the inflammation parameters NLR, and PLR to determine whether the fetal and placental inflammation that develops in isolated oligohydramnios and polyhydramnios causes an inflammatory response in the mother. Indeed, platelets was involved in a processes such as endothelial damage,[15] angiogenesis and hypoxia and increased platelet activity may conduced to the pathogenesis of IO. PLR levels were found increased in the oligohydramnios group than the control group. This is suggested that there is an inflammatory process in the pathophysiology of oligohydramnios and that a maternal inflammatory response may occur against this inflammation. Decreased uteroplacental flow may induce an augmentation in the appearance of chemokines and can overstimulate an inflammatory response.[16] Progressive decrease in uteroplacental flow causes activation of neutrophils in the fetus in the subsequent stages of pregnancy and increased chemokine release by resulting in chronic hypoxia.[17] These events lead to activation of neutrophils in the later stages of pregnancy.[18] Platelets, like neutrophils, increase the secretion of cytokines at the onset of inflammation, and increased cytokines contribute to increased inflammation by enhancing new neutrophil and platelet synthesis.[19] Otherwise a study involving 11,415 patients reported no significant difference in PLR and NLR values investigated in the first trimester between high-risk pregnancies and healthy pregnancies.[20]

The clinical importance of oligohydramnios derives cause of jeopardize fetal and neonatal wellness. More than one study has shown that increasing proinflammatory cytokine production may be caused hostile pregnancy effects such as uterine activation and prematurity.[21,22] It is unclear whether IO reflects an underlying pathological condition, for which reason the management of IO is still controversial These negative obstetric outcomes associated with oligohydramnios are assumed to be cause of placental insufficiency.[23] Therefore it may be helpful to reveal the relation between the inflammatory process in the mother with IO, inflammatory parameters, and obstetric outcomes. In the light of this information, first trimester hematological indices may be important in early diagnosis and prediction of IO. Previous studies have also investigated whether hematological parameters investigated in the first trimester can predict poor obstetric outcomes.[24] Recently, Kurt et al.[25] investigated that high RDW levels were related with both the constution and the degree of preeclampsia. It was reported that, in preeclampsia, hypoxic placenta is caused to elevated inflammatory events (eg, neutrophil, monocyte, and macrophage) and ended with the demolition of red blood cells by figuring with oxygen radicals and proteolytic enzymes.

Polyhydramnios could be idiopathic or related with different desease. About 40 percent of polyhydramnios is idiopathic.[26] However, an abnormality is diagnosed after birth in until 25 percent of cases that are considered prenatal idiopathic.[27] Persistent polyhydramnios has been related with an higher risk for adverse maternal and neonatal outcomes, in addition to poor outcomes with associated fetal morphological abnormalities.[28] The inflammatory process in the amniotic fluid or placenta may also be significant to detect cause of IP formation such as IO.

CONCLUSION

Consequently the reason for fulfilling this study was to asist for prediction of appropriate, convenient and cheaper examination of IO and IP by modest routine heamatologic markers. In summary, PLR increases in inflammation and were seen that risen in IO. This suggests that they could be used as markers in both the diagnosis of oligohydramnios and in predicting perinatal outcomes in suspected cases. It may be clinically helpful to determine prognostic parameters that would support the diagnosis of IO and enable to take precautions in terms of the risks. We think that IO can be identified early and neonatal morbidity and mortality reduced to a minimum by evaluating women's platelet values in the first trimester and PLR. We could not detect increases in inflammatory processes such as PLR, as in IO. More study is requered for the IP.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kayseri City Hospital Clinical Researches Ethics Committee (Date: 01.04.2021, Decision No: 376)

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The effect of nasal septum deviation type on the systemic inflammatory index and blood markers of inflammation

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ABSTRACT

Aims: The aim of this study was to identify the changes in blood markers of inflammation and the systemic immune inflammatory index in patients with nasal septum deviation (NSD). An additional aim was to determine if there was a significant difference in the findings according to septum deviation type.

Methods: This retrospective study included 321 patients diagnosed with NSD via physical examination and CT (NSD group), and 101 healthy controls. Standard complete blood count was performed. Main blood parameters and mean platelet volume (MPV), the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the systemic immune inflammatory index (SII) were recorded. NSD was classified as 3 types according to Dreher classification, and the types were compared to each other and the control group.

Results: The hemoglobin level, and platelet and neutrophil counts were significantly higher in the NSD group than in the control group ($P<0.001$ for each). The MPV, NLR, PLR, and SII were also significantly higher in the NSD group ($P<0.001$ for each). There was a significant increase in all blood markers of inflammation as the degree of septum deviation increased ($P<0.001$ for each).

Conclusion: Blood markers of inflammation are significantly elevated in patients with NSD. The SII value can indicate an inflammatory condition in these patients. The degree of septum deviation can affect the degree of inflammation.

Keywords: Nasal septum, nasal obstruction, mean platelet volume

INTRODUCTION

Nasal obstruction is among the most common complaints in otolaryngology practice. It is thought that 80% of the Turkish population has breathing problems.[1] Nasal septum deviation (NSD) is the most common cause of nasal obstruction. The nose is responsible for ~50% of total respiratory resistance; therefore, when there is nasal obstruction lung ventilation capacity decreases, and hypoxia and hypercapnia occur. Increased intrathoracic pressure causes lung and heart problems, and also negatively affects activation of the sympathetic and parasympathetic systems.[2] Hypoxia-induced erythropoiesis occurs due to chronic hypoxia and, therefore, diseases that cause chronic hypoxia increase the hemoglobin (Hb), hematocrit (Htc), and red blood cell (rbc) levels. Inflammation increases mean platelet volume (MPV), which is a potential risk for arterial thrombosis.[3] Research shows that there is an increase in MPV in patients with hypertension, hypercholesterolemia, diabetes mellitus, and acute ischemic attack.[4] Chronic inflammation causes an increase in the neutrophil to lymphocyte ratio (NLR) and the platelet to lymphocyte ratio (PLR).[5]

The systemic immune inflammation index (SII) is a novel marker calculated as the platelet count × NLR and includes 3 cell types. It provides information about the clinical outcome in patients with cancer and inflammatory diseases. A high SII in patients with acute myocardial infarction and heart failure is indicative of a poor prognosis. In recent years research on SII has been increasing, especially in patients with gastrointestinal and gynecological cancers.[6]

Studies show that these parameters increase significantly in cases of many diseases in which chronic hypoxia and inflammation occur. The present study aimed to determine if chronic inflammation due to NSD changes these parameters. In addition, patients with NSD were evaluated according to septum deviation type to determine whether there is a significant relationship between the degree of septum deviation and inflammation.

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METHODS

The study was carried out with the permission of Kırıkkale University Non-interventional Clinical Researches Ethics Committee (Date: 08.06.2022, Decision No: 2022.05.24). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This retrospective chart review study examined patient demographics, medical examination reports, and blood parameters. The NSD group included 321 patients that presented to Kırıkkale Yüksek İhtisas Hospital, Department of Otorhinolaryngology, and were diagnosed with NSD via anterior rhinoscopy, nasal endoscopy and paranasal CT between January 2018 and December 2022. The control group included 101 age-matched healthy individuals that presented to the otolaryngology department and did not have NSD based on otolaryngologic examination and paranasal CT.

NSD was classified according to Dreher et al.[7] as follows: type 1: mild deviation (deviation <50% of the distance from the midline septum to the lateral wall); type 2: moderate deviation (deviation >50% of the distance from the midline septum to the lateral wall); type 3: severe deviation (deviation touching the lateral wall) (**Figure 1-3**). NSD type was determined by evaluating paranasal CT scans. Paranasal CT scans were evaluated by the 2 authors separately. All participants underwent complete otorhinolaryngologic examination, including the nasopharynx, oropharynx, and larynx. Those with other possible causes of upper airway obstruction, such as concha hypertrophy, nasal polyposis, and adenotonsillar hypertrophy, were excluded from the study. Other exclusion criteria were as follows: active or chronic infection, cancer, systemic inflammatory disease, autoimmune disease, cardiac and lung disease, hypothyroidism, hyperthyroidism, hematological disease, and chronic renal or hepatic disease. Due to personal identity concealment and the retrospective nature of the study, written informed consent was not obtained.

Blood samples were collected before any treatment (surgical or non-surgical). The complete blood count was evaluated in both groups. The hemoglobin level, platelet, neutrophil, and lymphocyte counts, as well as the MPV, NLR, PLR, and SII were compared between the study and control groups, and between NSD types. All of the parameters examined in this study were measured in blood samples and were included in the complete blood count. As such, it is a method that can be used both to determine the severity of the disease and to evaluate the effectiveness of the surgery.

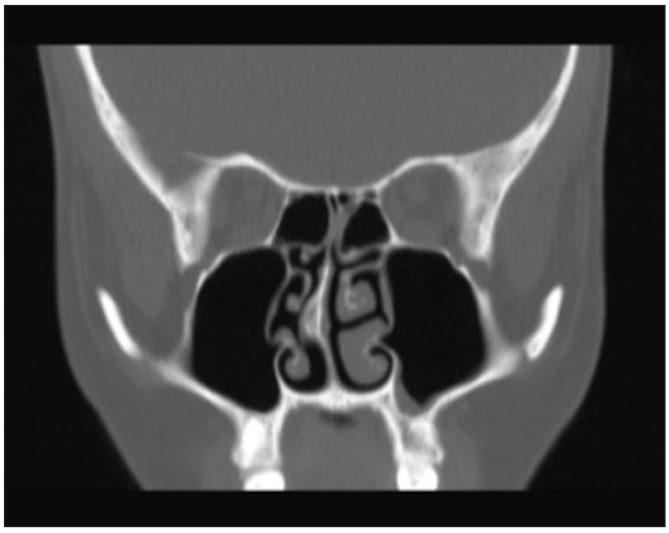

Figure 1. Type 1 nasal septum deviation (mild deviation: <50% of the distance from the midline septum to the lateral wall) (classified according to NSD, Dreher et al.)[7]

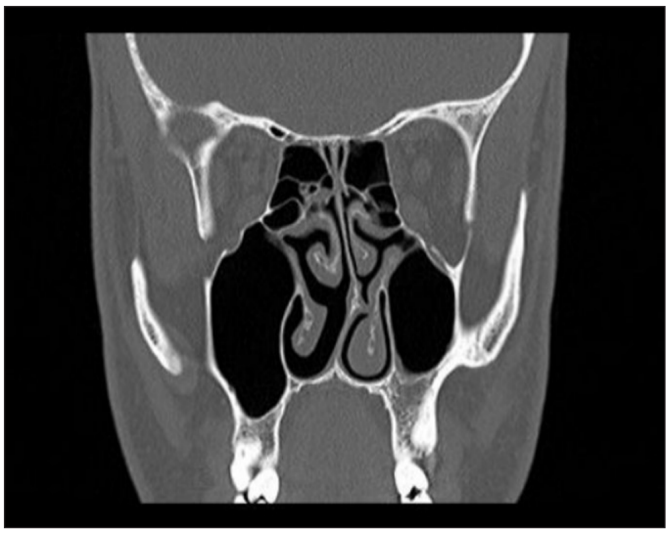

Figure 2. Type 2 nasal septum deviation (medium deviation: deviation >50% of the distance from the midline septum to the lateral wall) (classified according to NSD, Dreher et al.)[7]

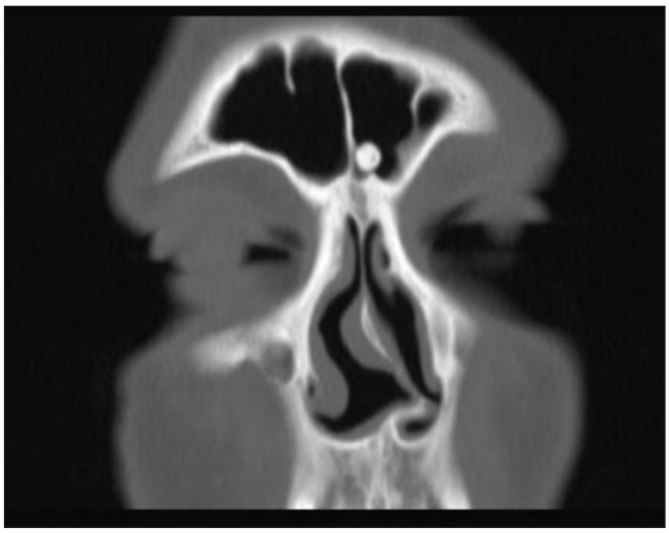

Figure 3. Type 3 nasal septum deviation (severe deviation touching the side wall) (classified according to NSD, Dreher et al.)[7]

Data were entered into Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Statistical analysis was performed using IBM SPSS Statistics for Windows v.25.0 (IBM Corp., Armonk, NY, USA). Descriptive analysis was performed, and the normality of the distribution of data was tested using the Kolmogorov-Smirnov normality test and normal distribution parameters. Categorical variables were compared using the chi-square test and Fisher's exact test for small-sample data (n<5). The Kruskal-Wallis test was used for non-normally distributed independent variables in multiple groups. The level of statistical significance was set at p<0.05; all reported p values are 2-sided.

RESULTS

The NSD group included 321 patients with NSD and the control group included 101 patients without NSD. In all, 42% of the control group were male and 58% were female, and 43.3% and 56.7% of the NSD group were male and female, respectively; there wasn't a significant difference in the distribution of genders between the 2 groups (p=0.76). In addition, there wasn't a significant difference in mean age between the 2 groups (p=0.11).

The mean hemoglobin level in the NSD group was 14.59±1.6 g dL-1, versus 13.8±1.6 g dL-1 in the control group; the difference was significant (p<0.001). The platelet and neutrophil counts, and the MPV, NLR, PLR, and SII differed significantly between the NSD and control groups (p<0.001 for all) (Table 1). These parameters were also compared between NSD types and between each NSD type and the control group. The parameters differed significantly between all NSD types and the control group, but they did not differ significantly difference between NSD types 2 and 3 (Table 2). The lymphocyte count was 2.42±0.64 10^3/μL in the NSD group, versus 2.5±0.6 10^3/μL in the control group; the difference was not significant (p=0.113).

Table 1. Comparison of the NSD group and control group

	NSD Group	Control Group	pa
Age, years	28.19±8.7	30.44±10.1	0.116
Hb	14.59±1.6 g/dl	13.8±1.6 g/dl	<0.001*
Platelet count	287.4±64.3 10^3/μl	254±48.4 10^3/μl	<0.001*
Neutrophil count	3.98±1.24 10^3/μl	3.3±0.9 10^3/μl	<0.001*
Lymphocyte count	2.42±0.64 10^3/μl	2.5±0.6 10^3/μl	0.113
MPV	10.4±0.82 fl	8.2±0.2 fl	<0.001*
SII	482.4±156.8	331.7±57.3	<0.001*
NLR	1.71±0.56	1.32±0.2	<0.001*
PLR	124.6±37.5	104.9±32.1	<0.001*

a Wilcoxon signed ranks test; *p< 0.05

DISCUSSION

The nose is responsible for ~50% of airway resistance. In cases of nasal obstruction respiratory resistance increases, lung ventilation decreases, and hypoxia and hypercapnia occur. Activation of the sympathetic and parasympathetic systems increases the risk of cardiac and pulmonary disease.[2] NSD is the most common cause of nasal obstruction. The literature includes multiple NSD classification systems. The shape of the deviation or the level of closure of the passage, and other concomitant nasal cavity pathologies are evaluated with these classification systems.[8] In the present study NSD was classified according to Dreher et al.[7] who classified NSD type according to the level of obstruction, as follows: type 1: the nasal cavity is <50% occluded; type 2: the nasal cavity is >50% occluded; type 3: the septum touches the lateral wall.

Erythropoiesis is stimulated in response to chronic hypoxia, with acceleration of platelet turnover, and increases the platelet count and volume. This increases the risk of arterial thrombosis. The parameter used to evaluate this response to hypoxia is the MPV. Unlu et al.[9] observed that there is a significant increase in MPV in patients with NSD. In the present study MPV was also significantly higher in the NSD group than in the control group. Ulu et al.[3] compared the Hb, Hct and Rbc values in patients with NSD, and reported a significant

Table 2. Comparison of NSD types and the control group

	NSD Type 1	NSD Type 2	NSD Type 3	Control	pa
Hb	14.27±1.59	14.57±1.74	15.03±1.7	13.88±1.62	<0.001*
Platelet	271±58.7	297±61.6	300±69.6	254±48.4	<0.001*
Neutrophil	3.7±1.1	4.1±1.2	4.1±1.2	3.3±0.9	<0.001*
Lymphocyte	2.5±0.6	2.3±0.6	2.3±0.5	2.5±0.6	0.023*
MPV	9.7±0.7	10.8±0.4	10.9±0.5	8.2±0.2	<0.001*
SII	410.7±144.8	524.2±150.3	540.5±140.7	331.7±57.3	<0.001*
NLR	1.54±0.5	1.8±0.5	1.85±0.5	1.32±0.2	<0.001*
PLR	112.6±33.1	131±37.7	134.8±38.7	104.9±32.1	<0.001*

a Wilcoxon signed ranks test; *p< 0.05

difference, as compared to the normal controls. Similarly, there was a significant difference in the Hb level between the NSD patients and controls. Varol et al.[4] reported that MPV is higher in patients with obstructive sleep apnea (OSA) than in controls, and that MPV increases as the apnea/hypopnea index and desaturation index increase. Importantly, they also reported the risk of cardiovascular disease is also increased in OSA patients.

In the case of chronic inflammation, an increase in the neutrophil, basophil, and platelet counts is observed, while there is a decrease in the lymphocyte count.[10] NLR and PLR are used by many medical disciplines as markers of inflammation. Karatas et al.[11] noted that there is a significant decrease in NLR after septoplasty in patients with NSD. In the present study there wasn't a significant difference in the lymphocyte count between the NSD patients and controls; however, NLR and PLR did differ significantly between the 2 groups. Hu et al.[12] described a novel marker of inflammation-SII. This marker has become very popular in recent years, as it evaluates 3 cell types simultaneously. SII is calculated as the platelet count×NLR and is used in the follow-up of patients with coronary atherosclerosis and chronic inflammatory diseases. Yang et al.[13] and Zhong et al.[14] showed in their meta-analyses that SII can be used as a prognostic indicator in patients with hepatocellular and gastrointestinal cancers.

Otolaryngologists have used SII for determining the prognosis in studies on oral cavity cancers, Bell's palsy, and chronic otitis media. In patients with oral cavity cancers it was observed that the SII is high before treatment, it significantly decreases after treatment, and it can be used to determine the prognosis.[15] Bell's palsy is another disease in which inflammation occurs, and it was reported that the SII is high in Bell's palsy patients.[16] A study that compared the SII in patients with mucosal chronic otitis media and squamous chronic otitis media noted that the SII was significantly higher in those with mucosal chronic otitis media (characterized by predominant inflammation) than in those with chronic otitis media originating from cholesteatoma.[17] The researchers concluded that the SII is an inexpensive and useful tool for differentiating the 2 diseases. To the best of our knowledge the literature is devoid of any reports on the relationship between NSD and the SII. In the present study the SII was compared between NSD patients and controls, and the difference was significant, suggesting that there is an increase in inflammation in patients with NSD. Many studies have shown that there is a relationship between NSD and cardiopulmonary diseases; however, the methods used to assess the risk of cardiopulmonary disease are costly and time consuming. In CT, the patient is exposed to high doses of radiation. Frequent CT is not appropriate during the follow-up period. In the present study the complete blood count, which is an inexpensive and easy method, was used and significant results were noted.

The present study has some limitations. Although the control group and the NSD group were similar in terms of age and gender, the control group was smaller; however, the similarity in age and gender in the 2 groups strengthens the reliability of the findings. The markers of inflammation used in the present study are also elevated in the presence of inflammation or oncological disease, and the presence or absence of such conditions could not be definitively excluded in the participants. Nevertheless, we think that the size of the NSD group minimizes this limitation. In addition, whether there was a significant change in values after septoplasty surgery in the NSD patients was not investigated. For this, a prospective study will be planned and long-term follow-up will make our hypothesis more meaningful.

In the present study patients with NSD were classified according to NSD type and compared to each other and to the control group. There was a significant difference in all the studied parameters, except the lymphocyte count, between those with type 1 and type 2 NSD, whereas there weren't any significant differences in any of the parameters between those with type 2 and type 3 NSD. Based on these findings, we think that the level of septum deviation has an effect on chronic hypoxia in patients with NSD. When the degree of septum deviation is >50% there is a significant increase in the level of inflammation; as such, not only the presence of NSD, but also the level of deviation is important in terms of cardiopulmonary diseases.

CONCLUSION

NSD causes chronic hypoxia, which leads to cardiopulmonary diseases and stimulation of erythropoiesis. The present findings show that there is an increase in markers of inflammation in patients with NSD, as compared to controls. The present findings indicate that chronic hypoxia is more likely and inflammation is higher in patients with NSD type 2 and 3, than in those with NSD type 1. The SII is a marker that can be used to determine the risk of cardiopulmonary disease in patients with NSD.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kırıkkale University Non-interventional Clinical Researches Ethics Committee (Date: 08.06.2022, Decision No: 2022.05.24).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Quality analysis of YouTube videos in the management of hyperlipidemia in adults

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ABSTRACT

Aims: We aimed to evaluate the quality of video content about hyperlipidemia and its treatment on YouTube which is used as an information source.

Methods: This study has a cross-sectional design. A hundred videos were reviewed. Journal of the American Medical Association (JAMA) score, Global quality score (GQS), modified DISCERN and Hyperlipidemia YouTube Score (HYS) were used for the quality analysis of the videos. Video duration (minutes), time since upload (months), Number of views/comments/likes were analyzed.

Results: GQS was 1.58±0.94 for Turkish videos and 2.28±1.21 for English videos ($p<0.001$). mDISCERN, JAMA and HYS scores were higher in English videos ($p<0.05$). 62% of English videos and 80% of Turkish videos were of low quality.

Conclusion: The overall quality of information on hyperlipidemia and its treatment on YouTube remains poor. The establishment of a control institution for preventive medicine and the inclusion of videos on YouTube about health issues to raise public awareness on this subject will be beneficial for accessing accurate and reliable information.

Keywords: Hyperlipidemia, YouTube, quality, public health, web-based health information

INTRODUCTION

Lipid metabolism or lipoproteins are of great importance for life. They are essential for the management and oversight of cellular functions, as well as forming essential components in cell membranes. Disturbance in cholesterol metabolism has been shown to be an independent predictor of many cardiovascular and cerebrovascular events globally.[1] An important mechanism of hyperlipidemia is atherosclerosis; encompasses both inflammatory and immunological responses. The most initial atherogenic event is the deposition of low-density lipoprotein (LDL) in the subendothelial matrix. This situation is optimal when circulating LDL amounts increase and high-density lipoprotein (HDL) decreases.[2] Therefore, the control of dyslipidemia has prognostic importance for myocardial infarction and strokes.[3]

It is possible to manage the risk factors associated with atherosclerosis and its accompanying cardiovascular diseases and to prevent the development of the disease. The cornerstones for hyperlipidemia treatment, including healthy diet and lifestyle behaviors, lower the LDL and triglyceride levels.[4] Dietary and lifestyle changes can prevent approximately 80% of cardiovascular disease mortality.[5] Additional pharmacological treatment may be recommended according to the severity of dyslipidemia and total cardiovascular disease risk score. The agents that alter the lipid level are statins, fibric acid derivatives, bile acid sequestrants, cholesterol absorption inhibitors and nicotinic acid.[6,7]

YouTube is the world's largest video website with 122 million active daily users and 5 billion daily watched videos.8 It has gained popularity as an online resource for medical information and a social networking platform for sharing health information among audiences. However, these health-related videos carry the risk of misleading and misdirecting information about important health topics.9 Individuals can shape their current treatments by watching these videos. Besides, it is important for public health to examine the authorship, quality, accuracy and validity of the information in the videos. Therefore, we aimed to evaluate the quality of the videos about hyperlipidemia and its treatment on YouTube, a large social networking platform.

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METHODS

All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The collected data included the review of videos available on the international social networking platform YouTube. Ethics committee approval was not obtained as there was no human/animal participated in the study and all videos used for the study were available on a public social media website.

Video Search on YouTube

The official page of the social media platform (https://www.YouTube.com) was used. The phrases "medical treatment of high cholesterol", "cholesterol management" for English videos and "high cholesterol treatment" for Turkish videos, were searched on the web between 01.04.2023 and 07.04.2023. Only English and Turkish videos were included in the study. There was no video time limit in the study. The 180 most watched videos in English were evaluated. 98 videos non-English, 4 video commercials, 4 videos duplicate videos, 17 videos herbal treatment suggestions, 1 video non-audio narration, 6 irrelevant videos were excluded from the study. 50 English videos were included in the study. The most watched 135 videos from Turkish videos were evaluated. 11 videos of commercials, 12 videos of duplicate videos, 3 videos of non-Turkish, 35 videos of herbal treatment suggestions, 2 videos of non-audio narration, 22 irrelevant videos were excluded from the study. 50 Turkish videos were included in the study (Figure 1).

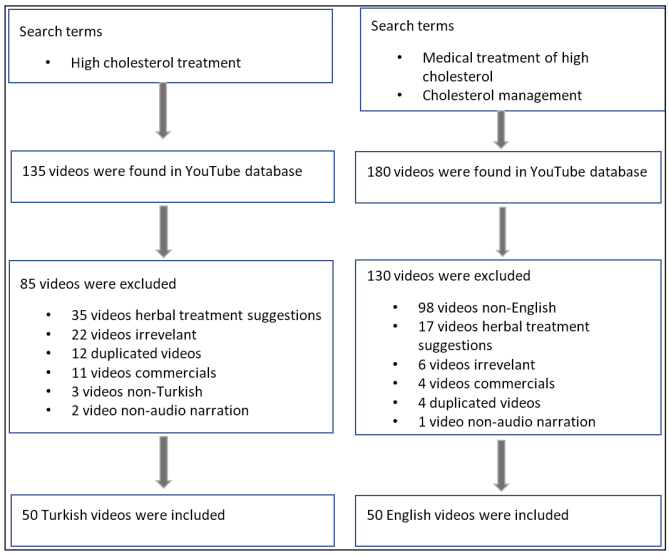

Figure I. Working flow

Video Characteristics

The characteristics of videos, including number of views, time since upload date (months), view ratio (views/day), duration, video source/uploader, number of likes and comments, were analyzed.

Video Sources

Video sources was categorized into physician or academic (authors/uploaders with university affiliations), medical sources (content from health-focused websites), pharmaceutical companies (with advertisement content), TV program.

Video Quality Analysis

Video contents were evaluated by two independent specialists (MT, CMC). A consensus was reached on the differently scored scores and a common score was given.

The Journal of the American Medical Association (JAMA) benchmark criteria, Global Quality Score (GQS) and modified DISCERN were used for each video to evaluate video quality. JAMA benchmarks are used to determine the reliability of online resources. It is formed by scoring criteria such as authorship, attribution, validity and explanation. A total score of '4' indicates high reliability, and '0' indicates low reliability. The GQS is a fivepoint Likert scale based on the quality of information, the flow of information found online, and ease of use, with 1 point very bad-5 points excellent quality. DISCERN is an information quality assessment tool created by Charnock et al.[10] It was modified as a questionnaire consisting of 5 questions by Singh et al in 2012.[11] The total score ranges from 0-5 points, with higher scores indicating greater reliability.

Hyperlipidemia YouTube Score (HYS) is a form created by us that includes definition, complications of hyperlipidemia, symptoms/signs, screening groups/risk factors, lifestyle changes, medical treatment and side effects. '1' if each criterion is deemed sufficient; If it was found insufficient, it was scored with '0'. However, similar tools exist in recent literature methods for assessing the overall educational quality of a video.[12,13]

Statistical Analysis

All statistical tests were performed using SPSS version 21 (IBM®, Chicago, USA). The normal and abnormal distribution of the variables was analyzed with the "Shapiro-Wilk test". Video characteristics, video reliability, and quality scores were analyzed through descriptive statistics Descriptive statistics were expressed as mean and standard deviation in normally distributed numerical data, median (minimum-maximum) in abnormally distributed data, number and percentage in nominal data. "Mann-Whitney U" and "Kruskal-Wallis test" were used in the analysis of non-normally distributed variables. Nominal data were compared using "Chi-square analysis". In correlation analysis, "Spearman's correlation analysis" was used between non-normally distributed data. P value of 0.05 was set to denote statistically significant findings.

RESULTS

50 Turkish and 50 English videos were analyzed. English and Turkish videos were compared in terms of definition, complications of hyperlipidemia, symptoms/signs, screening groups/risk factors, lifestyle changes, medical treatment and side effects. Recommendations regarding lifestyle changes were found to be significantly higher in English videos (p=0.013). There was no significant difference between the two groups in terms of other criteria (p≥0.05).

The characteristics and comparison of the English and Turkish videos are shown in Table 1.

Table 1. Descriptive characteristics and comparison of the English and Turkish videos

		English (n=50)	Turkish (n=50)	p value
Definition*	n (%)	30 (60)	24 (48)	0.229
Symptoms/signs*	n (%)	11 (22)	8 (16)	0.444
Complications of hyperlipidemia*	n (%)	42 (84)	40 (80)	0.603
Screening groups/risk factors*	n (%)	22 (44)	24 (48)	0.688
Lifestyle changes*	n (%)	42 (84)	31 (62)	0.013
Medical treatment*	n (%)	34 (68)	28 (56)	0.216
Side effects*	n (%)	12 (24)	9 (18)	0.461
Medication recommendation*	n (%)			0.911
Recommended		30 (60)	28 (56)	
Not recommended		4 (8)	4 (8)	
Not mentioned		16 (32)	18 (36)	

*Chi-square test

When English and Turkish videos were compared according to video characteristics, English videos had significantly higher number of views, view ratio (views/day), duration, number of likes and comments than Turkish videos (p<0.05). There was no significant difference between the two groups in terms of time since upload date (p=0.978). However, English videos had significantly higher scores when compared by quality index scores (GQS, mDISCERN, JAMA) (p<0.05). HYS scores were also significantly higher in English videos (p=0.049). In the classification according to GQS, 80% of Turkish videos were low quality, while 62% of English videos were low quality. (24&14%) medium quality and (14&6%) high quality for English and Turkish videos respectively. However, there was no significant relationship between the two groups.

There was no statistically significant difference in the number of follow-ups according to drug treatment recommendations (p=0.147). However, those who did not mention or recommend drug treatment had higher median values.

Video characteristics and quality scores of English and Turkish videos are shown in Table 2.

When English and Turkish videos are compared in terms of their sources, 50% of English videos were uploaded by professionals, while 38% of Turkish videos were uploaded by professionals. For English and Turkish videos, (32% vs 28%) medical resources and (14% vs 34%) TV programs were found to be the most important sources of information, respectively. 4% of the videos in English were uploaded by pharmaceutical companies. However, there was no significant relationship between the two groups.

Comparisons according to video sources are shown in Table 3.

Table 3. Comparisons according to video sources

		English (n=50)	Turkish (n=50)	p value
Physician or academic*	n (%)	25 (50)	19 (38)	0.068
Medical sources*	n (%)	16 (32)	14 (28)	
Pharmaceutical companies*	n (%)	2 (4)	-	
TV program*	n (%)	7 (14)	17 (34)	

TV: Television, *: Chi-square test

Table 2. Video characteristics and quality scores of English and Turkish videos

		English (N=50)	Turkish (N=50)	p value
Number of views**	Median (min-max)	276249.5 (486-4031490)	19737 (111-2034504)	≤0.001
Time since upload date (months)**	Median (min-max)	36 (1-120)	36 (3-132)	0.978
View ratio (views/day)**	Median (min-max)	224.95 (2.03-13866.67)	17.96 (0.08-5651.40)	≤0.001
Duration (minute)**	Median (min-max)	8.19 (1-70.18)	3.36 (0.43-49.17)	0.004
Number of likes**	Median (min-max)	4250 (0-46000)	107 (0-17000)	≤0.001
Number of comments**	Median (min-max)	219.5 (0-3000)	10 (0-418)	≤0.001
GQS**	Mean±sd	2,28±1.21	1.58±0.94	0.001
GQS group*	n (%)			0.132
Low quality (1-2)		31 (62)	40 (80)	
Moderate quality (3)		12 (24)	7 (14)	
High quality (4-5)		7 (14)	3 (6)	
mDISCERN **	Mean±sd	1.96±1.26	1.24±0.91	≤0.001
JAMA**	Mean±sd	1.58±1.05	1.04±0.66	0.002
HYS**	Mean±sd	3.86±1.35	3.28±1.34	0.049

GQS; Global Quality Score, JAMA; Journal of the American Medical Association, HYS; Hyperlipidemia YouTube Score. **Mann Whitney U Test; *Chi-square test.

In the correlation analysis, a significant positive correlation was found between video quality index scores and duration (p<0.001). Moreover, view ratio and GQS and mDISCERN were correlate. However, no correlation was found between other parameters. When quality index scores were analyzed, JAMA, mDISCERN, GQS showed significant positive correlations with each other (p<0.001). There was a significant positive correlation between HYS, mDISCERN and GQS, while no correlation was found between JAMA and HYS (p=0.149).

The correlation analysis between quality index scores and video characteristics is shown in Table 4.

Table 4. The correlation analysis between quality index scores and video characteristics

		GQS	JAMA	mDISCERN	HYS
Duration¥	rho	0.630	0.411	0.607	0.474
	p	<0.001	<0.001	<0.001	<0.001
Views¥	rho	0.146	0.114	0.166	0.071
	p	0.147	0.261	0.100	0.481
View ratio¥	rho	0.205	0.143	0.221	0.112
	p	0.040	0.157	0.027	0.267
Likes¥	rho	0.173	0.163	0.181	0
	p	0.086	0.105	0.072	0.998
Comment¥	rho	0.083	0.079	0.098	-0.012
	p	0.410	0.432	0.330	0.906
GQS	rho	-	0.533	0.814	0.557
	p	-	<0.001	<0.001	<0.001
JAMA	rho	0.533	-	0.662	0.145
	p	<0.001	-	<0.001	0.149
mDISCERN	rho	0.814	0.662	-	0.490
	p	<0.001	<0.001	-	<0.001
HYS	rho	0.557	0.145	0.490	-
	p	<0.001	0.149	<0.001	-

GQS; Global Quality Score, JAMA; Journal of the American Medical Association, HYS; Hyperlipidemia YouTube Score. ¥Spearman correlation analysis

When the quality of the videos was analyzed according to their sources, there was no significant relationship between the groups in terms of GQS and HYS, but there was a significant difference in terms of mDISCERN and JAMA. In pairwise comparisons, there was a significant difference between physician/ academic and medical sources for JAMA (p<0.001) and mDISCERN (p=0.027) scores, but not for HYS (p=0.812) and GQS (p=0.184) scores. There was a significant difference between physician/ academic and TV program in GQS (p=0.036) and mDISCERN (p=0.029) scores, but not in HYS (p=0.109) and JAMA (p=0.218) scores. There was a significant difference between TV programs and medical sources in terms of JAMA (p=0.019) scores; however, no significant association was found for HYS (p=0.129), GQS (p=0.320) and mDISCERN (p=0.529). The number of videos with the content of pharmaceutical companies was not included in the statistical analysis due to two videos.

Quality analysis of videos by source is shown in Table 5.

Table 5. Quality analysis of videos by source

		Physician/ academic	Medical sources	TV program	p value
GQS	Mean±sd	2.22±1.29	1.80±0.99	1.58±0.92	0.085
JAMA	Mean±sd	1.63±0.99	0.86±0.86	1.29±0.62	0.001
mDISCERN	Mean±sd	2±1.38	1.30±0.59	1.29±1.08	0.025
HYS	Mean±sd	3.75±1.55	3.70±1.11	3.16±1.27	0.212

GQS; Global Quality Score, JAMA; Journal of the American Medical Association, HYS; Hyperlipidemia YouTube Score

DISCUSSION

YouTube, a very popular video site worldwide, has a wide range of health information. Being a free online resource, it has a large user base with easy uploading, downloading and commenting on videos by participants. However, the reliability and guidance of the information in these videos has raised concerns among many health experts. Recently, there have been numerous studies in the literature examining the reliability and quality of YouTube videos.[13-17] However, to the best of our knowledge, there is no study evaluating hyperlipidemia videos on YouTube in the literature.

The present study showed that both Turkish and English videos were of low quality with the rate of 80% and 62%, respectively. Similar to our results, Akkus et al.[13] reported that English videos on Trichotillomania were of low quality with a rate of 68.6%. In a study evaluating the content of exercise videos released during the COVID-19 pandemic, the reliability and quality of most videos were classified as "very poor".[16] Tastemur et al.[14] stated that rate of poor quality of the videos on kidney transplantation were 72.6%. Another study evaluating videos on adhesive capsulitis also found low video quality with DISCERN of 2.73 and GQS of 2.38.[18] Also, in a study comparing English and Turkish videos on Alzheimer's disease in Turkey, English videos had significantly higher scores in quality analysis using GQS and mDISCERN.[19] Similiarly, the GQS, mDISCERN, JAMA and HYS scores of English videos were higher in our study. Therefore, the scarcity of high-quality information is of concern because it affects patients'access to correct information and self decision-making processes.

In the literature, the length of videos was found to be between 5.85 and 10.37 minutes on average in many studies on different topics.[16,20,21] In our study, the average duration of the videos was 8.19 minutes, and the duration of English and Turkish videos were similar. reported similar results. However, Kaşıkçı et al.[19] reported that Turkish videos were longer.

The quality of the videos may vary depending on the uploader. Wilkens et al.[22] conducted quality analysis with DISCERN and HONcode and found no correlation between the quality scores of video uploader resources.

However, Tang et al.[18] found the highest quality scores in academic sources. Similarly, in our study, the highest scores belonged to academic sources. In addition, 50% of the videos uploaded in English are uploaded by academic sources, this rate was 38% in Turkish videos. This is in accordance with the studies in the literature.[19]

The present study revealed that lifestyle changes, which are the cornerstone of hyperlipidemia treatment, were mentioned 84% of the time in English videos, but this rate was significantly lower in Turkish videos with 62%. In addition, 8% of the videos in both languages did not recommend the use of medication, 32% and 36% did not mention it at all.

HYS is a scoring system that we developed. It has not been used in any other study before. It includes definition of hyperlipidaemia, complications, symptoms/signs, screening groups/risk factors, lifestyle changes, medical treatment and side effects. '1' if each criterion is considered adequate; '0' if found inadequate. HYS was found to be higher in English videos. Correlation analysis showed a significant positive correlation between HYS, mDISCERN and GQS, while no correlation was found between JAMA and HYS. This result suggests that English videos are more scientific and useful, but there is no similar study to compare our results. Our results are therefore open to interpretation.

Duration, number of likes, number of comments, views and quality rating scores were higher than the characteristics of English videos. There are limited studies in the literature comparing English and Turkish videos. Similar results were found by Kaşıkçı et al.[19] This seems to be due to the fact that English is a globally accepted language. The important factor here is the language considered.

Our study had some limitations. The number of videos watched may be partially insufficient. In future studies, it may be recommended to watch more videos with more keywords according to relevance.

CONCLUSION

In this study, we analyzed YouTube videos on hyperlipidemia, and we found that both English and Turkish videos were of lower quality. The high number and viewership rates of low quality, incomplete and misinformed videos support our justified concerns on this issue. Better quality and reliability of these videos, which guide the public on all kinds of health issues, may increase our chances of patient treatment. Authorized institutions can also improve the quality of YouTube videos through audits or awareness-raising campaigns. We think it is a necessary measure for preventive medicine.

ETHICAL DECLARATIONS

Ethics Committee Approval: The collected data included the review of videos available on the international social networking platform YouTube. Ethics committee approval was not obtained as there was no human/animal participated in the study and all videos used for the study were available on a public social media website.

Informed consent: Informed consent was not obtained as there was no human/animal participated in the study and all videos used for the study were available on a public social media website.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

HEALTH SCIENCES
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The role of carbondioxide insufflation in preventing postoperative peritoneal adhesions in rats

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ABSTRACT

Aims: Adhesion is the pathological connections that occur during the healing with scar formation of peritoneal surface defects. CO_2 is used the most frequently in laparoscopic operations for insufflation. It is believed that it causes to changes in the inflammatory reply of the pneumo-peritoneum, defects in acid-base balance and decrease in peritoneal macrophage functions. CO_2 is the only gas whose immunologic effects have been shown. It has been proven in experimental studies that the CO_2 insufflation causes to local peritoneal acidosis without affecting the systemic status. Moreover, it has also been shown that it decreases the pneumo-peritoneum TNF-α and IL-6 production; however, increases the IL-10 production which is an anti-inflammatory cytokine. In the literature, the relation between the laparoscopy and the postoperative adhesions has always been explained by taking the suggestion of its causing to less tissue trauma as a basis when compared with the open surgery. The inflammatory reply of the CO_2 has been less dealt with. In this study, we wanted to find the answer to the question whether the capno-peritoneum has a role in preventing the postoperative adhesion formation only by using CO_2 without a a laparoscopic operation.

Methods: 30 female Wistar Albino type rats whose weights varied between 250±20 were used in the study. The rats were divided into 5 groups. Each group had 6 rats. Rats were placed in standard polycarbon cages in groups of 6. The room temperature was kept in 21°C. The rats were fed with standard pellet food during the study and tap water was provided to them. The operational anesthesia was performed by injecting intramuscular Ketamine Hydrochloride (Ketalar, Parke Davis and Eczacıbaşı, İstanbul) 50 mg/kg and Xylazine hydrochloride (Rompun, Bayer HealthCare) 5 mg/kg.

Results: A meaningful difference ($p<0.05$) was determined between the inflammation results of the groups. The inflammation findings become lighter as moved from Group 1 to Group 5. A meaningful difference ($p<0.05$) was determined between the fibrosis results. The fibrosis findings become lower as moved from Group 1 to Group 5. A meaningful difference ($p<0.05$) was determined between the adhesion results of the groups. The adhesion findings become lower as moved from Group 1 to Group 5. A statistically meaningful difference was not determined ($p>0.05$) between the PAI values of the groups. A statistically meaningful difference was not determined ($p<0.05$) between the MDA values of the groups. The difference stems from Group 1 and Group 5. The MDA values of Group 1 is relatively higher than those of other groups; while the MDA values of Group 5 is found to be lower when compared with the other groups

Conclusion: Our results suggest that CO_2 pneumo-peritoneum has positive effects in postoperative intraperitoneal adhesion development. Since we formed a scraping model in our study, we cannot suggest that the adhesion formation is decreased with mechanical effect. The patho-physiological and molecular bases of the postoperative adhesion formation have been documented and described well. However, we consider that the capno-peritoneum and postoperative adhesion formation is prevented with anti-inflammatory effect. We need to conduct more studies to examine this mechanism.

Keywords: Postoperative adhesion, laparoscopy, capnoperitoneum

INTRODUCTION

Adhesion (being stick together) is the pathological connections that occur during the healing with scar formation of peritoneal surface defects. Adhesions may be congenital or acquired later. Congenital adhesions occur after the abnormal development of peritoneal cavity (vitello-intestinal bands etc.). Acquired adhesions are divided into two classes as inflammatory and postoperative. Inflammatory adhesions occur due to appendicitis, cholecystitis, diverticulitis, pelvic inflammatory disease, or after the use of intrauterine contraception devices.[1,2]

- When the physiopathology of adhesion formation is considered, the following situations are observed;
- Serosal damage,
- Increase in local inflammatory reply, increase in vascular permeability and inflammatory exudate,
- Formation of free radicals such as superoxide, peroxidase and hydroxyl radicals, and these formations causing to defects in cell membrane,
- Activation of the coagulation system (fibrin formation) and the fibrinolytic system (fibrinogen degradation)

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and the mechanisms that move one within the other during the formation of imbalance intraperitoneal adhesions that occur in the balance between these two elements[3-6]

Menzies reported that intraabdominal adhesion developed in 79% and 93% of the patients who received intraabdominal operational procedure is his study.[2] The major morbidities caused by intraabdominal adhesions are intestinal obstruction infertility, difficult and risky re-explorations, ectopic pregnancy and chronic stomachache. Laparoscopy technique is used widely in surgical practices.

Laparoscopic operations mean less trauma in the peritonea, less intraoperative bleeding, polite manipulations to intraabdominal organs and tissues, less tissue damage in areas other than the operation area, less exposure to glove powder, gauze, compress and operational tools, and postoperative earlier recovery. These characteristics make the laparoscopy technique superior to conventional surgical methods in terms of adhesion formation status.

Carbon dioxide (CO_2) is used the most frequently in laparoscopic operations for insufflation; and with its biological characteristics, it is still the most suitable gas in this field. It is believed that it causes to changes in the inflammatory reply of the pneumo-peritoneum, defects in acid-base balance and decrease in peritoneal macrophage functions. CO_2 is the only gas whose immunologic effects have been shown. It has been proven in experimental studies that the CO_2 insufflation causes to local peritoneal acidosis without affecting the systemic status. Moreover, it has also been shown that it decreases the pneumo-peritoneum TNF-α and IL-6 production; however, increases the IL-10 production which is an anti-inflammatory cytokine. In the literature, the relation between the laparoscopy and the postoperative adhesions has always been explained by taking the suggestion of its causing to less tissue trauma as a basis when compared with the open surgery. The inflammatory reply of the CO_2 has been less dealt with. In this study, we wanted to find the answer to the question whether the capno-peritoneum has a role in preventing the postoperative adhesion formation only by using CO_2 without a laparoscopic operation.

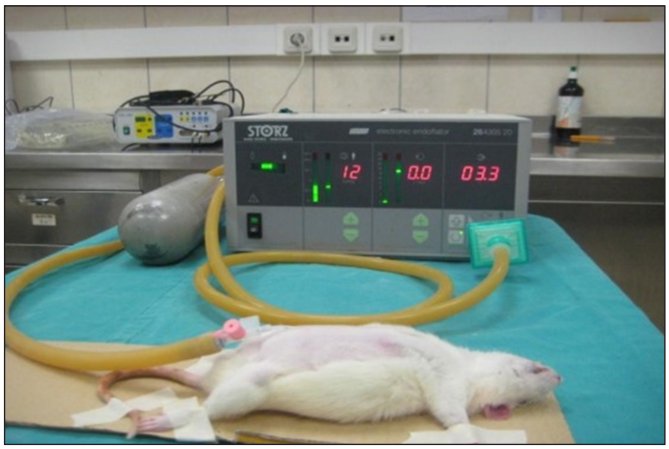
METHODS

This experimental study was carried out with the permission of Gazi University Animal Experiments Local Ethics Committee (Date: 04.09.2011, Decision No: B.30.2.GÜN.O.EU.00.00/38-5869.30). The study adhered to the animal research guidelines of the National Institute of Health.

30 female Wistar Albino type rats whose weights varied between 250±20 were used in the study. The rats were divided into 5 groups. Each group had 6 rats. Rats were placed in standard polycarbon cages in groups of 6. The room temperature was kept in 21°C. The rats were fed with standard pellet food during the study and tap water was provided to them. The operational anesthesia was performed by injecting intramuscular Ketamine Hydrochloride (Ketalar, Parke Davis and Eczacıbaşı, İstanbul) 50 mg/kg and Xylazine hydrochloride (Rompun, Bayer HealthCare) 5 mg/kg.

Operational Procedure

The necessary sterile conditions were provided after the anesthesia, and the rats were fixed to the operation board from their four extremities, and abdominal front walls of the rats were shaved and cleaned with povidone iodine. The cannula was connected to an electronic insufflator (Storz and Co., Tutthufen, Germany) and the CO_2 was insufflated so as it remained in 6 mmHg (**Picture 1**). After the operation, the rats were put in the cages in groups. The rats were then observed for 10 days in standard feeding and living conditions. Tissue samples were collected and frozen with liquid nitrogen, and kept in -80 °C until the analysis time.



Picture 1. CO_2 was insufflated so as it remained in 6 mmHg

Groups

Group I: The Sham Group (n: 6): Laparotomy was performed from the middle line, and closed without any interventions with 3/0 vicril.

Group II: The Control Group (n: 6): Laparotomy was performed from the middle line, and the petechial bleedings were performed with sterile dry sponge over the cecum, and the ileo-cecal artery was clamped for 1-2 minutes to form the scraping model. After this process, the incision was closed with 3/0 vicril.

Group III (n: 6): Laparotomy was performed from the middle line after 15 minutes carbon dioxide insufflation. Then petechial bleedings were performed with sterile dry sponge over the cecum, and the ileo-cecal artery was

clamped for 1-2 minutes to form the scraping model. After this process, the incision was closed with 3/0 vicril.

Group IV (n: 6): Laparotomy was performed from the middle line after 15 minutes carbon dioxide insufflation. Then petechial bleedings were performed with sterile dry sponge over the cecum, and the ileo-cecal artery was clamped for 1-2 minutes to form the scraping model. After this process, the incision was closed with 3/0 vicril. Then carbon dioxide insufflation was performed for 45 minutes.

Group V (n: 6): Laparotomy was performed from the middle line. Then petechial bleedings were performed with sterile dry sponge over the cecum, and the ileo-cecal artery was clamped for 1-2 minutes to form the scraping model. After this process, the incision was closed with 3/0 vicril. Then carbon dioxide insufflation was performed for 45 minutes.

Parameters

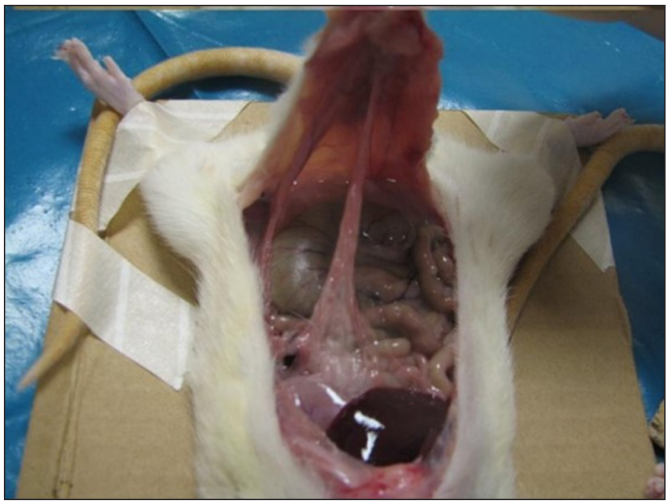
Tissue rat malondialdehyde (MDA) level measurement: The quantitative measurement of the MDA was performed with the ready-made Csabio ® tPA (American Diagnostica Inc.) kit according to the introductions of the producer company with the ELISA (Enzyme Linked-Immuno-Sorbent Assay) method. The values recorded were as pg/ml.

Tissue plasminogen activator inhibitor-1 (PAI-1) level measurement: To measure the amount of the Plasminogen activator inhibitor-1 quantitatively, the Cusabio® Plasma PAI-1 (American Diagnostica Inc.) ready-made kit was used according to the introductions of the producer company with the ELISA (Enzyme Linked-Immuno-Sorbent Assay) method. The values were recorded as pmol/ml.

Intraabdominal adhesion scoring: After 10 days, the rats were anesthetized with 75 mg /kg ketamine hydrochloride. An incision with the shape of a reverse-U was performed in the front wall of the abdomen to obtain a better vision, and all the adhesions in the abdomen were examined, recorded and classified (Picture 2). The Mazuji Classification was used in macroscopic classification.[7] The formed adhesions were taken out without damaging them, and put in 10% formalin solution, and sent to the Gazi University Faculty of Medicine Pathology Department for histopathological assessment. Some tissue samples were also sent to the biochemistry laboratory for MDA and PAI level measurements. After the exemplification, the rats were sacrificed under anesthesia by taking intracardiac blood and hypotensive collapse.

Histopathological Examination

The specimens, dehydrated and embedded in paraffin, were dyed with H&E in 1 mm thickness, and assessed in light microscope in terms of fibrosis and inflammation (Table 1,2). The fibroblast cell density (Table 1) and inflammation scoring systems (Table 2) were used in histopathological examination.[8]



Picture 2. Limited adhesion in an area thick (group 2 adhesion)

Table 1. Macroscopic adhesion evaluation scoring (Mazuji et al.)

Score	Evalution
0	No fibrosis
1	Minimally, loose
2	Moderate
3	Fluoride,intense

Table 2. Fibrosis assessment scoring (Hooker et al.)

Score	Evaluation
0	Not inflammation
1	Giant cells, scattered mononuclear inflammatory cells (Chronic inflammation)
2	An increased number of giant cells mixed with lymphocytes, neutrophils, eosinophils plasma cells (acute inflammation)
3	Many mixed inflammatory cells, presence mikroapse (acute suppurative inflammation)

Statistical Analysis

he data were entered into the SPSS 11.0 package program. In determining the difference between the groups, the Kruskal-Wallis variance analysis was used; and in determining the groups with the differences, the Mann-Whitney U non-parametric test was used. The $p<0.05$ value was accepted as statistically meaningful. The averages were given as average±standard deviation.

RESULTS

A meaningful difference ($p<0.05$) was determined between the inflammation results of the groups. The inflammation findings become lighter as moved from Group 1 to Group 5 (Table 3). A meaningful difference ($p<0.05$) was determined between the fibrosis results. The fibrosis findings become lower as moved from Group 1 to Group 5 (Table 4). A meaningful difference ($p<0.05$) was

determined between the adhesion results of the groups. The adhesion findings become lower as moved from Group 1 to Group 5 (Table 5). A statistically meaningful difference was not determined (p>0.05) between the PAI values of the groups. A statistically meaningful difference was not determined (p<0.05) between the MDA values of the groups. The difference stems from Group 1 and Group 5. The MDA values of Group 1 is relatively higher than those of other groups; while the MDA values of Group 5 is found to be lower when compared with the other groups.

Table 3. Comparison of the rate of inflammation between groups

Group	Inflammation				P value
	0 n (%)	1 n (%)	2 n (%)	3 n (%)	
1	-	-	-	6 (100)	0.0001
2	-	-	6 (100)	-	
3	-	4 (66.7)	2 (33.3)	-	
4	-	6 (100)	-	-	
5	4 (66.7)	2 (33.3)	-	-	

Table 4. The comparison between groups fibrosis

Group	Fibrosis			P value
	0 n (%)	1 n (%)	2 n (%)	
1	-	1 (16.7)	5 (83.3)	0.0001
2	-	5 (83.3)	1 (16.7)	
3	-	3 (50.0)	3 (50.0)	
4	-	6 (100)	-	
5	6 (100)	-	-	

(%)*: Row percent, Kruskall Wallis Test was used.

Table 5. Comparing the adhesion between groups

Group	Adhesion				P value
	1 n (%)	2 n (%)	3 n (%)	4 n (%)	
1	-	2 (33.3)	2 (33.3)	2 (33.3)	0.022
2	-	1 (16.7)	2 (33.3)	3 (50.0)	
3	1 (16.7)	3 (50.0)	2 (33.3)	-	
4	1 (16.7)	4 (66.7)	1 (16.7)	-	
5	2 (33.3)	3 (50.0)	1 (16.7)	-	

(%)*: Row percent, Kruskall Wallis Test was used.

A statistically meaningful difference was not determined (p>0.05) between the PAI values of the groups. A statistically meaningful difference was not determined (p<0.05) between the MDA values of the groups. The difference stems from Group 1 and Group 5. While the MDA values of Group 1 are relatively higher than those of other groups, the MDA values of Group 5 are found to be lower than those of other groups. A positive linear relation in medium level was determined between the PAI and MDA values (correlation coefficient: 0,381, p<0.05). As the PAI values increase, so do the MDA values. As the inflammation findings become severe, so do the severeness of fibrosis findings. A positive linear relation in medium level was determined between the inflammation findings and MDA values (correlation coefficient: 0,382, p<0.05). As the inflammation findings become severe, the MDA values increase. A strong positive linear relation was determined between the fibrosis findings and PAI values (correlation coefficient: 0,513, p<0.05). As the fibrosis findings become severe, the PAI values increase. A positive linear relation in medium level was determined between the fibrosis findings and MDA values (correlation coefficient: 0,399, p<0.05). As the PAI values increase, so do the MDA values. As the fibrosis findings become severe, the MDA values increase. A positive linear relation in medium level was determined between the adhesion findings and PAI values (correlation coefficient: 0,324, p<0.05). As the PAI values increase, so do the MDA values. As the adhesion findings become severe, the PAI values increase (Table 6).

DISCUSSION

Peritoneal adhesions occur after the single-layered mesothelial cells that form the peritonea are harmed due to some reasons (mechanical, ischemic, chemical, infective, inflammatory, etc.).

Table 6. Inflammation, fibrosis, adhesion, PAI 1 and MDA value relationship between

	Inflammation	Fibrosis	Adhesion	PAI	MDA
Inflammation					
Correlation composition (tau)	1	0.454*	0.193	0.111	0.382*
n	30	30	30	30	30
P	.	0.005	0.238	0.433	0.007
Fibrosis					
Correlation composition (tau)	0.454*	1	0.193	0.513*	0.399*
n	30	30	30	30	30
P value	0.005	.	0.238	0.0001	0.007
Adhesion					
Correlation composition (tau)	0.294	0.193	1	0.324*	0.051
n	30	30	30	30	30
P	0.114	0.238	.	0.024	0.719

The general incidence of re-application to the hospital due to adhesion when compared with operation procedures was reported as 4.6%.[9,10]

Various models such as damaged uterus horn model, peritonea damage model, colon anastomose model, ileal transection model, bacterial peritonitis model and clamping model were formed for experimental adhesion examination purposes. The clamping model that is applied in our study is proper for its defects being two-staged. The first stage is forming mechanical serosal intestine wall damage with direct sponge application; the second stage is forming ischemic damage after the vein is clamped. The reasons for our preference of this model is due to its similarity to surgical methods in the abdomen.

We did not determine a statistically meaningful difference among the inflammation results, the adhesion results and the fibrosis results in groups. We found that the inflammation findings became less severe as moved from Group 1 to Group 5. We found that the fibrosis findings became less severe as moved from Group 1 to Group 5. We also found that the adhesion findings became less severe as moved from Group 1 to Group 5. The findings of the study conducted by Scott-Coombes et al.[11] support our findings. In their studies, they determined PAI-1 in high density in peritoneal tissues of the patients with adhesion. We did not determine a statistically meaningful difference between the PAI-1 values among the groups in our study. We did not determine a statistically meaningful difference between the MDA values in groups. The difference stems from Group 1 and Group 5. The MDA values of Group 1 is higher than those of other groups, while the MDA values of Group 5 are lower than those of other groups. As the PAI-1 values increase, so do the MDA values. As the inflammation findings become severe, so do the severeness of fibrosis findings. As the inflammation findings become severe, the MDA values increase. As the fibrosis findings become severe, the PAI-1 values increase. As the fibrosis findings become severe, the MDA values increase. As the adhesion findings become severe, the PAI-1 values increase. As the adhesion findings in Group 1 become severe, the PAI-1 values increase. As the adhesion findings become severe in Group 3, the MDA values decrease. No relation was determined among the other findings and values in other groups.

The history of laparoscopy dates back to one century ago.[12] In early 1900s, endoscopy was used mainly in bladder, rectum, larynx, esophagus examinations; in 1901, a Russian Gynecologist named Dimitri Von Ott reported that he had entered to the peritoneal cavity with a minimal incision and examined the inside of the abdomen, and this process was later called as Ventroscopy by him.[13,14]

Laparoscopy is related with a small entry and manipulation injuries; however, pneumo-peritoneum is also responsible for the decrease in acute phase reply.[15] Laparoscopic surgery in abdominal explorations ensures that the blind dissection and retractors and compresses are used less. Moreover, when compared with traditional surgery, it decreases the washing and drying of peritonea, and contact of operational objects, tissue damage, and bleeding in operational area.[16,17] For these reasons, it is possible to suggest that laparoscopy causes to less adhesion. Carbon dioxide is the most frequently used gas in today's world.[18] The advantage of carbon dioxide is that it is not inflammable and explosive; its diffusion to the tissues is fast, and it is relatively cheaper. Gas embolism is the most important complication of the gasses that are used for pneumo-peritoneum. A gas such as carbon dioxide that is absorbed fast causes less persistent airway obstructions in right ventricular outlet. The most important disadvantage of carbon dioxide is that it dissolves in solutions quickly; and its causing to unwanted biological effects such as hypercapnia and acidosis less.[19] However, these effects are in minimal level in people who do not have further stage respiration problems and cardiac pathology. For these reasons, carbon dioxide is the most frequently preferred gas today. CO_2 is known to change the systemic immune reply in a proper way during laparoscopic surgery of the pneumo-peritoneum. It has been proven that CO_2 insufflations cause to local peritoneal acidosis. CO_2 is a strong anti-inflammatory agent.[20-22] It has been proven that CO_2 decreases the systemic inflammatory reply and capno-peritoneum macrophage function.[22,23] In case the peritoneal pH decreases to 6,1 after the CO_2 insufflation, the macrophage intracellular pH values also decrease.[24] It has been shown that superoxide production as well as phagocyte and cytokines are suppressed with the decrease in intracellular pH.[25,26] The decreasing pro-inflammatory cytokine production after the laparoscopy in peritoneal tissues may be responsible for the decrease in postoperative pain and adhesion formation.[25-27]

Adhesion formation is a complicated process. Macrophages, fibroblasts and mesothelial cells have major roles in the stages of adhesion formation. The studies conducted so far have shown that there is a dynamic balance between the pro- and anti-inflammatory cytokines in these stages of adhesion formation.[28,29] Surgery, inflammation and other similar factors cause to the collapse of this balance. It is known that when to defective sides come together, intraperitoneal adhesion formation is triggered. Miyano et al.[30] and Jansen et al.[31] reported that CO_2 insufflation and the mechanical effect of the pneumo-peritoneum decrease the contact between the defective sides. They determined that PAI-1 concentrations increased in the laparoscopic group as

a secondary process to the CO_2 insufflation.[32,33] In our study, as the severeness of the inflammation and fibrosis increased, so did the PAI-1 concentration.

Miyano et al.[30] reported that CO_2 insufflation decreased the contact between the damaged surfaces. It was suggested that this was due to the increase in the pressure after the pneumo-peritoneum duration and insufflation pressure causing to an increase in adhesion in hypoxia.[33,34]

Corona et al.[35] conducted a study and reported that adhesion and inflammation increased in CO_2 models. Unlike this study, it was determined in our study that capno-peritoneum decreased the adhesion and inflammation especially in the long-term group.

We did not observe a clinical side effect or histopathological abnormality due to CO_2 in our study and this result supports the findings of Miyano et al.[30] Bustorff-Silva et al.[36]

CONCLUSION

Our results suggest that CO_2 pneumo-peritoneum has positive effects in postoperative intraperitoneal adhesion development. Since we formed a scraping model in our study, we cannot suggest that the adhesion formation is decreased with mechanical effect. The patho-physiological and molecular bases of the postoperative adhesion formation have been documented and described well. However, we consider that the capno-peritoneum and postoperative adhesion formation is prevented with anti-inflammatory effect. We need to conduct more studies to examine this mechanism.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gazi University Animal Experiments Local Ethics Committee (Date: 04.09.2011, Decision No: B.30.2.GÜN.O.EU.00.00/38-5869.30).

Informed Consent: This experimental animal study does not require informed consent.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Preoperative pulmonary rehabilitation in medical inoperable patients with early stage non-small cell lung cancer and postoperative results

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ABSTRACT

Aims: The impact of postoperative complications after surgery for lung cancer is substantial, with the increasing age of patients and the presence of comorbidities. Impairment in exercise capacity is a potential modifiable risk factor for postoperative complications. This study aimed to assess the contribution of preoperative pulmonary rehabilitation (PR) for increasing operability conditions in non-small cell lung cancer (NSCLC) for patients with limited pulmonary functions and postoperative results.

Methods: The patients with NSCLC who had preoperative pulmonary rehabilitation and underwent surgical resection in our clinics between 2010-2019 were evaluated retrospectively. The patients enrolled in a comprehensive, multidisciplinary, supervised outpatient 10-day duration PR program preoperatively, consisting of bronchial hygiene, breathing control, energy conservation techniques, exercise training (endurance and strength), psychological support, and nutritional support. Exercise capacity and VO2 peak were evaluated by using an incremental shuttle walk test (ISWT).

Results: Eighteen patients who underwent surgery due to NSCLC and had a pre-operative pulmonary rehabilitation program were evaluated. All the cases were male; the mean age was 66.2 (53-77) years. The squamous cell/adenocarcinoma ratio was 2.6 (13:5), and the mean tumor size was 4.6 (8-18) cm. The postoperative hospital stay was 12.7 (4-42) days, and they were followed up for an average of 30.2 (2-83) months.

Conclusion: Complete surgical resection is the most effective curative treatment for lung cancer. However, many patients with lung cancer also have severe COPD, increasing their risk of postoperative complications and their likelihood of being considered "inoperable." Preoperative pulmonary rehabilitation (PR) has been proposed as an intervention for risk modification and to decrease surgical morbidity and mortality. The results of our study also revealed the importance of preoperative pulmonary rehabilitation in centers where lung cancer surgery was performed.

Keywords: Non-small cell lung cancer, surgery, preoperative pulmonary rehabilitation

INTRODUCTION

Complete surgical resection is currently the superior curative treatment for lung cancer. The histopathologic tumor-node-metastasis (TNM) stage is known to be the most significant prognostic factor for surgically treated patients with NSCLC. Nevertheless, patient comorbidities are also important prognostic factors for survival. Age and smoking are strongly associated with comorbidities such as chronic obstructive pulmonary disease (COPD) and cardiovascular disease, which generally coexist with NSCLC. These comorbidities may have independent negative impacts on survival and influence the outcomes of NSCLC treatment, such as surgery or adjuvant therapy. Therefore, several comorbidities may significantly impact the prognosis of high-risk patients with NSCLC than the cancer stage.[1] Even without complications, lung cancer surgery is associated with significant reductions (approximately 10%-18%) in functional capacity (FC). Poor FC strongly predicts postoperative complications, mortality, and long-term survival in lung cancer.[2]

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Many patients with lung cancer have severe COPD, increasing their risk of postoperative complications and the probability of being considered "medically inoperable."[3] Advanced age, cancer stage, associated diseases and impaired cardiorespiratory fitness (CRF) are predictive factors of major postoperative complications and long-term survival.[4] Cardiopulmonary exercise testing (CPET) describes the accepted standard for evaluating CRF and the response to a rehabilitation program.

Peak oxygen consumption (peakVo2) deliberates the ability of the pulmonary capacity to deliver oxygen to the working skeletal muscles maximally. Cutoff values for peakVo2 of approximately 16 mL/kg per minute and a 10- to 12-mL/kg per minute anaerobic threshold (AT) have been shown helpful in discriminating between patients at low risk and those at higher risk of major postoperative complications.[5]

In patients with lung cancer, peakVo2 is, on average, 25% to 30% lower than in age- and sex-matched individuals without cancer.[4] No intervention has been proven to reduce the risk of postoperative complications in patients with resectable lung cancer and poor lung function.

Pulmonary rehabilitation (PR) is a procedure to increase exercise capacity in severe COPD. It is recommended as preoperative adjunctive therapy to reduce the risk of postoperative pulmonary complications.[3] To our best knowledge, a limited number of studies reveal the effect of preoperative multidisciplinary comprehensive 10-day duration PR on postoperative results in patients with limited exercise capacity and pulmonary functions.

This study aimed to evaluate the contribution of preoperative pulmonary rehabilitation (PR) to improving operability conditions in non-small cell lung cancer (NSCLC) for patients with limited pulmonary functions and postoperative results.

METHODS

This retrospective study was carried out with the permission of the Ankara Atatürk Sanatorium Training and Research Hospital Ethics Committee (Date: 24.08.2023, Decision No. 2012-KAEK-15/2760). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

The patients with non-small lung cancer who had preoperative pulmonary rehabilitation and underwent surgical resection in our clinics between 1st January 2010- 31st December 2019 were evaluated retrospectively. Inclusion criteria were The patients who completed Pulmonary Rehabilitation, Operable Non-Small Cell Lung Cancer patients, and those who underwent surgery in our Thoracic Surgery Department. Exclusion criteria were: A previously received neoadjuvant/adjuvant treatment, incomplete pulmonary rehabilitation, refused or had an inadequate performance for surgery, and operated on in other clinics after PR were excluded.

Outcome Parameters

The demographic and clinical characteristics included age, sex, smoking status, pulmonary function, echocardiography findings, tumor histology, pathological stage, surgery type, and adjuvant therapy history. The histopathological staging was determined using the eighth edition of the TNM classification.[6] The surgical procedures were classified as sub-lobar, lobectomy, or extended resection. After completing pulmonary rehabilitation, all patients were operated by the same surgery team. All patients were followed up in the intensive care unit for one day after surgery. Postoperative hospital stay, number of 30-day emergency visits and readmissions, complications and survival status data of the patients were recorded from the hospital database.

The demographic and clinical characteristics included age, sex, smoking status, pulmonary function, echocardiography findings, tumor histology, pathological stage, surgery type, and adjuvant therapy history. The histopathological staging was determined using the eighth edition of the TNM classification. The surgical procedures were classified as wedge resection, sub-lobar resection, lobectomy, pneumonectomy or extended resection.

Preoperative Pulmonary Rehabilitation Program

The program was multidisciplinary, comprehensive, and compact (5 consecutive days a week) and lasted two weeks, ten sessions. In patients with transportation problems, the program was undertaken in an inpatient manner. PPR program consisting of (a) educational support, medication advice, bronchial hygiene techniques, breathing control techniques, energy conservation, relaxation, and dietary advice. Two chest physicians, two physical therapists, a dietician, one respiratory nurse, and a psychologist delivered educational sessions. (b) exercise training, (c) a nutritional intervention, and (d) psychological counseling, if needed. The exercise training program was individually tailored to each patient. A physical therapist supervised all exercise training sessions. Exercise included cycle ergometer training (15 min), treadmill training (15 min), upper and lower extremity strength training (5-10 min), breathing therapies (10-20 min), and relaxation therapies (5-10 min) for total 50-70 min/day. Patients underwent both cycle

ergometer and treadmill training. Both workloads for cycling and walking speed for the treadmill ergometer were calculated from incremental shuttle walking test (ISWT) results using formulations. St. George's Respiratory Questionnaire (SGRQ) is a questionnaire that evaluates the quality of life in patients with chronic obstructive pulmonary disease (COPD) and includes three parts (symptoms, activity, and impacts) divided into 76 items. The questionnaire is self-administered but may be read to illiterate persons. BORG dyspnea scores 4-6 were also used for prescribing exercise. Patients were trained at 50% of peak workload and 50-80% of peak VO2. Quadriceps resistance training was applied using free weights for five consecutive days a week according to a 1-repetition maximum starting at 50% for three sets and ten repetitions per set in the ten sessions. Upper extremity training consisted of one set, ten repetitions per set for ten sessions. Loads were recorded as kilograms.[7]

Postoperative hospital stay, number of 30-day emergency visits and readmissions, complications and survival status data of the patients were recorded from the hospital database.

Statistical Analysis

All data obtained during the study and recorded in the study form were analyzed using the IBM SPSS 20.0 (Chicago, IL, USA) statistical program. After evaluating whether the data were normally distributed, the Shapiro-Wilk test, histogram, and Q-Q plot were used to express the mean±standard deviation (SD) for normally distributed data. The median quartiles were used for non-normally distributed or ordinal data. The categorical variables were presented as the number of cases and the percentage. A paired test t-test compared two dependent groups regarding customarily distributed continuous variables. The 95% confidence intervals (95% CIs) were also calculated when appropriate, and a p-value less than 0.05 was considered statistically significant.

RESULTS

Eighty-three patients diagnosed with lung cancer who were admitted to a multidisciplinary pulmonary rehabilitation program were analyzed retrospectively. Forty-three patients completed the preoperative pulmonary rehabilitation program, and 18 of them who underwent surgery due to Non-Small Lung Cancer were evaluated. Demographics of the patients are shown in Table 1. All the cases were male; the mean age was 66.2 (53-77) years. All patients had a smoking history; their average consumption was 58.55 packs/year (19-110), and 27.7% were active smokers preoperatively.

When measurements and gains were evaluated before and after pulmonary rehabilitation, FEV1 increased from 54.06±12.9 to 57.89±11.19 (p: 0.23), FVC increased from 65.72±16.907 to 68.28±16.03 (p: 0.46), ISWT increased from 328.89±119.79 to 392.78±123.61 (p: 0.001) and SGRQ score increased from 54.2±17.7 to 29.05±8.7 (p<0.01), (Table 2).

Table 1. Demographics of the patients

Patient no	Age	Surgery	Histopathology	Stage	Complication	Hospital stay	Mortality	Mortality reason	Follow-up
1	67	LLL	A	IIIA	-	9	-	-	83
2	77	R BiL Inf	SCC	IIB	-	18	-	-	18
3	77	RUL	SCC	IIB	-	16	-	-	13
4	53	LSLL	SCC	IIIA	-	9	22nd month	Esophageal Fistula	22
5	69	LP	SCC	IA	-	7	-	-	64
6	55	RUL	SCC	IIB	Atelectasis	18	-	-	75
7	74	RLL	SCC	IIIA	VRE Pneumonia, PTE	42	42nd day	Sepsis	1
8	63	LP	SCC	IIB	-	8	-	-	11
9	59	LUL	A	IB	-	7	-	-	24
10	67	R BiL Inf	SCC	IIIA	Atelectasis, PAL	15	-	-	25
11	57	LSLL	SCC	IIA	-	8	-	-	43
12	72	RLL	SCC	IVA	-	8	5th month	Pneumonia	5
13	72	LV-UW	SCC	IA	-	4	-	-	6
14	65	LLL	SCC	IIIA	-	19	-	-	31
15	59	RUL	A	IB	Atelectasis, PAL	15	-	-	32
16	73	RV-LL	A	IA	Subcutaneous Emphysema	7	-	-	39
17	63	RUL	A	IB	-	10	-	-	24
18	69	RUW	SCC	IIB	-	9	-	-	8

LLL: Left Lower Lobectomy, R Bil Inf: Right bi-lobectomy Inferior, RUL: Right Upper Lobectomy, LSLL: Left Sleeve Lower Lobectomy, LP: Left Pneumonectomy, RLL: Right Lower Lobectomy, LUL: Left Upper Lobectomy, LV-UW: Left VATS Upper Wedge, RV-LL: Right VATS Lower Lobectomy, RUW: Right Upper Wedge Resection, A: Adenocarcinoma, SCC: Squamous Cell Carcinoma, PAL: Prolonged Air Leakage

Table 2. Outcome measures before and after the PR program

Parameters	Before PR	After PR	Mean differences with 95%CI	P value
FVC	65.72±16.907	68.28±16.03	-2.55 (-9.7 to 4.6)	0.46
FEV_1	54.06±12.9	57.89±11.19	-3.8 (-10.4 to 2.7)	0.23
ISWT	328.89±119.79	392.78±123.61	-63.8 (-95.9 to -31.7)	0.001
SGRQ	54.2±17.7	29.05±8.7	25.1 (18.05 to 32.2)	<0.001

Complications and death were determined as a composite outcome (5 patients had a composite outcome.) The amount of change in patients with and without a composite result was compared in terms of relevant data. There was no significant relation between FEV1 (p:0.737), FVC (p:0.358), ISWT (p:0.534) and Saint George's Respiratory Questionnaire (SGRQ) (p:0.220) with composite outcomes.

Surgical approaches were lobectomy for 50% (n: 9), wedge resection for 16.7% (n:3), pneumonectomy for 11.1% (n:2), Sleeve lobectomy for 11.1% (n:2), and bi-lobectomy inferior for 11.1% (n:2) of patients. The postoperative hospital stay was 12.7 (4-42) days.

The squamous cell/adenocarcinoma ratio was 2.6 (13:5), and the mean tumor size was 4.6 (8-18) cm. The participants were followed up for an average of 28.5 (1-83) months, and mortality occurred in three patients (16.66%) on the 42nd day, 5th month and 22nd month of follow-up. No mortality occurred in the first 30 days postoperatively. However, one patient died on the 42nd day due to Vancomycin-resistant Enterococci pneumonia and pulmonary thromboembolism. One patient who underwent cranial metastasectomy (Stage IV-A) was deceased due to pneumonia after chemotherapy in 5th month of the surgery. The other patient who underwent sleeve left lower lobectomy with Squamous Cell Ca (Stage III-A) died of esophageal fistula in the 22nd month.

Complications developed in 5 patients (27.77%). Atelectasis occurred in three cases (16.66%), prolonged air leakage occurred in two cases (11.11%), subcutaneous emphysema occurred in 1 (5.55%), pneumonia occurred in one (5.55%), pulmonary thromboembolism occurred in one patient (5.55%). A 55-year-old male who underwent a right upper lobectomy had atelectasis and was treated with repeating bronchoscopies. A 67-year-old male who underwent bi-lobectomy inferior and a 59-year-old male who underwent right upper lobectomy had prolonged air leakage and atelectasis. Bronchoscopy was performed for both patients. Pleurodesis was used for one of the patients, and the other underwent secondary chest tube insertion for prolonged air leakage. Subcutaneous emphysema occurred, and a fasciotomy was needed in a 73-year-old patient who underwent a right lower lobectomy with VATS.

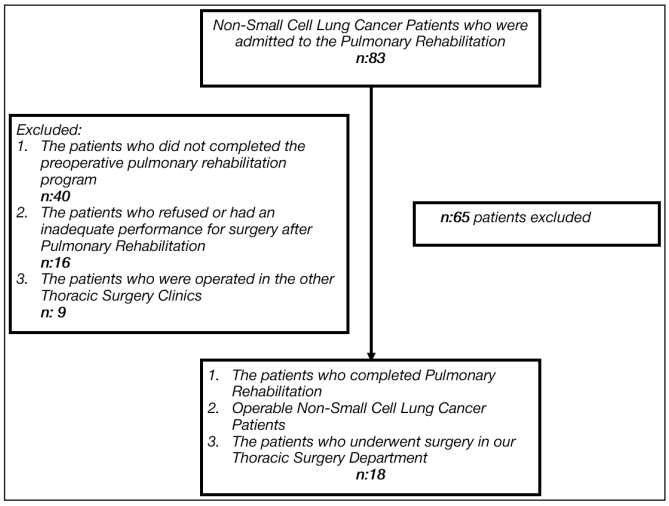

Figure 1.

DISCUSSION

This study showed a significant improvement in the incremental shuttle walk test (ISWT) and SGRQ with preoperative pulmonary rehabilitation in patients with Lung Cancer with COPD coexistence. Patients were safely taken to surgical treatment after pulmonary rehabilitation.

Lung cancer is the leading cause of cancer death worldwide, and the most effective treatment in the early stages of NSCLC remains complete surgical resection.[8,9] Elder age, cancer stage, co-morbidities, and impaired cardiorespiratory fitness (CRF) are predictive factors of major postoperative complications and long-term survival.[10] Even without complications, lung cancer surgery is associated with significant reductions (approximately 10%-18%) in functional capacity (FC). Poor FC strongly predicts postoperative complications, mortality, and long-term survival in lung cancer.[2] Enhancing FC by the exercise of the individual to enable them to withstand an incoming stressor such as surgery has been termed prehabilitation. Preoperative exercise training has significantly improved physical function before thoracic surgery approaches, with fewer pulmonary complications, shorter hospital stays, and better quality of life. Additionally, patients with lung cancer are at nutritional risk due to reduced food intake and underlying metabolic derangements leading to delayed recovery and mortality. Furthermore, these patients often experience psychological stress, such as anxiety and depression, after the diagnosis.[2,11]

Varela et al.[12] used a cross-sectional configuration with historical controls to consider the cost-effectiveness of chest physiotherapy following lobectomy. One hundred nineteen subjects undergoing lobectomy, more commonly by video-assisted thoracoscopic surgery (VATS) than by thoracotomy, received intensive chest physiotherapy one day before surgery and continued until hospital discharge. They were compared with a historical control group of 520 patients who had a lobectomy, more commonly by muscle-sparing thoracotomy than VATS at the same hospital. Nosocomial pneumonia and atelectasis rates were higher in the control group. However, only atelectasis rates demonstrated a significant difference (2% vs. 7.7%, odds ratio 0.20; 95% CI 0.05-0.86). The median length of stay was 5.7 days (range 3-22 days) in the physiotherapy group and 8.3 days (range 3-40 days) in the control group (p<0.0001). Cost analysis indicated a reduction in overall cost for hospital treatment in the physiotherapy group, but this did not include out-of-hospital charges caused by complications. The authors, therefore, refute that the difference between the groups could be accounted for by the more extensive use of the VATS approach in the physiotherapy group.[12,13] In our study, atelectasis occurred in three cases (16.66%), and the median length of stay was 12.7 (4-42) days. VATS lobectomy was performed in two patients (11.11%).

Li et al.[14] 2019 executed a meta-analysis of 404 patients from 7 randomized controlled trials (RCTs) on the effect of preoperative exercise therapy on surgical outcomes in lung cancer patients, with or without COPD. In 5 studies that reported postoperative pneumonia, there was no decrease in postoperative pneumonia frequency in those receiving preoperative exercise before lung cancer surgery. The length of stay in patients who underwent preoperative exercise training was shorter, with a standardized mean difference of minus 4.23 days (p=0.02).[15] Additional subgroup analysis of patients with COPD and lung cancer simultaneously found no advantage of preoperative exercise intervention in the incidence of PPC but a similar reduction in length of stay. Patients who received preoperative exercise before surgery reported an improved exercise capacity with an increase in 6-minute walking distance (6MWM) and a higher VO2 peak; however, no significant difference in pulmonary function was observed.

Lai et al.[16] presented a randomized trial of 68 patients with NSCLC undergoing VATs lobectomy to examine the impact of preoperative physical training. In those who underwent physical training preoperatively, the study found that preoperative physical training can improve cardiopulmonary tolerance, reduce PPCs and shorten in-hospital LOS. In Laurent et al.'s[17] study, 26 patients eligible for NSCLC resection were evaluated for the impact of preoperative respiratory muscle endurance training. Those in the intervention group (n=14) were found to have improved exercise capacity with increased minute ventilation [+15(±)16 vs. −2(±)17, p=0.004] and increased endurance time [+299(±)199 vs. −5 (±)371, p=0.001]. Those in the respiratory muscle endurance training group had fewer PPCs (2 vs. 10, p=0.037). However, there was no difference in LOS between groups.

Lee et al.[1] evaluated 471 patients with NSCLC who underwent surgery, and they were classified into high-risk (HR) (n=77) and standard-risk (SR) (n=394) groups according to the American College of Surgeons Oncology Group criteria (Z4099 trial).[18] In Lee et al.'s[1] study, the patients in the HR group experienced more postoperative complications (p≤0.001), hospital stays were significantly longer in the HR group than in the SR group and mortality was seen more often without disease recurrence. Pneumonia was the most frequent complication in HR patients, and its incidence was significantly higher than in SR patients. However, HR patients without postoperative complications had a survival rate similar to that of SR patients. In the present study, complications occurred in 5 patients (27.77%). The complications were atelectasis, prolonged air leakage, subcutaneous emphysema, pneumonia, and pulmonary thromboembolism. Early mortality occurred in a patient on the 42nd day due to pneumonia and pulmonary thromboembolism.

Pouwels et al.'s[19] systematic reviews (11 studies; 916 participants) investigated the effect of preoperative exercise therapy on patients undergoing lung surgery. Meta-analysis was not possible due to study heterogeneity. However, the review showed that a preoperative moderate to intense exercise program improved aerobic capacity, physical fitness, and quality of life, with a possibility that it might reduce postoperative complications and length of hospital stay. Preoperative physical therapy for cardiac surgery patients was evaluated in Hulzebos et al.'s[20] meta-analyses. Preoperative physical therapy that includes mixed interventions such as aerobic exercises, breathing exercises, and inspiratory muscle training compared with no treatment or sham therapy reduced postoperative atelectasis after elective cardiac surgery in four trials and pneumonia in five trials, but not the necessity for mechanical ventilation extended than 48 hours or mortality. The postoperative hospital stay was significantly faster for those in the physical therapy groups. A meta-analysis of 65 randomized controlled trials concluded that pulmonary rehabilitation was more effective than standard community-based care for functional exercise capacity.[21] The six-minute walk distance was greater following pulmonary rehabilitation than with community care. It transcended the clinical significance threshold (mean

difference 43.93 meters, 95% CI 32.64 to 55.21). Maximal exercise testing in participants assigned to pulmonary rehabilitation compared with usual care revealed increased maximal workload. Our study evaluated measurements and improvements before and after pulmonary rehabilitation. FEV1 increased from 54.06±12.9 to 57.89±11.19 (p: 0.23), FVC increased from 65.72±16.907 to 68.28±16.03 (p: 0.46), ISWT 328.89±119.79 from 392.78±123.61 (p: 0.001) and SGRQ score increased from 54.2±17.7 to 29.05±8.7 (p<0.01).

CONCLUSION

Preoperative pulmonary rehabilitation improves exercise capacity in patients undergoing surgical resection to manage lung cancer. Many studies support that preoperative exercise significantly reduces postoperative pulmonary complications and hospital length of stay. Referrals to exercise programs should be considered in patients awaiting lung resection, especially those patients considered borderline for suitability for surgical resection. Further studies are needed to investigate the effect of preoperative exercise on mortality and the cost/benefit of this intervention.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Ankara Atatürk Sanatorium Training and Research Hospital Ethics Committee (Date: 24.08.2023, Decision No. 2012-KAEK-15/2760).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluation of anxiety, psychological resilience and codependency in nurses during the COVID-19 pandemic

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ABSTRACT

Aims: In this study, it was aimed to evaluate anxiety levels in nurses during the COVID-19 pandemic and to examine the relationship between psychological resilience and codependency levels and anxiety levels.

Methods: The study was conducted on 152 nurses actively working in a pandemic hospital during the COVID-19 outbreak. Sociodemographic Information Form, Beck Anxiety Inventory, Spann-Fischer Codependency Scale, Brief Resilience Scale were used as data collection tools.

Results: As a result of the study, anxiety and codependency scores of nurses working in high-risk pandemic units during the pandemic were higher and psychological resilience scores were lower than those working in low-risk units. When the relationship between anxiety, psychological resilience and codependency in the research group was examined; a negative and highly significant relation was found between psychological resilience and anxiety and between psychological resilience and codependency, and a positive and highly significant relation was found between anxiety and codependency ($p<0.001$ for all). According to the results of regression analysis, it was found that anxiety levels decreased as psychological resilience levels increased in nurses, and anxiety levels increased as codependency levels increased.

Conclusion: It was concluded that the anxiety levels of nurses working in high-risk units during the COVID-19 pandemic were higher, and the levels of psychological resilience and codependency affected the level of anxiety experienced by nurses. Interventions to increase psychological resilience are important in order to protect nurses, who work with great devotion on the front line during disasters and pandemics, from anxiety and the development of codependency, which is an important problem that reduces the quality of care and decreases work performance.

Keywords: COVID-19, nurse, anxiety, resilience, codependency

The research was presented as an oral presentation at the 1st International Nursing Studies Congress to be held in Ordu/Turkey on July 14, 2023

INTRODUCTION

The novel coronavirus disease (COVID-19) pandemic has been reported to have negative effects on the mental health of healthcare workers, leading to various psychological distress such as depression, anxiety, and post-traumatic stress disorder.[1] Nurses are a group of health professionals who have to work in close contact with patients for long periods of time. In the COVID-19 pandemic, nurses were negatively affected by the process due to difficult working hours and conditions, having to stay away from their relatives and families, being in close contact with infected patients, having the risk of disease transmission, and witnessing suffering and dying patients.[2]

Studies have found that nurses exhibited high levels of anxiety during the COVID-19 pandemic.[3,4] Although a small amount of anxiety has protective and motivating beneficial effects, when it becomes excessive and continuous, it has negative effects on people's work performance, psychological and physical health.[2]

Psychological resilience is the ability to overcome negative symptoms that occur in stressful situations and to adapt positively.[5] Psychological resilience is important for individuals to cope with the anxiety and difficulties they experience due to stress factors. Previous studies have shown that resilience in nurses has a protective role in natural disasters and disease outbreaks.[6] Likewise, during the coronavirus pandemic, the importance of psychological resilience in nurses' coping with the stress caused by the pandemic was emphasized.[2,7] Therefore, it is important to take initiatives to increase nurses' resilience in managing and coping with stress in stressful situations such as epidemics.[2] Research has shown that low levels

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of resilience are significantly associated with high levels of anxiety, with a negative relation between them.[8,9] Fradelos et al.[10] reported that anxiety and resilience had a negative, statistically significant correlation in nurses.

Many definitions have been made for the 'codependency', but no clear consensus on the definition has been reached. According to the most widely accepted definition made by the American National Council on Codependence, codependency is a learned behaviour expressed by painful dependence on people and objects other than oneself in an effort to find self-worth, security and identity. Ancel[11] defines codependency as a form of pathological relationship that develops between the person dependent on another person's care and the caregiver, where individuals mutually support, increase and sustain each other's dependence. Codependency, which is emotional and social overdependence on a person who is dependent on someone else's care and characterized with mental occupation can become pathological.[12] Codependency is a trait that can lead to physical and psychological problems. It is reported in the researches performed that codependency is more commonly seen in healthcare workers, especially in nurses than in other occupational groups.[13,14]

It is inevitable that nurses, who work with great devotion on the front line in the struggling against epidemics and disasters, will be psychologically affected in these challenging processes. Hence, it is important to explore anxiety, one of the most commonly observed psychological symptoms during the COVID-19 outbreak, especially in nurses who are in direct contact with coronavirus patients for a long time and to reduce anxiety by determining the factors that may be related. The aim of this study was to evaluate anxiety levels in nurses during the COVID-19 pandemic and to examine psychological resilience and codependency, which are factors associated with anxiety.

METHODS

The study was initiated with the approval of the Amasya University Non-interventional Clinical Researches Ethics Committee (Date: 11.06.2020, Decision No: 44). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This cross-sectional and single center study was conducted between July 2020 and September 2020. The sample of the study consisted of nurses actively working in a pandemic hospital in Turkey during the COVID-19 outbreak. A total of 152 nurses were included in the study. 77 of these nurses were actively working in high-risk pandemic units and 75 were actively working in low-risk pandemic units. Voluntary nurses younger than 55 years of age, without neurological, psychiatric disorder or communication disabilities were included in the study. Nurses with any neurologic, psychiatric disorder or history of psychiatric medication use were excluded from the study. In the data collection phase; Sociodemographic Data Form, Beck Anxiety Inventory, Spann-Fischer Codependency Scale and Brief Resilience Scale were used.

Sociodemographical Data Form

This form created by the researcher and contains 5 questions about age, gender, marital status, years of working in the health field, and whether they worked in the pandemic unit.

Beck Anxiety Inventory (BAI)

The scale was developed by Beck et al.[15] to measure the severity and frequency of anxiety. It was adapted into Turkish by Ulusoy et al.[16] Cronbach's alpha internal consistency score of the scale was 0.93 and it was determined to bear sufficient reliability and validity.[16] The BAI is a 21-item self-assessment scale scored between 0 and 3. A high score on the scale indicates that the level of anxiety felt by the individual is high.

Spann-Fischer Codependency Scale (SFCDS)

It was developed by Fischer, Spann, and Crawford[17] to measure the codependency of individuals. Turkish standardization of the scale conducted by Tanhan and Mukba.[18] It is a scale scored between 1-6 and consists of 16 items. The 5th and 7th items of the scale have the feature of "reverse item". High scores obtained from the scale provide information about the high level of addiction of the person.

Brief Resilience Scale (BRS)

Smith et al.[19] developed the scale in order to measure the self-recovery potential and resilience of individuals, and it was adapted into Turkish by Doğan.[20] It is a 6-item 5-point Likert type self-report scale. In the scale, items 1,3,5 are coded correctly and the other items are coded in reverse. High scores on this scale indicate a high level of psychological resilience, whereas low scores indicate a low level of psychological resilience.

Statistical Analysis

Statistical analysis of data SPSS 23.0 (IBM Corp. Released 2015. IBM SPSS Statistics for Windows,Version 23.0. Armonk, NY: IBM Corp.) statistics package program. Normalization of data distribution was analyzed by Kolmogorov Smirnov test. Categorical variables were analyzed using chi-square test. In the comparison of quantitative data normally distributed according to pandemic risk status, independent two sample t test was used. Pearson correlation test was used in the analysis of

the relation between normally distributed scale scores. Multivariate linear regression model was used to analyse the dependent variables affecting the anxiety scores.The results of the analyses were shown as mean±standard deviation and frequency (percentage). Significance was evaluated at p<0.05 level.

RESULTS

It was found that 50.7% of the nurses worked in high-risk pandemic units, 63.8% were women, 65.8% were married (Table 1). The mean age of all nurses was 36.5±7.9 years, the mean experience in the health sector was 14.3±8.1 years, and the mean total scale scores were 17.4±14.2 for BAI, 19.0±6.0 for BRS, and 52.5±16.5 for SFCDS, respectively (Table 2).

Table 1. Frequency distribution of the nurses (n=152)

	Frequency (n)	Percentage (%)
Pandemic risk state		
High-risk units	77	50.7
Low-risk units	75	49.3
Sex		
Female	97	63.8
Male	55	36.2
Marital status		
Married	100	65.8
Single	52	34.2

Table 2. Descriptive statistics of the nurses

	Mean	SD
Age	36.5	7.9
Years of work in health sector	14.3	8.1
BAI total score	17.4	14.2
BRS total score	19.0	6.0
SFCDS total score	52.5	16.5

BAI: Beck anxiety inventory, BRS: Brief resilience scale, SFCDS: Spann-Fischer codependency scale, SD: Standart deviation

When the relationship between the scale scores for all participants was examined; there was a highly significant negative correlation between BRS and BAI mean scores (r=-0.737, p<0.001), a highly significant positive correlation between SFCDS and BAI scores (r=0.706, p<0.001), and a highly significant negative correlation (r=-0.652, p<0.001) between SFCDS and BRS scores (Table 3).

Table 3. Examination of correlation analysis between scale scores

		BAI total score	BRS total score
BRS total scores	r p	-0.737 <0.001	-0.652
SFCDS total scores	r p	0.706 <0.001	<0.001

BRS: Brief resilience scale, SFCDS: Spann-Fischer codependency scale, r: Pearson correlation coefficient

When the nurses working in high-risk pandemic units and nurses working in low-risk units were compared in terms of scale mean scores, the total scores of anxiety, resilience and codependency of nurses working in high-risk pandemic units were significantly higher than those of nurses working in low-risk units (p<0.001 for all). The findings are presented in Table 4.

Table 4. Comparison of quantitative variables according to working status in the pandemic unit

	High-risk unit Mean±SD	Low-risk unit Mean±SD	p-value
BAI total scores	28.92±9.9	5.6±5.7	<0.001
BRS total scores	15.62±5.33	22.51±4.43	<0.001
SFCDS total scores	63.78±11.83	40.89±12.02	<0.001

BRS: Brief resilience scale, SFCDS: Spann-Fischer codependency scale , SD: Standart deviation

When the effect of the Brief resilience scale and the Spann-Fischer codependency scale scores on Beck anxiety inventory score of nurses was examined by linear regression, the regression model was statistically significant (F=127.756, p<0.001). As the total score of brief resilience scale increases, the anxiety score of nurses decreases, and when the total score of resilience scale increases by one unit, the anxiety score decreases by 1.146 (p<0.001). As the total Spann-Fischer codependency scale score increases, the anxiety score increases, and when the total score of codependency scale increases by one unit, the anxiety score increases by 0.337 (p<0.001) (Table 5).

Table 5. Regression analysis results for the total score of Beck Anxiety Inventory

	Beta*	Standard error	Standardized Beta (%95 CI)	p value
Constant	21.547	5.43	(10.816 - 32.277)	<0.001
BRS total scores	-1.146	0.156	-0.482 (-1.454 - -0.838)	<0.001
SFCDS total scores	0.337	0.056	0.391 (0.225 - 0.448)	<0.001

BRS: Brief resilience scale, SFCDS: Spann- Fischer codependency scale, *Not standardized, F: 127,756, p<0,001, R^2=0,632, Corrected R^2=0,627

DISCUSSION

The objective of the present study was to investigate codependency and psychological resilience, which are factors associated with anxiety in nurses during the COVID-19 pandemic in Turkey. The results showed that nurses working in high-risk pandemic units had high levels of anxiety and codependency compared to nurses working in low-risk pandemic units, and their psychological resilience was lower.

To the best of our knowledge, this is the first study to evaluate codependency among healthcare workers during the COVID-19 pandemic in Turkey. Our study, consistent

with the literature, indicates that the nursing profession is associated with anxiety[21] and additionally, it shows the relationship between the concept of codependency and psychological resilience with anxiety. Our findings highlight that during periods of high stress such as disasters and pandemics, which significantly impact the mental well-being of healthcare workers, especially nurses, anxiety emerges as a crucial psychological issue that needs to be addressed and treated as necessary.

The concept of psychological resilience is the ability to maintain balance in the face of stress and to adapt positively to adversity.[22] Therefore, it is thought to be a concept that should come to mind in terms of protecting the mental health of healthcare professionals working in hospitals and especially in pandemic units during epidemic periods. In this study, a high negative correlation was found between anxiety and resilience in the research group. There are studies compatible with this finding.[23,24] In the present study, it was found that the psychological resilience of all nurses participating in the study was at a moderate level. Similar to studies conducted in Turkey during the COVID-19 pandemic, it has been shown that nurses directly involved in the care and treatment of COVID-19 patients also have a moderate level of psychological resilience.[25,26] Karabulak and Kaya[25] found a significant negative relationship between perceived stress and psychological resilience in nurses working during the COVID-19 pandemic in Turkey. On the other hand, Bayat and Polat Olca[26] reported that the high levels of psychological resilience among nurses play a protective role against depression and anxiety.

The relationship between codependency, which is defined as the learned behavior of excessive emotional and social dependence on external individuals and events, and psychological symptoms is known. One of the psychological symptoms associated with codependency is anxiety. In this study, a highly significant correlation was found between codependency and anxiety. In their study, Cullen and Carr[27] reported that people with high levels of codependency had more anxiety and depressive symptoms. Similarly, Fischer et al.[17] found out that anxiety was positively associated with codependency. The development of codependency is an important problem that can cause burnout in nurses, reduce the quality of care, decrease work performance, and over time result in loss of professional identity and disengagement from work.[12,28,29] Therefore, preventing the formation of codependency in the nursing profession is important in terms of both protecting mental health and sustaining the profession.

The results also revealed the presence of a high level and negative relation between codependency and psychological resilience. In the literature, there is limited data assessing the relationship between psychological resilience and codependency. Knapek et al.[30] reported that codependent individuals had lower resilience scale scores compared to healthy individuals, but they also indicated a higher presence of early maladaptive schemas. Early maladaptive schemas are dysfunctional patterns of memories, sensations, and physical sensations that develop throughout childhood and adolescence and that harm an individual's interpersonal relationship. Psychological resilience is one of the factors that help prevent maladaptive schemas that negatively affect the individual's functionality. Studies have reported that early maladaptive schemas are associated with psychological resilience and an increase in these schemas is linked to a decrease in resilience.[31,32] The inverse relationship between codependency and psychological resilience obtained as a finding of this study can also be explained by the role of early maladaptive schemas. However, more studies are needed to evaluate the relationship between codependency and psychological resilience.

In the present study, it was determined that the levels of psychological resilience and codependency are factors that influence anxiety. The results of the conducted regression analysis revealed that higher levels of psychological resilience were associated with a decrease in anxiety, while higher levels of codependency were associated with an increase in anxiety. These findings are consistent with the literature, which suggests a positive relationship between codependency and anxiety, and a negative relationship between higher resilience scale scores and lower anxiety.[17,33-35] Thus, it is essential to pay special attention to the mental well-being of nurses working on the frontline during situations like a pandemic. A detailed assessment of anxiety and related factors is necessary, and supportive and therapeutic interventions must be provided when needed.

There were some limitations in this study, such as the number of participants and single center experience.

CONCLUSION

The results of this study emphasise that psychological resilience and codependency are associated with the level of anxiety experienced by nurses during the coronavirus outbreak. In this case, the higher the psychological resilience of nurses and the lower their codependency, the lower their anxiety levels will be.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Amasya University Non-interventional Clinical Researches Ethics Committee (Date: 11.06.2020, Decision No: 44).

Informed consent: Written consent was obtained from the nurses participating in this study.

Referee Evaluation Process: Externally peer reviewed.

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

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Predictors for axillary lymph node metastasis in primary neuroendocrine carcinomas of the breast and neuroendocrine differentiated breast cancers

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ABSTRACT

Aims: Primary neuroendocrine carcinomas of the breast (NEC) and neuroendocrine differentiated breast cancers are rare entities. The aim of this study was to investigate clinical and histopathological findings and predictors for axillary lymph node metastasis (ALNM) in primary neuroendocrine carcinomas of the breast (NEC) and neuroendocrine differentiated breast cancers (NEBC).

Methods: Patients with a diagnosis of breast cancer with histopathological neuroendocrine features between the years 2015 and 2022 were retrospectively screened. The patients were divided into two main groups, the NEC and NEBC groups. The two groups were evaluated in terms of their clinical and histopathological characteristics and predictive factors for axillary lymph node.

Results: A total of 35 patients [NEBC group: 24 patients, NEC group: 11 patients) were evaluated. At the time of diagnosis, the median age was 57 (NEC: 49 years, NEBC: 57.5). Of the 35 patients, 15 (57.1%) had ALNM, and lymphovascular invasion was detected in 16 (45.7%). When the whole patient population was evaluated for ALNM, it was found that lymphovascular invasion had an effect on ALNM (p=0.005). In the NEBC group, the rate of ALNM was associated with an increase in tumor diameter (p=0.035). Additionally, the tumor diameter was found to be predictive of ALNM in the ROC analysis (AUC: 0.753, 95% CI: 0.557-0.950, cut-off: 2.35 cm, p=0.035). Analyses of correlation revealed a low-level correlation between age and Ki-67 in the study cohort (ρ= -0.341, p=0.45).

Conclusion: NECs and NEBCs of the breast are uncommon tumors with a high ALNM potential. Patients with lymphovascular invasion and a large tumor diameter should be carefully evaluated for ALNM. Further research is required to determine the most appropriate treatment strategy for these rare subtypes of breast cancers.

Keywords: Neuroendocrine, breast cancer, axillary lymph node metastasis, nodal status

INTRODUCTION

Neuroendocrine tumors originates from submucosal neuroendocrine cells in the gastrointestinal tract and lungs. They are categorized as high-grade tumors with metastasis potential.[1,2]

Neuroendocrine breast tumors are rare tumors. Neuroendocrine breast tumors are detected by immunohistochemical staining with neuroendocrine markers (chromogranin A, synaptophysin, insulinoma-associated protein 1, neuron-specific enolase, and CD56). Neuroendocrine carcinoma of the breast (NEC) was first described in 1963. It was included in the World Health Organization (WHO) classification in 2003. Later, in 2012, WHO defined neuroendocrine neoplasms (NEN) into 3 subcategories: well-differentiated neuroendocrine tumor, poorly differentiated neuroendocrine carcinoma, and neuroendocrine differentiated breast cancer (NEBC).[3] WHO proposed a uniform classification including neuroendocrine neoplasms in different anatomical regions with breast NECs. According to this classification, breast cancers with >90% of tumor cells showing neuroendocrine differentiation were included in this group. Breast cancers with ≤90% of tumor cells showing neuroendocrine differentiation were considered as neuroendocrine differentiated breast cancer (NEBC) and were excluded it on the assumption that neuroendocrine differentiation in breast cancers has no therapeutic value.[4,5] In the National Comprehensive Cancer Network (NCCN) guidelines, NEBC is evaluated in the same category as invasive ductal carcinomas with non-specific types (BC-NST), and the same treatment strategies are recommended.[6]

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NECs represent 1% of all NECs and less than 5% of all breast tumors.[2,7-9] NEBC are more prevalent than NEC. The incidence rate of all invasive breast cancers varies between 0.1% and 30%, although the figures reported in the literature vary depending on the studies.[4,8,10-12] Due to frequent changes in diagnostic criteria and the lack of routine use of neuroendocrine markers, it is challenging to ascertain the true incidence.[4,7,10]

Axillary lymph node metastasis (ALNM) is a strong and independent negative prognostic factor in breast cancers.[13,14] The presence of axillary lymph node metastases is crucial for the management of breast cancer and the selection of surgical/neoadjuvant therapy. Determination of predictors for axillary lymph node metastasis of this rare tumor will guide surgeons and oncologists in determining prognosis and in treatment decision. This study aims to discuss the factors that influence the clinical, histopathological, and axillary lymph node metastasis of NEBCs and NECs through our case series analysis.

METHODS

The study was initiated with the approval of the Gazi University Non-interventional Clinical Researches Ethics Committee (Date: 08.05.2023, Decision No: 395). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

1002 patients who underwent surgical treatment for breast cancer at Gazi University Faculty of Medicine, Department of General Surgery, between 2015-2022 were evaluated retrospectively. The study included 35 patients with histopathologically confirmed cases of neuroendocrine breast cancer (NEC) and neuroendocrine differentiated breast cancer (NEBC). The inclusion criteria were: primary breast cancer with neuroendocrine differentiation; primary breast neuroendocrine carcinoma; and having undergone axillary lymph node sampling or dissection. The exclusion criteria were having distant metastases (M1) and receiving neoadjuvant therapy. Since the histopathological features of the tumors in patients with multifocal tumors were the same, they were not considered separate tumors, and the largest tumor diameter was used (TNM, 8th edition, 2018). Demographic, clinical, and histopathological data of patients who met the inclusion criteria were recorded. Next, factors affecting axillary lymph node metastasis in the NEC and NEBC subgroups of the entire patient population were evaluated.

Statistical Analysis

The data of our study was analyzed using the SPSS 21.0 program (IBM Inc, Chicago, IL, USA). The categorical data obtained were expressed as percentage and frequency (N), and the quantitative data as median (median) (IQR). Due to the small sample size and non-normal distribution of the data, non-parametric tests were used. A paired group (independent) comparison was performed using the Mann-Whitney U test. The relationship between binary categorical groups was examined using Pearson's chi-square test or Fisher's exact test. Spearman analyses revealed the correlations between the quantitative parameters. Diagnostic values, including predictive values, sensitivity, and specificity, were analyzed using ROC. In the study, an α (type-I error) value of 0.05 (5%) was used, and the p significance value was accepted as 0.05 for interpretation.

RESULTS

In this study, 35 patients who met the inclusion criteria were evaluated, 11 of whom had NEC and 24 of whom had NEBC. The clinical, histopathological features and performed surgical procedures of the study cohort are presented in **Tables 1**, **2** and **3**. The median age at diagnosis was 49 in the NEC group (min: 35, max: 80), 57.5 in the NEBC group (min: 32-max: 85), and 57 in the entire study cohort (min: 32, max: 85). Twelve patients (34.3%) had T1, 17 patients (48.6%) had T2, and 6 patients (17.1%) had T3 tumors. Of the 35 patients, 15 (57.1%) had ALNM, and lymphovascular invasion was detected in 16 (45.7%). The most common molecular subtype was Luminal B, detected in 16 patients (45.7%), followed by the Her-enriched subtype in 11 patients (31.4%) and 8 patients (22.9%) Twenty-one patients (60.0%) were positive for chromogranin A (CgA) (**Table 3**).

Analysis of quantitative and categorical parameters according to axillary lymph node metastasis status of the study cohort, NEC and NEBC group are presented in **Table 4**, **5** and **6**. When evaluating the study cohort for ALNM, it was found that lymphovascular invasion had an effect on ALNM (p=0.005) (**Table 4**). In the NEBC group, ALNM was associated with increased tumor size (p=0.035) (**Table 6**). ROC analysis data for quantitative parameters for axillary lymph node metastasis for the study cohort and NEBC group, tumor size was found to be predictive of ALNM (AUC: 0.753, 95% CI: 0.557-0.950, cut-off: 2.35 cm, p=0.035). Analyses of correlation revealed low-level correlation between age and Ki-67 in the study cohort (ρ= -0.341, p=0.45).

Table 1. Clinicopathological features in the study cohort.

Parameters	NEC group		NEBC group		Total	
	n	%	n	%	n	%
Age at diagnosis						
< 50	6	54.5	6	25.0	12	34.3
50-69	1	9.1	15	62.5	16	45.7
≥ 70	4	36.4	3	12.5	7	20.0
T-Stage						
T1	2	18.2	10	41.7	12	34.3
T2	7	63.6	10	41.7	17	48.6
T3	2	18.2	4	16.6	6	17.1
Tumor focality						
Unifocal	10	90.9	19	79.2	29	82.9
Multifocal	1	9.1	5	20.8	6	17.1
Tumor types						
Neuroendocrin carcinoma	11	100	-	-	11	31.4
Invasive ductal carcinoma	-	-	17	70.8	17	48.6
Mucinous carcinoma	-	-	5	20.8	5	14.3
Papillary carcinoma	-	-	2	8.4	2	5.7
Ductal carcinoma insitu component						
Yes	4	36.4	11	45.8	15	42.9
No	7	63.6	13	54.2	20	57.1
Ductal carcinoma insitu grade						
Grade 1	0	0.0	3	27.3	3	20.0
Grade 2	4	100	6	54.5	10	66.7
Grade 3	0	0	2	18.2	2	13.3
Axillary lymph node metastasis						
Positive	3	27.3	12	50.0	15	57.1
Negative	8	72.7	12	50.0	20	42.9
Grading						
Grade 1	4	36.4	2	8.3	6	17.1
Grade 2	5	45.4	17	70.9	22	62.9
Grade 3	2	18.2	5	20.8	7	20.0
Lymphovascular invasion						
Positive	5	45.4	11	45.8	16	45.7
Negative	6	54.6	13	54.2	19	54.3

DCIS: Ductal carcinoma insitu

Table 3. Distribution of histopathological, molecular, and receptor-related parameters in the study cohort

Parameters	NEC group	NEBC group	Total
			Frequency (%)
Chromogranin			
Positive (+)	7 (63.6%)	14 (58.3%)	21 (60.0%)
Negative (-)	4 (36.4%)	10 (41.7%)	14 (40.0%)
Snaptophysin			
Positive (+)	11 (100%)	24 (100%)	35 (100.0%)
Negative (-)	0 (0.0%)	0 (0.0%)	0 (0.0%)
CD-56			
Positive (+)	0 (0.0%)	2 (8.3%)	2 (22.2%)
Negative (-)	3 (27.3%)	4 (16.7%)	7 (77.8%)
Unknown	9 (81.7%)	18 (75.0%)	
ER			
Positive (+)	11 (100%)	24 (100%)	35 (100.0%)
Negative (-)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PR			
Positive (+)	11 (100%)	21 (87.5%)	32 (91.4%)
Negative (-)	0 (0.0%)	3 (12.5%)	3 (8.6%)
Cerb-b2			
Positive (+)	0 (0.0%)	11 (45.8%)	11 (31.4%)
Negative (-)	11 (100%)	13 (54.2%)	24 (68.6%)
Ki-67			
≤%14	5 (45.5%)	5 (20.8%)	10 (28.6%)
>%14	6 (54.5%)	19 (79.2%)	25 (71.4%)
Molecular Subtypes			
Luminal A	5 (45.5%)	3 (12.5%)	8 (22.9%)
Luminal B	6 (54.5%)	10 (41.7%)	16 (45.7%)
HER2-enriched	0 (0.0%)	11 (45.8%)	11 (31.4%)

ER: estrogen receptor, PR: progesterone receptor

Table 2. Surgical procedures performed in the study cohort

Surgical procedure	NEC Group		NEBC Group		Total	
	n	%	n	%	n	%
SM + SLNB	5	45.5	9	37.5	14	40
Modified radical mastectomy	2	18.2	8	33.3	10	28.6
BCS + SLNB	2	18.2	4	16.7	6	17.1
SM + ALND	1	9.1	2	8.4	3	8.6
BCS + ALND	1	9.1	1	4.2	2	5.7
Total:	11	100	24	100	35	100

SM: Simple mastectomy, BCS: Breast conserving surgery, SLNB: Sentinel lymph node biopsy, ALND: Axillary lymph node dissection

Table 4. Analysis of quantitative and categorical parameters according to axillary lymph node metastasis status of the study cohort

Median (min-max) [a]	Axillary Lymph Node Metastasis		p
	Negative (n=20, 57.1%)	Positive (n=15, 42.9%)	
Age (year)	51 (32-80)	58 (43-85)	0.142
Age at diagnosis			0.059 [b]
< 50	10 (83.3%)	2 (16.7%)	
50-69	6 (37.5%)	10 (62.5%)	
≥70	4 (57.1%)	3 (42.9%)	
Quadrants, n (%)			0.247 [b]
Upper-outer	7 (50.0%)	7 (50.0%)	
Upper-inner	3 (100%)	0 (0%)	
Lower-outer	8 (72.73%)	3 (27.27%)	
Lower-inner	1 (33.33%)	2 (66.67%)	
Central	1 (25.0%)	3 (75.0%)	
Focality, n (%)			0.481 [c]
Unifocal	16 (55.17%)	13 (44.83%)	
Multifocal	4 (66.67%)	2 (33.33%)	
Tumor size (cm)	2.35 (0.5-7.0%)	2.5 (0.7-11.0%)	0.359
Chromogranin, n (%)			0.486 [c]
Negative (-)	7 (50.0%)	7 (50.0%)	
Positive (+)	13 (61.9%)	8 (38.1%)	
Ki-67. n (%)			0.458 [b]
≤ %14	7 (70%)	3 (30%)	
> %14	13 (52%)	12 (48%)	
Ductal carcinoma insitu component n (%)			0.693 [c]
No	12 (60%)	8 (40%)	
Yes	8 (53.33%)	7 (46.67%)	
Cerb-b2. n (%)			0.99 [b]
Negative (-)	14 (58.33%)	10 (41.67%)	
Positive (+)	6 (54.55%)	5 (45.45%)	
Molecular subtypes, n (%)			0.611 [b]
Luminal A	6 (75.0%)	2 (25.0%)	
Luminal B	8 (50.0%)	8 (50.0%)	
HER2-enriched	6 (54.55%)	5 (45.45%)	
Lymphovascular invasion, n (%)			0.005 [b]
Negative	15 (78.95%)	4 (21.05%)	
Positive	5 (31.25%)	11 (68.75%)	
Grading, n (%)			0.89 [b]
Grade 1	4 (66.67%)	2 (33.33%)	
Grade 2	12 (54.55%)	10 (45.45%)	
Grade 3	4 (57.14%)	3 (42.86%)	
T-Stage, n (%)			0.99 [b]
T1	7 (58.33%)	5 (41.67%)	
T2	10 (58.82%)	7 (41.18%)	
T3	3 (50.0%)	3 (50.0%)	

a Mann Whitney U test; parameters (min-max), IQR= Interquartile Range, b Fisher's exact test, c Pearson chi-square analysis

Table 5. Analysis of quantitative and categorical parameters according to axillary lymph node metastasis status of the NEC group

Median (min-max) [a]	Axillary Lymph Node Metastasis		p
	Negative (n=8, %72.7)	Positive (n=3, %27.3)	
Age (year)	48.5 (35-80)	58 (43-72)	0.918
Age at diagnosis			0.41 [b]
< 50	5 (83.3%)	1 (16.7%)	
50-69	0 (0%)	1 (100.0%)	
≥70	3 (75.0%)	1 (25.0%)	
Quadrants, n (%)			0.121[b]
Upper-outer	1 (33.33%)	2 (66.67%)	
Upper-inner	1 (100%)	0 (0%)	
Lower-outer	5 (100%)	0 (0%)	
Lower-inner	0 (0%)	1 (100%)	
Central	1 (100%)	0 (0%)	
Focality, n (%)			0.99 [b]
Unifocal	7 (70.0%)	3 (30.0%)	
Multifocal	1 (100%)	0 (0%)	
Tumor size (cm)	2.95 (1.5-7%)	2.1 (1-2.8%)	0.153
Chromogranin, n (%)			0.491 [b]
Negative (-)	2 (50.0%)	2 (50.0%)	
Positive (+)	6 (85.71%)	1 (14.29%)	
Ki-67. n (%)			0.99 [b]
≤ %14	4 (80.0%)	1 (20.0%)	
> %14	4 (66.67%)	2 (33.33%)	
Ductal carcinoma insitu component n (%)			0.99 [b]
No	5 (71.43%)	2 (28.57%)	
Yes	3 (75.0%)	1 (25.0%)	
Cerb-b2. n (%)			
Negative (-)			
Positive (+)			
Molecular subtypes, n (%)			0.99 [b]
Luminal A	4 (80.0%)	1 (20.0%)	
Luminal B	4 (66.67%)	2 (33.33%)	
HER2-enriched	0 (0.0%)	0 (0.0%)	
Lymphovascular invasion, n (%)			0.424 [b]
Negative	5 (83.33%)	1 (16.67%)	
Positive	3 (60.0%)	2 (40.0%)	
Grading, n (%)			0.99 [b]
Grade 1	3 (75.0%)	1 (25.0%)	
Grade 2	3 (60.0%)	2 (40.0%)	
Grade 3	2 (100%)	0 (0%)	
T-Stage, n (%)			0.99 [b]
T1	1 (50.0%)	1 (50.0%)	
T2	5 (71.43%)	2 (28.57%)	
T3	2 (100%)	0 (0%)	

a Mann Whitney U test; parameters (min-max), IQR= Interquartile Range, b Fisher's exact test, c Pearson chi-square analysis

Table 6. Analysis of quantitative and categorical parameters according to axillary lymph node metastasis status of the NEBC group

Median (min-max) [a]	Axillary Lymph Node Metastasis		p
	Negative (n=12, %50.0)	Positive (n=12, %50.0)	
Age (year)	54.5 (32-80)	60.5 (47-85)	0.112
Age at diagnosis			0.188
< 50	5 (83.3%)	1 (16.7%)	
50-69	6 (40.0%)	9 (60.0%)	
≥70	1 (33.3%)	2 (66.7%)	
Quadrants, n (%)			0.34
Upper-outer	6 (54.55%)	5 (45.45%)	
Upper-inner	2 (100%)	0 (0%)	
Lower-outer	3 (50.0%)	3 (50.0%)	
Lower-inner	1 (50.0%)	1 (50.0%)	
Central	0 (0%)	3 (100%)	
Focality, n (%)			0.99 [b]
Unifocal	9 (47.37%)	10 (52.63%)	
Multifocal	3 (60.0%)	2 (40.0%)	
Tumor size (cm)	1.6 (0.5-5.0%)	3.25 (0.7-11.0%)	0.035
Chromogranin, n (%)			0.99 [c]
Negative (-)	5 (50.0%)	5 (50.0%)	
Positive (+)	7 (50.0%)	7 (50.0%)	
Ki-67. n (%)			0.99 [b]
≤ %14	3 (60%)	2 (40%)	
> %14	9 (47.37%)	10 (52.63%)	
Ductal carcinoma insitu component n (%)			0.68 [c]
No	7 (53.85%)	6 (46.15%)	
Yes	5 (45.45%)	6 (54.55%)	
Cerb-b2. n (%)			0.682 [c]
Negative (-)	6 (46.2%)	7 (53.8%)	
Positive (+)	6 (54.5%)	5 (45.5%)	
Molecular subtypes, n (%)			0.742 [b]
Luminal A	2 (66.67%)	1 (33.33%)	
Luminal B	4 (40.0%)	6 (60.0%)	
HER2-enriched	6 (54.55%)	5 (45.45%)	
Lymphovascular invasion, n (%)			0.004 [c]
Negative	10 (76.92%)	3 (23.08%)	
Positive	2 (18.18%)	9 (81.82%)	
Grading, n (%)			0.99 [b]
Grade 1	1 (50.0%)	1 (50.0%)	
Grade 2	9 (52.94%)	8 (47.06%)	
Grade 3	2 (40.0%)	3 (60.0%)	
T-Stage, n (%)			0.663 [b]
T1	6 (60.0%)	4 (40.0%)	
T2	5 (50.0%)	5 (50.0%)	
T3	1 (25.0%)	3 (75.0%)	

a Mann Whitney U test; parameters (min-max), IQR= Interquartile Range, b Fisher's exact test, c Pearson chi-square analysis

DISCUSSION

Despite neuroendocrine differentiation in breast cancers was initially described in 1963, it was not recognized as a distinct subtype by the WHO until 2003. Even though significant advances in breast cancer research and treatment in recent years, the exact prevalence, clinical behavior, and treatment standards for this rare subset of breast cancers have not been well established. This is likely a result of their low frequency and evolving definitions.[15]

All patients included in our study were diagnosed with neuroendocrine neoplasm in the breast according to WHO 2012 criteria. However, definitions of neuroendocrine neoplasms of the breast were changed again in 2012 and 2019. Lastly, WHO includes primary breast neuroendocrine tumors in the same classification as neuroendocrine tumors in other anatomical locations. They define neuroendocrine differentiated breast cancer as a non-specific subtype. Nonetheless, neuroendocrine differentiation in breast cancer has been associated with a number of distinct clinical characteristics.[9,11,12,16-24] Inclusion of breast neuroendocrine tumors in distinct classifications and the therapeutic value of neuroendocrine differentiation in breast cancers have not been adequately addressed as of yet. Debates regarding the WHO classification of neuroendocrine neoplasms of the breast are still ongoing and it is emphasized that it needs further adjustments regarding morphological and immunohistochemical criteria.[25] In particular, well-differentiated NECs of the breast are treated in the same way as invasive breast carcinoma. However, there is limited data on whether treatment modalities similar to this treatment for invasive breast carcinoma are effective for neuroendocrine neoplasm of the breast. Yang et al.[26] reported that current treatment protocols did not improve survival in breast NENs. Due to this confusion in diagnostic classification and treatment protocols, it is clear that new studies on the behavior of these tumors are necessary. Axillary lymph node metastasis is an important prognostic indicator of breast cancer. Therefore, the NEC and NECB subgroups of the patients included in our study were evaluated for axillary lymph node metastasis. Furthermore, it was intended to contribute to the body of knowledge with a large number of cases.

The median age at first diagnosis in our study cohort was 57, which was consistent with the median age at diagnosis of non-specific type breast cancers reported in the literature.[27] There are studies that do not indicate a difference in age at diagnosis between breast NEN and BC-NST.[16,21,23] Nevertheless, several studies conducted with large cohorts have reported that breast NENs are significantly older than BC-NST patients.[9,17,19,24] These different results may also be attributable to the non-standard diagnostic criteria employed in the studies. The majority of studies meeting WHO 2003 criteria indicate that breast NEN patients are significantly older than BC-NST patients.[9,17,19,24]

Most of the patients in our cohort (65.2%) had ≥T2 tumors. ALNM was present in 57.1% of our patients. Previous similar studies have also reported that neuroendocrine neoplasms of the breast have ALNM at the time of diagnosis. Wang et al.[9] showed in their study, which included 142 breast neuroendocrine neoplasms, that it had a higher rate of ALNM (28.8%) than other non-specific types. Krawczyk et al.[10] reported the ALNM rate as 37% in their series, in which they included 27 NEBCs. Cloyd et al.[20] reported the ALNM rate as 63.2% in breast NENs. In their series of 128 cases, Bogina et al.[23] reported the rate of ALNM as 33% in NEN patients and did not observe a significant difference with BC-NST. On the contrary, some studies have reported similar TNM stages at diagnosis in breast cancer cases with and without neuroendocrine differentiation.[16,17,21,28]

In our study, all of the patients were ER-positive, and 91.4% were PR-positive. Similar to previous studies, the majority (68.6%) had ER-positive, HER2-negative tumors.[9,16,18,22] However, when we classified them according to molecular subtypes, Luminal B (45.7%) was predominant due to high Ki-67 ratios. Previous research has demonstrated a significant association between neuroendocrine differentiation and the presence of positive hormone receptors and a negative HER2-status.[17,19,21,23,24]

ALNM is a strong and independent negative prognostic factor for breast cancers. Among women without metastatic disease, the five-year survival rate is 99 percent for those with a without ALNM and 85 percent for those with a with ALNM.[13] In addition, the presence of lymphovascular invasion appears to be a poor prognostic indicator, particularly in higher-grade tumors. In our study, we also showed that lymphovascular invasion has an effect on ALNM in breast NENs and NEBCs (p=0.005; p=0.004). Based on this result and in consideration of the high ALNM potential of breast NENs, we conclude that patients with lymphovascular invasion should be treated with caution when it comes to axillary lymph node management.

Tumor size was recognized early as an important prognostic factor in breast cancer. Tumor size is correlated with ALNM, but the prognostic value of the two factors is independent.[14] Interestingly, in our study, tumor size was found to be higher in ALNM-negative cases in the NEC group, although it was not statistically significant (2.95 cm vs 2.1 cm, p=0.153). This suggests that the NEC group exhibits a different biological behavior for ALNM than

other breast cancers. In our study, we also showed that increased tumor size in the NEBC group was associated with ALNM (AUC: 0.753, 95% CI: 0.557-0.950, cut-off: 2.35 cm, p=0.035). Hence, axillary lymph nodes should be carefully evaluated, especially in patients with NEBC tumors larger than 2 cm.

In our study, 62.9% of the patients had Grade-2 tumors, and in 71%, Ki-67 was higher than 14%. Similarly, Krawczyk et al.[10] reported that Grade-2 (78%) and Ki-67 >30% tumors were the most prevalent in their study. In other series in the literature, it has been shown that Grade-2 tumors are more prevalent among NEN patients than in BC-NST patients.[17,23] The relationship between Ki-67 and age is an issue that has not been clarified in the literature.[29] We also determined that increasing age was associated with lower Ki-67 rates in the cohort of our study (ρ=-0.341 p=0.45). Since Ki-67 is an important prognostic marker, we think that NECs and NEBCs diagnosed at younger ages may have a worse prognosis.

Study Limitations

Our study has some limitations. Patients were identified retrospectively through a review of clinical records. A retrospective analysis of 1002 cases resulted in the identification of 35 cases (3.49%). In our study, we found a prevalence that is consistent with the 2-5% estimated by WHO.30 Due to the lack of a systematic morphological and immunohistochemical reassessment and the lack of routine use of neuroendocrine markers, we believe that the true prevalence is higher. The prevalence of neuroendocrine differentiation ranges from 0.1% to 20% in published studies.[9,28] The reason for this is the variable diagnostic criteria on the one hand and the NEN definition criteria used in published studies on the other. In our study, breast neuroendocrine neoplasms were also divided into NEC and NEBC subgroups for analysis. The limited number of patients included in the study may have also prevented the achievement of statistical significance in certain analyses.

CONCLUSION

The importance of primary neuroendocrine tumors of the breast and neuroendocrine differentiation in breast cancers in determining treatment strategies is still not clearly clarified. This tumor group has a high incidence of axillary lymph node metastases, which play an important role in the treatment strategy for breast cancer. In patients with lymphovascular invasion and a large tumor size, extra attention should be paid to axillary lymph node metastases. As previous research has shown that breast NECs and NEBCs are associated with poor clinical outcomes, further research is required to determine the optimal treatment strategy for this subtype of breast cancer.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Gazi University Non-interventional Clinical Researches Ethics Committee (Date: 08.05.2023, Decision No: 395).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Prevalence of adrenal incidentaloma in patients performed thorax computed tomography for suspected COVID-19 infection

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ABSTRACT

Aims: The primary purpose of this study is to make a retrospective evaluation of adrenal incidentaloma (AI) prevalence in patients performed thorax computed tomography (TCT), along with determining whether the diagnosed AIs were assessed functionally for adrenal gland hyperfunction and to detect the rate of hyperfunctional AI.

Methods: The patients who applied to with the suspected COVID-19 and performed TCT between January 2020 and December 2021, were included in the present study. However, the patients who were followed-up due to a known adrenal mass and a malignant tumor were excluded.

Results: TCT imaging, including adrenal glands, was performed on 2580 patients. The mean age for these patients was 54±16.8. However, when the patients were separated into groups in terms of adrenal pathology (AP), the mean age for the patients with and without AP was 61.4±11 and 53.8±16.9, respectively ($p<0.001$). The number of patients detected with AP, AI, and adrenal hyperplasia was 68 (2.6%), 60 (88.2%), and 7 (10.3%), respectively. The mean mass diameter was 17 (11-41). Bilateral adrenal hyperplasia was not observed in any patients; however, 13 of the patients (18.1%) detected with AI and adrenal hyperplasia were evaluated functionally. Hyperfunction was not observed in any of the assessed patients.

Conclusion: The prevalence of AI found in our study was similar to other studies in the literature; however, functional evaluations of AIs detected via TCT performed due to suspected COVID-19 remained low. Although they are rare, it is important to define whether AIs are functional or malignant due to the comorbid conditions they create. For this reason, we believe patients with AI should be directed to an endocrinology clinic for a practical examination and follow-up plan.

Keywords: Adrenal incidentaloma, adrenal hyperplasia, COVID-19, prevalence

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INTRODUCTION

Adrenal incidentaloma (AI) is defined as an adrenal mass larger than 10 mm detected by coincidence throughout radiological examination for reasons other than an adrenal disease.[1] Adrenal masses may be found incidentally when computed tomography (CT) scans or magnetic resonance imaging (MRI) are performed for other reasons. Nowadays, AI is more common due to the widespread use of imaging methods. Once these masses are detected, radiological and biochemical evaluations are required to determine the risks of malignancy and whether there is excessive hormone secretion or not. Nonetheless, most of the incidentalomas are not followed.[2] The prevalence of AI varies regarding data sources such as autopsy series-radiological series and patient selection such as general population-specific patient categories.[3] The prevalence of AI determined by imaging methods differs in various studies. While a study reported a 4.4% of prevalence for abdominal CT,[4] another study found the prevalence to be 0.98% for abdominal CT and 0.81% for thorax computed tomography (TCT).[4,5]

Over the course of the COVID-19 pandemic, which started in 2019 and influenced almost all the world, TCT was performed for the diagnostic and therapeutic purposes. The main aim of this study is to make a retrospective evaluation of AI prevalence in patients performed TCT due to suspected COVID-19 infection. The secondary reason behind this study is to determine whether the diagnosed AIs were assessed functionally for adrenal gland hyperfunction and to detect the rate of hyperfunctional AI.

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METHODS

The study was initiated with the approval of the Dışkapı Yıldırım Beyazıt Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date: 19.04.2021, Decision No: 109/10). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The present study was designed retrospectively, and the patients having applied to due to suspected COVID-19 and performed TCT between January 2020 and December 2021 were involved. However, those who were followed up due to a known adrenal mass and a malignant tumor were excluded. AI was defined as adrenal masses larger than 1 cm discovered throughout the radiological examination for reasons other than investigating an adrenal disease 6. Adrenal hyperplasia was considered enlarged on the condition that the axis of the body of the adrenal gland was >10 mm or the diameter of a limb was >5 mm. The diagnosis of AI was based on TCT findings, and it was noted down whether the identified AIs were functionally evaluated. The radiology report of each TCT was reviewed, and the patients whose reports indicated an adrenal gland abnormality were also registered. The radiological images of the patients with a detected adrenal mass were assessed by a second specialist, and the reports were verified. The endocrinologic evaluation was made in the patients with AI regarding the guidelines: overnight 1-mg dexamethasone suppression test or 24-hour urine cortisol measurement for hypercortisolism, 24-hour urinalysis or plasma for catecholamines and metanephrines, and measurement of plasma renin activity and plasma aldosterone concentration for hypertension (HT) or hypokalemia.[1,6] The presence of HT and diabetes mellitus (DM) diagnosis was recorded based on the patients' files.

RESULTS

TCT imaging, including adrenal glands, was performed on 2876 patients with suspected COVID-19. The number of the patients involved in the present study considering inclusion criteria was 2580, and 1327 of them (51.4%) were female. The mean age was 54±16.8. The number of the patients with HT and type 2 DM was 662 (25.7%) and 423 (16.4%), respectively.

Once the patients were classified in terms of adrenal pathology (AP), the mean age for the patients with and without AP was 61.4±11 and 53.8±16.9, respectively (p<0.001). On the other hand, the number of female patients with and without AP was 43 (63.2%) and 1284 (51.1%), respectively. HT and DM frequency was measured in the patients with AP to be 14 (20.6%) and 18 (26.5%), whereas 648 (25.8%) and 405 (16.1%) in the patients without AP (p=0.399 and p=0.03, respectively). Additionally, 18 (4.2%) of the patients with DM had AP.

AP was detected in 68 (2.6%) patients. Sixty (88.2%) patients were with AI, and 7 (10.3%) were with adrenal hyperplasia. Angiomyolipoma was found in only 1 patient (1.5%) on TCT imaging. Forty-two (70%) of AIs were on the left, and 14 (23.3%) were on the right. However, in two cases, 4 (6.7%) were bilateral. The mean mass diameter was 17 (11-41). One (14.3%) of adrenal hyperplasias was on the right side, and 5 (71.4%) were on the left. Bilateral adrenal hyperplasia was observed in 1 (14.3%) patient. Thirteen (18.1%) of the patients with AP were evaluated functionally; however, no patient was functionally active among the assessed patients (**Table 1**).

Table. Characteristic of adrenal lesions (n=68)

Age, years	61.4 ± 11
Gender	
Female, n (%)	43 (63.2%)
Male, n (%)	25 (36.8%)
Findings	
Adrenal incidentaloma, n (%)	60 (88.2%)
Adrenal hyperplasia, n (%)	7 (10.3%)
Angiomyolipoma	1 (1.5%)
Adrenal mass size, mm	17 (11-41)
Adrenal incidentaloma	
Left, n (%)	42 (70%)
Right, n (%)	14 (23.3%)
Bilateral, n (%)	4 (6.7%)
Adrenal hyperplasia	
Right, n (%)	1 (14.3%)
Left, n (%)	5 (71.4%)
Bilateral, n (%)	1 (14.3%)

When the patients with AP were functionally evaluated, the mean age for those with functional evaluation was similar to those with no functional evaluation (59.5±7.6 and 61.3±11.6, p=0.605, respectively). Mass sizes were also similar (19.5 (11-33) and 17 (11-41), p= 0.492, respectively). There was no difference between both groups considering DM and HT rates (p=0.309 and p=0.955, respectively).

Statistical Analysis

Visual (histograms and probability plots) and analytic methods (Kolmogorov-Smirnov and Shapiro-Wilk's test) were used for the normally and non-normally distribution of the variables. Normally distributed variables were compared via the independent sample's T-test. The Chi-square test or Fisher's exact test (when Chi-square test assumptions do not hold due to low expected cell counts) was used to compare the proportions in patients with and without AP. The continuity correction was used on the condition that the expected count was from 5 to 25. Median and minimum-maximum presented descriptive analyses for non-normally distributed variables and numbers and percentages for categorical variables. A p-value less than 0.05 was considered statistically significant.

DISCUSSION

In our study, we found the rate of AI to be 2.6% in patients performed TCT with suspected COVID-19 infection. This rate is consistent with other studies in the literature. As expected, patients with AI had a higher mean age. The number of patients with functional evaluation was minimal, and hyperfunction was not observed in any of them.

Factors such as technologically advanced diagnostic imaging, the aging population, and the awareness of preventive maintenance have led to a substantial increase in the number of AP diagnosis.[7] In a study conducted in 1982,[8] the prevalence of CT and AI was detected to be 0.6%. However, the studies revealed a higher AI rate in the following years. On the other hand, some recent studies have provided similar results to our research. Taya et al.[9] indicated a 2.3% AI rate in their study conducted on 42,575 abdominal CT, whereas this rate was found to be 2.1% in another study.[9,10] In addition to the studies mentioned above, there are various studies detecting higher AI prevalences, which were found to be 4.4% and 5.1%.[11]

The reason for these differences in a study may be the inclusion of elderly patients screened for lung cancer or the combined evaluation of abdominal and TCT scans, not just the thorax.[4,11] A large study 12 encompassing all hospitals in West Sweden reported that the AI frequency was only 0.9% at the initial radiological evaluation, then increased to 4.5% after the central radiological revision. The difference is particularly striking in TCT evaluations. In addition, another reason may be the assessment of TCT in patients of all ages with suspected COVID-19 infection. Considering autopsy series, in a study analysing the world literature on the prevalence of AI, the median value for all research was found to be 3%, close to our study.[7]

The prevalence of AI increases with age and reaches the highest rate between the 5th and 7th decades.[7] As stated previously, when the patients were separated into groups regarding AI, the mean age for the patients with AI was found to be higher as expected. On the other hand, we did not detect any difference between the patients in regards to gender factor. However, a wide range of research indicates that the prevalence of AI in females is higher than in males, which has not been observed in autopsy studies.[7,12-14] Nevertheless, some studies show that gender distribution is almost equal, similar to our research.[11] Another finding of our study was that the HT rate was the same in patients with and without AI, whereas the DM rate was high. The reason behind this finding may be that the mean age for the patients with AI was found to be higher. Nonetheless, in studies evaluating similar mean age, the DM rate was higher in patients with AP, like our study.[15]

The size and lateralization of AI have been investigated in many previous studies. In our study, the mean mass diameter was 17 mm. In a recent study16 that screened 1149 patients, the mean tumor size was 18 mm. In another recent study,[17] the mean tumor size was found to be 17 mm, similar to our study. Interestingly enough, in a study conducted in our country, the tumor diameter was found to be 48 mm.[18] This difference may be related to the study population or the number of patients.

Our study detected 70% of AIs on the left and 23.3% on the right. Bilateral AI was present in 6.7% of the cases. Similar lesion distribution in the right and left adrenal glands was reported in autopsy studies and CT series.[7] However, some studies provide a higher prevalence of left-sided adrenal tumors detected on imaging.[17,19] This observation may reflect a detection bias, as left-sided adrenal tumors are more easily observed by radiologists.[7]

We did not detect any difference among the patients with AP in terms of age, tumor size, and the rate of comorbidities (DM and HT). In a study conducted by Maher et al.[10] the patients with AI were compared based on their follow-up conditions. In discordance with our research, the age average was found to be low, whereas the tumor size was found to be larger in those patients who were followed up.

Many AIs were non-hyperfunctioning; only 10-15% of hyperfunctioning were reported.[13,20] A review presented that 89.7%, 6.4%, 3.1%, and 0.6% of the AIs were non-functioning, subclinical Cushing's syndrome, pheochromocytoma, and primary aldosteronism, respectively. Hyperfunction was not detected in any of the 13 patients examined by endocrinologists in our research. On the other hand, a study in Türkiye 21 conducted on 376 patients with AI revealed that 10.9%, 4%, 5.3%, and 4% of the patients were detected with subclinical Cushing's syndrome, Cushing's syndrome, pheochromocytoma, and primary hyperaldosteronism, respectively. The point to be noted here is that all of these patients were evaluated by endocrinologists. In the study by Davenport et al.[22] AI was detected in 75 out of 4028 patients on their abdominal CT scans. It was determined that only 13 (17%) of the patients with AI were referred for specialist review. Eighty percent of AI patients were not evaluated biochemically and followed up. However, all of the patients referred to an endocrinologist were evaluated appropriately, and adrenalectomy was indicated for 3 of these patients. Accordingly, in our study, only 13 (18.1%) patients were referred for endocrinological evaluation.

CONCLUSION

Considering the aforementioned studies, the prevalence of AI was detected between 0.3% and 5.1%. When analyzing the studies evaluating AI prevalence through only TCT, it differs between 0.81% and 4.4%; however, it was found to be 2.6% in our research. Even though our findings were similar to the results of other studies in the literature, the rate of functionally evaluated AIs detected via TCT due to suspected COVID-19 remains low. Although they are rare, it is important to define whether AIs are functional or malignant due to the comorbid conditions they create. In this regard, we consider that patients with AI should be directed to an endocrinology clinic for a practical examination and follow-up plan.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Dışkapı Yıldırım Beyazıt Training and Research Hospital Non-interventional Clinical Researches Ethics Committee (Date: 19.04.2021, Decision No: 109/10).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

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Comparative analysis of laparoscopic inguinal hernia surgical training videos on WebSurg vs YouTube platforms: a quality evaluation

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ABSTRACT

Aims: Minimally invasive surgical techniques, particularly laparoscopic methods for inguinal hernia repair, have gained popularity due to their benefits such as reduced postoperative pain and faster recovery. This study aimed to compare the educational quality of laparoscopic inguinal hernia surgical training videos between YouTube and WebSurg platforms.

Methods: Using the keyword "laparoscopic inguinal hernia" we selected and analyzed top 20 videos based on popularity on both platforms. The study examined video sources, duration, likes, views, upload year, coverage of the entire surgical procedure, and evaluation scores (Global Quality Scale-GQS, American Medical Association-JAMA scores, modified DISCERN score, LAP-VEGaS criteria).

Results: YouTube predominantly featured private hospitals/organizations (30%) and physicians (45%), while WebSurg showcased academic institutions (75%). Notably, YouTube hosted mostly edited/abbreviated videos (95%), whereas WebSurg presented a balanced distribution of full-length (50%) and edited/abbreviated (50%) videos. While engagement metrics were comparable, WebSurg consistently achieved higher evaluation scores across various criteria, including modified DISCERN, GQS, JAMA, and LAP-VEGaS ($p < 0.001$, $p < .0.001$, $p = 0.002$ and $p < 0.001$ respectively).

Conclusion: This study is the first to compare WebSurg and YouTube videos in laparoscopic hernia surgery education, revealing that WebSurg provides higher-quality content, highlighting the importance of platform choice for advancing surgical training.

Keywords: Laparoscopy, inguinal hernia, YouTube, WebSurg

INTRODUCTION

Minimally invasive surgical techniques are gaining popularity, because of their benefits like reduced postoperative pain and quicker recovery. Laparoscopic procedures for inguinal hernia repair have become safe options. These laparoscopic methods were initially introduced in 1992 for treating inguinal hernias. The transabdominal preperitoneal and total extraperitoneal approaches are now widely accepted as the standard laparoscopic techniques for hernia repair.[1]

YouTube, a widely popular platform, has emerged as a primary source for accessing surgical educational videos due to its vast collection of freely available content. However, it's worth noting that the quality and accuracy of these videos lack expert review before being shared online.[2-5] In contrast, WebSurg stands as a dedicated platform for delivering educational resources, with a specific focus on minimally invasive surgical techniques within ongoing medical education. Esteemed for its comprehensive array of high-quality surgical educational videos contributed from diverse regions, WebSurg is acknowledged as a pioneering force in the realm of continuous surgical education and online training. Examining the website's statistics reveals a remarkable growth in its user engagement. The number of members has increased by an astonishing 1980%, visitors have surged by 740%, and video views have experienced a remarkable surge of 3300% between 2004 and 2010. These statistics clearly underscore the significant appeal of this virtual university within the surgical community.[6]

This study aimed to compare the quality and educational aspects of laparoscopic inguinal hernia surgical training videos on YouTube and WebSurg platforms. This examination seeks to assist surgical experts in making more informed choices regarding a more effective educational resource.

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METHODS

This study analyzed readily accessible online videos intended for a general audience. Notably, there were no human or animal participants involved in the research. All videos were publicly available on widely accessible platforms such as YouTube and WebSurg. Given that no patient data was utilized and the analyzed videos were already accessible to the public, the requirement for ethical approval was waived. Thus, since no data pertaining to humans or animals was employed, and the videos under scrutiny were already accessible to the public on open platforms, obtaining ethical approval was deemed unnecessary.

In our research on YouTube, conducted on May 1, 2023, using the keyword 'laparoscopic inguinal hernia' we sorted the videos based on their popularity. We analyzed videos that encompassed various surgical techniques and educational content related to the topic of laparoscopic inguinal hernia."

Concurrently, we conducted additional research on WebSurg® using the same keyword. The videos were sorted by popularity, and the top twenty videos were selected for inclusion in the study.

Repetitive, commercially-driven, or unrelated videos, as well as those lacking surgical educational intent, were excluded from the study. Videos were evaluated by a surgeon who has performed over 100 laparoscopic inguinal hernia surgeries.

In the study, video sources used in both groups were identified. These sources included academic institutions, private hospital clinics, expert doctors, and health information channels. The collected data were evaluated based on the duration of videos, number of likes, number of views, upload year, whether the video covered the entire surgical procedure, Global Quality Scale (GQS), American Medical Association (JAMA) scores, modified DISCERN score, and LAParoscopic surgery Video Educational GuidelineS (LAP-VEGaS) video assessment tool.[7]

The educational content quality of the videos was assessed using the GQS. The GQS ranges from 1 (indicating poor quality) to 5 (reflecting excellent coherence and quality). Assessments of video credibility were conducted using criteria from the JAMA scores. The JAMA score assigns 1 point for each of the specified elements: authorship, attribution, disclosures, and currency. The modified DISCERN score was used to evaluate both the reliability and quality aspects of the videos score comprised of five questions, with responses marked as either yes (1 point) or no (0 points). Higher scores indicate increased reliability, in line with previous research findings.[8-11]

Within the scope of the study, the content of the videos was thoroughly evaluated based on the LAP-VEGaS criteria. Initially, the presentation of the video by the author and the introduction of the content were examined. Subsequently, the case presentation where the surgical procedure was performed was analyzed in detail. The video provided a step-by-step demonstration of the surgical procedure, clearly presenting procedural details. The outcomes and success of the surgical intervention were meticulously assessed. Additionally, attention was given to whether the video conveyed educational content. This content evaluation was supported by a peer review of the video by experts in the field. Finally, the potential utilization of the videos in surgical education programs was considered. Employing these criteria, the videos were categorized using the LAP-VEGaS score ranging from 1 to 18. All these criteria collectively allowed for a comprehensive analysis of the content quality.[7]

Baseline clinical data underwent statistical analysis: continuous data via t-tests/Mann-Whitney U and categorical data through Fisher's exact test/chi-square test. SPSS v22.0 was employed. Descriptive statistics summarized data (mean, standard deviation, median, frequency, percentage, minimum, maximum). One-way ANOVA compared normally distributed quantitative variables across groups. Tests were two-tailed; $P<0.05$ indicated significance.

RESULTS

A total of 20 videos from each platform were subjected to evaluation across various parameters. Regarding video sources, distinctive patterns emerged. On YouTube, video sources included academic institutions (5%), private hospitals/organizations (30%), physicians (45%), and health information websites (20%). Conversely, WebSurg predominantly featured videos from academic institutions (75%), with a smaller representation from private hospitals/organizations (25%). This difference was found to be statistically significant ($p<0.001$).

The differentiation extended to the types of surgical videos as well. YouTube predominantly hosted edited and abbreviated versions (95%), whereas full-length surgical videos constituted only 5% of the content. In contrast, WebSurg demonstrated a more balanced distribution with 50% full-length surgical videos and 50% edited/abbreviated videos. This divergence was statistically significant ($p=0.001$).

Despite these variations, there was no statistically significant difference in video duration between the platforms. The median duration of videos on YouTube was 14.68 minutes, and on WebSurg, it was 14.39 minutes ($p=0.299$).

Table 1. Comparative analysis of laparoscopic inguinal hernia surgery videos on YouTube and WebSurg

Parameters	YouTube (n=20) (%)	WebSurg (n=20) (%)	P value
Video source Academic institutions Private hospitals, organizations Physicians Health information website	 1 (5) 6 (30) 9 (45) 4 (20)	 15 (75) 5 (25) 0 (0) 0 (0)	<0.001
Full-length surgical videos Edited and abbreviated surgical videos	1 (5) 19 (95)	10 (50) 10 (50)	0.001
Duration (median, min.) (min-max)	14.68 (4.02-35.26)	14.39 (6.16-51.16)	0.299
Likes (median) (min-max)	114 (0-2950)	107.50 (44-1167)	0.162
Views (median) (min-max)	16576 (1600-371365)	9715 (5023-47539)	0.018
Duration since video upload date, days (median) (min-max)	1185.50 (220-43988)	1832 (9-4603)	0.215
GQS score (mean±SD)	2.55±1.09	3.60±0.99	0.003
JAMA score (mean±SD)	2±0.64	2.90±0.85	0.001
DISCERN score (mean±SD)	2.90±0.78	3.75±0.78	0.002
LAP-VEGaS (mean±SD)	8.20±3.13	12.65±2.23	<0.001

Table 2. Comparative analysis of full-length vs. edited/abbreviated surgical videos on YouTube and WebSurg

	Full-length surgical videos	Edited and abbreviated surgical videos	P value
Website (n, %) YouTube WebSurg	 1 (5) 10 (50)	 19 (95) 10 (50)	0.001
Duration (median, min.) (min-max)	35.26 (13.17-51.16)	12.08 (4.02-34.56)	<0.001
Likes (median) (min-max)	150 (0-1167)	108 (13-2950)	0.515
Views (median) (min-max)	14747 (6574-279237)	13488 (1600-371365)	0.747
Duration since video upload date, days (median) (min-max)	1678 (802-4231)	1102 (9-4603)	0.537
GQS score (mean±SD)	4.09±0.53	2.68±1.10	<0.001
JAMA score (mean±SD)	3.27±0.78	2.13±0.78	<0.001
DISCERN score (mean±SD)	4±0.63	3.06±0.84	0.002
LAP-VEGaS (mean±SD)	13.90±1.30	9.10±3.15	<0.001

Engagement metrics, encompassing likes and views, did not exhibit noteworthy differences. Median likes were 114 on YouTube and 107.50 on WebSurg (p=0.162). However, median views demonstrated a statistically significant difference, with 16576 on YouTube and 9715 on WebSurg (p=0.018).

Furthermore, the duration since the video upload date did not present a significant variance between platforms. The median duration since the video upload date on YouTube was 1185.50 days, while on WebSurg, it was 1832 days (p=0.215).

Evaluation scores, including the modified DISCERN score, in addition to the GQS, JAMA score, and LAP-VEGaS score, unveiled noteworthy distinctions. WebSurg videos consistently garnered higher scores across these metrics with statistically significant differences (Modified DISCERN: p<0.001, GQS: p=0.003, JAMA: p=0.001, LAP-VEGaS: p<0.001). The comparative analysis of full-length vs. edited/abbreviated surgical videos on YouTube and WebSurg platforms reveals distinct differences between these video categories.

Regarding the distribution across websites, 5% of full-length surgical videos were found on YouTube, while 50% of edited/abbreviated videos were hosted on the same platform. Conversely, 95% of full-length videos and 50% of edited/abbreviated videos were present on WebSurg. This distribution discrepancy is highly significant (p=0.001), illustrating the differing content strategies of the platforms.

Examining video duration, full-length surgical videos had a median duration of 35.26 minutes (ranging from 13.17 to 51.16 minutes), whereas edited/abbreviated videos had a median duration of 12.08 minutes (ranging from 4.02 to 34.56 minutes). This contrast is statistically significant (p<0.001), underscoring the substantial variance in video length.

Engagement metrics like likes displayed a median value of 150 for full-length videos and 108 for edited/abbreviated videos, without significant distinction (p=0.515). Similarly, median views for full-length and edited/abbreviated videos were 14,747 and 13,488, respectively, without a statistically significant difference (p=0.747).

The median duration since the video upload date was 1678 days for full-length videos and 1102 days for edited/abbreviated videos, without a significant difference (p=0.537). Evaluation scores—GQS, JAMA, modified DISCERN, and LAP-VEGaS—exhibited noteworthy variations. Full-length videos obtained a mean GQS

score of 4.09±0.53, while edited/abbreviated videos scored 2.68±1.10 (p<0.001). Mean JAMA scores for full-length and edited/abbreviated videos were 3.27±0.78 and 2.13±0.78, respectively (p<0.001). Full-length videos achieved a mean modified DISCERN score of 4.00±0.63, while edited/abbreviated videos scored 3.06±0.84 (p=0.002). In addition, LAP-VEGaS scores demonstrated a significant difference, with full-length videos scoring 13.90±1.30 and edited/abbreviated videos scoring 9.10±3.15 (p<0.001).

DISCUSSION

This study holds the distinction of being the first to compare surgical education aspects of WebSurg and YouTube videos in laparoscopic hernia surgery. The comparative analysis clearly highlights variations in video sources, types, engagement metrics, and evaluation scores between YouTube and WebSurg platforms. These findings underscore the potential impact of platform choice on the accessibility and educational quality of laparoscopic inguinal hernia surgery videos. The obtained results emphasize the significance of selecting the appropriate platform for the advancement of medical education and surgical practice. In parallel with our study, numerous researchers have examined medical videos uploaded on YouTube for quality and accuracy. Moreover, most of these studies have revealed that many videos exhibit lower quality and accuracy than anticipated. The absence of references and data sources in YouTube videos, coupled with the lack of a review process to assess information reliability, has prompted researchers to question the academic competency of YouTube videos.[3,12-16]

Furthermore, the fact that videos uploaded by academic professionals and published after academic review on WebSurg could indicate potentially higher quality and reliability of education. Moreover, published studies have emphasized that videos uploaded by healthcare professionals are of elevated quality and are considered reliable.[17,18]

In our study, evaluations conducted regarding content quality and educational value revealed higher scores for videos on the WebSurg platform. This disparity can be attributed to WebSurg's provision of unique and comprehensive content, including videos and educational materials produced by experts. In light of these findings, a study conducted in 2020 examined 50 inguinal hernia surgery videos on the YouTube platform. The results elucidated that online surgical videos on YouTube are not reliable sources for demonstrating best practices in minimally invasive inguinal hernia repairs.[19] Furthermore, in another study that assessed videos on the WebSurg platform concerning parathyroid surgery, it was determined that while these videos do assist surgeons in incrementally learning the procedure prior to performing the surgery, they nonetheless fall short of meeting expected quality standards.[20] A similar trend was observed in a study focused on sleeve gastrectomy operations. In that study as well, the challenges of meeting stringent academic publishing criteria on YouTube, given its open-access nature, were emphasized. Furthermore, it was noted that WebSurg videos attained higher DISCERN, JAMA, and GQS scores compared to YouTube videos. This difference might stem from WebSurg's emphasis on delivering more academic content.[21]

When comparing full-length and edited/abbreviated surgical videos that encompass the entirety of the surgical procedure, significant differences emerge between the YouTube and WebSurg platforms. The comparative analysis of these two categories reveals pronounced distinctions in GQS, JAMA, modified DISCERN, and LAP-VEGaS evaluation scores. Full-length videos tend to receive higher scores based on these evaluation criteria.

In a study examining videos of the Total Extraperitoneal Hernia (TEP) technique on the WebSurg platform, a positive correlation was observed between video publication duration, video duration, and video popularity. However, no significant correlation was found between educational and surgical technical quality scores. These findings underscore the intricate interplay between video duration, popularity, and educational quality.[22]

In response to this, the findings from our study demonstrate that full-length videos, due to their comprehensive coverage of the entire surgical procedure, have the potential to better showcase various challenges encountered during the operation and better prepare the viewer. Significantly, the comprehensive portrayal of each procedural phase in full-length videos offers viewers an authentic understanding of the surgical process, catering to those striving to grasp and evaluate diverse operation facets. Conversely, edited or abridged videos may efficiently deliver concise information, yet at the expense of limiting opportunities for thorough learning.

This study bears limitations; our evaluations might be deemed surface-level due to the lack of a dedicated tool for topic-specific video analysis. Furthermore, being conducted by a single surgeon, divergent assessments by varied healthcare experts could yield disparate outcomes. In conclusion, this pioneering study juxtaposes WebSurg and YouTube videos in laparoscopic hernia surgery education, with WebSurg showcasing superior content quality. These findings underscore the pivotal role of platform selection in medical education, with potential implications for enhanced surgical training.

CONCLUSION

This study presents the first comparison of WebSurg and YouTube videos within the realm of laparoscopic hernia surgery education. The outcomes indicate that WebSurg delivers superior content quality. These findings emphasize the critical role of platform selection in medical education, potentially augmenting the refinement of surgical training.

ETHICAL DECLARATIONS

Ethics Committee Approval: Because no patient data was used and all videos are publicly accessible on YouTube and WebSurg, ethical approval wasn't required.

Informed consent: As this study solely utilized publicly accessible online videos and did not involve any direct interaction or data collection from human or animal subjects, the concept of informed consent was not applicable. The videos under examination were already shared on openly accessible platforms and did not involve the use of personal or sensitive information. Consequently, the study did not encompass the conventional process of obtaining informed consent from participants.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Evaluation of the relationship between mitral annular calcification and triglyceride-glucose index

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ABSTRACT

Aims: Mitral annular calcification (MAC) is an echocardiographic condition with a multicomponent etiopathogenesis, one of which is insulin resistance. Triglyceride-glucose (TyG) index is an excellent biochemical parameter that has proven itself in determining insulin resistance in recent years. This study aims to reveal the relationship between the TyG index and the presence of MAC.

Methods: The study included 159 patients with MAC and 167 control group. The control group has similar demographic characteristics such as age, gender, presence of hypertension (HT), and diabetes mellitus with the MAC group. TyG index was calculated as a formula: ln[fasting triglyceride (mg/dl) × fasting plasma glucose (mg/dl)/2].

Results: The mean age of the patients was 74.2 and 48.2% of the patients were male. Coronary artery disease (CAD) (p: 0.031), glucose (p:0.001), total cholesterol (p:0.009), low-density lipoprotein (LDL) (p:0.004), triglyceride (TG) (p <0.001) levels and TyG index (p <0.001) were higher in MAC group. In the multivariate regression analysis, TG (p:0.004) and TyG index (p<0.001) were found to be independent risk factors. As a result of the ROC analysis, the cut-off value for estimating MAC was found to be 8.81 [(sensitivity: 77.3%, specificity: 76.5%, AUC (95% CI) 0.756 (0.704-0.807) p<0.001)].

Conclusion: In this study, a high TyG index was found to be an independent risk factor for MAC. The TyG index was found to be a better biomarker than TG and glucose alone in predicting MAC. Further extensive studies are necessary to determine the importance and use of TyG in MAC.

Keywords: Mitral annular calcification, insulin resistance, triglyceride-glucose index

INTRODUCTION

Mitral annular calcification (MAC) is defined as not only a local, chronic and degenerative process characterized by the precipitation of calcium and phosphate in the fibrous tissue of the mitral ring but also as an active and regulated molecular process related to lipid and mineral metabolism, hemodynamic stress and inflammation.[1,2] The mitral annulus is one of the most common sites of calcification after the coronary arteries.[3] Although its name suggests that calcification is limited to the mitral annulus only, it has been observed in surgical specimens that calcification may also extend to the chordae tendinea, papillary muscles, and left ventricle.[4] Major risk factors for the development of MAC are age, female gender, obesity, hypertension (HT), diabetes mellitus (DM), dyslipidemia, chronic kidney disease and smoking.[5,6]

The most common radiographic method for the diagnosis of MAC is echocardiography. MAC appears as an irregular and echo-dense structure, and its size increases with the severity of calcification.[7] The most specific diagnostic test is computed tomography.[7,8] Although it varies depending on the imaging method and the population, the prevalence of MAC varies between 5% and 47%.[1,9]

In clinical practice, MAC is associated with not only mitral valve dysfunction which causes systemic diseases but also cardiovascular diseases.[2] MAC, which is associated with atherosclerosis, heart failure, stroke, and atrial fibrillation, has also been independently associated with all-cause and cardiovascular mortality.[10-12] This strong link encouraged researchers to design studies by emphasizing MAC's pathophysiology and clinical value.

Despite its importance in cardiovascular pathologies, the mechanism of MAC formation still needs to be fully understood. Previous data have shown that inflammation, oxidative stress, dyslipidemia, and dysregulation in bone mineral metabolism are

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essential in developing MAC in pathogenesis.[2,13,14] In addition to all these processes, it is known that insulin resistance also plays a role in the mechanism of MAC formation, such as coronary calcification.[15] However, it is unclear whether insulin resistance affects calcification formation beyond metabolic syndrome components and cardiovascular risk factors.[16] However, measuring insulin resistance requires complex techniques, a more straightforward method, the Hemostasis Model Evaluation (HOMA), is routinely used. On the other hand HOMA is a relatively expensive method requiring glucose and insulin measurement. In recent years, the triglyceride-glucose (TyG) index, based on fasting glucose and triglyceride (TG) measurements, has been a reliable marker with high sensitivity in the assessment of insulin resistance.[17] The relationship of the TyG index with coronary and peripheral arterial diseases, stroke, and HT has been demonstrated.[18-20] Until now, there is not enough data about the importance of the TyG index in estimating MAC in the literature.

This study aims to evaluate the importance of the TyG index as a screening parameter of the early diagnosis of MAC by examining the relationship between the TyG index, which has proven to be a good biochemical parameter in demonstrating insulin resistance, and MAC.

METHODS

The study was initiated with the approval of the Ankara City Hospital No: 1 Clinical Researches Ethics Committee (Date: 19/07/2023, Decision No: AEŞH-EK1-2023-273). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

This single-center study retrospectively scanned the files of adult patients who underwent echocardiography between January 2019 and June 2023. A study group with 159 patients with MAC and a control group with 167 patients with similar demographic characteristics such as age, gender, DM and HT were included. The participants' demographic, laboratory and echocardiographic data were recorded from the hospital system.

Patients with rheumatic heart valve disease, prosthetic heart valve disease, acute and chronic inflammatory disease, advanced renal failure (stage 4-5), liver failure, active malignancy, disease related to calcium metabolism, and patients receiving steroid therapy for any reason were excluded.

Laboratory & Imaging Data

Laboratory parameters, including triglyceride and glucose were recorded from the hospital records. Blood samples were taken from the patients by venous route after 8 hours of fasting. TyG index was calculated as ln (fasting triglyceride × fasting glucose/2).[21]

Transthoracic echocardiography was performed by the same cardiologist with the Philips Healthcare iE33 xMATRIX echocardiography (Philips Medical Systems, MA, USA) device with an S5-1 transducer. MAC; parasternal long or short axis was defined as a dense echocardiographic structure with localized reflective features at the junction of the atrioventricular groove and the anterior or posterior leaflet of the mitral valve on apical two- or four-chamber views.[22]

Statistical Analysis

Continuous variables are mean ± standard deviation, and categorical data are shared percentages and numbers (n). Student's t-test was used for normally distributed data, and the Mann-Whitney U test was used for non-normally distributed data. Categorical data were compared with the chi-square test. Univariate regression analysis was performed to find predictive factors for developing MAC. Multivariate regression analysis was applied to the parameters that were significant in the univariate test and independent risk factors for MAC development were determined. Receiver operating characteristic (ROC) analysis was used to estimate the optimum cut-off value of the TyG Index to indicate MAC. Sensitivity, specificity, and area under the curve (AUC) were calculated. Two-sided $p<0.05$ was considered significant. Data were analyzed with the SPSS 23.0 statistical program (SPSS Inc., NY, USA).

RESULTS

48.2% (n=157) of the patients were male. The mean age of the patients was 71.4 years. There was no difference between age, gender, HT, DM, smoking and EF. Only coronary artery disease (CAD) was higher in patients with MAC (n=79 (49.7%) vs. n=65 (38.9%), p: 0.031). No difference was found between hemoglobin, WBC, platelet, creatinine and high-density lipoprotein (HDL). In MAC patients, glucose ($p<0.001$), total cholesterol (p:0.009), Low-density lipoprotein (LDL) (p:0.004), TG ($p<0.001$) and TyG index (8.90±0.44 vs. 8.43±0.49, $p<0.001$) were higher than control group (**Table 1**).

As a result of univariate regression analysis performed to determine the variables predicting the development of MAC, glucose ($p = <0.001$), total cholesterol ($p = 0.010$), TG ($p=<0.001$) and TyG index ($p= 0.001$) were found to be predictive factors for the development of MAC. As a result of multivariate regression analysis, TG ($p = 0.004$) and TyG index ($p= <0.001$) were found to be independent risk factors (**Table 2**).

Table 1. Basal demographic and laboratory characteristics of the patients and control group.

	MAC (n=159)	Control group (n=167)	p
Age, years	71.9 ±6.8	70.8±6.5	0.134
Male, n (%)	72 (45.3)	85 (50.9)	0.183
HT, n (%)	50 (31.4)	46 (27.5)	0.258
DM, n (%)	66 (41.5)	74 (44.3)	0.354
CAD, n (%)	79 (49.7)	65 (38.9)	0.031
Smoking, n (%)	50 (31.4)	46 (27.5)	0.258
EF, %	62.4±11.2	63.1±10.8	0.376
Hemoglobin, g/dl	12.7±2.4	12.9±2.6	0.481
WBC, 10^3/mm³	7.8±1.9	7.7±2.4	0.205
Platelet, 10^3/mm³	276.3±75.5	279.6±54.7	0.662
Glucose, mg/dl	91.5±5.8	87.7±8.9	<0.001
Creatinin, mg/dl	1.17±0.28	1.16±0.32	0.728
Total cholesterol, mg/dl	207.2±38.7	196.4±38.2	0.009
LDL, mg/dl	126.3±31.9	121.4±33.8	0.004
HDL, mg/dl	50.5±6.6	51.9±5.8	0.236
TG, mg/dl	168.3±63.5	131.2±49.4	<0.001
TyG Index	8.90±0.44	8.43±0.49	<0.001

MAC: Mitral annular calcification, HT: Hypertension, DM: Diabetes mellitus, CAD: Coronary artery disease, EF: Ejection fraction, WBC: White blood cell, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TG: Triglycerides, TyG: Triglycerides-Glucose

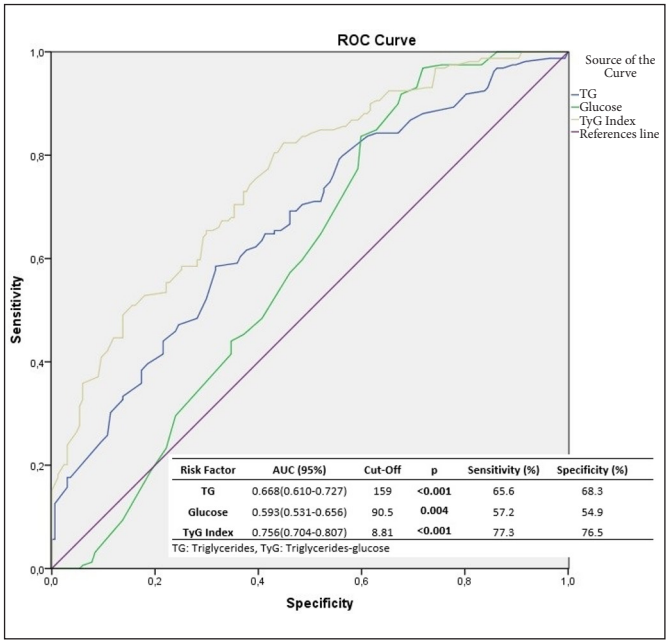

Figure 1. Receiver operating characteristic analysis results for TG, glucose, and TyG Index.

The ROC analysis performed to determine the optimal cutoff values of the TG, glucose and TyG index parameters in predicting the MAC development revealed the optimal cut-off values for TG, glucose and TyG index as 159 [sensitivity: 65.6%, specificity: 68.6%, AUC (95% Confidence Interval (CI)) 0.668 (0.610-0.727) p<0.001], 90.5 ((sensitivity: 57.2%, specificity: 54.9%, AUC (95% CI) 0.593(0.531-0.656) p:0.004), and 8.81 ((sensitivity: 77.3%, specificity: 76.5%, AUC (95% CI) 0.756 (0.704-0.807) p<0.001), respectively (Figure 1).

DISCUSSION

In this study, the relationship between MAC and TyG index was evaluated. It was observed that there was a significant relationship between the triglyceride-glucose index and MAC. In multiple regression analysis, high TG and TyG index levels were found to be an independent risk factor in predicting MAC formation. In addition, MAC was more common in patients with CAD and high glucose, LDL and total cholesterol levels. This study is important as it is the first study to evaluate the relationship between MAC and the TyG index, as far as we know.

Mitral annular calcification is an echocardiographic finding that clinically focuses primarily on the degree of accompanying valve dysfunction. It has been proven to be strongly associated with cardiovascular morbidity and mortality in recent years.[23] It is frequently seen in the elder age, as in many other MAC studies; the elderly population was found to be the majority in our study.[5,6] The biological mechanisms explaining the relationship of MAC with cardiovascular diseases have not yet to be fully elucidated. However, it is known that the histopathology of MAC is similar to coronary atherosclerosis. These two diseases share cardiovascular risk factors (HT, DM, dyslipidemia, smoking, etc.) and common pathophysiological mechanisms.[23-25] However, contrary to the atherosclerosis paradigm, MAC is more common in women. In our study, the female sex ratio was predominant in the MAC group, similar to previous clinical studies.[25,26] In addition, the rate of CAD was higher in patients with MAC in our study. This result supported previous studies.[9]

Table 2. Predictors of mitral annular calcification.

	Univariate analysis OR (95% CI)	p	Multivariate analysis OR (95% CI)	p
CAD	1.550(0.998-2.406)	0.510	**	**
Glucose	1.051(1.025-1.076)	<0.001	0.999(0.992-1.006)	0.706
Total cholesterol	1.008(1.002-1.014)	0.010	0.999(0.992-1.006)	0.784
LDL	1.002(0.989-1.005)	0.181	**	**
TG	1.012(1.007-1.016)	<0.001	1.016(1.005-1.027)	0.004
TyG Index	1.126(1.072-1.219)	<0.001	1.088(1.012-1.195)	<0.001

CAD: Coronary artery disease, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, TG: Triglycerides, TyG: Triglycerides-Glucose

Although the physiopathogenesis of mitral annular calcification is not clear, it is known to occur due to multifactorial mechanisms such as dyslipidemia, inflammation, oxidative stress, bone mineral metabolism disorders and insulin resistance.[2,14] Previous studies revealed results proving the relationship between inflammation and MAC, one of these mechanisms.[3,6,26] The relationship between insulin resistance and MAC has been relatively less studied. Recently, Grigorescu et al.[6] showed that patients with MAC have higher insulin resistance with higher HOMA C-peptide and C-peptide index. On the other hand, Tison et al.[16] showed a positive and graded relationship between insulin resistance and extra-coronary calcification, including in patients with MAC, based on HOMA level. In our study, we tried to prove the existing relationship by using the non-insulin level-based TyG index, which was obtained simply as an indicator of insulin resistance. At the same time, the relationship between the development of MAC, which is a multi-component process, and inflammation is known, and studies have revealed that systemic inflammation is more common in patients with insulin resistance.[6] Our results suggest that the higher detection of MAC in patients with high TyG index may be both a result and a cause. Thus, there may be a bidirectional relationship between the TyG index MAC and systemic inflammation.

The TyG index used in our study was first studied as an insulin resistance marker candidate and proved to be a good biochemical parameter in demonstrating insulin resistance.[17,27] Today, this index has not been used routinely due to the lack of studies that will improve its capacity and standardize it in detecting insulin resistance. However, the fact that it is a cheaper and more accessible parameter than insulin level-based methods that show insulin resistance has created an advantage in using this index in various epidemiological and clinical studies.[28] In recent studies, TyG is associated with cardiovascular diseases such as coronary artery disease, peripheral vascular disease, stroke, HT, the risk of developing type 2 DM and metabolic syndrome.[18,19] Although the detailed mechanism of the relationship between cardiovascular diseases and the TyG index has not been fully demonstrated, the TyG index has been accepted as a valuable indicator associated with insulin resistance and cardiovascular disease.[20] We also determined a positive relationship with MAC, whose relationship was not examined before. Although the exact reason for this relationship is unclear due to the nature of the study design.

In ROC analysis, the TyG index predicts MAC; obtained better results than the TG and glucose parameters it contains alone. The sensitivity and specificity values of the TyG index were higher than glucose and TG. This suggests that the TyG index is a more reliable and helpful biomarker.

Limitations

Our study has several limitations. The most important limitation of the study is that it is a single-center and retrospective study. This situation needs to make it possible to explain the causality of the relationship between MAC and the TyG index. Secondly, a selection bias is possible when initially forming the control group. The results should be interpreted with caution and further scrutinized in larger multicenter studies. Finally, since our study is retrospective, there is no data or classification regarding the severity of MAC.

CONCLUSION

This study showed a significant positive correlation between MAC and the TyG index, which can be calculated in routine blood parameters. A higher TyG index independently predicted a higher rate of MAC. Patients with a high TyG index can be observed more closely regarding MAC formation in clinical practice. In this group, index-reducing treatments may slow down the occurrence of MAC. However, multicenter, randomized, prospective studies are needed to support and clarify these hypotheses.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Ankara City Hospital No:1 Clinical Researches Ethics Committee (Date: 19.07.2023, Decision No: AEŞH-EK1-2023-273).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The leuko-glycemic index can predict multivessel disease in the elderly acute myocardial infarction population? a retrospective cohort study

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ABSTRACT

Aims: Cardiovascular diseases are still the leading cause of death, as the cause of approximately 30% of all deaths in the world and half of all deaths due to cardiovascular diseases also consist of individuals aged 70 and older. The leukoglycemic index (LGI) is a new parameter associated with mortality, complication, and prognosis in cardiovascular diseases. It can be applied easily at the bedside, has a low cost, and consists of a combination of leukocytes and glucose. In our study, we aimed to evaluate the predictive effect of leukoglycemic index on multivessel disease in elderly patients over 65 who were hospitalized with acute myocardial infarction.

Methods: In our retrospective cohort study, patients over 65 who were hospitalized with the diagnosis of acute myocardial infarction were included. LGI was calculated with the formula: blood glucose × white blood cell/1000. All datas about patients were collected from the electronic hospital information system, patient files and our hospital's archive.

Results: The patients were divided into two groups: single-vessel disease and multivessel disease. The laboratory parameters of the patients were compared, and LGI (1532.5 (577.7-3770.3) vs 2077.9 (646.6-5301); p<0.001) were found to be significantly different between the two groups. According to unadjusted univariate log regression analysis, LGI was statistically significant in predicting multivessel disease (OR: 1.724, 95% CI: (1.222-2.432), p=0.002). After adjusting the model by adding clinically and statistically significant variables, LGI remained an independent predictor of multivessel disease (OR: 1.599, 95% CI (1.086-2.357), p=0.018). The discrimination ability of LGI was analyzed with ROC curve analysis. The LGI AUC value was 0.619 (95% Cl, 0.544-0.695, p=0.003).

Conclusion: Our study showed that high LGI is an independent predictor of multivessel coronary artery disease in elderly patients with acute myocardial infarction.

Keywords: Blood glucose, coronary artery disease, elderly, leukocyte, myocardial infarction

INTRODUCTION

Cardiovascular diseases are still the leading cause of death, as the cause of approximately 30% of all deaths in the world and half of all deaths due to cardiovascular diseases also consist of individuals aged 70 and older.[1]

While the EU population over the age of 65 was about 12% in the 1950s, according to recent data, it is seen that the proportion of individuals over the age of 65 is about 19.2% today, and it is estimated that this will reach 36% in 2050.[2,3] When the data and statistics are analyzed, the elderly population will increase even more over the years, and we will encounter the elderly patient population more. More studies on the elderly population, where diagnosis and treatment management are difficult, comorbidity is high, and relatively few studies are available, can be considered the main factor in creating diagnosis, treatment and follow-up algorithms.

The leukoglycemic index (LGI) is a new parameter associated with mortality, complications, and prognosis in cardiovascular disease. It can be applied easily at the bedside, has a low cost, and consists of a combination of leukocytes and glucose.[4-7] For the first time, 101 ST-elevated patients, LGI was evaluated by Quiroga Castro et al.[7] and was shown to be independently associated with both in-hospital mortality and complications. Systematic review and meta-analysis of eleven studies by Roxana Sadeghi et al.[4] stated that LGI is independently associated with mortality and complications after acute myocardial infarction. Identifying patients at high risk

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for coronary artery disease (CAD) can guide physicians in terms of preventive measures, follow-up planning and treatment intensity. For this purpose, many studies have been conducted on biomarkers and scoring. Our study was designed to determine the prognostic value of this index in different cardiovascular patient populations.

In our study, we aimed to evaluate the predictive effect of the leukoglycemic index on multivessel disease in elderly patients who were hospitalized with acute myocardial infarction.

METHODS

The study was initiated with the approval of the Kutahya Health Science University Non-interventional Clinical Researches Ethics Committee (Date: 10.07.2023, Decision No: 2023/08). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

In our retrospective cohort study, patients over 65 who were hospitalized with the diagnosis of acute myocardial infarction (AMI) and underwent coronary angiography between January 2022 and June 2023 were included.

Patients with chronic coronary syndrome, active malignancy, active infectious disease, severe hepatic and renal failure, active or recent internal bleeding, and other significant comorbidities were excluded from the study. As a result, 22 patients were excluded (Figure 1). The patient's data were analyzed retrospectively through the hospital's electronic data information system and patient files.

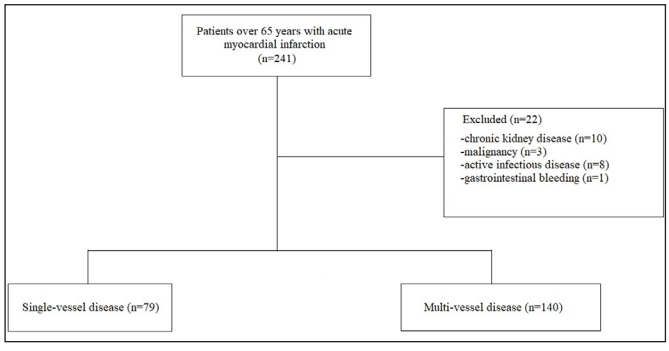

Figure 1. Diagram of the patient flowchart

We analyzed peripheral venous blood samples from the patients in the hematology laboratory, and complete blood count parameters were calculated by an automated blood counter (Beckman Coulter, Brea, CA). Blood glucose levels, urea, creatinine, glomerular filtration rate, albumin, alanine aminotransferase, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, troponin I were analyzed in the biochemistry laboratory for all patients on admission.

Demographic characteristics, medical history and clinical and laboratory data of patients were recorded. Echocardiographic and angiographic images were analyzed from the hospital's electronic imaging system, patients files and our hospital's archive.

Multivessel coronary artery disease was defined as 50% or more stenosis in at least two coronary arteries. The patients were divided into multivessel and single coronary artery disease. LGI was calculated with the formula: blood glucose x white blood cell/10^3.[7]

Statistical Analysis

We analyzed the data with SPSS, version 21.0 (IBM). The normality of distribution for parametric data was evaluated with the Shapiro-Wilks test. Levene's test analyzed the homogeneity of the data. For continuous variables with a normal distribution, means accompanied by standard deviations (±SD) were employed, while for variables without a normal distribution, minimum and maximum values were utilized. The differences between the categorical variables of the groups were analyzed using the chi-square test. The differences in the numerical variables for the independent groups were evaluated with the t-test or Mann-Whitney U test.

The discriminative abilities and cut-off points of the leuko-glycemic index in predicting multivessel disease were compared using the area under the ROC curve. Univariate and multivariate logistic regression models were used to evaluate the independent association of the leuko-glycemic index with multivessel disease; odds ratios are reported with their respective 95% confidence intervals. Clinically and statistically significant variables were included in the variants in the logistic regression analysis. The level of significance was set at $p<0.05$.

RESULTS

Our study included 219 patients, 72 (32.9%) women and 147 (67.1%) men, hospitalized with acute miyocardial infarction and underwent coronary angiography. According to the study design, all patients were over 65, and the mean age was 71.2±5.7 years. The patients were divided into two groups: single-vessel disease and multivessel disease.

It was observed that a history of hypertension (HT) (32.9%, vs. 47.9%; p=0.032) and diabetes mellitus (DM) (27.8%, vs. 44.3%; p=0.016) was more frequent in multivessel disease. The laboratory parameters of the patients were compared, and total cholesterol (172.2 (72-291) vs 185.1 (78-319); p= 0.049) triglyceride (114.3 (41-533) vs 141.9 (42-533); p=0.030) and LGI (1532.5 (577.7-3770.3) vs 2077.9 (646.6-5301); p<0.001) were found to be significantly different between the two groups. Baseline clinical, demographic and laboratory characteristics of the study population according to the multivessel disease are shown in Table 1. It was observed that gender and age

were not associated with multivessel disease over 65 years of age. There were no significant differences in AMI type, albumin, alanine aminotransferase, white blood cell, hemoglobin, platelet, and troponin parameters between the two groups on admission.

According to univariate logistic regression analysis, LGI was statistically significant in predicting multivessel disease (Odds Ratio: 1.724, 95% CI: (1.222-2.432), p=0.002). After adjusting the model by adding clinically and statistically significant variables (HT, total cholesterol, DM, LGI), LGI was still an independent predictor of multivessel disease (Odds Ratio: 1.599, 95%CI: (1.086-2.357), p=0.018) (Table 2).

The discrimination ability of LGI was analyzed with ROC curve analysis. The LGI AUC value was 0.619 (%95 Cl, 0.544-0.695, p=0.003). The cut-off value for LGI was 1471.65, with sensitivity of 55.7% and specificity of 55.7% (Figure 2).

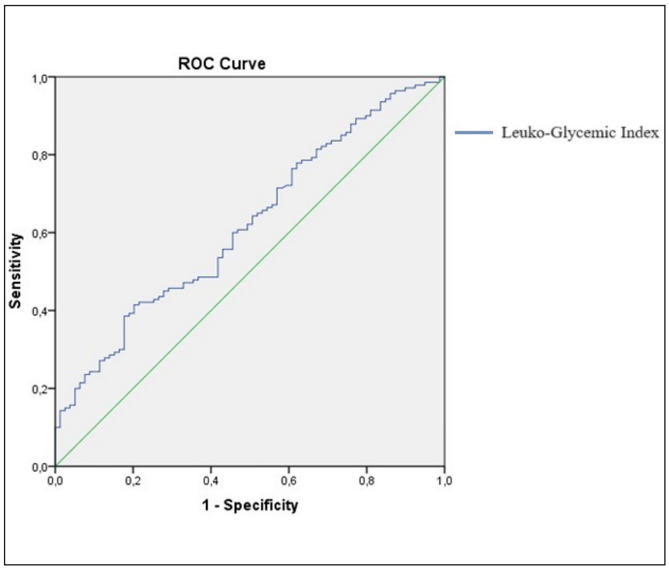

Figure 2. Receiver-operating characteristic (ROC) curve analysis plot to determine the cut-off value of LGI in the prediction of multivessel diseae. AUC: 0.619 (95% Cl, 0.544-0.695, p=0.003)

Table 1. Baseline and laboratory characteristics according to single vessel vs multivessel coronary artery disease.

Characteristics	Single-vessel CAD (n=79)	Multi-vessel CAD (n=140)	p value
Age (years), mean ± SD	71.4 ± 5.6	71.2 ± 5.8	0.796
Sex, male %	68.4	66.4	0.771
Diabetes mellitus, %	27.8	44.3	0.016
Hypertension, %	32.9	47.9	0.032
Prior cerebrovascular event, %	0	5.6	0.306
Peripheral artery disease, %	2.5	1.4	0.826
MI type (STEMI), %	49.4	45	0.534
Ejection fraction (%), median (min-max)	48.4 (25-65)	47.3 (25-70)	0.521
Serum creatinine (mg/dl), median (min-max)	1.04 (0.3-1.89)	1.06 (0.55-2.0)	0.803
Total cholesterol (mg/dl), median (min-max)	172.2 (72-291)	185.1 (78-319)	0.049
LDL-C (mg/dl), median (min-max)	110 (25-198)	117.2 (49-251)	0.178
HDL-C (mg/dl), median (min-max)	42.6 (18-79)	41.2 (10-73)	0.385
Triglyceride (mg/dl), median (min-max)	114.3 (41-533)	141.9 (42-533)	0.030
WBC (×10³ µl), median (min-max)	10.4 (5.3-19.7)	10.8 (5.3-26.4)	0.417
Hemoglobin (g/dl), median (min-max)	13.5 (6.7-18.8)	13.4 (9.3-17.6)	0.646
Platelet (×10³ µl), median (min-max)	243.5 (94-566)	235.8 (60-630)	0.475
Neutrophyl (×10³ µl), median (min-max)	7.7 (2.7-18)	7.8 (0.4-23.1)	0.853
ALT (U/L), median (min-max)	31.8 (8-360)	32.2 (6-410)	0.951
Albumin (g/L), median (min-max)	40.7 (28-49.2)	39.2 (28-47.7)	0.871
Troponin (ng/ml), median (min-max)	13205 (0.40-95000)	4508.7 (0.50-82000)	0.142
LGI, median (min-max)	1532.5 (577.7-3770.3)	2077.9 (646.6-5301)	<0.001

ALT, alanine aminotransferase; CAD, coronary artery disease; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; LGI, leuko-glycaemic index; MI, myocardial infarction; STEMI, ST elevated myocardial infarction; TG, triglyceride; WBC, white blood cell

Table 2. Univariate and multivariate regression analysis of significant parameters for multivessel disease

Variables	Univariate		Multivariate	
	OR (95% Cl)	p value	OR (95% Cl)	p value
HT	1.870 (1.053-3.320)	0.033	1.729 (0.930-3.215)	0.084
DM	2.059 (1.137-3.731)	0.017	1.100 (0.545-2.218)	0.790
Total cholesterol	1.006 (1.000-1.013)	0.054	1.006 (0.999-1.013)	0.074
LGI	1.724 (1.222-2.432)	0.002	1.599 (1.086-2.357)	0.018

DM, diabetes mellitus; HT,hypertension; LDL, low density lipoprotein; LGI, leuko-glycaemic index.

DISCUSSION

In this study, we showed that leukoglycemic index is an independent predictor of multiple coronary artery disease in elderly patients who are hospitalized with the diagnosis of AMI.

The LGI consists of two parameters: leukocyte count and blood glucose level on admission. Leukocytes are one of the primary mediators of inflammation and are also used in the clinic as a marker of inflammation. It has been shown that peripheral leukocyte count is closely related to in-hospital mortality, cardiogenic shock, and heart failure in patients with acute myocardial infarction.[8,9] With increasing evidence over the years, it is known that chronic inflammation also has a main role in atherosclerosis. Various inflammatory biomarkers, such as leukocytes and C-reactive protein, have been used to predict the risk of coronary artery disease.[10,11] At the same time, previously published studies have shown that the total leukocyte count is also independently associated with the presence and severity of coronary artery disease and plaque calcification.[12,13]

During acute stress, glucose metabolism is affected by inflammatory mediators, independent of diabetes mellitus, and hyperglycemia occurs.[14] Stress hyperglycemia is defined by the American Diabetes Association (ADA) as having a random glucose level higher than 140 mg/dl in hospitalized patients at any time. Hyperglycemia, which can be seen as an inflammatory response regardless of diabetes mellitus, is seen in 58% of patients with acute coronary syndrome.[15] A previous study showed a significant association between hyperglycemia and high leukocyte count in AMI patients.[16] Admission hyperglycemia has been shown in many studies to be associated with both in-hospital and long-term increased adverse outcomes in ACS patients, independent of diabetes mellitus.[14,17,18]

In the study published by Ling-Yao Qi et al.[5] 1256 AMI patients from multiple centers were included in the observational study. The patients were divided into two groups: diabetic and non-diabetic. Optimal cut-off values were determined as 3593 mg/dl mm^3 for diabetics and 1402 mg/dl mm^3 for non-diabetics. The study concluded that LGI is an independent predictor of all-cause mortality in hospital and MACE at follow-up in nondiabetic patients, but not in diabetics. In our study, diabetic and nondiabetic patients were also included. It was shown that history of diabetes mellitus is not a independent predictor, but LGI is an independent predictor for multivessel disease in the elderly population.

In the study of Oguz Kilic et al.[6] the relationship between LGI and the severity of CAD in patients with chronic coronary syndrome has been investigated. The severity of CAD was evaluated by the Gensini score. It has been shown that LGI is an independent predictor of the severity of CAD, and LGI was correlated with the Gensini score in patients with chronic coronary syndrome. In addition, according to the results, it was observed that age was an independent predictor. In our study, we evaluated the severity of coronary artery disease in elderly AMI patients and observed that while age was not significant, LGI was an independent predictor.

Despite the significant reduction of mortality in coronary artery disease with the development of new antiaggregant drugs, mortality-reducing treatments, new stent technologies, and techniques, acute coronary syndromes (ACS) still rank first in the causes of death in worldwide.[19] Cardiovascular diseases account for 82% of the causes of death in patients over 65.[20] However, because studies on elderly patients are mostly subgroup analyses and few randomized studies, the data on elderly patients can still be considered insufficient.[21,22] It is known that age alone is an important risk factor for cardiovascular diseases. Furthermore, it has also been stated that individuals over 60 can be considered very high-risk.[23] Age is also directly related to ACS patients' mortality, which increases sharply, especially after 70.[21,24] In addition, coronary artery disease is more often accompanied by multi-vessel disease, left main coronary artery disease, coronary calcification, and heart failure in elderly patients.[1,25,26] Other cardiac problems that occur with increasing age are structural and functional changes such as myocyte loss and left ventricular hypertrophy, increased fibrocalcification in the valves, cell loss in the sinoatrial node, changes in systolic and diastolic functions and volumes.[2,27,28]

The increase in comorbidities such as hypertension, chronic kidney diseases, neurocognitive diseases with advancing age, and the more frequent atypical clinical presentation cause errors in the diagnosis process and more defensive treatment approaches against elderly fragile patients. 80% of people over 65 have at least one chronic condition, and 68% have two or more chronic diseases. It should be known that elderly patients are also not homogeneous. Therefore their prognosis is different, and their life expectancy may change.[1,2] The treatment process in elderly patients also requires a multidisciplinary approach due to polypharmacy, drug interactions, side effects, incompatibilities, cognitive disorders, and organ failures.

In the registry published by Kochar et al.[29] AMI patients over 65 were evaluated, and 8-year mortality was found to be 65%. It has even been reported that the long-term mortality of patients who lived for one year after index MI coronary revascularization exceeded 45%. In the Global Registry of Acute Coronary Events (GRACE), it has been

reported that although ST-elevation myocardial infarction is observed more frequently in the younger age population, the frequency of non-ST elevation myocardial infarction increases with increasing age. In the follow-up, it was observed that bleeding complications, cardiogenic shock, and in-hospital mortality were higher in elderly patients.[30]

The multivessel disease has been defined as 50% or more in at least two coronary arteries and associated with a poor prognosis in ACS patients. Although the issue of multiple coronary artery revascularization strategy in ACS patients is controversial, evaluation should be made according to patient and lesion characteristics, and gradual total revascularization for ischemic lesions is recommended.[22,31] In Angiocardio registry published by Cantarelli et al.[32] 16320 patients were analyzed, and independent risk factors in multivessel coronary artery disease (more than 50% stenosis in more than one coronary artery) were evaluated. Age, diabetes mellitus and chronic kidney disease were stated to be the most substantial risk factors for multivessel coronary artery disease.

Considering the above studies, combining leukocyte and blood glucose levels as a new parameter with the prevalence of multivessel coronary artery disease in patients with AMI is reasonable and feasible. Especially in elderly patients with insufficient studies, these scores can give an idea about patient prognosis, follow-up, and even treatment intensity, and a strategy can be planned.

Limitations

There are several limitations of this study. Initially, the study was designed as a retrospective and single-center study. Our number of patients is relatively insufficient. There is a need for more patient numbers and prospective studies on this subject. Another limitation is that the medication information of patients cannot be accessed retrospectively, and the proBNP, CRP, etc. levels of patients cannot be included in the study due to the fact that they are not routinely analyzed during hospitalization. The results cannot be generalised, considering the number of patients and their data.

CONCLUSION

Our study showed that high LGI is an independent predictor of the multivessel coronary artery disease in elderly patients with AMI.

The use of this inexpensive and simple index in combination with other cardiac tests in elderly patients may also provide some information about the severity of coronary artery disease. Prospective studies are needed to show the cardiovascular prognostic relationship between LGI and multiple coronary artery disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Kütahya Health Sciences University Non-interventional Clinical Researches Ethics Committee (Date: 10.07.2023, Decision No: 2023/08).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Individualized fluoroscopic lateral femoral neck view for fixation of hip fractures in the lateral decubitus position

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ABSTRACT

Aims: The aim of this study is to evaluate whether displaced hip fractures can be reduced and nailed properly in the lateral decubitus position without using a traction table with Proximal Femoral Nail Antirotation as a fixation device with inlet flouroscopic view.

Methods: In this retrospective study, 58 patients with hip fractures who were treated with Proximal Femoral Nail Antirotation in a single center were evaluated to determine the efficacy of the inlet fluoroscopic image. The postoperative X-rays of the patients underwent a comprehensive evaluation including the tip-apex distance, the quality of fracture reduction, and the positioning of the screw. The length of hospital stay, postoperative, and total hospital stays were evaluated. We propose personalized fluoroscopy positioning method for the reduction and internal fixation of hip fractures, eliminating the need for a traction table.

Results: The mean age of the patients was 78.43±11.67 years. By inlet viewing of the hip the most common placement of the integrated compression screws on postoperative radiographs was found to be 63.8% in Cleveland zone 5 and an increase in the femoral neck angle mean was 133,6° resulted in a significant decrease in the postoperative hospital stay of which mean was 3.95 days . The tip-apex distance was 19 mm as a mean.

Conclusion: The nailing of proximal femoral fractures using a lateral decubitus position and neutral fluoroscopy view may not achieve optimal quadrant placement of the nail. However, by repositioning the C-arm fluoroscopy with a 45-degree inlet angulation from the initial reference point, aligned with the femur and considering individual adduction, an enhanced lateral visualization of the femoral neck can be achieved, which will also help reduce potential complications during surgery.

Keywords: Flouroscopy, hip fracture, lateral decubitus position, proximal femoral nailing

INTRODUCTION

Hip fracture is widely recognized as the leading cause of hospital admission among the elderly population.[1,2] Addressing this prevalent issue is crucial to improving the quality of the patient's life, minimizing hospital stays, facilitating a prompt return to pre-fracture normalcy, and ultimately reducing the burden on healthcare systems.[2] To optimize treatment outcomes, significant advancements have been made in the development of both intramedullary and extramedullary implants within the field of surgery.[3] Among the various implant options available, cephalomedullary nails have emerged as the preferred choice for hip fracture management, encompassing fractures in the intertrochanteric, subtrochanteric, and, in specific instances, the basilar neck regions.[4-6]

Hip fractures are commonly treated through reduction and nailing in the supine position, typically with the aid of a traction table.[7,8] However, it is important to note that not all hospitals are equipped with a traction or fracture table.[9] Factors such as the patient's body structure (e.g., obesity or limited mobility) and health conditions (e.g., certain cardiovascular diseases) may necessitate alternative surgical positions as preferred by the surgeon.[10] Additionally, the use of traction tables has been associated with various complications, including pudendal nerve palsy, erectile dysfunction, and perineal sloughing.[10-12]

There have been limited reports on the use of proximal femoral nail antirotation (PFNA) for hip fractures treated in the lateral decubitus position (LDP).[9,13,14] The LDP has gained preference as the treatment position due to its advantages, such as providing easy access to the patient's hip and proximal femur and allowing for convenient visualization of both the anterior and posterior regions, particularly in obese patients. Additionally, this position offers better visibility of the trochanteric region.[14]

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Mechanical complications are the most common complications associated with implant failure and subsequent revision surgery.[1,6,15,16] Among these complications, the cutout of the neck-head by fixation device (e.g., lag screw, integrated compression screws, helical blade) has been identified as the most frequent type of mechanical failure.[17] Consequently, the positioning of the nail or proximal fixation on the femoral head plays a crucial role in addressing this issue.[1,2,6] However, when utilizing the LDP for hip fracture surgery, intraoperative imaging of the PFNA and its associated head screw can pose challenges, resulting in variations and a lack of consensus regarding the lateral imaging techniques to use.[17] It is worth noting that anatomical studies have revealed the obliquely elliptical cross-sectional morphology of the femoral neck, with a higher incidence of defects observed in the posterosuperior and anteroinferior regions.[7]

The importance of obtaining accurate lateral imaging in the supine position has been widely acknowledged, prompting research into various fluoroscopic positions to achieve different lateral views.[7,8] While some researchers have explored techniques involving alterations in the position of the fractured hip in the LDP to obtain fluoroscopic images of the lateral femoral neck, which may result in reduction loss, others solely rely on vertrical views without direct contact with the affected hip.[12,14] Despite the recognized significance of lateral imaging as a prognostic factor impacting both radiological and functional outcomes, the existing discrepancies highlight a lack of consensus and effectiveness in this area.[18]

The objective of the present study was to evaluate the feasibility and efficacy of using PFNA as a fixation device in the LDP without the need for a traction table, by an inlet fluoroscopic view. By addressing the limitations and uncertainties surrounding lateral imaging techniques, this research aimed to enhance the reduction and proper fixation of hip fractures in the LDP, ultimately improving surgical outcomes and patient care in doing so, it also helps to reduce complications during and after surgery.

METHODS

The study was initiated with the approval of the Kastamonu University Clinical Researches Ethics Committee (Date: 19.04.2023, Decision No: 2023-KAEK-48). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The present retrospective study was conducted at the Department of Orthopaedic and Trauma Surgery of Kastamonu Education and Research Hospital, including patients who presented between September 1, 2017, and November 1, 2020, with acute peritrochanteric hip fractures and subsequently underwent surgical intervention utilizing PFNA in the LDP. The study focused on patients who had undergone surgical procedure with individualized lateral femoral neck imaging technique. The analysis involved a comprehensive review and examination of hospital records and electronic data. Notably, other surgical techniques for hip fractures were specifically excluded from the analysis.

Before applying the technique we performed a saw bone model imaging study to explain which angle of lateral imaging is more reliable. Different images were obtained in different inlet positions of the lateral femoral neck in the neutral hip position (no adduction) as seen in **Figure 1**. The most suitable angle was found to be 45°. At other entry angles, the metal object did not show itself in the correct position situated has not correctly demonstrated outside the joint. This suggests that improper placement of the proximal fixation material due to improper positioning of the flouroscopy give rise to potential complications.

After finding 45° as the most suitable angle in sawbone models we applied this to patients with hip fractures in the LDP, just like the positions called "groin lateral view," "horizontal beam lateral view," and "cross-table lateral view" in supine positions which are used to obtain lateral hip radiographs in the supine position by directing the tube angle at a 20-45° angle toward the groin area[19,20] without touching or moving the hip, by positioning the

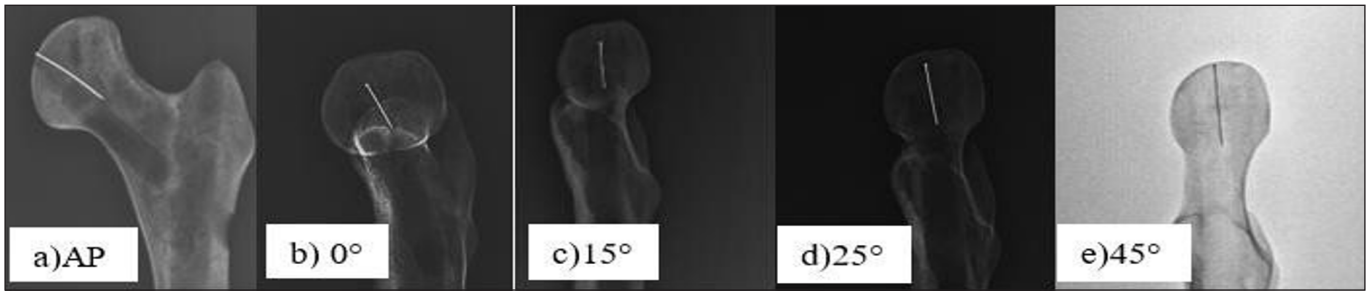

Figure 1. Lateral femoral neck fluoroscopic images of the metal located on the articular surface in the neutral hip position (corrected adduction). It is observed that the correct image is obtained at a 45-degree angle. In the images at other angles, the metal on the articular surface appears to be in a safe area as if it is not on the joint surface a: Hip AP view in internal rotation view. The metal tip is seen on the articular surface b: 0° inlet view. c: 15° inlet view. d: 25° inlet view. e: 45° inlet view.

image intensifier of the fluoroscopy device (or the X-ray tube section in obese patients) above the patient in the inlet position. This allowed us to obtain a true lateral femoral neck image in LDP.

The initial diagnosis of fractures was made via direct radiography and clinical evaluation for all enrolled patients. Pertinent patient data, including age, gender, fracture type, and length of hospital stay, were meticulously documented. Subsequently, immediate postoperative radiographs to evaluate femoral neck-shaft angle (NSA) were assessed to evaluate the quality of fracture reduction, which was categorized as good (<5° varus/valgus and/or anteversion/retroversion), acceptable (5-10° varus/valgus and/or anteversion/retroversion), or poor (>10° varus/valgus and/or anteversion/retroversion). The screw's placement was assessed using the tip-apex distance (TAD) methodology, originally defined by Baumgaertner et al.[6] which involves measuring the combined distance in millimeters from the lag screw's tip to the femoral head's apex on both anteroposterior and lateral radiographs. Furthermore, the positioning of the screw within the femoral head was documented according to the Cleveland and Bosworth classification system, which partitions the cephalic circumference into nine segments and sequentially labels them from left to right and top to bottom.[1,9]

The exclusion criteria for the present study were patients who did not undergo hip fracture surgery in the LDP because of unpositioning of the patient due to thoracic trauma or other extremity trauma, and those who opted for a different fixation method, such as cables or alternative implants. Patients with pathological fractures, open fractures, or cases requiring open surgery were also excluded.

Preoperatively, each patient underwent a comprehensive evaluation conducted by a medical consultant and an anesthesiologist. The surgical procedure was performed by the same senior orthopedic surgeon, taking into consideration the patient's comorbidities and preoperative treatment regimen, unless there was a medical condition that necessitated a delay in the surgery. Prophylactic measures for thromboembolic diseases included the administration of enoxaparin once daily for 4 weeks. Antibiotic prophylaxis was implemented using a first-generation cephalosporin at a 2 g dose administered prior to the surgical intervention and continued for 24 h, as per the standard protocols.[1]

The surgical procedures were conducted by a single surgeon on a radiolucent table. The patients were positioned in the LDP and securely immobilized. Specifically, the affected hip was maintained in an extended position, while the non-affected hip was flexed. The fixation device was meticulously inspected to ensure appropriate fluoroscopic visualization. During the surgical intervention, three scrubbed individuals, all wearing protective lead coats and neck collars underneath their sterile dressings, were present in the operating room. This team consisted of a primary surgeon, a registered nurse's first assistant, and a scrub nurse, all actively involved in the procedure, adhering to the principles of sterility and safety measures. Reduction of the fracture was accomplished through either manual longitudinal traction and internal rotation of the affected leg or utilization of a limited open approach, as shown in **Figure 2**. To validate the quality of the reduction achieved, images were obtained using an image intensifier for both anteroposterior (AP) and inlet flouroscopic position for true lateral view, as shown in **Figures 3A** and **3B**, respectively.

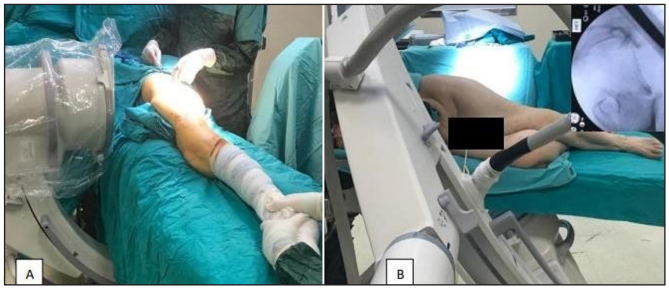

Figure 2. a: Fluoroscopic position for hip anteroposterior (AP) view **b:** Fluoroscopic position for hip lateral view and the corresponding image at the same moment.

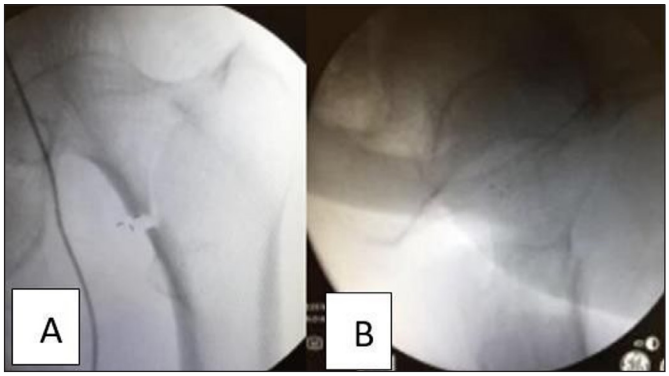

Figure 3. Preoperative fluoroscopic images of the patient in the lateral decubitus position. **a:** Hip anteroposterior (AP) view **b:** Hip inlet lateral view.

The Trigen-Intertan Intertrochanteric Antegrade Nail with integrated compression screws for the proximal fixation, a 130°, 20 cm nail specifically designed for proximal femoral nailing, was used in the present study. This brand was selected due to the radiolucency of the drill guide handle, which facilitated the attainment of improved lateral imaging during PFN application.

When we apply fluoroscopic view before or during the operation patient's lower extremity is in adduction by itself without any further positioning due to the surgical

position (Figure 4, line A). To determine the appropriate inlet angle, the initial position of the corrected fluoroscopic image relative to the lateral femoral cortex in the hip should be considered. Assuming the starting 0° position of the fluoroscopy in an almost vertical position (Figure 4, B line) at the beginning in the patients' placed position for the surgery, for the correct lateral view of the femoral neck visualization, the fluoroscopy should be adjusted to a 45° inlet position according to starting 0° (Figure 4, C line). The actual inlet angle differs for each individual. The main reasons for this variability include differences in pelvic height-width, femoral length, and thigh thickness. Therefore, in the LDP patients exhibit varying degrees of adduction, necessitating personalized adjustment of the fluoroscopic angle to achieve optimal lateral visualization of the femoral neck.

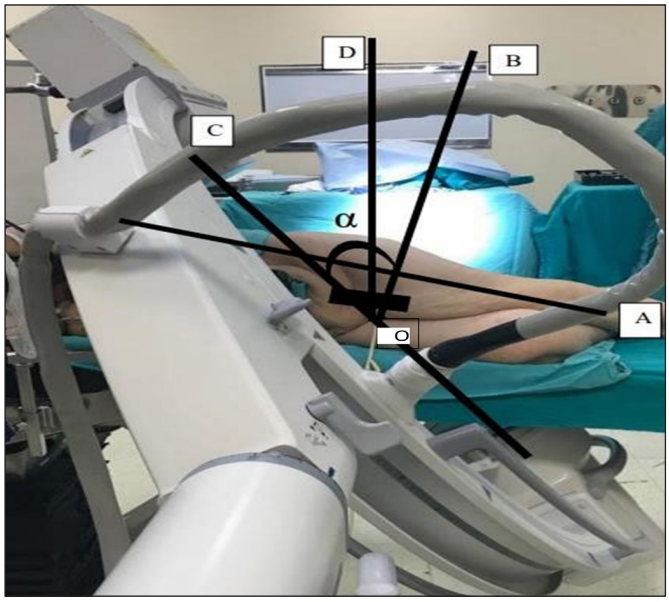

Figure 4. Position of the C-arm fluoroscope in real-time in the operating room to obtain the lateral view of the femoral neck. Line A represents the axis of the femur. Line B represents the perpendicular line to line A, indicating the corrected starting point - "0" point. Line C represents the necessary inlet position for obtaining the true lateral femoral neck view. Line D refers to the upright position of the C-arm fluoroscopy commonly used for classic femoral neck lateral imaging. The inlet angle (between B and C lines- BOC angle) has been determined as 45°

Considering that the fluoroscopy beam, which needs to be perpendicular to the femoral neck for the true hip lateral view (Figure 5), we believe that described inlet femoral neck view is the most suitable approach for lateral hip imaging in the LDP.

Following the selection of an appropriate-diameter nail based on preoperative planning, the nail was inserted through a classical 4 cm entry incision located 4-5 cm proximal to the trochanteric tip. To obtain the AP view, the guide pin was driven into the neck-head region, and the C-arm was rotated in the opposite direction, either under or above the table, as shown in Figure 2A. The lateral view, as shown in Figure 2B, was obtained without rotating or physically manipulating the hip joint. Instead, fluoroscopy was achieved with a corrected adduction angle of 45° inlet, considering that the affected leg was in adduction due to the lateral decubitus position, as shown in the figure. The position and TAD of the guide wire were assessed in both the AP and lateral projections, as shown in Figure 6A. Once the optimal position of the guide wire was confirmed, the appropriate length was determined and applied, as shown in Figure 6B. Subsequently, the near cortex was predrilled, and integrated compression screws were inserted, as shown in Figure 7A AP view and in Figure 7B lateral view. Finally, a single static distal locking screw was introduced through the drill guide handle of the nail to complete the fixation process.

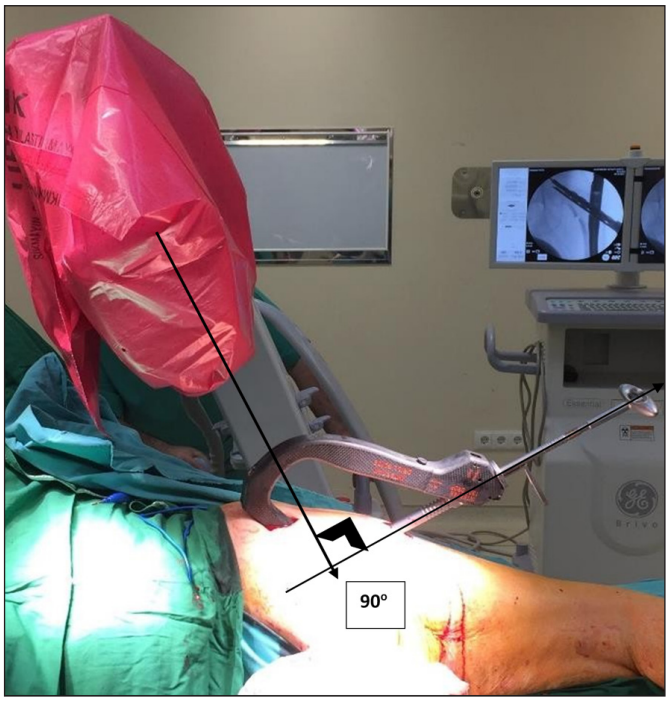

Figure 5. The angle between the K-wire/screw-blade going to the hip and the axis of the scope is 90°

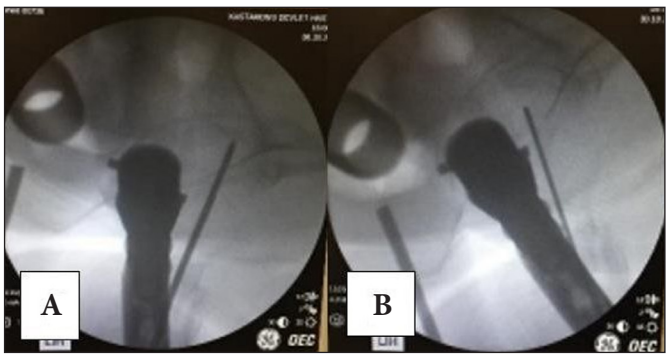

Figure 6. Intraoperative fluoroscopic images of the patient in the lateral decubitus position **a:** Excessive anterior deviation observed in the lateral view after insertion of the guide wire **b:** Re-alignment of the guide wire direction after correction.

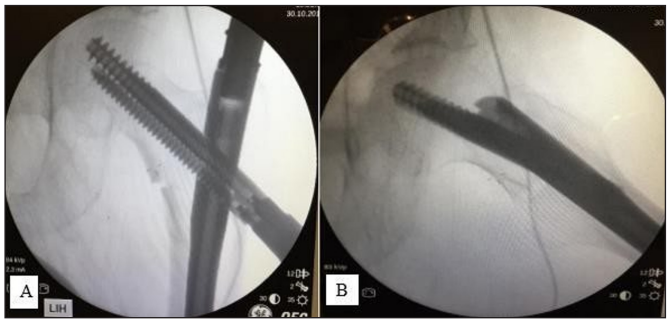

Figure 7. Final evaluations after PFNA (Proximal Femoral Nail Antirotation) procedure a: Hip anteroposterior (AP) view b: Hip lateral view

Statistical Analysis

The distribution of the data was examined using the Shapiro-Wilk test. The statistical relationships between the variables were analyzed using Spearman's correlation analysis. The impacts of the independent variables on the dependent variables were evaluated using linear regression analysis with the enter method. The descriptive statistics of the data were presented as mean±standard deviation or frequency (percentage). All statistical analyses were performed using IBM SPSS Statistics 26.0 software and analyzed and reported at a significance level α=0.05.

RESULTS

The present study included 58 patients. Subtrochanteric fracture was observed in 13 (22.4%) of these patients, while intertrochanteric fracture was present in 41(70.7%) and basicervical fracture 4 (6.9%) of them. Among the patients, 36 (62.1%) were female and 22 (37.9%) were male. The mean age of the patients was 78.43±11.67 years, the TAD score was 19±4.25, NSA 133.64±5.48°. The average value of the angle between the vertical position of fluoroscopy and the target inlet position when reached in patients was 23.5±2.9. The average length of stay in the hospital is 5.55 days while after surgery this average is 3,95 days.

The descriptive statistics for the demographic data of the patients are presented in Table 1.

The most frequent placement of the tip of the integrated compression screws on postoperative radiographs was in Cleveland zone 5 (center-center), observed in 37 cases (63.8%). The second most common location was in zone 8 (central-inferior), accounting for seven cases (12.1%). The third most common location is zone 2 central-superior region, with four cases (6.9%). In the other zones, one or two cases were observed.

The postoperative quality of fracture reduction was described as good for all the patients. It can be observed that there are no statistically significant relationships (p>0.05) between TAD and NSA and hospital stay duration. However, there is a statistically significant, negative, and weak relationship between NSA and postoperative total time (r=-0.327; p=0.012), indicating that as the femoral neck angle increases, the postoperative total time decreases. There are no significant correlations between femoral neck angle and other variables (p>0.05). However, there is a significant, moderate, positive relationship between hospital stay duration and time between admission and surgery (r=0.670; p<0.001), as well as a significant, moderate, positive relationship between postoperative total time and hospital stay duration (r=0.532; p<0.001). In the linear regression analysis, TAD and gender had no statistically significant effects on postoperative hospital stay duration (p=0.508).

Table I. Data of the patients and measurements on X-rays

n=58	
Gender	
Female	36 (62.1%)
Male	22 (37.9%)
Age	78.43±11.67
Fracture type	
Subtrochanteric	13 (22.45)
Intertrochanteric	41 (70.7%)
Basicervical fracture	4 (6.9%)
Tip apex distance	19±4.25
Femoral neck-shaft angle	133.64±5.48
Postoperative hospital stay	3.95±1.49
Hospital stay duration	5.55±2.13
Fluoroscopy from perpendicular to the ground inlet angle[1]	23.5±2.9

[1]The angle between the vertical position of fluoroscopy and the target inlet position when reached, line D-C / DOC angle in Figure 4

DISCUSSION

The current investigation pertains to the implementation of an intraoperative fluoroscopic lateral projection of the femoral neck, which augments the meticulous evaluation of the placement of fixation materials and their relative proximity to the joint. The precise localization of the metallic fragment exterior to the joint was successfully attained by employing a 45° inlet view without adduction during the fluoroscopic assessment of the sawbone model. Consequently, two crucial factors were emphasized in the intraoperative visualization of this true hip lateral angle: (1) the consideration of adduction caused by the patient's LDP for fluoroscopy, which necessitates the calculation of the appropriate starting point; and (2) the acquisition of the 45° inlet view, as demonstrated in the sawbone model. Given the variation in adduction angles among LDP patients, we believe that the individualized assessment of this imaging technique is essential for each patient as it facilitates the prevention of complications and ensures the accurate placement of blades/screws, ultimately leading to effective treatment outcomes. When nailing proximal femoral fractures, three critical criteria

should be met: avoiding varus reduction, achieving an appropriate TAD, and inserting the helical blade/screw in the correct quadrant.[1,21,22]

In the the present study, based on the information we were able to visualize and obtain through intraoperative C-arm fluoroscopy, we demonstrated that the fluoroscopic lateral view of the femoral neck helps in achieving the desired values in the three crucial criteria for proximal fixation in PFN applications. In the surgical treatment of femoral fractures, although PFN is an effective method, the choice between supine decubitus positions and LDPs remains a controversial issue.[9,13,14] It is known that incorrect positioning of the proximal fixation material can lead to inadequate fracture reduction stability and negatively affect the patient's functional outcomes.8 Therefore, obtaining adequate true AP and lateral fluoroscopic images during the intraoperative period is crucial.[21-24] Better visualization of the implant placement during surgery, makes fracture fixation easier by closed reduction and preventing the need for transitioning to an open reduction with additional incisions.

Numerous studies have described different imaging techniques in this regard.[7,8,23,24] Aibinder et al.[23] proposed the use of sequential fluoroscopic rollover images to reliably identify the absence of posterosuperior screw in-out-in application in cases of femoral neck fractures. However, due to a significant increase in the number of fluoroscopic shots, this approach is considered impractical.[7] Schep et al.[24] in their 2002 study on hip region validation of fluoroscopy, emphasized the importance and difficulty of obtaining true AP and lateral images and highlighted the potential complications associated with virtual images. They reported that obtaining a lateral image could be achieved only by taking perpendicular shots in the lateral view during the sawbone study. In our study we ensured that the fluoroscopy position for the femoral neck lateral image was appropriately set at a 90° perpendicular angle. In the supine position, 150° oblique tangential and 30° oblique tangential fluoroscopic images have been described as enhancing the visibility of the lateral view of the hip.[7,8] Apart from the study by Bishop et al.[25] where better hip lateral images were obtained by directing the imaging perpendicular to the ground and tilting posteriorly by 20-30° due to femoral neck anteversion, no studies have been conducted for the LDP.

Studies have shown that a TAD of less than 25 mm is an effective indicator, especially for cutout complications.[18,26,27] Nikoloski et al.[26] reported that only 54% of the cases had a TAD of 25 mm or less, with an 18.6% implant-related complication rate and a 6.2% cutout rate. They found a significantly higher incidence of cutout complications when the TAD was above 30 mm. Herman et al.[21] applied the standard surgical technique for PFN and found an average TAD of 20.3 in the complication-free group, while the group with cutout complications had an average TAD of 24.0. Sadic et al.[27] reported an average TAD of 25.6, with only 53% of the cases having a TAD below 25 mm.

Sonmez et al.[9] determined the average TAD to be 25.04 mm and Turgut et al.[14] reported an average TAD of 29.2 mm by positioning the hip at 90° flexion and 30-40° abduction in lateral- mode fluoroscopy in the LDP. In the present study, the average TAD was found to be 19 mm, by using inlet fluoroscopic position and only four cases (6.9%) had a TAD of 25 mm or above. Although Nikoloski et al.[26] mentioned the possibility of encountering cutout complications despite an appropriate TAD, the present study demonstrated that inappropriate intraoperative or postoperative hip lateral radiographs obtained with pain could yield a misleading TAD, as observed in angular imaging models performed with sawbones (**Figure 1**). According to Turgut et al.[17] to avoid complications in PFN, the NSA should be greater than 130°, and avoiding varus reduction is among the most crucial factors preventing cutout complications. In the present study, the average NSA was 133.64±5.48. A statistically significant correlation was found between the NSA and the patients' postoperative length of stay. Based on this information, it is suggested that NSA not only plays a significant role in the risk of cutout but also affects the patient's functional outcome.

Cleveland and Bosworth divided the femoral head and neck into nine quadrants.[1,9,28] For mechanical strength, insertion of the proximal fixation device into the central- central or inferior-central quadrant is recommended.[1,16,17,29] The superior-posterior quadrant is considered the most unsuitable position.[29] The importance and correlation of TAD and quadrant position are evident, highlighting the increasing significance of obtaining a true lateral projection during the operation. By determining the individual's optimal fluoroscopy angle and inlet view, a clearer and more accurate image than the conventional position will be achieved. According to the Cleveland zone, among our patients, 63.8% had a central-central quadrant placement, and 12.1% had a central-inferior quadrant placement. Thus, 75.6% of our patients achieved the most suitable position. Aguado-Maestro et al.[1] reported a total rate of 77.3%, Nikoloski et al.[26] reported 72.5%. and Sacid et al.[27] found 50% of the cases in the recommended quadrants. The present study demonstrated similar or better results in this regard.

Hengg et al.[31] highlighted that the graph obtained from the patient's position in the upper extremity can lead to incorrect treatment and surgical outcomes. It would be inappropriate to assume that the same applies to the lower

extremity. Specifically, during hip surgery, obtaining a high-quality lateral image necessitates adjusting the fluoroscope angle to make it perpendicular to the femoral neck.[24] However, due to the wide range of tube angles in standard imaging practices for the hip, it is believed that accurate data may not be obtained.[19,20] Consequently, the importance of individualized X-ray tube angles in the diagnosis and treatment of patients is increasing.[32,33]

In this paper, we emphasize the need to use individualized X-ray tube angles instead of standard angles in the assessment of functional and clinical outcomes related to the accurate placement of the femoral neck implant in conditions such as hip fractures. Such an individualization not only has the potential to shorten the surgical duration, which is particularly crucial in the elderly patient group but also holds clinical significance by reducing radiation exposure time for healthcare workers, prioritizing their health.

The present study had some limitations. First, it was a retrospective study, which could have introduced bias and limitations in the data collection and analysis. Second, the sample size was relatively small, and while our findings hold promise, their generalizability to a larger and more diverse patient population may be somewhat constrained. Third, the use of only the sawbone model for evaluating the central position may not fully represent the clinical scenario and the variability that can occur in actual patients. These constraints should be kept in mind while interpreting the results of the current study, emphasizing the need for future research endeavors with expanded sample sizes and prospective designs to substantiate the findings presented in this study.

CONCLUSION

The conventional nailing technique of proximal femoral fractures using an LDP and neutral fluoroscopy view may not achieve optimal quadrant placement. However repositioning of fluoroscopy with a 45° inlet angulation from the initial reference point aligned with the femur, considering individual adduction, provides improved lateral visualization of the femoral neck. We also emphasize the importance of capturing lateral images with the same position in the postoperative X-rays. This ensures that the graph is taken with the correct positioning, thus providing valuable information for post-surgical evaluation.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Kastamonu University Clinical Researches Ethics Committee (Date: 19.04.2023, Decision No: 2023-KAEK-48).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The effects of frailty on quality of recovery and complications in older adults undergoing major abdominal surgery: a prospective cohort study

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ABSTRACT

Aims: There is an increase in the number of older people who have undergone surgical intervention in proportion to the aging of the global population. This situation creates the need to adapt surgical care according to the pathophysiological profile of older people. Recently, it has been argued that chronological age alone does not explain biological age, and frailty will be an appropriate parameter in organizing surgical care of older people. This study aimed to determine the effect of frailty on 30-day postoperative complications and quality of recovery in older people undergoing major abdominal surgery.

Methods: A prospective cohort study was conducted in the General Surgery Department of the Erciyes University Medical Center in Türkiye. Overall, 222 patients aged 65 and over underwent major abdominal surgery between February 2021 and 2023. Frailty was determined using Fried Frailty Index. 30-day postoperative complications were evaluated using the Clavien Dindo Classification. Quality of recovery- 40 (QoR-40) was filled three days after surgery to determine the quality of recovery of the patients. Receiver-operating characteristic curves analysis was used to evaluate the ability to predict 30-day complications of frailty. Univariate linear regression analysis was performed to determine frailty to be an independent predictor of the QoR-40.

Results: The majority of the participants were male (56.8%), the mean age was 71.41±5.29 years, and 50% of patients were frail. Frail patients (28.1%) showed a higher rate of major complications compared to non-frail patients (9.3%). The Fried frailty index score significantly predicted 30-day postoperative complications (AUC=0.653, 95%=0.565-0.741). The total mean score of the QoR-40 scale was 147.09±15.82. Univariate linear regression analysis found frailty (OR -3.81, 95% CI -4.79- -2,83), age (OR -0,46, 95% CI -0,79- -0,12), Charlson comorbidity index (OR -2,40, 95% CI -3,23- -1,57), and operation time (OR -0,04, 95% CI -0,06- -0,02) as independent predictors of quality of recovery.

Conclusion: Frailty is a significant predictor for 30-day postoperative complications and quality of recovery in older people undergoing major abdominal surgery.

Keywords: Frailty, abdominal surgery, quality of recovery, postoperative complication, older people

INTRODUCTION

Older adults are the fastest growing group of the population and the proportion of the population aged 65 years or over in the total population is increasing all over the world.[1,2] In proportion to the aging of the global population, the number of surgeries performed on older adults tends to increase. Surgical interventions, considered contraindicated for the aging population in the historical process, can be applied much more frequently and safely thanks to today's techniques and technology.[3] It is reported that 23% of all surgical procedures are performed on older adults.[4] It is estimated that one in five people over the age of 75 will have surgery in the UK and an estimated £2.7 billion will be spent by 2030.[5]

Due to the effects of aging, older adults are prone to postoperative complications and long-term recovery. While the incidence of postoperative complications in patients undergoing abdominal surgery ranges from 34.4% to 66.9%[6,7], older adults are approximately 2.5 times more likely to experience postoperative complications than younger patients.[7] Older adults constitute the majority of the "high risk" surgical population. For all these reasons, there is a need to adapt surgical care according to the pathophysiological profile of older adults.[5]

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A comprehensive preoperative risk assessment is necessary for older adults undergoing surgery for the appropriate management of postoperative care. There is a growing recognition that frailty is a risk factor for postoperative complications in older adults.[8,9] Frailty is a condition characterized by a multidomain decline in physiological reserve and function, which leads to negative consequences such as increased morbidity and mortality in the perioperative period. Not surprisingly, seven out of ten frail patients have multimorbidity.[2,8] Although current guidelines recommend assessing frailty using a valid tool for older adults, the role of frailty in the decision-making on surgery and its importance in assessing the benefits and risks of surgery is often ignored.[8] Recently, it is thought that chronological age is not enough in surgical decision making for older adults. On the contrary, frailty reflecting biological age will be an appropriate parameter for an accurate and individualized decision.[8,10] In a meta-analysis study conducted with patients undergoing major abdominal surgery, frail older adults have a twice greater risk of developing major postoperative morbidity and six times postoperative mortality compared to non-frail.[10]

Based on the studies, it would not be wrong to state that traditionally, the postoperative recovery is usually measured by parameters such as complication and death rates, length of hospital stay, and cost of hospital stay.[6,7,10] These outcomes suggest that the patient's perception is ignored and the patient-reported outcome measures are not focused on. Patient-reported outcome measurement tools should be used to evaluate postoperative recovery, which is defined as patients' return to normal state after surgery. For this purpose, the self-reported quality of recovery scale (QoR), which integrates the physical, emotional, and social aspects of recovery, is the most frequently used tool in postoperative follow-up.[11,12] This study was conducted to investigate the effect of frailty on 30-day postoperative complications and QoR in older adults undergoing major abdominal surgery.

METHODS

The study was carried out with the permission of Erciyes University Faculty of Medicine Clinical Researches Ethics Committee (Date: 07.01.2021, Decision No: 2021-480). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design and Participants

This study was a prospective, cohort study. It was conducted in the general surgery department, at a university hospital with 1200 beds in Türkiye.

The inclusion criteria were: patients undergo a major abdominal surgery, were aged ≥65 years as well as being able to speak Turkish. Exclusion criteria included patients having cognitive impairment, and/or neuropsychiatric disease and patients who refused to participate, and who were unable to undergo frailty assessment. Drop out criteria include patients who wanted to withdraw from the study and who were out of reach within 30 days after the operation.

Among 466 patients consecutively admitted in the major abdominal surgery unit between February 2021 and February 2023, 219 were excluded initially: 201 patients were less than 65 years old, 14 patients did not speak Turkish, one patients had a dementia and three patients refused to participate in the study. Additionally, 18 patients' operations were cancelled or postponed, and 7 patients could not be reached within 30 days follow-up (**Figure 1**). Thus, 222 patients were recruited for the study. Written consent forms were obtained from all participants.

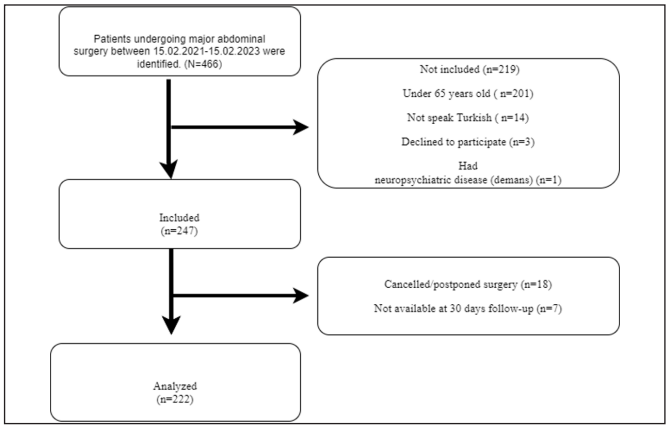

Figure 1. Flow Chart

The variables such as age, gender, diagnosis, malignancy, type of surgery, smoking, weight, height, body mass index (BMI), NRS-2002 score,[13] comorbidities, medications, Charlson comorbidity index,[14] and American Society of Anesthesiologists (ASA) physical status were included, as well as operative variables such as the types of operation (open or laparoscopic), operative time, and wound-type.

After obtaining informed consent for the study, we applied the Fried frailty index,[15] which consists of five questions: unintentional weight loss; weakness or poor handgrip strength; self-reported exhaustion; slow walking speed; and low physical activity. From the Fried Frailty Index, which is a categorical index, 0 (no) or 1 (yes) point is received for each question. Based on the Fried criteria, the three stages of frailty are listed as follows; A score of 0 indicates that the person is robust, a score of 1 or 2 indicates that the person is pre-frail, and a score of 3-5 indicates that the person is frail. In the present study, we divided our sample into two groups based on the scale and classified them as frail, not frail (pre-frail and robust).

Effect Size

The post-hoc power analysis was performed on data which comparison of the QoR-40 mean score of the frail and non-frail older adults was used in G*Power 3.1.9.4. Power (1-β) was found to be 0.97 in the power analysis made with the sample size (222), effect size (0,48), α (0,05).

Outcome Measures

This study primarily measured 30-day postoperative complications. These complications were collected from the hospital medical records and patient interviews. Patients were followed up for thirty days after the operation by researchers using the Clavien-Dindo classification. The Clavien Dindo classification, originally described in 2004, is used to rank the severity of a complication which occurs because of surgical procedure. The scale consists of five grades (Grade I, II, IIIa, IIIb, IVa, IVb, and V) (Figure 2). Clavien-Dindo classification is a very useful method for reporting outcomes of complications after major abdominal surgery.16 The higher grade represents the severity of the complication. Grade I complications are usually mild, but Grade V means the death of a patient.[17] In patients developing more than one complication, the most severe complication was recorded. In addition to 30-day postoperative complications, the length of stay in the intensive care unit (ICU) and hospital stay were followed.

Grade I:	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic, and radiological interventions Allowed therapeutic regimens are drugs as antiemetics, antipyretics, analectics. diuretics, electrolytes, and physiotherapy. This grade also includes wound infections opened at the bedside.
Grade II:	Requiring pharmacological treatment with drugs other than such allowed for grade I complications Blood transfusions and total parenteral nutrition are also included
Grade III:	Requiring surgical, endoscopic or radiological intervention
Grade IIIa:	Intervention not under general anesthesia
Grade IIIb:	Intervention under general anesthesia
Grade IV:	Life-threatening complication (including CNS complications)* requiring IC/ICU management
Grade IVa:	Single organ dysfunction (including dialysis)
Grade IVb:	Multiorgan dysfunction
Grade V:	Death of a patient

Figure 2: Classification of surgical complications according to Clavien Dindo

*Brain hemorrhage, ischemic stroke, subarrachnoidal bleeding, but excluding transient ischemic attacks. CNS, central nervous system; IC, intermediate care; ICU, intensive care unit.

Additionally, we applied the QoR-40 scale to the patients to determine postoperative recovery on the third day after the operation. The Quality of Recovery Scale (QoR-40) was filled in by interviewing the patient twenty-four hours after the surgical procedure. This scale was developed by Myles et al.[18] (2000) to measure the quality of recovery after the operation. The QoR-40, a 40-item questionnaire, is scored on a five-point Likert-type scale with a total score ranging from 40 (poor QoR) to 200 (excellent QoR). An increase in the scale total score means that the postoperative physical and emotional well-being increase, and a decrease in the score means that they are negatively affected.[18,19]

Statistical Analysis

Analyses were performed using SPSS 22 software (IBM SPSS Statistics Standard Concurrent User ver. 22). Descriptive statistics were expressed as statistical units (n), percent (%), mean (X), standard deviation (SD). The Shapiro-Wilk test was used to examine continuous variables depending on the variable distribution, and Levene test was used to check the homogeneity of the variances. In the comparison of frail and non-frail older adults, two independent samples t-test for numeric variables and chi-square test for categorical variables were used. The chi-square test and relative risk were applied to estimate the risk of developing minor and major complications of frailty according to the Fried frailty index. Multiple logistic regression analysis was used to analyze the associated factors of QoR after major abdominal surgery. $p<0.05$ was considered statistically significant.

RESULTS

Characteristics of the Patients

Among the participants, 56.8% were male, with an mean age of 71.41±5.29 years. The mean BMI of the patients was 26.67±4.97, 77.5% had a chronic illness, and 65.8% were continuously taking medication. The mean CCI was 5.54±1.98 years (Table 1).

There was a relationship among being frail, gender, and chronic disease ($p=0.018$). Although the gender of frail patients was close to each other, it was seen that non-frail individuals are mostly (64.8%) male. Frail older adults had more chronic diseases than non-frail older adults (84.2% vs 70.4%). There was a relationship between hypertension, antihypertensives and frailty ($p<0.05$). Frail patients had significantly higher age and CCI score and lower BMI than non-frail patients ($p<0.05$) (Table 1).

Of the patients, 62.2% underwent colorectal surgery, 88.3% had malignancy, 98.2% had elective and open surgery. The mean operation time was 182.70±74.90, and the mean postoperative length of stay was 11.04±5.64. 51.4% of the older adults were frail (Table 2). There was a relationship between the urgency of the surgery (0.020) and frailty. Frail patients stayed in the hospital longer time after surgery than non-frail ($p=0.014$). The difference was significant between frailty and some laboratory results; in preoperative period, hemoglobin ($p<0.001$) and albumin ($p=0.013$) levels were lower, while blood urea nitrogen levels were significantly higher in frail older adults ($p=0.033$). In the postoperative period, albumin ($p<0.001$) and total protein ($p<0.001$) levels were similarly lower in frail individuals (Table 2).

Table 1. The characteristics of the patients undergoing major abdominal surgery

Characteristics	Overall n (%)	Frail n (%)	Non-frail n (%)	Statistical test; p
Sex				X^2=5,564; p=0,018
Female	96 (43.2)	58 (50.9)	38 (35.2)	
Male	126 (56.8)	56 (49.1)	70 (64.8)	
Chronic disease				X^2=6.088; p=0.014
Yes	172 (77.5)	96 (84.2)	76 (70.4)	
No	50 (22.5)	18 (15.8)	32 (29.6)	
DM	70 (31.5)	42 (36.8)	28 (25.9)	X^2=3.061; p=0.080
HT	120 (54.1)	74 (64.9)	46 (42.6)	X^2=11.124; p=0.001
Cardiovascular diseases	36 (16.2)	22 (19.3)	14 (13.0)	X^2=1.638; p=0.201
Thyroid disorders	24 (10.8)	8 (7.0)	16 (14.8)	X^2=3.497; p=0.061
Chronic pulmonary diseases	34 (15.3)	18 (15.8)	16 (14.8)	X^2=0.041; p=0.840
Regular medication use				
Yes	146 (65.8)	80 (70.2)	66 (61.11)	X^2=2.024; p=0.155
No	76 (34.2)	34 (29.8)	42 (38.9)	
Antihypertensive	114 (51.5)	70 (61.4)	44 (38.5)	X^2=9.478; p=0.002
OAD	50 (22.5)	30 (26.3)	20 (18.5)	X^2=1.932; p=0.165
Insulin	22 (9.9)	14 (12.3)	8 (7.4)	X^2=1.475; p=0.225
Anticoagulant	24 (10.8)	12 (10.5)	12 (11.1)	X^2=0.20; p=0.888
Bronchodilator	30 (13.5)	14 (12.3)	16 (14.8)	X^2=0.305; p=0.581
	$\bar{x}$±SD			
Age	71.41±5.29	73.00±5.64	69.74±4.34	t=-4.839; p<0.001
BMI	26.67±4.97	26.03±5.08	27.35±4.79	t=1.979; p=0.049
CCI	5.54±1.98	5.84±1.69	5.24±2.24	t=-2.277; p=0.024

DM: Diabetes mellitus, HT: Hypertension, OAD: Oral antidiabetic, CCI: Charlson comorbidity index, $\bar{x}$:mean, SD: standard deviation X^2:Chi-square test, t:Independent t test

Complication Rates of the Patients and Relationship between Frailty and Complications

In Table 3, 30-day postoperative complications of frail and non-frail older adults were presented. This study demonstrated the significant relationship between frailty and postoperative complications (p<0.001). While Grade 1 complications including simple complications had more common in non-frail patients than in frail patients (40.7% vs. 15.8%), Grade 2 and 3 complications were more common in frail individuals. It was found that frail patients experienced anemia (p=0.005), electrolyte disturbance (p=0.000), abdominal distension (p=0.001), hypertension (p=0.012), and nausea and vomiting (p=0.004) more than the non-frail patients. When the complications related to the surgical site problems of the patients were analyzed, the frail patients experienced more complications of seroma (p=0.003) and wound infection (p=0.002) than the non-frail patients (Table 3).

Major complications occurred in 28.1% of the frail patients compared to only 9.3% of the non-frail patients. In bivariate analysis, frail and non-frail older adults undergoing major abdominal surgery showed a significant difference in terms of the incidence of minor or major 30-day post-operative complications (RR 3.824, 95% CI 1.774-8.246; p<0.001). According to the ROC curve analysis shown in Figure 3, the Fried Frailty Scale score effectively predicted the severity of postoperative complications (AUC=0.653, 95%=0.565-0.741) (Table 4).

Table 3. 30-day postoperative complications associated with the frailty of the patients

Complication	Non-Frail n (%)	Frail n (%)	Statistical test, p
Clavien Dindo Classification			X^2=25.843 p<0.001
1	44 (40.7)	18 (15.8)	
2	54 (50.0)	64 (56.1)	
3	6 (5.6)	28 (24.6)	
4-5	4 (3.7)	4 (3.5)	
Anaemia	50 (46.3)	74 (64.9)	X^2=7.795 p=0.005
Electrolyte disorders	70 (64.8)	102 (89.5)	X^2=19.325 p<0.001
Abdominal distension	16 (14.8)	38 (33.3)	X^2=10.332 p=0.001
HT	54 (50.0)	76 (58.6)	X^2=6.348 p=0.012
Hyperglycaemia	16 (14.8)	26 (22.8)	X^2=2.309 p=0.129
Requiring blood transfusions	18 (16.7)	18 (15.8)	X^2=0.31 p=0.859
Seroma	16 (14.8)	36 (31.6)	X^2=8.689 p=0.003
Nausea	36 (33.3)	60 (52.6)	X^2=8.415 p=0.004
TPN requirement	22 (20.4)	36 (31.6)	X^2=3.601 p=0.057
Wound infection	6 (5.6)	22 (19.3)	X^2=9.503 p=0.002
Ex	0 (0.0)	2 (1.8)	X^2=1.590 p=0.207

HT: Hypertension, TPN: Total Parenteral Nutrition, Ex: Exitus, X2:Chi-square test, Percentages show patients who was developed postoprative complications.

Table 2. The characteristics of patients related to surgery

Characteristics	Overall n (%)	Frail n (%)	Non-frail n (%)	Statistical test, p
Type of surgery				X^2=5.479; p=0.242
Colorectal surgery	138 (62.2)	72 (63.3)	66 (61.1)	
Gastrectomy	70 (31.5)	38 (33.3)	32 (29.6)	
Pancreatectomy	8 (3.6)	2 (1.8)	6 (5.6)	
Liver resection	4 (1.8)	2 (1.8)	2 (1.9)	
Whipple	2 (0.9)	0 (0.0)	2 (1.9)	
Malignancy				X^2=0.318; p=0.573
Yes	196 (88.3)	102 (89.5)	94 (87.0)	
No	26 (11.7)	12 (10.5)	14 (13.0)	
ASA				X^2=0.695; p=0.404
ASA I-II	180 (81.1)	90 (79.0)	90 (83.3)	
ASA III-IV	42 (18.9)	24 (21.0)	18 (16.7)	
Urgency				X^2=5.401; p=0.020
Emergency	4 (1.8)	4 (3.5)	0 (0.0)	
Elective	218 (98.2)	104 (96.5)	108 (100)	
Surgical approach				X^2=0.003; p=0.956
Open	218 (98.2)	112 (98.2)	106 (98.1)	
Laparoscopic	4 (1.8)	2 (1.8)	2 (1.9)	
Stay in the ICU				X^2=2.502; p=0.114
Yes	90 (40.5)	52 (45.6)	38 (35.2)	
No	132 (59.5)	62 (54.4)	70 (64.8)	
		$\overline{x}$±SS		
Operation time	182.70±74.90	180.43±67.55	185.09±82.20	t=0.462; p=0.645
Postoperative length of stay (day)	11.04±5.64	12.01±6.05	10.00±5.56	t=-2.475; p=0.014
Preoperative laboratory testing				
Hgb	12.21±1.97	11.63±1.90	12.82±1.86	t=4.697; p<0.001
Alb	4.09±0.79	3.97±1.01	4.23±0.41	t=2.512; p=0.013
BUN	15.75±5.46	16.51±5.46	14.95±5.37	t=-2.145; p=0.033
CR	1.04±0.88	1.03±0.87	1.05±0.90	t=0.096; p=0.924
AST	22.13±9.7	21.31±7.91	22.99±11.29	t=1.283; p=0.201
ALT	16.65±11.89	14.99±8.13	17.41±14.70	t=1.157; p=0.235
Total protein	6.82±0.78	6.63±0.87	7.02±0.61	t=3.895; p<0.001
Postoperative laboratory testing				
Hgb	10.91±2.23	10.69±2.63	11.15±1.70	t=1.534; p=0.127
Alb	3.19±0.62	3.01±0.62	3.37±0.57	t=4.521; p<0.001
BUN	16.46±14.00	17.71±18.49	15.15±6.37	t=-1.397; p=0.165
CR	0.85±0.40	0.87±0.51	0.83±0.23	t=-0.715; p=0.476
AST	37.54±62.74	33.63±52.73	41.67±71.84	t=0.954; p=0.341
ALT	28.14±39.03	26.44±37.76	29.93±40.42	t=0.666; p=0.506
Total protein	5.47±0.79	5.29±0.83	5.66±0.70	t=0.206; p<0.001

ICU: Intensive care unit, ASA: American Society of Anesthesiologists physical status classification system, Min: Minute, Hgb: Haemoglobin, Alb: Albumin, BUN: Blood urea nitrogen, CR: Creatine, AST: Aspartat aminotransferaz, ALT: Alanin aminotransferaz, $\overline{x}$:mean, SD: standard deviation, X^2:Chi-square test, t:Independent t test

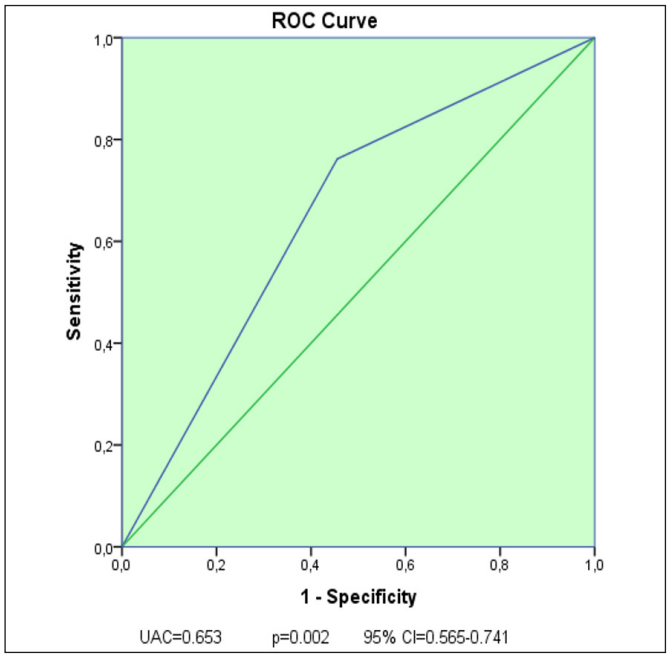

Figure 3. Roc analysis

Table 4. Bivariate Analysis between Frailty Impact on 30-day Major Abdominal Surgery Complications

Frailty	Complications		Relative Risk	95% CI	p*
	Minor	Major			
Non-frail	98 (90.7)	10 (9.3)	3.824	1.774-8.246	<0.001
Frail	82 (71.9)	32 (28.1)	Ref.		

*Chi-square test

The Relationship between Patients' Quality of Recovery Scale Scores and Frailty

In Table 5, QoR-40 scores of older adults who underwent major abdominal surgery are presented. The mean QoR-40 total score of the patients were 45.63±5.18 for the comfort subscale, 33.93±4.35 for the emotional state subscale, 9.77±3.58 for physical independence, 29.00±4.30 for the psychological support subscale, 28.75±3.41 for the pain subscale, and 147.09±15.82 for the total score.

Total and sub-scale scores of the QoR-40 of frail patients were lower than the non-frail patients, and this difference was statistically significant (p<0.05) (Table 5).

The model constructed to determine the risk factors affecting the quality of recovery in elderly individuals after major abdominal surgery explained 45% of the QoR score (R2=0.451, F=46.338, p<0.001). Multiple logistic regression analysis showed that the Fried Frailty Scale was associated with postoperative complications (OR -3.81, 95% CI -4.79- -2.83). In addition, QoR decreased as the age increases (OR -0.46, 95% CI -0.79- -0.12), CCI (OR -2.40, 95% CI -3.23- -1.57), and operation time (OR -0.04, 95% CI -0.06- -0.02) (Table 6).

Table 6. Factors affecting quality of recovery after major abdominal surgery

Model	OR	95 % CI	p
Fried frailty scale	-3.816	-4.796- -2.837	<0.001
Age	-0.460	-0.793- -0.127	0.007
CCI	-2.403	-3.232- -1.575	<0.001
Operation time	-0.042	-0.063- -0.021	<0.001

CCI: Charlson comorbidity index, OR: Odss ratio, Cl: confidence interval, *Multiple logistic regression

DISCUSSION

Considering the increase in the number of surgical interventions and the high prevalence of frailty in the geriatric population, studies on predicting and improving geriatric patient outcomes have become a necessity. Much research has been devoted to the prediction of surgical complications and its demonstrated relationship with many healthcare quality indicators such as length of hospital stay and healthcare costs.[10,20-22] In both clinics and studies, parameters such as the quality of recovery based on patient's self-assessment, in which the traditional approach is adopted, appear to be an overlooked outcome of surgical care. To our knowledge, the present study is the first study to examine the relationship between frailty and quality of recovery in older adults undergoing major abdominal surgery. Our findings demonstrated the effect of frailty on predicting postoperative complications and quality of recovery in older adults undergoing major abdominal surgery.

Table 5. Quality of recovery scale scores of the patients undergoing major abdominal surgery

QoR-40	Min-Max	Overall $\bar{x}$±SS	Frail $\bar{x}$±SS	Non-frail $\bar{x}$±SS	p
Comfort	34-71	45.63±5.18	44.66±5.44	46.64±4.71	t=2.891; p=0.004
Emotional state	24-45	33.93±4.35	32.03±3.68	35.99±4.11	t=7.466; p<0.001
Physical independence	5-23	9.77±3.58	8.12±2.21	11.51±3.92	t=7.887; p<0.001
Psychological support	13-35	29.00±4.30	27.54±4.51	30.53±3.47	t=5.511; p<0.001
Pain	18-34	28.75±3.41	28.17±3.02	29.37±3.69	t=2.629; p=0.009
Total scale	109-136	147.09±15.82	140.54±14.01	154.01±14.69	t=6.993; p<0.001

QoR-40:quality of recovery-40 scale, $\bar{x}$:mean, SD: standard deviation, t:Independent t test

First, given the results of the present study regarding postoperative complications, frailty is significantly associated with the severity of 30-day postoperative complications following major abdominal surgery. It has been revealed that frail individuals have three times higher risk of developing major complications (Clavien Dindo Classification 3-5) than the non-frail individuals (28.1% vs 9.3%). In a meta-analysis study focusing on older adults undergoing emergency abdominal surgery, frailty was identified as a risk factor for 30-day mortality. Frail patients have four times greater risk of 30-day mortality compared to the non-frail patients (OR 4.3, 95% CI 2.25-8.19).[23] In a prospective cohort study of patients (n=245) undergoing major thoracic and abdominal surgery, Han et al.[24] (2019) found frailty to be an effective predictor of postoperative complications. Moreover, the area under the curve (AUC) for frailty for prediction of postoperative complications was 0.762 (95% CI 0.703-0.814). Similarly, in our study, frailty was predicted as a tool to assess the risk of complications (AUC 0,653, %95 0,565-0,741). Aceto et al.[25] (2021) conducted a prospective cohort study (n=105) to determine the effect of frailty in predicting pulmonary complications after major abdominal surgery. It was demonstrated that frail patients were exposed to a higher risk of pulmonary complications after major abdominal surgery, and frailty was an important factor in predicting postoperative pulmonary complications (AUC 0,90, %95 Cl 0,565-0,741). In a prospective cohort study to examine the effect of frailty after emergency abdominal surgery in older adults (n=109), loss of functional independence at first year was researched using two frailty measures (Fried and Frailty index-11). In the study, Fried frailty Index was significantly associated with loss of functional independence at first year (OR 13.00, 95% CI 2.21-76.63).[26] In another study (n=104) in which frailty was evaluated as a predictor for postoperative complications in older adults undergoing major abdominal surgery, frail patients showed significantly longer hospital stays, ICU admissions, readmissions, and higher mortality rates. In addition, frailty was identified as an independent predictor for 30-day perioperative complications (AUC 0.75).[21] In another study, the Fried frailty index were found to be predictive of one-year mortality in major abdominal surgery.[27] The findings of our study corroborate the findings of the previous studies.

Considering the existing literature, there is no common tool used in assessment of frailty. More than 70 documented frailty measurement tools have been reported in the literature, ranging from short questionnaires to long assessments and patient examinations.[23] It has also been observed that the FRAIL scale,[24] frailty scale,[25] modified Rockwood frailty index,[21] fried frailty index,[27] and clinical frailty scale[28] are used in patients undergoing major abdominal surgery. It is important to agree on a common frailty tool in the assessment of frailty in surgical patients.

In our study, the mean score of the QoR-40 on the postoperative third day of the older adults who had major abdominal surgery was 147.09±15.82. According to these results, it can be stated that the recovery of quality of the patients is above the medium level. Oreskov et al.[29] (2020) used the QoR-15 scale in a prospective observational cohort study in which they evaluated the quality of recovery of patients undergoing emergency major abdominal surgery. In the study, it was reported that patients showed poor and moderate quality of recovery in the first seven days postoperatively. It is considered that this may be because patients who are taken to emergency surgery have worse patient outcomes in the preoperative period. In another cross-sectional study (n=105), which was conducted to examine the relationship between frailty level and quality of recovery in elderly patients hospitalized in the neurosurgery clinic, the mean QoR-40 score of the patients was found to be 134.49±11.09.[30] When studies on the quality of recovery are examined, there is a limited number of studies involving orthopedic surgery,[31] day surgery,[32] oncologic surgery,[33] and/or all elective surgery patients,[34,35] in the literature. It would not be wrong to claim that the quality of recovery is a patient outcome criterion that is less valued in the post-surgical follow up of patients.

In the model established to determine the quality of recovery in older adults undergoing major abdominal surgery; age, CCI, frailty, and operation time were significantly associated with the quality of recovery. Our results show that as patients' Fried frailty index score increases, their Q0R-40 total score decreases (OR -3.81, 95% CI -4.79- -2,83). In an observational prospective study (n=138), which was conducted to determine the predictors of poor-quality recovery after cancer surgery, one-third of the patients consisted of gastrointestinal cancer patients. In the study, there is a relationship between poor healing quality and frailty, and it was reported that patients with poor healing quality were frailer.[36] In a prospective cohort study evaluating frailty's ability to predict the quality of postoperative recovery in patients with gynecological cancer, Liu et al.[37] (2023) determined that frailty was a significant predictor of the 3-day QoR-15 score (OR 11.69, 95% CI 4.26-32.08). Günel and Özşaker[30] (2021), in their study dealing with the neurosurgery clinic, stated that there was a negative and weak correlation between frailty and the total score of the QoR-40. (r=-0,336, p<0,05). Despite the limited number of studies, it may be possible to say that frailty is a determinant of poor quality of recovery.

Limitations

There are some limitations in this study. First, it may be that the study included results from a single center. In addition, not following the long-term complications of the patients is a limitation of the study.

CONCLUSION

In this study, frailty is a significant predictor for 30-day postoperative complications and poor quality of recovery older adults undergoing major abdominal surgery. The Fried frailty index can be used in preoperative risk examination because of its ability to predict postoperative complications and quality of recovery. In both clinical and studies, there is a need for using more patient outcome parameters based on patient self-assessment. Long-term studies are recommended to search the effect of frailty on the long-term patient outcomes of older adults undergoing major abdominal.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Faculty of Medicine Clinical Researches Ethics Committee (Date: 07.01.2021, Decision No: 2021-480).

Informed consent: Informed consent was obtained from the participants.

Referee Evaluation Process: Externally peer reviewed.

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

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Author Contributions: All the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The relationship between serum soluble ACE 2 protein level and the clinical course of COVID-19 disease

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ABSTRACT

Aims: The angiotensin converting enzyme 2 (ACE 2) molecule, which mediates the entry of the virus into the cell, plays a very important role in the pathogenesis of COVID-19 disease. However, its effect on prognosis has not been fully explained. In this study, it was aimed to investigate the relationship between soluble ACE 2 (sACE 2) levels in the blood and the course of the disease.

Methods: sACE 2 levels at 0, 3 and 5 days were measured in patients with mild, moderate and severe COVID-19 pneumonia who were hospitalized between March 15, 2020 and August 30, 2020.

Results: 69 patients, 35 (51.5%) female and 34 (49.3%) male, with a mean age of 64.3±2.1 were included in the study. 42.0% of the patients had mild, 30.4% moderate, 27.5% severe pneumonia. Clinical follow-up of 7 patients resulted in death. There was no statistically significant difference between sACE 2 levels and gender, severity of pneumonia, initial hospitalization, presence of intubation and mortality.

Conclusion: sACE 2 levels were not associated with disease severity and inflammatory markers. Studies in larger patient populations are needed to explain the relationship between sACE 2 activity and SARS-CoV-2 infection and to develop new treatment strategies.

Keywords: COVID-19, angiotensin converting enzyme 2, prognosis

INTRODUCTION

Coronavirus-associated disease 19 (COVID-19), caused by a novel type of beta coronavirus, "Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)", has triggered a pandemic shortly after the first cases appeared in China in December 2019. The disease can cause a variety of clinical manifestations ranging from an asymptomatic course to mild upper respiratory tract infection, severe pneumonia, multiple organ failure, and death.[1-3]

Unfortunately, personal protective measures such as wearing masks, social distancing, hand hygiene, and lockdown measures have not proven effective enough in containing the outbreak. The molecule angiotensin converting enzyme 2 (ACE 2), which plays a key role in the pathogenesis of the disease and mediates viral entry into cells, has been the focus of most of the vaccine and drug development efforts, which are proceeding at a dizzying pace.[4] In the human body, ACE 2 is expressed in the central nervous system, lungs, cardiovascular system, adipose tissue, intestines, and kidneys.[5] The expression and distribution of ACE 2 in the human body may indicate potential infection pathways for SARS-CoV-2, so organs whose cells express high levels of ACE 2 should be considered high-risk areas for SARS-CoV-2 infection.[6] The large surface area of alveolar epithelial cells, particularly in the lung, may explain the susceptibility of this organ to the consequences of viral invasion. SARS CoV-2 uses the spike (S) protein to invade human alveolar epithelial cells. S protein binds to the ACE 2 receptor upon host cell invasion.[7,8] Therapeutic monoclonal antibodies targeting neutralizing epitopes on the S protein and vaccine-induced antibodies block viral binding to ACE 2, thereby preventing viral entry into cells.[9-11]

ACE 2, a transmembrane glycoprotein, is also an important component of the renin-angiotensin system (RAS).[4] ACE 2 reduces inflammation, fibrosis, and thrombosis mainly by converting Ang (angiotensin) I and Ang II to Ang 1-9 and Ang 1-7, respectively, and shows a protective effect against tissue damage in the organs where it is expressed.[12] Acute respiratory distress syndrome (ARDS) is a condition associated with high clinical mortality and ACE 2 has a protective effect on this type of acute lung injury.[13,14]

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Circulating ACE 2 levels in healthy individuals are very low and difficult to detect.[15] ACE 2 in the cell membrane can be cleaved and excreted into the circulation by both a disintegrin and metalloproteinase 17 (ADAM17) and a type II transmembrane serine protease (TMPRSS2). ADAM17 cleavage is a normal pathway that results in the production of circulating ACE 2 (soluble ACE 2, sACE 2).[5,13] Since soluble ACE 2 contains the virus binding site, it can also bind to the virus and thus facilitate virus entry.[4] Circulating ACE 2 can prevent severe pathological conditions and protect organs during SARS-CoV-2 infection. Meanwhile, cleavage by TMPRSS2 results in elevated SARS-CoV cell entry, allowing SARS-CoV-2 to invade cells in the lungs and intestines. The TMPRSS2-division pathway can block the ADAM17 division pathway. If the immune system fails to combat the virus, SARS-CoV-2 invades cellular ACE 2, where it proliferates greatly and destroys host cells in the lung and intestine.[13] The physiological and pathological effects of sACE 2 on particular organs or tissues are being investigated at present, but a good understanding of the mechanism behind these effects has not yet been established.[4]

Recent studies have demonstrated that the poor prognosis of COVID-19 patients is associated with sex (male), age (>60 years), underlying diseases (hypertension, diabetes, and cardiovascular disease), secondary ARDS, and other relevant factors. Owing to the protective effects of ACE 2 on these chronic underlying diseases and ARDS, the development of drugs that increase ACE 2 activity may be one of the most promising approaches for the treatment of COVID-19 in the future.[13,14] This subject, however, requires further research.

In this study, we aimed to investigate the effects of serum soluble ACE 2 levels on the clinical course of COVID-19 disease.

METHODS

The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Researches Ethics Committee (Date: 11/09/2020, Decision No: 2020/432). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was designed retrospectively. It was planned to study serum soluble ACE 2 protein levels by using Elisa kits from the samples taken for routine examinations on the day of hospitalization, on the 3rd and 5th days of hospitalization from patients with the mild, moderate, and severe clinical course of COVID-19 pneumonia, who were hospitalized in the infectious diseases pandemic ward and intensive care unit of Afyonkarahisar Health Sciences University Hospital between March 15, 2020, and August 30, 2020, as well as to retrospectively analyze the patient records, to examine the relationship of serum soluble ACE 2 protein levels with laboratory and clinical parameters affecting prognosis and to evaluate the effect of serum soluble ACE 2 protein levels on patient prognosis.

Participants

Patients who were found SARS-CoV-2 positive using the reverse transcriptase polymerase chain reaction (RT-PCR) method and who were found to be compatible with viral pneumonia in the lung tomographic examination were included in the study. According to the chest CT interpretation of the patients at the time of presentation, the presence of a ground glass density of less than 3 cm in all three foci was classified as mild pneumonia, while the presence of ground glass density or consolidation in more than three foci or greater than 3 cm was classified as moderate pneumonia and when there was involvement in all lobes of both lungs with at least three of the lesions larger than 3 cm were classified as severe pneumonia.[16]

Laboratory Method

Venous blood samples were collected from the patients in a 10 ml gel Vacutainer SST tube (Becton Dickinson, France). After waiting for 30 min, it was centrifuged at 2000 g for 10 min at 4°C. The serum samples remaining after centrifugation were separated into Eppendorf tubes and stored at -80°C until the tests were performed.

ACE 2 was measured in serum using BT-LAB Human ACE 2 Elisa kit (BT Lab Bioassay Technology Laboratory, Zhejiang, China). Absorbance reading was performed on a ChemWell 2910 Elisa reader (Awareness Technology, Inc. Martin Hwy. Palm City, USA). Results were expressed in ng/ml.

Statistical Analysis

In descriptive statistics, mean, standard deviation, median, and distance between quartiles were used for continuous variables, while numbers and percentages were used for nominal variables. Normality distributions of continuous variables were analyzed via Kolmogorov Smirnov and Shapiro-Wilk tests. The differences between the measurements of ACE 2 and other continuous variables on days 0, 3, and 5 were determined by Friedman's analysis of variance test and posthoc Dunn test (Bonferroni correction) in dependent measures. Differences in ACE 2 values between independent groups were analyzed via Mann-Whitney U-test in paired groups and Kruskall-Wallis tests in groups of more than two. The correlation between age and ACE 2 levels was determined by Spearman Correlation analysis. The results were considered significant at $p<0.05$. Statistical analyzes were conducted via SPSS 28.0 software.

RESULTS

A total of 69 patients were included in the study. The mean age of the patients was 64.3±2.1 years and 35 (51.5%) were female. Sixty-five percent of the patients had a history of chronic disease. The first hospitalization place of 79.7% of the patients was the ward, and 6 patients were transferred from the ward to the intensive care unit (ICU). Of the patients, 42.0% had mild pneumonia, 30.4% had moderate, and 27.5% had severe pneumonia. Ten patients had been intubated and seven patients had died. The characteristics of the patients are presented in Table 1.

Table 1. Characteristics of the patients

Characteristics **Age (n=69)**	**(Mean±SD)** **64.3±2.1** **n**	**Median (IQR)** **69 (23)** **%**
Sex (n=69)		
Female	35	50.7
Male	34	49.3
Place of first hospitalization (n=69)		
Ward	55	79.7
Transferred from ward to ICU	6	8.7
ICU	14	20.3
Pneumonia Severity (n=69)		
Mild	29	42.0
Moderate	21	30.4
Severe	19	27.5
Intubated (n=69)	10	14.5
Prognosis (n=69)		
Healing	62	89.9
Death	7	10.1
Smoker (n=68)	7	10.3
Comorbidities (n=69)		
Any chronic disease	45	65.2
Hypertension	33	47.8
Diabetes	32	46.4
Heart failure	17	24.6
Renal failure	14	20.3
COPD	10	14.5
Malignancy	10	14.5
Liver failure	2	2.9

No significant difference was found between ACE 2 levels. However, there was a significant decrease in Hgb (p=0.036), and Neu/Lym (0.004) levels on day 5 compared to baseline values (Posthoc Dunn test with Bonferroni adjustment). On the other hand, no significant difference was found between the measurements according to days in terms of other parameters (Table 2).

There was no significant correlation between sex, severity of pneumonia, place of first hospitalization, presence of intubation, mortality status, and all three ACE 2 measurements (Table 3). There was no significant correlation between the ACE 2 measurement made during hospitalization and the patient's age, whereas a significant negative correlation was determined in the measurements on the 3rd and 5th days.

DISCUSSION

In this study, sACE 2 levels were evaluated in blood samples taken on the day of admission, on the 3rd and 5th days of hospitalization of 69 patients with COVID-19 pneumonia who were hospitalized in the pandemic ward and intensive care unit of Afyonkarahisar Health Sciences University Hospital between March 15, 2020, and August 30, 2020.

Two recent studies have shown that sACE 2 binds to SARS-COV-2 and then mediates its entry into cells.17,18 These findings suggest that ACE 2 scattering and sACE 2 have a role in SARS-CoV-2 infection. However, it is not fully understood what effect they have on the clinical course of the disease, either positively or negatively.[4] ADAM17 inhibition is predicted to reduce ACE 2 trimming, viral entry, and cytokine production. Clinical grade recombinant human ACE 2 (rhACE 2), a type of exogenous soluble form of ACE 2, binds to SARS-COV-2 in human tissues and inhibits virus infection.[19] Thus, it would be very useful to develop treatment regimens for the inhibition of ADAM 17, which is involved in the producer mechanism of sACE 2 in COVID-19, and for the therapeutic use of rhACE 2 in COVID-19. In the literature, there are very few studies that address this topic.

Table 2. ACE 2 level measurements, complete blood counts, and some biochemical values of the patients

Day		**P***	**(Mean±SD)**	**Median (IQR)**		**P***	**(Mean±SD)**	**Median (IQR)**
0	ACE 2	0.933	2.48±1.87	1.82 (1.77)	Neu/Lym	0.005	10.50±22.39	3.97 (5.08)
3			2.72±2.24	1.96 (2.07)			9.18±17.41	4.28 (5.26)
5			2.71±2.21	1.91 (1.77)			6.58±6.85	3.79 (5.00)
0	Wbc	0.796	8.21±5.16	6.68 (6.03)	D-Dimer	0.694	3.14±8.53	1.20 (2.23)
3			7.97±4.53	6.83 (4.86)			2.13±3.04	1.22 (2.32)
5			7.63±4.39	7.04 (4.59)			1.95±2.20	1.54 (2.16)
0	Hgb	0.028	11.03±2.53	10.50 (4.40)	Crp	0.339	54.91±68.62	24.36 (78.99)
3			10.82±2.45	10.30 (3.90)			48.97±63.41	24.40 (69.55)
5			10.43±2.61	10.20 (3.25)			40.94±48.31	17.94 (62.99)
0	Plt	0.148	209.42±102.17	200.00 (88)	Ferritin	0.137	445.79±524.33	272.80 (417.05)
3			206.14±116.68	185 (121)			440.68±453.37	314.90 (395.80)
5			221.25±125.33	195 (143)			515.05±529.78	345.30 (511.35)
0	Lym	0.694	1.15±0.66	0.93 (0.91)	Procalcitonin	0.152	4.99±20.45	0.08 (0.24)
3			1.20±0.72	1.07 (0.89)			2.79±10.77	0.09 (0.33)
5			1.37±1.45	1.12 (1.05)			1.34±4.59	0.07 (0.32)

*Friedman's Two Way Analysis of Variance

Table 3. ACE 2 levels by different parameters

	ACE 2 (Baseline)		ACE 2 (Day 3)		ACE 2 (Day 5)	
	(Mean±SD)	Median (IQR)	(Mean±SD)	Median (IQR)	(Mean±SD)	Median (IQR)
Sex						
Female	2.59±2.08	1.82 (1.75)	2.84±2.43	2.09 (2.32)	2.66±2.23	1.90 (1.77)
Male	2.36±1.65	1.78 (1.79)	2.58±2.05	1.91 (1.93)	2.76±2.21	2.01 (1.86)
P*	0.810		0.783		0.924	
Age						
p, r**	0.094, -0.203		0.009, -0.313		0.025, -0.270	
Severity of pneumonia						
Mild	2.29±1.70	1.68 (1.18)	2.47±2.18	1.91 (1.36)	2.33±1.88	1.63 (0.98)
Moderate	2.68±2.28	2.10 (2.54)	3.00±2.43	2.51 (4.26)	2.85±2.40	2.42 (2.42)
Severe	2.53±1.69	1.98 (1.86)	2.78±2.20	2.22 (2.41)	3.14±2.46	2.66 (1.70)
p***	0.724		0.777		0.354	
Place of the first hospitalization						
Ward	2.53±1.94	1.83 (1.72)	2.73±2.19	1.96 (1.83)	2.76±2.24	1.91 (1.62)
ICU	2.27±1.60	1.76 (1.98)	2.67±2.51	1.88 (2.57)	2.50±2.14	2.16 (2.11)
p*	0.676		0.633		0.665	
Intubation						
No	2.54±1.91	1.83 (1.72)	2.69±2.14	2.02 (1.91)	2.70±2.19	1.91 (1.63)
Yes	2.13±1.66	1.75 (1.49)	2.84±2.90	1.66 (2.93)	2.78±2.40	2.25 (2.63)
p*	0.485		0.733		0.959	
Prognosis						
Healing	2.54±1.88	1.83 (1.70)	2.82±2.30	2.06 (1.99)	2.81±2.28	1.94 (1.81)
Death	1.88±1.77	1.24 (1.13)	1.76±1.34	1.27 (2.83)	1.79±0.98	1.26 (2.04)
p*	0.218		0.190		0.330	

*Mann-Whitney U test, **Spearman Correlation, ***Kruskall-Wallis test

Many of the symptoms of COVID-19 patients are associated with the interaction of the virus with the ACE 2 protein at relevant sites, including cardiovascular and pulmonary complications.[20] There have been numerous proposals for therapeutic options related to the novel coronavirus, and a substantial number of them are based on the interaction between ACE 2 and the viral spike protein. Hydroxychloroquine, for example, one of the first medications approved for COVID-19, blocks the binding of the virus' spike protein by altering part of the ACE 2 molecule. Using soluble ACE 2 (sACE 2) to block the ACE 2 receptor and to bind to and neutralize viral spike protein are further examples of how this interaction can lead to therapeutic strategies. Moreover, changes in ACE 2 expression due to diabetes, cardiac and renal disease, and aging may also contribute to the greater susceptibility of these patient populations to SARS-CoV-2.[21] In the present study, although 65.2% of the patients had any chronic disease, no significant difference was observed between ACE 2 levels in those with comorbidities.

In 38 patients followed up with the diagnosis of COVID-19 in Iran, sACE 2 levels were measured on the 0^{th}, 3^{rd}, and 7^{th} days of hospitalization. It was determined that sACE 2 levels could be useful in predicting the need for mechanical ventilation, but it was underscored that further studies with a larger sample size are needed to make a definite conclusion on this issue.[21]

In a study, it was suggested that upregulation of ACE 2 expression may reflect the severity of the disease in COVID-19, and it was also reported that sACE 2 activity at the time of admission did not reflect disease severity.[21] Consistent with these findings, in our study, no significant difference was found between the sACE 2 levels of patients with mild, moderate, and severe COVID-19 pneumonia on the day of hospitalization. The fact that ACE activity levels were similar in patients with different disease severity is quite remarkable in this respect.

In the study, no correlation was found between biochemical parameters and sACE level and our findings are consistent with the existing literature data. Meanwhile, in the study by Robertson et al.[22] CRP and ferritin levels showed an inverse correlation with sACE 2 concentration, and no significant difference was observed in sACE 2 levels for any of the comorbidities. In a study conducted in Turkey, sACE 2 levels were examined in 55 patients and 18 healthy control subjects, and no significant difference was found in serum ACE 2 activity between patients and healthy subjects. In the same study, a comparison of sACE 2 activity according to disease outcome showed no significant difference between patients with good or poor prognosis, and no correlation was found between serum ACE activity and inflammatory markers including ferritin, CRP, procalcitonin, fibrinogen, and IL-6 levels.[23]

There is a major limitation to this study, in that there are a limited number of patients, which can be countered by conducting studies with larger patient populations to obtain more reliable results.

CONCLUSION

Serum sACE 2 levels were not associated with disease severity and did not correlate with inflammatory markers. Further studies in this area are needed to address the lack of information on the relationship between sACE 2 activity and SARS-CoV-2 infection and to develop novel treatment strategies based on this information.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Researches Ethics Committee (Date: 11/09/2020, Decision No: 2020/432).

Informed consent: Because the study was designed retrospectively, no written informed consent from was obtained from the patients.

Referee Evaluation Process: Externally peer reviewed.

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SOURCE WRITING EXAMPLES

Excerpt from journals;

Cesur S, Aslan T, Hoca NT, Cimen F, Tarhan G, Cifci A. Clinical importance of serum neopterin level in patients with pulmonary tuberculosis. Int J Mycobacteriol 2014; 3: 15-8 (not 15-18).

Excerpt from the book;

Tos M. Cartilage tympanoplasty. 1st ed. Stuttgart-New York: Georg Thieme Verlag; 2009.

Excerpt from the book, which is the only author and editor;

Neinstein LS. The office visit, interview techniques, and recommendations to parents. In: Neinstein LS (ed). Adolescent Health Care. A practical guide. 3rd ed. Baltimore: Williams & Wilkins; 1996: 46-60.

Excerpt from the book with multiple authors and editors;

Schulz JE, Parran T Jr.: Principles of identification and intervention. In: Principles of Addicton Medicine, Graem AW. Shultz TK (eds). American Society of Addiction Medicine, 3rd ed. Baltimore: Williams & Wilkins; 1998: 1-10.

If the editor is also the author of the chapter in the book;

Diener HC, Wilkinson M (editors). Drug-induced headache. In: Headache. First ed., New York: Springer-Verlag; 1988: 45-67.

Excerpt from PhD/Undergraduate Thesis;

Kilic C. General Health Survey: A Study of Reliability and Validity. phD Thesis, Hacettepe University Faculty of Medicine, Department of Psychiatrics, Ankara; 1992.

Excerpt from an internet site;

Site name, URL address, author names, access date should be given in detail.

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Joos S, Musselmann B, Szecsenyi J. Integration of complementary and alternative medicine into the family market in Germany: Result of National Survey. Evid Based Complement Alternat Med 2011 (doi: 10.1093/ecam/nep019).

For other reference styles, see "ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References".

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