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Evidence-based health: mathematical strategies for translating scientific findings into routine clinical care

André Pontes-Silva[1]*

Currently, in the health area, several experimental studies (investigating an intervention versus placebo/control) attempt to demonstrate the relevance of outcomes through statistically significant results[1]. However, statistical significance (i.e., p<0.05) does not indicate clinical relevance (Figure 1)[2,3]. In fact, it is possible to find a statistically significant result with no clinical relevance, just as it is possible to find a statistically significant result with clinical relevance[4].

A challenge in longitudinal studies (e.g., clinical trials) is the difficulty in translating numbers (outcome) into something applicable to the clinical context (real world) because the p-value (<0.05 or >0.05) only indicates statistical significance[5,6], in which interpretation only translates a hypothesis test governed by a previously defined probability of error alpha (H0 versus H1)[7]. The language of health is biostatistics[8,9], but patients are not numbers[10]. Therefore, numerical conclusions should be translated into applicability to routine clinical care[11]. As such, science should be combined with clinical context so that patients could receive optimal treatment[12]. How to solve it? It could be done simply by evaluating the clinical relevance of the results[10].

One way to verify the clinical relevance of results is through health economic evaluations[13], effect size assessments[14], or estimates of minimal clinically important differences and minimal detectable change[15]. I suggest that new studies describe Cohen's effect size[14,16] (e.g., d-value or w-value). Cohen's d can be used to assess effect sizes when comparing two means (0.2=small effect, 0.5=moderate effect, and 0.8=large effect)[17] and the Cohen's w can be used to assess effect sizes using a chi-squared test (0.1=small effect, 0.3=moderate effect, and 0.5=large effect)[18].

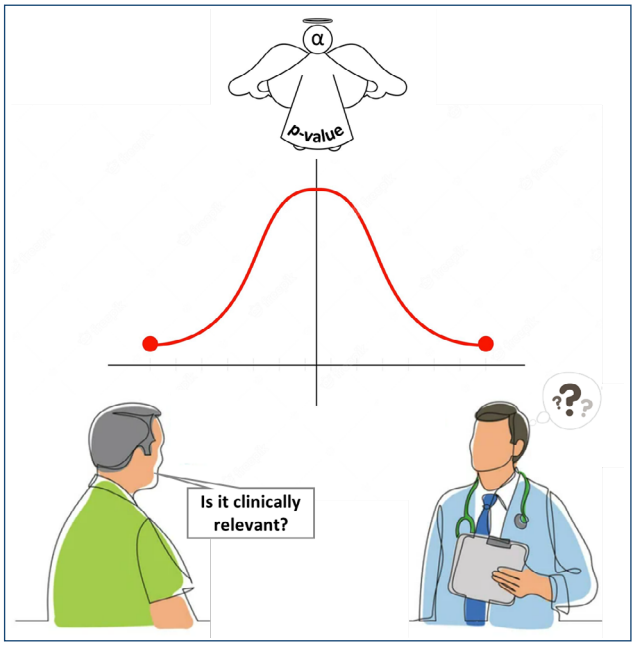

Figure 1. Statistical significance (i.e., p≤0.05) does not show clinical relevance.

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ORIGINAL ARTICLE

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Monitoring of antibody levels in healthcare workers after inactivated coronavirus disease 19 vaccination

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SUMMARY

OBJECTIVE: Because of the coronavirus disease 19 pandemic, studies on vaccination are being conducted in our country as well as across the world. In this study, the antibody levels in healthcare workers vaccinated with two doses of inactivated vaccine and the factors affecting these levels were investigated.

METHODS: Randomly selected volunteers from healthcare workers, who had been vaccinated with two doses of inactivated vaccine in January to February 2021, were included in the study. Blood samples were drawn twice, 1 month and 6 months after the second dose vaccine (CoronaVac:Sinovac Life Science Co, Ltd, Beijing, China). The antibody levels were determined by the chemiluminescence microparticle immunoassay method using kits for quantitative detection of immunoglobulin class G antibodies to severe acute respiratory syndrome coronavirus 2.

RESULTS: The mean antibody levels of 129 volunteers were 1232.5 (min: 103 to max: 7151) AU/mL in the first month and 403.5 (min: 23 to max: 4963) AU/mL in the sixth month. According to the survey results, 91 (71%) volunteers had not been diagnosed with coronavirus disease 19 before vaccination. The antibody levels 1 month and 6 months after the second dose of vaccination were significantly higher in those who had been diagnosed with coronavirus disease 19 before vaccination than in those who had not. It was found that age, gender, fast food, or healthy nutrition had no effect on antibody levels.

CONCLUSION: Vaccines are very important both to protect against coronavirus disease 19 and to experience only a mild form of the disease. Immunoglobulin class G levels formed after vaccination may be affected by many factors and may decrease over time.

KEYWORDS: Antibody. COVID-19. Healthcare workers. Vaccination.

INTRODUCTION

In late December 2019, a new virus from the coronavirus family was isolated in a group of patients with lower respiratory tract symptoms in Wuhan City, China[1]. This clinical condition was named coronavirus disease 19 (COVID-19) and its causative agent was severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2]. The disease spread across the world in a short period of time and was declared a pandemic by the World Health Organization on March 12, 2020. The first case in Turkey was detected on March 10, 2020.

In the diagnosis of COVID-19, polymerase chain reaction (PCR) testing is used considering epidemiologic history and symptoms. Especially in asymptomatic cases, additional serological tests are also beneficial to demonstrate the acquiring of immunity in the patient[3]. Antibody tests indirectly support the diagnosis of COVID-19 and determine seroprevalence. At a certain period of time after SARS-CoV-2 infection, antibodies (IgA, IgM, and IgG) are detected in the serum, which have developed against the virus depending on the patient's immune system. SARS-CoV-2 IgG, indicating exposure to the virus, begins to form on the seventh day of the incubation period after contact with the virus, and its level in the serum gradually increases in the second and third weeks. How long the antibodies produced remain at a high level or when they start to decrease is still unclear. It is not yet clear which type of antibodies affect the severity of the disease or to what extent the antibodies are affected.

The study of vaccination, which is an important means of providing immunity, is progressing rapidly worldwide. Many vaccines have been developed since the onset of the pandemic. Sinovac-CoronaVac, developed by Sinovac/China National Pharmaceutical Group, is an inactivated vaccine for COVID-19[4]. The SARS-CoV-2 vaccination program in Turkey was launched on January 11, 2021, with priority given first to healthcare workers and then

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to high-risk groups. In this program, CoronaVac 600 U/0.5 mL (Sinovac Life Science Co., Ltd, Beijing, China) was used, and two doses of the inactivated vaccine were administered intramuscularly 28 days apart[5]. It is known that the inactivated vaccine administration is safe, induces humoral and cellular responses in vaccinated individuals from different age groups, and significantly reduces hospitalization and mortality rates[6].

The aim of this study was to quantify the antibody levels in the first and sixth months after vaccination with an inactivated vaccine, in healthcare workers, and also to investigate various factors that may affect the antibody levels including personal characteristics such as nutritional habits, body mass index (BMI), age, and gender.

METHODS

After obtaining the approval from the Duzce University Non-Interventional Health Research Ethics Committee dated 01.02.2021 and with the number 2021/16, volunteers who agreed to answer the questionnaire were determined from healthcare workers who had been vaccinated with two doses of the inactivated COVID-19 vaccine (CoronaVac:Sinovac Life Science Co, Ltd, Beijing, China) in January to February 2021. All study participants completed, signed, and returned an informed consent form. Blood samples were drawn from the volunteers twice, in March 2021 and in August 2021, 1 month and 6 months after the second dose of the two vaccine doses that were administered 28 days apart. The blood samples were examined in the Duzce University Faculty of Medicine Medical Microbiology Laboratory. A survey, including their sociodemographic characteristics, whether or not they had been previously diagnosed for COVİD-19, and their nutritional habits, was supplied to the volunteers. Volunteers who did not complete the survey and were vaccinated with the third dose of the COVID-19 vaccine without having a blood sample drawn at 6 months to measure the antibody levels were excluded from the study.

First of all, healthcare workers included in the study were divided into two groups according to whether or not they had been COVID-19 diagnosed, and the difference between antibody levels was investigated.

Determination of the antibody levels

After the collection of blood samples, the serum samples were separated and stored at –20°C until the study was performed. After the serum samples had been placed at room temperature, there was a possibility of binding IgG antibodies, including antibodies against the receptor-binding site of the spike protein S1 subunit of SARS-CoV-2.

The antibody levels were detected (AU/mL) by chemiluminescent microparticle immunoassay (CMIA) (Architect i2000, Abbott, USA) using kits that quantitively determine antibodies.

Statistical analysis

Healthcare workers were selected using a simple random sampling technique. The SPSS 23 program was used for statistical analysis of the data. All data from the study were calculated according to type and using appropriate descriptives (mean, standard deviation, median, width between quarters, and percentage). The Mann-Whitney U test was used to evaluate the factors that might influence the antibody levels in the first and sixth months. The $p<0.05$ was considered significant.

RESULTS

A total of 129 healthcare workers who had been vaccinated with only two doses of inactivated vaccine COVID-19 were included in the study. The flowchart of the cases is shown in Figure 1.

In the study, 76 (59%) healthcare workers were women and 53 (41%) were men, and the mean age was 36.2 (SD 7.6) (min: 20 to max: 60) years. Mean BMI was determined as 25.4 (SD 4.5) (min: 17.6 to max: 40.5). The mean antibody levels of the 129 volunteers included in the study were 1232.5 (min: 103 to max: 7151) AU/mL in the first month and 403.5 (min: 23 to max: 4963) AU/mL in the sixth month. According to the survey results, 91 (71%) volunteers reported that they were not diagnosed with COVID-19 before vaccination. It was found similar ($p=0.439$ and $p=0.299$, respectively) in the mean age and the mean BMI of those diagnosed and not diagnosed with COVID-19, before the first dose of the vaccine. The antibody levels 1 month and 6 months after the second vaccine dose in those diagnosed with COVID-19 before vaccination were significantly higher than those not diagnosed (Table 1).

Some factors that might affect the antibody levels of 91 healthcare workers who were not diagnosed with COVID-19 and had been vaccinated with two doses of the inactivated vaccine were evaluated based on the responses in the survey used (Table 2).

In terms of nutrition, while it was found that the antibody levels were similar in those fed on more fast food and high carbohydrate, the antibody levels were lower in those fed on high probiotic and prebiotic foods than those fed on low probiotic and prebiotic foods.

DISCUSSION

Immunoglobulin G antibodies can be detected in individuals who have been vaccinated against COVID-19 or have been diagnosed with COVID-19. In studies of the antibody levels

formed after both inactivated and mRNA vaccination, IgG antibody levels were reported to be significantly higher in those who have been diagnosed with COVID-19 than in those who have not been diagnosed[7-10]. Yalçın et al., found that IgG antibody levels were higher in people who had COVID-19 and a single dose of vaccine than in people who did not have COVID-19 but were vaccinated with two doses of the vaccine[11]. There are studies reporting that anti-spike IgG antibodies remain stable for 6 months in patients who have had the disease in the past[12]. Similarly, in our study, anti-spike IgG antibody levels were found to be significantly higher in patients who had the disease before vaccination than in patients who had not. This indicates that the antibody response, occurring in those who have undergone the disease, remains positive for some time. In studies on the antibody levels detected approximately 1 month after two doses of vaccine in healthcare workers who were vaccinated with inactivated vaccine without undergoing the disease, Dinç et al., found a mean IgG level of 707.1, and Tekol et al., found this

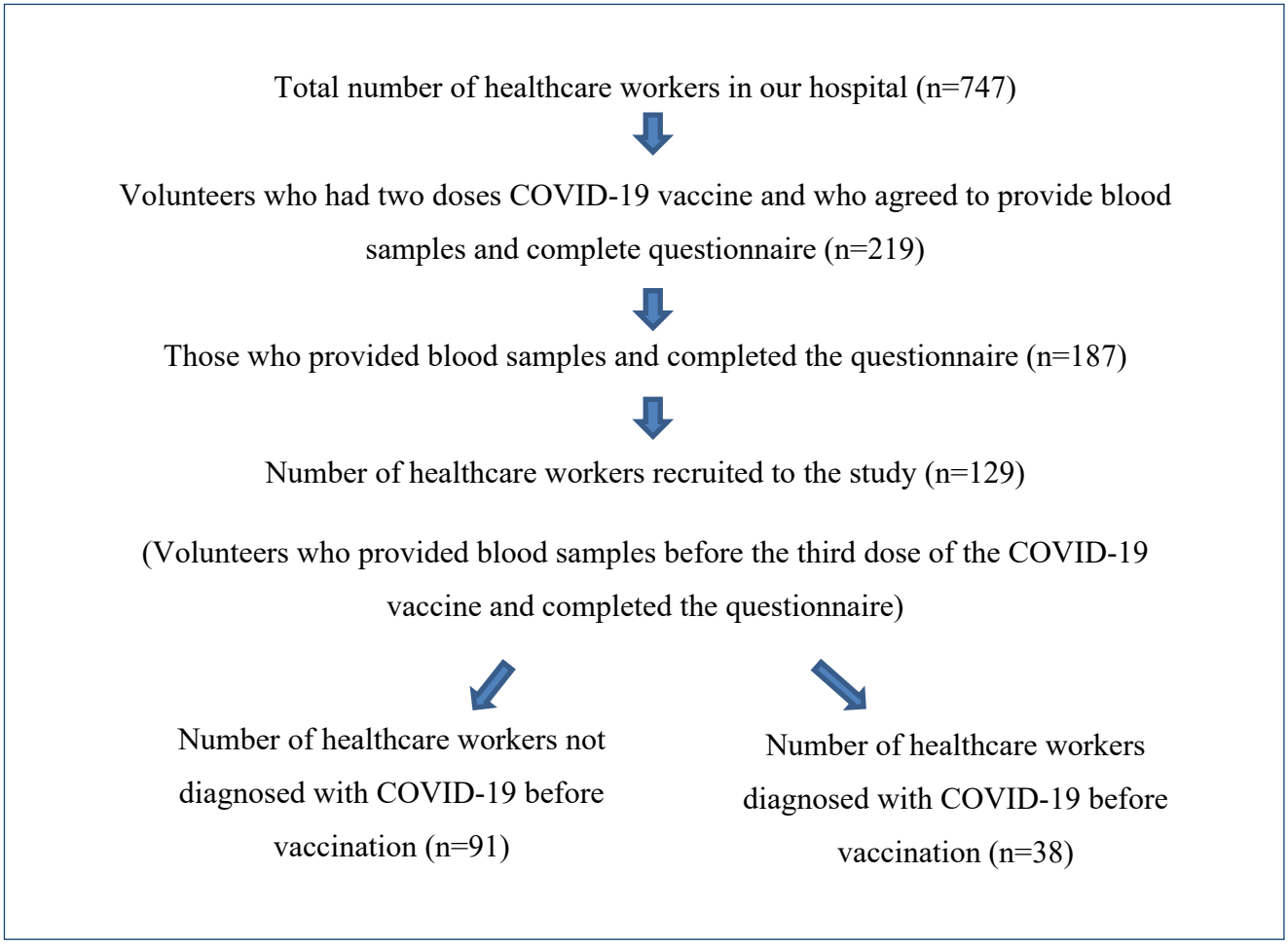

Figure 1. The flowchart of cases.

Table 1. Antibody levels (AU/mL) in those with and without diagnoses with coronavirus disease 19 before vaccination.

Groups	One month after the second vaccine dose			Six months after the second vaccine dose		
	Median	IQR	p-value	Median	IQR	p-value
Diagnosed with coronavirus disease 19 before vaccination (n=38)	1076.5	1383.2	**0.004**	446.0	519.4	**<0.001**
Not diagnosed with coronavirus disease 19 before vaccination (n=91)	805.0	768.7		140.8	155.7	

IQR: interquartile range. Significant p-value are indicated in bold.

level to be 171.3 and 89.85 4 months after the second dose[10-13]. In our study, the mean IgG level after 1 month was 805 AU/mL, and after 6 months, it was 140.8 AU/mL. The detected antibody levels showed a decrease over time.

It is known that there are many risk factors that determine being infected or the severity of the disease. For example, male gender, age over 50 years, chronic diseases such as hypertension, heart disease, diabetes, malignancy, chronic lung disease, kidney disease, living in nursing and rehabilitation centers, and staying in crowded environments such as schools, prisons, and immigrant camps are risk factors for COVID-19[14]. Vural et al., reported a decrease in the antibody levels with age, compared with vaccinated individuals under 40 and over 40 years of age[7]. Yiğit et al., also found that the younger the age of vaccinated healthcare workers, the higher the anti-SARS-CoV-2 immunoglobulin G level[15]. Bayram et al., studied the

Table 2. In those with no diagnosis of coronavirus disease 19 before vaccination, antibody levels 1 month and 6 months after vaccination, and the relationships with certain factors.

Groups	One month after the second vaccine dose			Six months after the second vaccine dose		
	Median	IQR	p-value	Median	IQR	p-value
Profession						
Doctor (n=48)	642.7	761.9	0.469	153.7	149.4	0.994
Others (n=43)	864.1	806.7		138.4	168.0	
Age group						
Below 35 years (n=39)	801.6	745.3	0.873	136.7	155.7	0.854
35 years and above (n=52)	818.9	831.4		143.4	160.4	
Gender						
Female (n=55)	891.8	860.9	0.403	135.1	179.3	0.792
Male (n=36)	632.8	664.2		153.7	113.8	
Body mass index						
Thin-normal (n=50)	860.3	788.2	0.661	133.0	158.1	0.873
Overweight-obese (n=41)	653.2	771.0		161.5	167.0	
Use of antibiotics in the past 6 months						
Yes (n=72)	844.7	697.9	0.257	155.8	151.9	0.232
No (n=19)	632.2	833.4		122.7	185.5	
Using vitamins (one or more of vitamin C, vitamin D, and fish oil)						
Yes (n=45)	805.0	799.0	0.353	123.9	122.4	0.079
No (n=46)	829.1	859.5		170.9	188.8	
Presence of chronic disease						
Yes (n=25)	958.0	653.5	0.722	182.6	208.6	0.513
No (n=66)	651.5	760.6		268.8	117.3	
Fed on carbohydrate						
Below average (n=61)	939.8	723.9	0.098	140.8	177.9	0.960
Above average (n=30)	598.6	631.3		149.9	298.5	
Fed on fast food						
Below average (n=53)	811.0	692.9	0.803	140.8	127.9	0.907
Above average (n=38)	803.3	846.9		141.0	193.3	
Fed on probiotic and prebiotic food						
Below average (n=58)	**905.4**	658.0	**0.019**	171.4	168.0	0.073
Above average (n=33)	**513.3**	817.6		111.7	114.0	

IQR: interquartile range. Values with statistically significant differences were bolded.

post-vaccination antibody levels in healthcare workers aged 18–34 years and found that it was higher in the older age groups[9]. Dinç et al., in their study examining post-vaccination antibody levels in healthcare workers who had not yet been diagnosed with COVID-19, found that the antibody levels were slightly lower in those over 40 years of age than in those under 40 years of age[10]. In our study, healthcare workers in the over and under 35 age groups were found to have similar antibody levels (Table 2). It is suggested that these levels were similar because all healthcare workers included in our study were under 60 years of age.

Although there are studies in which the antibody levels were higher in women than in men[11,15,16], studies with similar rates have also been reported as in our study[10]. This suggests that gender alone may not be an indicator.

Obesity has been reported to cause lower levels of antibodies to COVID-19 compared with healthy-weight individuals[17]. Pellini et al., found that the antibody levels were significantly higher in thin and normal-weight individuals than in overweight and obese individuals[16]. Franca et al., also reported a negative correlation between BMI and antibody levels[18]. Similar to our study, Dinç et al., found that the antibody levels did not differ in normal weight and obese healthcare workers[10]. These results indicate that more comprehensive studies are needed to determine the effects of obesity on antibody levels. It is known that several chronic diseases such as obesity also affect antibody levels. Bayram et al., and Dinç et al., found that patients with chronic diseases and hypertension had lower IgG levels against COVID-19 compared with healthy individuals[9,10]. Although no statistically significant difference was found in the patients with chronic diseases in our study, it was observed that the antibody levels were lower, especially in the sixth month. These results indicate that chronic diseases may have an inhibitory effect on the immune system of individuals.

It is reported that the type of nutrition and use of vitamin supplements have no effect on COVID-19 infection, but consumption of water and adequate and balanced nutrition are important for the treatment of disease[19]. It is known that the use of vitamin D and vitamin C may also be beneficial in prophylaxis and treatment[20,21]. Nutrition is shown to be important in reducing mortality from COVID-19 because high-carbohydrate nutrition leads to obesity, which negatively affects the prognosis of the disease. In our literature search, we could not find any study on how the antibody levels formed after vaccination are affected by nutrition. In our study, no statistically significant difference was found between the antibody levels of those who were fed high carbohydrate and more fast food compared with those who were fed less of these foods (Table 2). Those who consumed more foods containing probiotics (e.g., yogurt, cabbage, and kefir) and prebiotics (e.g., garlic, onion, and fruit) were found to have lower antibody levels. As IgG levels alone cannot be an indicator of the overall immune system, it was considered that more comprehensive studies are also needed in which cellular immunity parameters can be determined by assessing nutrition habits.

Therefore, vaccines serve as the most important shield in protecting people against COVID-19 and in alleviating the disease. Post-vaccination IgG levels can be affected by many factors, especially the presence of chronic disease, and they decrease over time. As there is no definitive value for the level of protective antibodies, it is important to continue vaccinations with differently produced technologies and not to forget reminder doses in order to maintain protection. More comprehensive studies on the effects of nutrition on antibodies are needed.

Limitations of the study

As third-dose vaccinations were not being considered at the time the ethics committee approval of the study was obtained, the study was initiated to monitor the antibody levels of two doses of vaccine for 1 year. However, due to the introduction of the third dose of the vaccine in August 2021, the antibody levels generated by two doses of the inactivated COVID-19 vaccine could only be monitored for 6 months rather than the planned 12 months.

ETHICS

Approval of the Duzce University Non-Interventional Health Research Ethics Committee dated 01.02.2021 and with the number 2021/16.

AUTHORS' CONTRIBUTIONS

EÇ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **CEÖ**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **ŞÖ**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **NI**: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **DY**: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **GK**: Data curation, Formal Analysis, Supervision, Writing – review & editing. **PD**: Data curation, Formal Analysis, Supervision, Writing – review & editing. **İŞ**: Writing – review & editing.

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ORIGINAL ARTICLE

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The Brazilian version of the telehealth usability questionnaire (telehealth usability questionnaire Brazil): translation, cross-cultural adaptation, and psychometric properties

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SUMMARY

OBJECTIVE: The objectives of this study were to translate and cross-culturally adapt the telehealth usability questionnaire into Brazilian Portuguese and to evaluate its psychometric properties.

METHODS: This was a methodological validation study carried out in two phases. In phase 1, the telehealth usability questionnaire was cross-culturally adapted with 10 participants comprising the expert committee members, including 5 healthcare professionals with theoretical and practical knowledge of telehealth, 1 methodologist, and 4 translators. This phase was performed at Universidade Federal de Juiz de Fora Physiotherapy Clinic School. In phase 2, the psychometric properties of telehealth usability questionnaire Brazil were analyzed. This phase included in-person assessments at Márcio Cunha Hospital, Minas Gerais. The recruitment period for both phases was from April 2020 to February 2021. Content validity, reliability, internal consistency, and criterion validity were analyzed. The criterion validity was evaluated using correlation with a validated instrument: the system usability scale.

RESULTS: The telehealth usability questionnaire was adequately translated and cross-culturally adapted. The telehealth usability questionnaire Brazil presented an excellent content validity index of 0.96 with percentages of understanding higher than 90%. The telehealth usability questionnaire Brazil demonstrated great internal consistency (α=0.94 and ω=0.94), excellent intra-rater reliability (intraclass correlation coefficient=0.85, 95%CI 0.75–0.91), no difference between the test and retest [T (0.425), p>0.673], and no proportional bias (p=0.205). There was a moderate correlation between telehealth usability questionnaire Brazil and the system usability scale (r=0.52, p<0.0001).

CONCLUSION: The telehealth usability questionnaire was adequately translated and cross-culturally adapted into Brazilian Portuguese and showed adequate psychometric properties for use in telehealth clinical practice and research in Brazilian-Portuguese-speaking individuals.

KEYWORDS: Validation study. Surveys and questionnaires. Telemedicine.

INTRODUCTION

Telehealth provides healthcare using telecommunication and information technologies, including mobile phones, smartphones, and other communication devices[1]. The use of telehealth to provide healthcare remotely has become increasingly frequent with the development of technologies[1,2]. Telehealth emerges as an alternative and complementary strategy for managing patients when it is difficult to reach the traditional health services infrastructure or when face-to-face contact is not feasible. It may include diverse teleconsultation systems, telediagnosis, telemonitoring, tele-education, and telerehabilitation[2]. An exponential increase in telehealth use started in 2020 when systems were expanded globally in response to the coronavirus disease 2019 (COVID-19) pandemic[2].

Telehealth systems must be helpful for both users and healthcare professionals. Good usability has several benefits, including fewer frequent errors, reduced user training time, acceptance, and greater efficiency and productivity when operating

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a particular system[3]. Although the access, acceptability, quality, and cost of telehealth systems have been evaluated across varying devices, the assessment of systems usability still needs to be improved. However, no telehealth-specific instrument is available for the Brazilian Portuguese-speaking population[4] despite the growth of telehealth systems use in the country[4].

The telehealth usability questionnaire (TUQ) was developed in 2016 and combined items from existing telehealth-specific and general computer usability questionnaires[5]. The TUQ assesses the healthcare professional and patient's usability experience and covers factors influencing usability, including usefulness, interface friendliness, effectiveness, reliability, and satisfaction with varying telehealth devices[5]. The TUQ is freely available and the most used tool for telehealth usability assessment[6]. Due to the broad TUQ applicability in clinical practice and research on telehealth, the objectives of this study were (1) to translate and cross-culturally adapt the TUQ into Brazilian Portuguese and (2) to evaluate its psychometric properties regarding content and criterion validity, test-retest reliability, and internal consistency.

METHODS

Study design and participants

This was a cross-sectional, methodological study approved by the Human Research Ethics Committees of the Universidade Federal de Juiz de Fora and Hospital Márcio Cunha, Minas Gerais (approvals: 28613719.8.0000.5147 and 28613719.8.3002.8147, in April 3, 2020, and January 8, 2021). All participants signed an informed consent form before participating in the study. This study had two phases. First, the cross-cultural adaptation of the TUQ was carried out following the recommended methodology[7]. Second, analyses of the TUQ Brazil psychometric properties followed the recommendations of the Consensus-based Standards for selecting health Measurement Instruments (COSMIN)[8]. A convenience sample of 10 participants was invited to comprise the expert committee members: 5 healthcare professionals with theoretical and practical knowledge of telehealth, 1 methodologist[9], and 4 translators. The expert committee members' recruitment period was from April 10, 2020, to July 16, 2020.

In evaluating the TUQ Brazil psychometric properties, a convenience sample of individuals with recent telehealth experience within the last 6 months before participation in the study, either as a health professional or a patient, was included. Participants with difficulty with the Brazilian-Portuguese language were excluded for reasons of understanding the instruments used in the study. Participants were recruited in this phase from January 10, 2021, to February 21, 2021.

Instruments

Telehealth usability questionnaire

The TUQ assesses the utility and usability of the technology[5]. TUQ is easy to apply and contains 21 statements ranked on a one-to-seven-level Likert-type scale from "strongly disagree" to "strongly agree" or "not applicable." The TUQ score is determined by the average score of (1–7) responses, excluding the non-applicable items. The higher the response average, the greater the usability of the telehealth system. The questionnaire is freely available on the Internet (https://ux.hari.pitt.edu/v2/portal/#/about).

System usability scale

The system usability scale (SUS) was applied to test the TUQ Brazil concurrent criterion validity. The SUS is a widely used and valid self-reported instrument to assess the usability of varying technology interfaces. The SUS consists of 10 statements scored on a five-level Likert-type scale with anchors for "strongly agree" and "strongly disagree." Using a simple formula, its final score ranges from 0 to 100. Higher scores indicate better system usability[10].

Cross-cultural adaptation of the telehealth usability questionnaire

The author of the TUQ original version was contacted, and permission to cross-culturally adapt to the Brazilian-Portuguese language was sought. The translation and cross-cultural adaptation were performed in five steps[7]. (i) Translation of the instrument by two bilingual independent translators who were not aware of the objectives of the study. One translator was a health professional to provide a clinical perspective in the translated version that represents the language used by the target population. The second translator was unfamiliar with the topic addressed, thus being better able to detect different meanings and possible ambiguities[7]. (ii) Two authors carried out synthesizing translated versions for consensus, evaluating semantic, idiomatic, conceptual, linguistic, and contextual discrepancies, and analyzing the structure, layout, instrument instructions, and the scope and adequacy of the expressions contained in the items. This phase aimed to reach a single combined version. In case of divergences, adaptations would be made until a consensus on the translation was reached. (iii) Reverse translation was conducted by two native English speakers translators without prior access to the TUQ. This step aimed to assess the extent to which the

translated version reflects the content of the items, as proposed in the original version of the questionnaire. The authors and the reverse translators analyzed discrepancies between the two versions and compared the back-translated consensus version with the original instrument in English. (iv) An expert committee of 10 participants[7,9,11] conducted the content validity assessment. The expert committee comprised five health professionals and a methodologist previously contacted with theoretical and practical knowledge about telehealth, in addition to the four translators who participated in the previous steps. This step consolidated all questionnaire versions and developed a preliminary, pre-final version. The expert committee analyzed the semantic, idiomatic, conceptual, linguistic, and contextual discrepancies and the questionnaire structure, layout, and user instructions[7,9,11]. The committee members also answered questions about the comprehension of items. The agreement was verified quantitatively using the content validity index (CVI)[12]. The CVI was calculated using a four-point Likert-type scale, in which the sum of responses scored as three and four of each committee member are divided by the total number of responses. (v) The evaluation of the final version or pilot study was performed, when telehealth users were interviewed for the understanding of each TUQ Brazil item[9,13]. The participants were instructed to complete the questionnaire. They were interviewed to investigate their perception of each item and the answer they chose, being asked about their understanding of each statement in the instrument and the justification for the difficulty in understanding. Items that presented 10% or more of "non-comprehension" would be modified, based on the participants' responses, to reach the highest understanding in the final version until a pre-established percentage of adjustment (comprehension) in all items was reached (≥90%)[9,13].

Psychometric properties of the telehealth usability questionnaire Brazil

Data collection included online and face-to-face assessments to evaluate the psychometric properties during the cross-cultural adaptation process. The analysis of psychometric properties was conducted for internal consistency, and the test-retest method used repeated evaluation of the TUQ Brazil version 7–14 days after the first assessment for reliability. In addition, participants were asked to answer the SUS in the retest session.

Statistical analysis

Data were stored and analyzed using the SPSS® version 22.0 and Jamovi® 2.3.26. The content validity was tested using the CVI's quantitative agreement among expert committee members[12]. A CVI ³0.80 was evidence of content validity[12]. Test-retest reliability was investigated using the intraclass correlation coefficient (ICC)[14] and paired t-test. ICC³0.80 were accepted as a standard of reliability[15]. Studies recommend a minimum of 50 participants as the sample size required to test reliability[16]. The level of agreement of the test-retest was also verified using Bland-Altman plotting[14]. Internal consistency was assessed by Cronbach's alpha and McDonald's omega. Cronbach's alpha index range is 0–1[17], and values between 0.75 and 0.95 were considered appropriate[17]. The McDonald's omega ³0.7 was considered adequate internal consistency[14]. The concurrent criterion validity was tested using Spearman's correlation coefficient between the TUQ Brazil and the SUS. Correlation coefficients were interpreted according to Schober et al.[18]

RESULTS

The final version of TUQ Brazil kept a similar format to the original questionnaire and is available in the electronic repository: https://data.mendeley.com/datasets/p8d3xyvfnp/4. The questionnaire versions used for cross-cultural adaptation, the final version, and the summary with items of divergence are also available in the electronic repository. There was no denial by the invited experts, and the 10 invited members agreed to participate. The committee reported adequate operational equivalence with the original questionnaire format. The TUQ Brazil showed an excellent CVI of 0.96 with great agreement.

A convenience sample of 54 individuals with prior experience as telehealth systems users (i.e., 30 patients and 24 healthcare professionals) was included. Three individuals did not complete the study assessments due to loss of contact (dropout rate of 1.6%). Participants' demographic characteristics and telehealth modalities experienced are shown in Table 1. The TUQ Brazil

Table 1. Participants' characteristics and telehealth modalities experienced (n=54).

Age (years)		33 (21–63)
Sex	Female	48 (88.9)
Education level	Complete elementary school	2 (3.7)
	Complete secondary school	14 (25.9)
	Complete higher education	38 (70.4)
Telehealth modality	Telemonitoring	17 (31.5)
	Teleconsultation	16 (29.6)
	Teleconsulting	9 (16.7)
	Tele-education	8 (14.8)
	Telerehabilitation	4 (7.4)
	Telediagnosis	0 (0.0)

Data are presented as median (minimum to maximum) or absolute number and percentage, n (%).

items' comprehension percentage on the final version evaluation was above 98.7%. No item required modification following patients' and healthcare professionals' comprehension assessment, and no redesign was necessary. The TUQ Brazil demonstrated excellent internal consistency. The Cronbach's alpha after single-item removal ranged from 0.927 to 0.940, and no item influenced reliability when removed from the analysis. Furthermore, the McDonald's omega was regarded as adequate (0.941). The TUQ Brazil showed excellent intra-rater reliability (ICC=0.85, 95%CI 0.75–0.91). The paired t-test did not demonstrate significant differences between the test and retest [T (0.425), p>0.673]. The Bland-Altman plot showed agreement between test and retest assessments, and the data distribution was homoscedastic with no proportional bias (p=0.205) (Figure 1). The TUQ Brazil showed a moderate correlation (r=0.52, p<0.01) with the SUS (Figure 2).

DISCUSSION

This study translated and cross-culturally adapted the TUQ into Brazilian Portuguese and analyzed its psychometric properties.

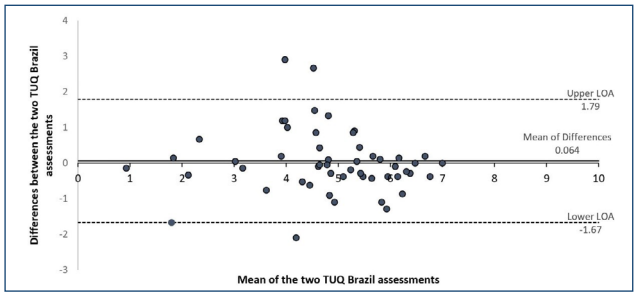

Figure 1. The Bland-Altman plot on the agreement between the two telehealth usability questionnaire Brazil assessments. LOA: limit of agreement, upper (bias+1.96×standard deviation) and lower (bias-1.96×standard deviation).

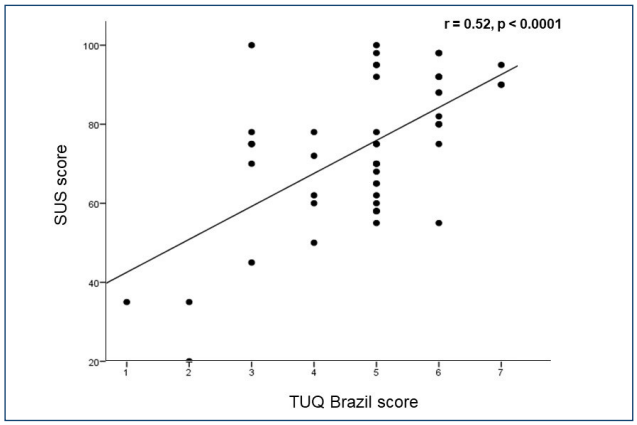

Figure 2. Spearman correlation plot between the telehealth usability questionnaire Brazil and the system usability scale.

The final version of the TUQ Brazil is the first telehealth usability assessment tool validated in Brazilian Portuguese and presented adequate operational equivalence to the original questionnaire format. The TUQ Brazil is a versatile questionnaire that may help identify telehealth systems limitations, guide new strategies to overcome the challenges while implementing new systems, and create a suitable configuration to implement and use telehealth services in Brazil successfully. In addition, the TUQ has no segmentation or domains and examines fewer items compared with other TUQs[6]; this may facilitate its use in population studies and services in public health.

During the translation and cross-cultural adaptation process, expert committee members modified or replaced no items from the original version. Despite the few divergences in the translations, the final version was approved with an excellent agreement, above the recommended CVI level[12]. The TUQ Brazil was easily understood according to the percentage of comprehension observed in the target population. This confirmed the questionnaire's adequacy in assessing the usability of telehealth systems among Portuguese-speaking Brazilians. Adequate understanding of the translated and cross-culturally adapted versions of the TUQ was also reported for its Spanish[19] and Turkish[20] versions.

The internal consistency of the TUQ Brazil items demonstrated the homogeneity of the questionnaire. The TUQ Brazil Cronbach's alpha range was similar to that found in another cross-culturally adapted TUQ version[20] and compatible with the one reported by Parmanto et al.[5] in the original version of the questionnaire (from 0.79 to 0.93). Of note, the TUQ Brazil internal consistency was close to the ones reported for other questionnaires on telehealth systems usability[21-23]. A score greater than 0.70 is rated positive for an instrument registering group data[15,17]. The TUQ Brazil also showed excellent intra-rater reliability (ICC>0.80), similar to values reported for the TUQ Turkish version[20] and the mHealth APP usability questionnaire (MAUQ), which is a usability questionnaire for telehealth applications[23].

The TUQ Brazil showed a moderate correlation with the SUS. The non-parametric distribution of the TUQ Brazil overall score may explain the absence of a more robust correlation; however, the strength of the correlation between the TUQ Brazil and SUS was close to those reported between questionnaires used to assess the usability of e-health questionnaires and the SUS, including the MAUQ (r=0.64) and the Post-Study System Usability Questionnaire (PSSUQ) (r=0.67)[23]. Conversely, a strong correlation was reported between the TUQ and the Telemedicine Satisfaction Questionnaire[24], as both are telehealth-specific questionnaires instead of a general technology usability scale like the SUS[20].

There are some study limitations to be addressed. A convenience sample of users with experience in varying telehealth systems modalities was included for external validity, which is an important practical feature of the TUQ. However, the lack of a uniform telehealth modality under assessment, such as teleconsultation, telediagnosis, or telemonitoring only, prevented the analysis of additional psychometric properties of the TUQ Brazil, including floor and ceiling effects, standard error of psychometric, and minimal detectable difference. Although a minimum sample of 50 participants recommended for reliability assessment was recruited[16], the subjects participated in evaluating the final version and analyses of the reported psychometric properties, as no item required modification following patients' and healthcare professionals' comprehension assessment, and no redesign was necessary from the pre-final to the final version of the TUQ Brazil.

CONCLUSION

The TUQ Brazil is a translated and cross-culturally adapted tool for assessing the usability of telehealth systems in the Brazilian-Portuguese-speaking population and presents adequate psychometric properties, including criterion validity, test-retest reliability, and internal consistency. Further studies must explore additional psychometric properties of the TUQ Brazil, including responsiveness to telehealth system usability modifications.

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AUTHORS' CONTRIBUTIONS

MRS: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. **ALSo:** Data curation, Project administration, Visualization, Writing – original draft. **LHGN:** Data curation, Project administration, Visualization, Writing – original draft. **CCO:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **LAC:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **BP:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AJ:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **ALSa:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **CM:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **LAS:** Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Writing – original draft.

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ORIGINAL ARTICLE

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Evaluation of respiratory bronchiolitis nodules with maximum intensity projection images

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SUMMARY

OBJECTIVE: Respiratory bronchiolitis is a disease associated with heavy smoking. Computed tomography in this disease often shows symmetrical and bilaterally ill-defined circumscribed centriacinar micronodular involvement in the upper-middle lobes. The maximum intensity projection method is a kind of image processing method and provides a better evaluation of nodules and vascular structures. Our study aimed to show whether maximum intensity projection images increase the diagnostic accuracy in the detection of micronodules in respiratory bronchiolitis.

METHODS: Two radiologists with different experiences (first reader: 10-year radiologist with cardiothoracic radiology experience and second reader: nonspecific radiologist with 2 years of experience) reviewed images of patients whose respiratory bronchiolitis diagnosis was supported by clinical findings. The evaluation was done independently of each other. Both conventional computed tomography images and maximum intensity projection images of the same patients were examined. The detection rates on conventional computed tomography and maximum intensity projection images were then compared.

RESULTS: A total of 53 patients were evaluated, of whom 48 were men and 5 were women. The first reader detected centriacinar nodules in 42 (79.2%) patients on conventional computed tomography and centriacinar nodules in all 53 (100%) patients on maximum intensity projection images. The second reader detected centriacinar nodules in 12 (22.6%) patients on conventional computed tomography images and in 48 (90.6%) patients on maximum intensity projection images. For the less experienced reader, the detection rate of micronodules in respiratory bronchiolitis in maximum intensity projection images increased statistically significantly ($p<0.001$).

CONCLUSION: Maximum intensity projection images in respiratory bronchiolitis increase the detectability of micronodules independently of the experience of the radiologist.

KEYWORDS: Bronchiolitis. Lung. Multiple pulmonary nodules. Smoking.

INTRODUCTION

Smoking is one of the leading causes of preventable death worldwide. Smoking mainly causes lung cancer and chronic obstructive pulmonary disease in the lung. In addition, smoking both increases the susceptibility to lung infection and accelerates lung fibrosis. Smoking also causes some non-neoplastic lung diseases such as desquamative interstitial pneumonia (DIP), respiratory bronchiolitis (RB), RB-associated interstitial lung disease (RB-ILD), and Langerhans cell histiocytosis (LCH)[1].

Respiratory bronchiolitis is one of the non-neoplastic lung diseases associated with smoking. Histopathological presence in the lungs of smokers was first demonstrated in 1974[2]. The main histological finding in RB is peribronchial pigmented macrophage accumulation. Although classical computed tomographic (CT) findings of RB are variable, ill-defined centrilobular micronodules and bronchial wall thickenings are frequently detected[3]. In addition, areas of varying degrees of ground glass density can be seen on CT. Hypersensitivity pneumonia should be considered in the radiological differential diagnosis of RB. The absence of a positive relationship between hypersensitivity pneumonia and smoking, the presence of a history of organic antigen exposure, and lymphocytosis in the bronchoalveolar lavage (BAL) fluid contribute to its differentiation from RB. Nonspecific findings are seen in BAL in RB[1].

Computed tomography technology has shown a rapid development process in recent years. The maximum intensity projection (MIP) method, which is a not very new reconstruction technique, is especially helpful in the evaluation of pulmonary vascular structures and micronodules[4]. In this technique, voxels with the highest attenuation value for each slice are projected onto a two-dimensional image. MIP and minimum intensity projection (MinIP) images are a simplified version of the volume rendering technique. MIP images are particularly suitable for analyzing superposed complex anatomical structures with small volumes. In the literature, there are studies comparing conventional CT and MIP images in

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the detection of micronodules[4,5]. However, there is no study in the literature showing the diagnostic contribution of MIP examination by radiologists with different experiences to a specific micronodular disease. The main indications for the use of MIP images in the lung window are that it is used to evaluate mild form micronodular lung infiltrates, bronchial diseases, pulmonary microcirculation anomalies, and mild form lung heterogeneity[6].

In our study, it is evaluated whether MIP images, which are a known technique in the detection of RB and RB-ILD, which are common in the community and cause a relatively mild clinical course associated with smoking, are more sensitive than conventional images.

METHODS

The study was carried out retrospectively and the study was started after the approval of the local ethics committee dated 11.03.2022 and numbered 22-KAEK-237. Our study was carried out according to the "Helsinki Declaration."

Case selection

Patients who underwent high-resolution thorax CT (HRCT) between January 2019 and September 2022 and who had a smoking history were included in our study. Lung biopsy was not performed on the patients. Patients with clinical and radiological findings suitable for RB and RB-ILD were included in the study. Conditions mimicking RB and RB-ILD images were not included in the study. For this purpose, cases with lymphocytosis in BAL fluid and with organic antigen exposure were not included in the study even if they had appropriate clinical and radiological findings. In addition, other diseases with micronodular involvement were not included in the study. For this purpose, clinical cases suitable for miliary tuberculosis were excluded. Also, cases of sarcoidosis were not included. In addition, due to the possibility of pneumoconiosis, attention was paid to occupational anamnesis, and cases with a history of exposure were excluded. In addition, patients whose HRCT examination was not of optimal quality (not breathing properly, patients in motion, imaging that did not cover the entire lung in the examination area) were not included in the study.

High-resolution thorax computed tomography technique and protocol

The images of the patients were obtained using a 64-slice CT device (GE Optima CT660) with a speed of 0.5 s in a helical scanning type bone algorithm with a section thickness of 0.625 mm. In technical parameters, it produced 350 milliampere-seconds (mAs) and 120 kilovoltage peak (kVp) X-ray. Collimation was of 1 mm thick. The mean X-ray exposure time was 3.13 s. Although the field of view ratio was manually adjusted from patient to patient, it was adjusted according to the Large Body option before the examination. The dose length product (DLP) body was set to 386.74 mGy×cm at 32 cm Phantom. The examination was obtained from the level of the thyroid gland in the neck region to the inferior of the costophrenic sinuses, while the patient was in the supine position and in deep inspiration. No contrast agent was used for the examination. Adjustment of MIP images was obtained by automatically reconstructing 10-mm slab thickness axial and coronal images in the workstation.

Image evaluation

The images were analyzed by two radiologists with different experiences (first reader with 10 years of experience in cardiothoracic radiology and second reader with 2 years of experience as nonspecific radiologist). Images were evaluated from a 21.3-inch 3MP IPS Screen medical monitor via Sectra IDS 7 PACS (Picture Archiving and Communication Systems). Both readers reviewed independently. First, whether they detected centriacinar nodules for each patient on conventional CT images was recorded. One month after the conventional CT images of all patients were examined, MIP images were examined. Then, whether they detected centriacinar nodules on MIP images was recorded. Conventional CT and MIP images were evaluated by both radiologists on axial and coronal images. Conventional and MIP images were evaluated at the same window level (WL) and same window width (WW) in the lung parenchyma window (WW=1500 HU, WL= -650 HU).

Statistical analysis

The Windows SPSS 20 package program was used for the statistical analysis of data. Categorical measurements were summarized as numbers and percentages, and continuous measurements were summarized as mean, deviation, and minimum-maximum. The normality of distributions was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilk W tests. The independent samples t-test was used as our data showed a normal distribution. $p<0.05$ was considered statistically significant. Detection rates of centriacinar nodules for the first and second readers were examined using the McNemar test.

RESULTS

A total of 53 patients were included in our study, of whom 48 (90.6%) were males and 5 (9.4%) were females. The mean

age of the patients was calculated as 61.6±11.6 (38–88 years). All of the patients were smokers, and the average smoking rate was 34±11.4 packs/year (20–80 packs/year). The mean age of the male patients was 61.2±11.6 years, and the mean smoking rate was 34.9±11.6 packs/year. The mean age of the female patients was 66±11.7 years, and the mean smoking rate was 26±5.4 packs/year (Table 1). There was no statistically significant difference between male and female patients for age and smoking variables in RB patients (p=0.386 and p=0.1, respectively).

The first reader (a cardiothoracic radiologist with 10 years of experience) detected centriacinar nodules in 42 (79.2%) of 53 patients on conventional CT images and centriacinar nodules in all 53 (100%) patients on MIP images (Figures 1A and B).

Table 1. Number of patients, smoking, and age.

	Number (%)	Age (years)±SD	Smoking (packs/year)±SD
Male	48 (90.6)	61.2±11.6	34.9±11.6
Female	5 (9.4)	66±11.7	26±5.4
Total	53 (100)	61.6±11.6	34±11.4

SD: standard deviation.

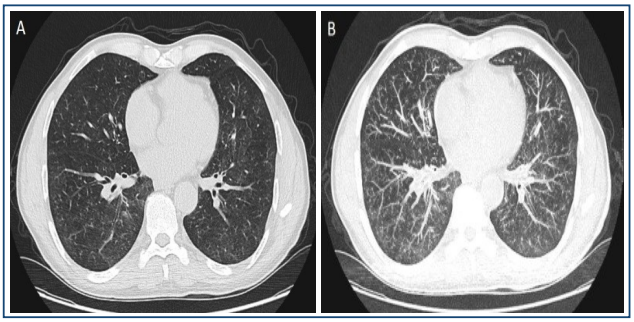

Figure 1. (A) In a 57-year-old male patient with a 35 packs/year smoking history, ill-defined centriacinar nodules were detected on a conventional axial computed tomography scan. (B) Centriacinar nodules are more demonstratively observed in the maximum intensity projection images of the same patient.

The second reader (a nonspecific radiologist with 2 years of experience) detected centriacinar nodules in 12 (22.6%) of 53 patients on conventional CT images and centriacinar nodules in 48 (90.6%) patients on MIP images (Table 2). When the first and second readers were compared in the detection rate of nodules in the evaluation of conventional images, the first reader was able to detect nodules at a statistically significant rate (p<0.001). In the evaluation of MIP images, a comparison of the first and second readers in the detection rate of nodules could not be made statistically because the first reader detected all nodules at a higher rate in MIP images. For the first reader, a statistical comparison could not be made in the detection of nodules in conventional and MIP images, as all nodules were detected in MIP images, but an increase in the detection rate of nodules in MIP images was observed. In the comparison of conventional and MIP images, it was observed that the detection rate of nodules for the second reader was statistically significantly higher in MIP images (p<0.001).

Ill-defined centrilobular micronodules were observed in all patients (100%). In addition, areas of ground glass density in 31 (58.4%) patients, mosaic perfusion areas (patch-like hypotenuation areas) in 26 (49%) patients, bronchial wall thickening in 35 (66%) patients, emphysema areas in 30 (56.6%) patients, and subpleural reticulations in 5 (9.4%) patients were observed. In the craniocaudal distribution of the findings, upper-middle zone dominance was observed in 41 (77.3%) patients, while zonal dominance was not detected in 12 (26.7%) patients. It was found that the involvement was bilateral in all patients (100%).

DISCUSSION

In our study, in which we examined the contribution of MIP images to the diagnostic accuracy in detecting micronodules in diseases such as RB and RB-ILD that form ill-defined

Table 2. Detection rates of first and second readers' micronodules on conventional computed tomographic images and maximum intensity projection images.

		Conventional images		MIP images		p-value
		Number	%	Number	%	
First reader	Detected	42	79.2	53	100	Could not be calculated
	Not detected	11	10.8	0	0	
Second reader	Detected	12	22.6	48	90.6	<0.001
	Not detected	41	77.4	5	9.4	

MIP: maximum intensity projection.

micronodules in the lung, we were able to show that MIP images increase the detection rate of radiologists regardless of experience. There are studies in the literature showing that MIP images have a higher rate of detecting nodules[4,7-9], and the importance of MIP images in detecting especially small nodules has been emphasized[10]. However, we have shown for the first time, independent of the radiologist's experience, that MIP images increase the detectability of micronodules for a specific disease such as RB.

Maximum intensity projection images consist of axial slab volumetric data and have two important advantages. The first is to ensure that nodular structures adjacent to vascular structures are clearly discerned in a single image. Second, although it reduces the number of slices available, it does not lose spatial resolution for a single MIP slab. However, it should not be forgotten that there is data loss in MIP reconstruction. In a study, 5 mm MIP slab thickness and 1 mm HRCT and conventional CT micronodule detection were compared. In this study, MIP images of 5 mm slab thickness detected micronodules at the highest rate with 100% sensitivity[5]. However, in another study, 30 mm slab thickness MIP images were evaluated, and the nodule detection rate decreased due to the superposition of pulmonary vascular structures to the nodules[11]. In our study, we evaluated 10 mm slab thickness MIP images. Both readers detected a higher rate of micronodules in MIP images than in conventional images. A statistically significant increase was found in MIP images, especially for the less experienced radiologist.

In the literature, the classic HRCT image of RB has been described as bronchial wall thickening, ill-defined centrilobular nodules, and ground glass areas[12-14]. Involvement in RB is more prominent in the upper zones. Lung fibrosis and honeycombing are uncommon. Findings are usually observed predominantly in the upper zone. Ground glass areas are bilateral, and patchy involvement is observed[15]. We also found similar findings on conventional CT images. We were able to show the nodules more demonstratively in MIP images. In RB, peripherally located ground-glass densities can be observed in the lower lobes with overlapping findings with DIP, another form of smoking-related bronchiolitis[1]. Due to the presence of ill-defined centrilobular nodules, RB is most often confused with subacute hypersensitivity pneumonia[16]. In differential diagnosis, a strong association between smoking history, nonspecific findings in BAL (lymphocytosis is observed in BAL in hypersensitivity pneumonitis), and the absence of organic antigen exposure is observed.

In some cases, other diseases with micronodular involvement may also be included in the differential diagnosis of RB. In this case, attention is paid to the patient's history, involved lung zone, symmetrical or asymmetrical involvement. In addition, attention is paid to the perilymphatic, centrilobular, and random involvement of micronodules. For example, inorganic material exposure is observed in pneumoconiosis and its subtype silicosis. In pneumoconiosis, the nodules are more sharply circumscribed and symmetrically located in the upper lobes. Nodules are frequently observed in the posterior parts of the lung. In miliary tuberculosis, unlike RB, the nodules are randomly distributed and all lobes are uniformly affected. In sarcoidosis, nodules are observed in the perilymphatic region and there is asymmetric involvement[17].

A strong relationship between RB and smoking is mentioned in the literature[1,12-14]. All of our patients have a strong smoking history. However, in the literature, besides smoking, soldering smoke, diesel smoke, and fiberglass are also included in the etiology[18,19].

The limitations of our study are as follows: the patients were not confirmed by biopsy, the number of patients studied was low, the number of radiologists who evaluated them was low, and MIP images with a thickness of 5 mm slabs were not studied in the search for nodules.

CONCLUSION

In patients presenting with a micronodular pattern such as RB, MIP images increase the detectability of micronodules independently of the experience of the radiologist. The inclusion of MIP images in the protocol will increase the accuracy of diagnosis when evaluating the HRCTs of the cases in which micronodules are investigated. We hope that the number of studies supporting our study will increase and the contribution of other advanced reconstruction techniques to the diagnosis of lung diseases will be investigated.

AVAILABILITY OF DATA

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTIONS

HAK: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **MB:** Conceptualization, Data curation, Supervision. **EG:** Formal Analysis, Supervision.

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ORIGINAL ARTICLE

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Electrophysiological study in chagasics with syncope and conduction disorder

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SUMMARY

BACKGROUND: Investigation of syncope involves the use of electrophysiological study, particularly in patients with cardiac conduction disorder. There is conflicting evidence about the role of electrophysiological study in patients with Chagas disease.

OBJECTIVE: The objective of this study was to evaluate the electrophysiological study findings in patients with Chagas disease and bundle branch block and/or divisional block presenting with syncope.

METHODS: This is a retrospective study of patients with Chagas disease and cardiac conduction disorder who underwent electrophysiological study from 2017 to 2021 for the investigation of syncope in a tertiary hospital in São Paulo, Brazil. Those with non-interpretable ECG, known coronary artery disease, and/or other cardiomyopathies were excluded. HV interval and electrophysiological study-induced malignant ventricular arrhythmias data were analyzed.

RESULTS: A total of 45 patients (60.2±11.29 years, 57.8% males) were included. The mean HV interval was 58.37 ms±10.68; 22.2% of the studied population presented an HV interval of ≥70 ms; and malignant ventricular arrhythmias were induced in 57.8% patients. The use of beta-blockers and amiodarone (p=0.002 and 0.036, respectively), NYHA functional class≥II (p=0.013), wide QRS (p=0.047), increased HV interval (p=0.02), Rassi score >6.5 (p=0.003), and reduced left ventricular ejection fraction (p=0.031) were associated with increased risk of inducible malignant ventricular arrhythmias.

CONCLUSION: More than half of the patients with Chagas disease, syncope, and cardiac conduction disorder have inducible malignant ventricular arrhythmias. Prolonged HV interval was observed in only 20% of population. Wide QRS, prolonged HV, reduced ejection fraction, and higher Rassi score were associated with increased risk of malignant ventricular arrhythmias.

KEYWORDS: Chagas disease. Electrophysiological study. Syncope. Bundle branch block.

INTRODUCTION

Chagas disease (CD) is an endemic disease in Latin America. It affects approximately 18–20 million individuals and is responsible for high rates of morbidity and early mortality[1]. Approximately 30–40% of the infected population develop the cardiac form, with a worse prognosis, which may manifest with heart failure (HF) symptoms, cardiac arrhythmias, or thromboembolism[2].

The chronic inflammatory disease caused by the presence of parasite may result in sinus dysfunction and cardiac conduction system abnormalities. Myocardial fibrosis is the substrate for reentrant circuits and the main mechanism of malignant ventricular arrhythmias (MVA) and sudden death (SD) even in patients without HF or severe left ventricular dysfunction. In patients with chagasic cardiomyopathy, syncope may be a consequence of ventricular tachycardia (VT), ventricular fibrillation (VF), atrioventricular block (AVB), sinus node dysfunction, or neuromodulated mechanisms[3].

In patients with unexplained syncope and bifascicular branch block, a permanent pacemaker (PM) is indicated in cases of HV interval≥70 ms and/or second- or third-degree AVB during atrial stimulation or pharmacological testing[4].

Investigation of syncope involves the use of electrophysiological study (EPS), particularly in patients with cardiac conduction disorder (CCD). There is conflicting evidence about EPS in patients with CD. The purpose of this study was to evaluate the EPS findings in patients with CD and bundle branch block and/or divisional block presenting with syncope.

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METHODS

All the participants signed a written informed consent form. In November 2021, the protocol number 4893/2018 was approved by the local ethics committee.

This retrospective study included consecutive patients with CD and CCD who underwent EPS for the investigation of syncope in a tertiary hospital in São Paulo, Brazil, from 2017 to 2021. HV interval and EPS-induced MVA. Clinical data, electrocardiographic, echocardiographic, and 24-h Holter findings were obtained.

Inclusion and exclusion criteria

Patients with CD at any age, syncope, and bundle branch block and/or divisional block who underwent EPS were included in the analysis. Those with non-interpretable ECG and/or other cardiomyopathies such as hypertrophic, valvopathy, and right ventricular dysplasia were excluded. Patients with coronary artery disease defined by either symptoms of angina pectoris and/or dyspnea on exertion associated with ≥50% obstruction of the vascular lumen of epicardial coronary arteries on cineangiocoronariography or myocardial Ischemia on non-invasive exam as well as patients with previous MI with reduced LV function/scar were also excluded.

For EPS, the following protocol was used: ventricular stimulation with two basic cycles and up to three extrastimuli as well as rapid ventricular stimulation (up to 250 ms or 2:1 ventricular capture) in both apex and right ventricular outflow tract. VMA was classified into sustained VT and VF according to the definitions of current guidelines[5]. HV interval was measured from the beginning of the His bundle potential deflection (H) to the earliest onset of ventricular activity (V).

Statistical analysis

Quantitative variables were presented by means, standard deviations, and minimum and maximum values, and categorical variables were presented by frequencies and percentages.

For univariate analysis, Fisher's exact test or chi-square test was used for categorical variables, and for those with a quantitative character, Student's t-test for independent samples or Mann-Whitney's non-parametric test was used. The normal condition of the quantitative variables was assessed using Kolmogorov-Smirnov's test.

As for the multivariate analysis, a logistic regression model was adjusted as explanatory variables showed significance in the univariate analysis. The stepwise backward method was used to reduce the model. For model adjustment, Hosmer-Lemeshow's test was applied and the value of the area under the C-statistic [receiver operating characteristic (ROC) curve] was estimated. $p<0.05$ was considered statistically significant. Data were analyzed using IBM SPSS Statistics for Windows, Version 19.0 (Released 2010, Armonk, NY: IBM Corp.) and R Core v3.6.3.

RESULTS

A total of 62 patients with syncope, Chagas disease, and CCD undergoing EPS were evaluated, of whom 17 were excluded due to incomplete records. Therefore, 45 patients were included in the analysis.

The mean age was 60.2±11.29 years, and 26 (57.8%) were males. Approximately 70% of patients had hypertension and 13.3% were diabetic. Most patients were in NYHA functional class I. The mean Rassi score was 8.53±5.86. The clinical characteristics of the studied population are presented in Table 1.

Most patients were in sinus rhythm, of whom 13.3% were in atrial fibrillation, and 4.4% had paced rhythm on ECG. Almost half of the patients had both right bundle branch block (RBBB) and left anterior fascicular block (LAFB). Left bundle branch block (LBBB) was observed in 20% of patients. The mean QRS duration was 144.8 ms (ranging from 80 to 210 ms). Notably, 51.1% of patients had frequent PVCs (>30/h), 40% had non-sustained VT, and 17.7% sustained VT on 24-h Holter monitoring (Table 1).

The mean LVEF was 45±15.7%, being 40% lower than 40%. Echocardiographic findings are presented in Table 1. The mean LVEF was 51.9 and 39.8% in the groups without and with MVA induction, respectively (p=0.013) (Table 2 and Figure 1).

The mean HV interval was 58.37±10.68 ms. In only 22.2% of patients, HV was ≥70 ms. Ventricular arrhythmias were induced in 57.8% of the sample.

In the univariate analysis, MVA predictors were use of beta-blockers and amiodarone (p=0.002 and 0.036, respectively), HV>70 ms (p=0.02), Rassi score >6.5 (p=0.003), and low LVEF.

For each 10-ms increase in the HV interval, there was a 51% increase in MVA inducibility (p=0.19).

For each 10-ms increase in QRS duration, there was a 29% increase in MVA inducibility (p=0.19). An ROC curve was performed to determine the cutoff point of the QRS interval associated with MVA induction. The value of 127 ms showed a sensitivity of 80.8% and a specificity of 36.8% [area under the curve (AUC) of 0.67 (p=0.04)] (Figure 1).

For each 10-unit decrease in LVEF, an increased risk of 75% in MVA was observed (p=0.01). An ROC curve was performed to determine the cutoff point of LVEF associated with VMA induction. The value of 48% showed a sensitivity of 73.1% and a specificity of 68.4% [AUC of 0.72 (p=0.01)] (Figure 1).

Table 1. Demographic characteristics.

Variable		Mean and N	%
Age (years)		60.27±11.29	
Male		26	57.8
Dyslipidemia		20	44.4
Systemic hypertension		32	71.1
Previous stroke		3	6.7
Diabetes mellitus		6	13.3
Coronary artery disease*		5	11.1
NYHA	I	28	62.2
	II	11	24.4
	III	6	13.3
	IV	0	0.0
Smoking	No	32	71.1
	Former smoker	12	26.7
	Yes	1	2.2
Rassi score		8.53±5.86	
ACEI/ARB		37	82.2
Beta-blockers		26	57.8
Amiodarone		27	60.0
Diuretic		29	64.4
Estatin		23	51.1
AAS		12	26.7
Warfarin		11	24.4
DOAC		3	6.7
Electrocardiographic findings			
Sinus rhythm		37	82.2
Atrial fibrillation		6	13.3
Pacemaker		2	4.4
Right bundle branch block		9	20.0
Left anterior fascicular block		5	11.1
RBBB+LAFB		22	48.8
LBBB		9	20
First-degree AV block		15	33.3
Second-degree AV block		2	4.4
Holter findings			
PVC>30/h		23	51.1
Non-sustained VT		18	40.0
Sustained VT		8	17.7
Echocardiogram findings			
LVEF mean (SD)		45%	15.7
Left ventricular thrombus		0	0
Left ventricular aneurysm		7	15.6
Left atrial volume≥32 mL/m²		43	95.5

ACEI/ARB: angiotensin-converting enzyme inhibitor/angiotensin receptor block; AV: atrioventricular; DOAC: direct oral anticoagulant; LAFB: left anterior fascicular block; LBBB: left bundle branch block; LVEF: left ventricular ejection fraction; PVC: premature ventricular contraction; RBBB: right bundle branch block; SD: standard deviation; VT: ventricular tachycardia. *This includes mild CAD patients (<50% obstruction of the vascular lumen of epicardial coronary arteries on cineangiocoronariography) or previous MI without compromised LV function/scar.

Finally, a Rassi score of 6.5 presented a sensitivity of 75.9% and a specificity of 75%, for VMA induction [AUC of 0.77 (p=0.003)] (Figure 1).

DISCUSSION

In this study of patients with Chagas disease and conduction disorder, MVA induction on EPS was the main factor associated with the occurrence of syncope. This finding might help physicians in the decision-making by indicating an ICD instead of a PM for this specific population.

Syncope in patients with CD and cardiac involvement is an alert situation, assuming that the main etiology is ventricular arrhythmia[6]. However, other causes such as paroxysmal AVB should be considered, with a more favorable prognosis[7].

Unlike other conditions, vasovagal syncope in patients with CD is not always benign, once cardiac dysautonomia is related to reduced baroreflex sensitivity and the occurrence of complex ventricular arrhythmias[8]. Autonomic dysfunction may occur before ventricular dysfunction in CD, and this was demonstrated by myocardial scintigraphy with Iodine-123-labeled metaiodobenzylguanidine (123I-MIBG). In a study of patients with CD and normal or slightly reduced LV function, the presence of ventricular arrhythmias was associated with more extensive areas of viable and denervated myocardium identified by 123I-MIBG[9].

The Rassi score is widely used for mortality prediction in CD. Its elaboration was based on a systematic review of 12 studies that did not include syncope[10]. Syncope inclusion as a risk factor would probably increase the score sensitivity without changing its simplicity. In this study, a Rassi score of 6.5 was associated with 75.9% sensitivity and 75% specificity for MVA induction.

Few studies have assessed the value of EPS in patients with CD. Leite et al.[11] analyzed chagasics with spontaneous sustained VT despite the use of class III antiarrhythmics. Those who presented unstable VT had a worse prognosis compared with patients in whom VT was either hemodynamically tolerated or not induced. In most individuals with preserved LVEF and either no spontaneous arrhythmias or NSVT on 24-h Holter monitoring, EPS does not provide relevant prognostic information[12,13]. In this study, patients underwent EPS for syncope investigation according to the recommendations of current guidelines[14], after inconclusive non-invasive evaluation.

In the study published by Silva et al., EPS-induced VMA was a predictor of arrhythmogenic death and all-cause mortality[15]. The pathophysiological mechanism involves the presence of regional fibrosis, particularly in the left ventricular posterior-lateral wall, and results in reentrant circuits[16-19]. In our study,

Table 2. Clinical characteristics and VMA induction.

	VMA induction		p-value
	No	Yes	
Male (26)	9 (34.6%)	17 (65.4%)	0.28
Smoking (13)	6 (46.2%)	7 (53.8%)	0.41
Dyslipidemia (20)	11 (55%)	9 (45%)	0.12
Hypertension (32)	15 (46.9%)	17 (53.1%)	0.32
Stroke (3)	0	3 (100%)	0.12
Diabetes mellitus (6)	2 (33.3%)	4 (66.7%)	0.64
CAD (5)*	1 (20%)	4 (80%)	0.29
NYHA			0.034
I (28)	16 (57.1%)	12 (42.9%)	0.013
II and III (17)	3 (17.6%)	14 (82.4%)	0.013
ACEI/ARB (37)	17 (45.9%)	20 (54.1%)	0.277
Beta-blockers (26)	6 (23.1%)	20 (76.9%)	0.002
Amiodarone (27)	8 (29.6%)	19 (70.4%)	0.036
Diuretic (29)	11 (37.9%)	18 (62.1%)	0.433
Statin (23)	11 (47.8%)	12 (52.2%)	0.436
Aspirin (12)	4 (33.3%)	8 (66.7%)	0.467
Warfarin (11)	6 (54.5%)	5 (45.5%)	0.341
DOAC (3)	2 (66.7%)	1 (33.3%)	0.375
Sinus rhythm (37)	14 (37.8%)	23 (62.2%)	0.4
Atrial fibrillation (6)	4 (66.7%)	2 (33.3%)	0.4
RBBB (31)	16 (51.6%)	15 (48.4%)	0.58
LBBB (9)	2 (22.2%)	7 (77.8%)	0.17
LAFB (27)	13 (48.1%)	14 (51,9%)	0.32
First-degree AV block (15)	5 (33.3%)	10 (66.7%)	0.39
Second-degree AV block (2)	1 (50%)	1 (50%)	0.82
PVC>30/H (23)	7 (30.4%)	16 (69.6%)	0.1
NSVT (18)	7 (38.9%)	11 (61.1%)	0.71
HV			0.02
<70 ms (31)	16 (51.6%)	15 (48.4%)	0.02
≥70 ms (10)	1 (10%)	9 (90%)	0.02
Left ventricular aneurysm (7)	2 (28.6%)	5 (71.4%)	0.43
Mean LVEF (%) SD	51.9% (±14.7)	39.8% (±14.6)	0.013
Left atrial volume			
≥32 (43)	19 (44.1%)	24 (55.8%)	0.747

ACEI/ARB: angiotensin-converting enzyme inhibitor/angiotensin receptor block; AV: atrioventricular; CAD: coronary arterial disease; DOAC: direct oral anticoagulant; LAFB: left anterior fascicular block; LBBB: left bundle branch block; LVEF: left ventricular ejection fraction; NSVT: non-sustained ventricular tachycardia; PVC: premature ventricular contraction; RBBB: right bundle branch block; SD: standard deviation; VT: ventricular tachycardia. *This includes mild CAD patients (<50% obstruction of the vascular lumen of epicardial coronary arteries on cineangiocoronariography) or previous MI without compromised LV function/scar.

more than half of the patients had VMA induction. This finding is in accordance with the data published by Martinelli et al., in which the most prevalent cause of syncope in chagasics was VMA (43%) followed by paroxysmal AVB (21%)[7].

In this study, the use of amiodarone and beta-blockers, NYHA functional class>I, reduced LVEF (<50%), QRS duration, and prolonged HV interval (>70 ms) were found to be predictors of VMA.

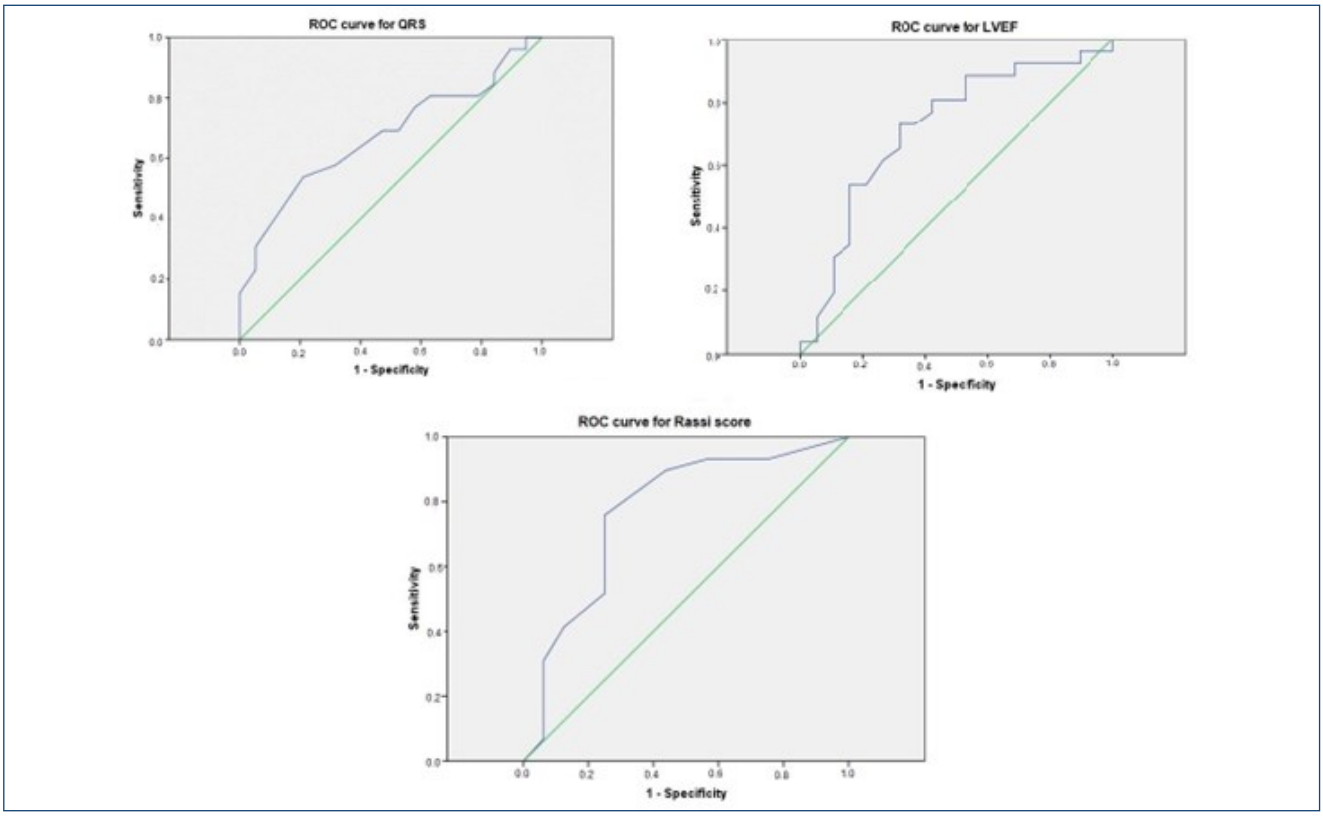

Figure 1. Receiver operating characteristic curve for QRS, left ventricular ejection fraction, and Rassi score values in the prediction of VMA.

The higher occurrence of VMA in patients under amiodarone treatment may reflect the previous diagnosis of ventricular arrhythmias and, consequently, the greater severity of these patients. The same finding was observed in the study of Cardinalli et al.[20], in which amiodarone therapy was an independent risk of VMA.

Ventricular dysfunction is also a predictor of ventricular arrhythmias in patients with CD. For each 10-unit decrease in LVEF, we found a 75% increase in the risk of VMA induction. LVEF of 48% had a sensitivity of 73% and a specificity of 68.4% for VMA induction. On the contrary, there was no association between the density of ventricular arrhythmias on 24-h Holter and VMA induction, which is different from the publication of Souza et al.[6], in which the presence of syncope, QT interval, ventricular dysfunction, and ventricular ectopies were the predictors of sudden cardiac death in patients with CD.

Wide QRS complex and prolonged HV interval are the markers of structural heart disease (fibrosis and/or ventricular dysfunction), which reflect slower and nonsynchronized ventricular depolarization, a substrate for reentrant circuits. For each 10-ms increase in QRS duration, we showed a 29% increase in VMA inducibility. QRS interval >127 ms was associated with a sensitivity of 80.8% of VMA induction. Although not statistically significant, for each 10-ms increase in the HV interval, there was a 51% increase in VMA inducibility.

Prolonged HV interval is a controversial risk factor for the development of AV block. Studies have shown that HV>70 ms is associated with a higher risk of AV block, especially in symptomatic patients. HV>100 ms identifies a group of very high risk of AV block (25% in 22 months)[21]. In the presence of RBBB with or without fascicular block, HV is normal as long as the conduction through the left bundle branch is unchanged. However, 50% of patients with RBBB are combined with anterior superior divisional block and 75% with LBBB have HV interval prolongation[22]. Although our sample was composed of patients with CCD, only 22.2% had prolonged HV interval (11% of patients with LBBB and 34.8% with RBBB). In these cases, the etiology of syncope is multifactorial and may be secondary to paroxysms of AVBs, sinus node disease, dysautonomia, or VMA.

The main limitations of this study are the inclusion of a single center, the retrospective nature, and the small sample. Despite these, we were able to demonstrate that, similar to previous publications, even in the presence of intraventricular conduction abnormalities, VMA is the main cause of syncope in patients with Chagas disease.

CONCLUSION

More than half of patients with Chagas disease, syncope, and CCD have inducible VMA. Prolonged HV interval was observed in only 20% of the population. Wide QRS, prolonged HV, reduced ejection fraction, and higher Rassi score were associated with an increased risk of VMA. Larger studies are needed to confirm the findings.

AUTHORS' CONTRIBUTIONS

AHD: Data curation, Investigation, Project administration, Writing – original draft. **LA:** Investigation, Project administration, Writing – review & editing. **DARM:** Writing – review & editing. **RDL:** Writing – review & editing. **BPV:** Writing – review & editing.

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ORIGINAL ARTICLE

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Critical assessment of resource waste in staging and follow-up of breast cancer

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SUMMARY

BACKGROUND: Breast cancer is a public health problem with both high incidence and cure rates. After treatment, patients are monitored for long periods of time due to the risk of recurrence. Thus, staging and follow-up strategies should consider not only the best results for the patient but also its costs for the public health system.

OBJECTIVE: The objective of this study was to quantify the waste of resources on breast cancer follow-up and evaluate its impact on the public health system.

METHODS: This is a retrospective analysis of consecutive medical records to identify the intervals between consultations and tests used for staging and during the first 2 years of follow-up of patients with breast cancer treated at a public hospital in Brazil. Data were compared with the guidelines of the main international consensus.

RESULTS: Medical records of 60 consecutive patients treated in 2018 were selected, of whom 52 had 2 or more years of follow-up, and 8 had only 1 year of complete follow-up. A total of 34 patients (56.67%) underwent excessive examinations for stating. During follow-up, 125 surplus consultations were performed (33.6%). In this phase, 111 surplus exams were also performed, representing an increase of 100.9%. A total of 423 laboratory tests were performed for 18 patients in the first year and 229 tests for 14 patients in the second year.

CONCLUSION: Excessive tests and consultations significantly burdened the Unified Health System without any benefit to patients. Better adherence to staging and follow-up recommendations could reduce costs and optimize the limited resources used in the public health system.

KEYWORDS: Breast cancer. Public health. Cancer care facilities. Continuity of patient care. Brazil.

INTRODUCTION

Breast cancer (BC) is a challenging public health problem. According to the International Agency for Research on Cancer (IARC), it is classified as the second highest in global incidence, with lung cancer being the first when both men and women are considered[1,2]. In 2018, BC had an incidence of 2,088,849 cases, which corresponds to more than 11% of the total number of cancer cases worldwide[2]. In Brazil, data from GLOBOCAN 2018 show that the incidence of BC was 62.9 per 1,00,000 inhabitants, which is second only to prostate cancer. By 2023–2025, the National Institute of Cancer estimates more than 73,000 new cases[2,3] in the country.

Brazil is well-known for the largest public health system in the world, which ought to provide free tests and treatment to everyone in the country, and it comes with a cost[4]. Regarding tests for BC diagnosis, which are usually performed in medium- and high-complexity centers, the Federal Court of Auditors disclosed the staggering amount of BRL 41,174,464,206.19 or USD 7,952,729,981.49 allocated for these procedures in 2018[5-7].

Given the limited resources—human and physical—presented in the public health setting, their poor distribution among national states, and their high costs, patients do not always have access to the best medical practice. Therefore, it is imperative to discuss cost-effective methods for conducting oncology patients, especially those treated with curative intent. Thus, this study aims to observe and quantify the use of resources outside the international recommendations and to evaluate the temporal and financial impact of such waste of resources in the public health system.

METHODS

Study design and data collection

Patients who underwent consultation with clinical oncology from January 1, 2018, to December 31, 2018, were consecutively selected in the hospital database using the International Code of Diseases (ICD-10) for BC (C50, C50.8, and C50.9)[8].

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Their records were consulted, and all descriptive and relevant data were collected. Patients receiving molecular targeted therapy or those with metastatic disease were excluded.

Initially, an analysis was performed on imaging tests requested for staging of each patient to verify whether they were in accordance with the main oncological guidelines[9]. Excess tests requested due to clinical complaints were excluded.

Then, the first and second years of follow-up were evaluated, and the frequency of medical consultations with the Oncology and Mastology teams was verified. Patients who needed to anticipate appointments and undergo tests due to clinical complications were excluded. Imaging tests requested during both years of follow-up were also analyzed. The requested exams were compared with those indicated by guidelines. Those exceeding the recommendations, except for the tests requested upon complaints or physical findings, were counted.

In addition, laboratory tests performed during both years of patient follow-up were also evaluated. Patients who needed to undergo additional tests or tests commonly requested due to specific oncological medications were excluded. Once laboratory tests are only indicated if symptoms are present, all tests performed outside this condition were considered surplus. Tests with positive findings that led to changes in conduct and those usually requested during follow-up for patients using certain oncological medications were disregarded.

Statistical analysis

A total of 522 consecutive medical records were evaluated, of which 462 were excluded for not meeting the inclusion criteria. The remaining 60 medical records were included. Data were tabulated using Microsoft Excel® and analyzed with descriptive statistics using the Stata/IC® software. Numerical values are expressed as mean and standard deviation when applicable. Comparisons between the number of exams and consultations recommended and performed were made with frequencies and absolute numbers. To test the alternative hypothesis that there was an actual difference between the ideal and real amount of exams and consultations performed, the Wilcoxon signed-rank test was used. Confidence level was set at 95%.

RESULTS

Sample characteristics

A total of 60 female patients aged between 40 and 95 years (mean±SD=62.68±12.51) were included. Table 1 shows the distribution of the relevant characteristics of the sample in absolute and percentage values.

Table 1. Characteristics of the sample.

Patients (n=60)	
Characteristics	**N (%)**
Age	
Up to 65 years	34 (57)
>65 years	26 (43)
Menopause	
Pre	23 (38)
Post	37 (62)
Clinical staging	
I	4 (7)
II	33 (55)
III	23 (38)
Molecular subtype	
Luminal A	10 (17)
Luminal B	33 (55)
Luminal Hybrid	7 (12)
Triple-negative	6 (10)
HER-2 +	4 (7)
Neoadjuvant therapy	
Yes	38 (63)
No	22 (37)
Length of follow-up (years)	
1	8 (13)
2 or more	52 (87)

Resources used for staging

In the absence of symptoms, clinical staging I and II are not indicated for requesting imaging tests; therefore, 121 surplus tests were performed for these groups. For stage III, imaging tests requested are in accordance with the BC guidelines. However, one of these patients had no information about staging tests in their medical records, and one patient underwent fewer tests than indicated.

First year of follow-up

A total of 237 consultations were performed with oncology and 145 consultations were performed with mastology. Disregarding 11 patients who needed additional consultations, a total of 288 consultations were performed for 49 patients, representing 5.88 consultations per patient or a surplus of 46.94%.

Of the 60 patients in the first year of follow-up, 6 underwent one excess mammogram but one of them was excluded from the analysis because she showed positive findings that justified its conduction. Thus, five mammograms were performed

in excess; however, as three patients did not undergo the recommended exam, the result was an increase of 3.39% over the ideal amount.

Regarding the performance of breast ultrasound exams, 59 exams were performed; of these, 3 were excluded because they were performed for patients with positive findings. Thus, 56 additional tests were performed.

There were 18 patients with data described in medical records who underwent a total of 423 surplus laboratory tests (Table 2).

Second-year follow-up procedures

A total of 171 consultations were performed with oncology and 106 consultations with mastology. Disregarding 8 patients who needed additional consultations, a surplus of 33 consultations was performed, which represents an increase of 18.75%.

Of the 52 patients in the second year of follow-up, there were 7 surplus mammograms, resulting in a 13.73% increase over the ideal number. Regarding breast ultrasound, one patient was excluded from the analysis because she had a positive finding that justified the performance of the additional examination—i.e., not only she had suggestive clinical symptoms, but also the imaging exam confirmed the disease. A total of 46 breast ultrasounds were performed in excess of the recommendations of the main guidelines.

There were 14 patients with data described in medical records who underwent a total of 229 surplus laboratory tests (Table 2).

Excessive follow-up expenses

For staging, 121 exams were performed in excess, resulting in a surplus of 101%. In the first year of follow-up, there was an increase of 98.3% in imaging tests and 46.9% in consultations. A total of 423 laboratory tests were performed. During the second year, the surplus was 103.9% in imaging tests and 18.75% in consultations, and 229 more laboratory tests were performed than expected (Table 2).

The ideal number of consultations and examinations recommended by BC guidelines and the amount performed in excess for the period are shown in Figure 1.

DISCUSSION

This study found a waste of public resources during the first 2 years of follow-up of BC patients. It is important to note that data were collected from a university service, where there should be a greater concern in following the main consensus and guidelines. Thus, it is reasonable to assume that costs may be even higher for services not linked to educational institutes.

Both the waste of resources and the time spent in consultations were higher than ideal at all times, with the greatest

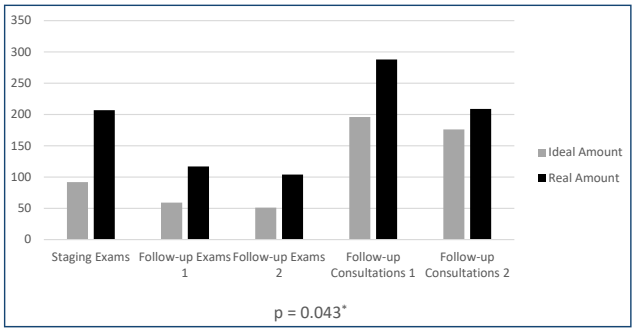

Figure 1. Actual vs. ideal quantity of staging exams, consultations, and exams performed in the first- and second-year follow-up. *Based on Wilcoxon signed-rank test.

Table 2. Optimal and actual number of consultations and complementary tests performed during the first 2 years of follow-up of breast cancer patients.

Procedures during the first year of follow-up	Optimal	Actual amount	Surplus	Surplus percentage
Consultations (n=60)*	196	288	92	46.49%
Mammography (n=57)*	59	61	2	3.39%
Breast ultrasound (n=48)*	0	56	56	–
Procedures during the second year of follow-up	**Optimal**	**Actual amount**	**Surplus**	**Surplus percentage**
Consultations (n=52)*	176	209	33	18.75%
Mammography (n=49)*	51	58	7	13.73%
Breast ultrasound (n=48)*	0	46	46	–
Surplus blood tests run during follow-up				**Absolute number**
First year (n=18)*				423
Second year (n=14)*				229

*Number of patients for whom surplus blood tests were performed.

difference seen at the staging phase, where imaging tests are recommended only for symptomatic patients or those in clinical stage IIIA or higher[10,11]. According to several studies and the American Society of Clinical Oncology, a complete diagnostic investigation for the detection of metastases is unnecessary for most newly diagnosed BC patients[12-15].

Breast cancer is a disease with high incidence and increasingly effective treatments, resulting in longer survival for affected patients. According to Tiezzi et al., the survival rate is 90% in 5 years; therefore, a good follow-up strategy is imperative, and the optimization of public resources to meet this growing demand is of great importance[16].

Besides, 40% of women are diagnosed with locally advanced or metastatic disease, partly due to a lack of guidance and preventive tests in some locations in the country. The lack of resources to acquire and maintain a mammography unit as well as a professional to operate it means that, despite the law to offer it to women as old as 40 years of age, only 30% of the 16 million Brazilian women in the recommended age range underwent mammograms between 2017 and 2018[17,18].

Furthermore, there is difficulty in implementing prevention strategies when primary care services are overloaded[19]. In this sense, this study showed no greater efficacy in performing tests more frequently than indicated in guidelines, as most asymptomatic patients also did not require a different medical approach. In the first year of follow-up, only 4 of 60 patients analyzed had positive imaging findings. In the second year, 1 of 52 patients had positive imaging findings. It should be noted that there is a risk of exposure to ionizing radiation used in imaging tests such as CT and mammography, which, although small, can be harmful[20]. Besides, more imaging does not equal better results. For instance, the routine use of breast ultrasound is not recommended because it is expensive, may not alter survival outcomes, and its addition to mammography may increase the diagnostic yield as well as false-positive rates even in a high-risk population[21,22].

In this study, special attention must be given to the frequency of consultations, which are often performed to evaluate the requested tests that frequently focus on health problems unrelated to cancer. The cost-benefit ratio of performing additional consultations and requesting tests should be evaluated frequently. Specialized medical evaluation is essential for treatment and unrelated conditions should be monitored in primary health care. Thus, the number of consultations could and should be improved to reduce waiting lists and provide better care for cancer patients. One way to do it would be by alternating follow-up consultations between specialties (clinical oncology and mastology) totaling four consultations per year, aiming for better use of the services.

Study limitations

This study was performed in only one service, with a small number of patients, and over a period of 1 year of care. Its retrospective nature also limits the information found, as only the exams available in the medical records were considered, and it is not possible to infer information that may exist but was not recorded.

CONCLUSION

This study showed that laboratory tests and imaging were performed at a higher frequency than recommended by both BC guidelines. Better adherence to staging and follow-up recommendations, regarding the number of consultations performed and tests requested, could reduce costs and optimize the limited resources used in the public health system to benefit a larger number of citizens.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by our institutional review board (Faculdade de Medicina do ABC) on May 4, 2021. It must be noted that only secondary data were gathered for this research, and therefore, the institutional review board waived the duty to apply consent forms for any participant. All methods were carried out ethically as approved by aforementioned the institutional review board.

AVAILABILITY OF DATA AND MATERIALS

All data will be available upon reasonable request to the corresponding author through the email danielcubero@me.com.

AUTHORS' CONTRIBUTIONS

JVBB: Conceptualization, Investigation, Methodology, Project administration. **CVMS:** Conceptualization, Investigation, Project administration. **PXS:** Conceptualization, Investigation, Project administration. **AG:** Conceptualization, Investigation, Project administration. **DIGC:** Conceptualization, Investigation, Project administration. **JHMS:** Methodology.

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ORIGINAL ARTICLE

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Clinical significance of sarcopenia in patients undergoing treatment for gastric cancer

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the impact of sarcopenia on prognosis in patients with gastric cancer in order to explore the relationship between sarcopenia and postoperative complications as well as durations of hospital stay and intensive care unit.

METHODS: A total of 175 patients who visited the oncology clinic between 2017 and 2022 with respect to their radiological images, demographic data, and laboratory parameters were perused. The OsiriX software was used to measure the skeletal muscle area that was divided by the body height in order to obtain the skeletal muscle index.

RESULTS: A total of 50.28% of 175 patients (41 females and 134 males, with a mean age of 63.5 years) who met the inclusion criteria in the study were sarcopenic. Significant differences appeared between sarcopenic and non-sarcopenic patients with respect to durations of both hospital stay ($p<0.01$) and intensive care unit stay ($p<0.01$) (multivariate analysis). Furthermore, patients with sarcopenia had significantly frequent postoperative complications in comparison with those without sarcopenia. Among the patients with sarcopenia, decreased levels of hemoglobin and albumin as well as lymphocytes were encountered in terms of inflammatory markers; nevertheless, no significant differences were determined among other inflammatory markers.

CONCLUSION: In patients undergoing treatment for gastric cancer, sarcopenia increases postoperative complications and prolongs hospital and intensive care stays during the treatment process.

KEYWORDS: Albumins. Biomarker. Intensive care units. Sarcopenia. Skeletal muscle. Stomach neoplasm.

INTRODUCTION

Gastric cancer is the fifth most common type of cancer in the world[1]. Gastric carcinoma (GC) ranks third in cancer-related deaths worldwide[2]. Despite the decrease in the incidence of GC in recent years as well as the increase in endoscopic detection and screening in the early phase, GC poses still a significant clinical challenge[3]. More than 950,000 new diagnoses are performed annually across the world[4]. Gastric cancer comprises 10.4% of cancer deaths worldwide[5]. Surgical resection remains the only potential curative treatment[2]. Chemotherapy is commonly used in addition to surgery in order to improve patient outcomes[3].

Gastric cancer can be assorted into three groups with respect to its etiology and localization: (a) distal type gastric cancer associated with chronic gastritis and *Helicobacter pylori* infection, (b) proximal type cancer associated with obesity and gastroesophageal reflux disease and being more aggressive, and (c) signet-ring cell type cancer that is diffusely infiltrative and not associated with gastritis[6].

Nowadays, due to the increase in life comfort and the development of screening methods, patients are generally diagnosed with endoscopy for symptoms such as dyspepsia and reflux[7]. However, patients may rarely present with advanced symptoms such as gastrointestinal bleeding, dysphagia, anorexia, weight loss, abdominal pain, and nausea[4,7].

Optimal diagnosis for gastric cancer appears to be a staging process with computed tomography (CT) after biopsy taken with endoscopy and diagnosed[2]. Sufficient surgical resection is the only curative option for gastric cancer[4]. Recent studies have substantiated a negative association between sarcopenia and postoperative complications as well as the duration of hospital stay, survival, and early- and long-term outcomes of patients after surgery[1].

Sarcopenia is defined as a condition characterized by the loss of skeletal muscle mass and strength[5]. It is commonly observed in elderly individuals, but it can also occur in younger ages with chronic diseases or prolonged bed rest[5]. European Working

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Group on Sarcopenia determines sarcopenia in elderly people as weak muscle mass, either as low muscle strength or as low physical performance[1]. Sarcopenia has been associated with an increased risk of complications following surgery and poorer outcomes in various types of cancers[1]. Recent studies have proposed that systemic inflammatory markers such as hypoalbuminemia, anemia, lymphopenia, thrombocytopenia, and neutrophil/lymphocyte ratio (NLR) have prognostic consideration in the course of the disease and adherence to treatment when evaluated together with sarcopenia[2]. The study published in the Annals of Surgical Oncology emphasizes that sarcopenic patients had a higher incidence of postoperative complications, such as surgical site infection, anastomotic leaks, and delayed gastric emptying, following surgery for gastric cancer, and cardiac and pulmonary complications[8]. Furthermore, it adverted that patients with sarcopenia had a longer hospital stay and a higher mortality rate than those without sarcopenia[8].

In sarcopenia diagnosis, the skeletal muscle index, which is derived from muscle mass area acquired by using CT, is utilized. Measurements of each patient were performed using contrast-enhanced abdominal CT scans.

The objective of this study was to assess the association between sarcopenia and postoperative complications as well as the durations of hospital stay and intensive care unit (ICU) in patients with gastric cancer.

METHODS

The study was conducted in accordance with the Declaration of Helsinki as well as reviewed and approved by the ethics committee of our hospital (Approval date and no: 2011-KAEK-25 2023/03-04).

The preoperative data of the patients who participated in the study, including medical records, age, gender, height, weight, body mass index, blood parameters, surgery date, use of neo-adjuvant chemotherapy, and patients' comorbidities, were collected from the hospital system. Patients who underwent surgery as well as those who were inoperable and received adjuvant therapy were screened. Sarcopenia indexes were calculated based on CT images taken before surgery or chemotherapy. Surgical methods, the extent of the disease, lymph node involvement during surgery, and surgical stages were surveyed. As postoperative information, the duration of hospital stay and cardio-pulmonary system complications after surgery were investigated.

Study population

We assessed 175 of 217 patients who visited the Oncology Department between September 2017 and December 2022 and who were diagnosed with pathologically gastric cancer.

Inclusion criteria for the study

(1) Patients diagnosed with gastric cancer by biopsy, (2) patients who had a CT examination in the hospital system within a maximum of 4 weeks before the surgery or chemotherapy, (3) patients with blood parameters taken within a maximum of 4 weeks before the surgery or chemotherapy present in the system, and (4) patients whose weight and height data present in the system within a maximum of 4 weeks before the surgery or chemotherapy were investigated.

Exclusion criteria

(1) Patients with significant subcutaneous and mesenteric edema, (2) patients with widespread metastases in muscle tissue and intra-abdominal adipose tissue, and (3) patients with widespread intra-abdominal implants were excluded from the study.

A total of 17 patients with subcutaneous and mesenteric edema, 17 patients with the absence of preoperative CT images, and 8 patients with the absence of preoperative blood parameters in the hospital system were excluded from the study. In 175 patients, measurements were executed and images were analyzed. Blood counts, body weight and height, and serum tumor markers were also collected.

Analyses of computed tomography images and skeletal muscle mass measurement

All abdomen CT scans were performed by using a 128-slice multi-detector-row CT scanner (Toshiba Aquillion, Japan). All CT images were acquired at deep inspiration in the supine position, and all thoracic and abdominal sections were scanned in the soft tissue window, using thin section and contrast-enhanced scans. CT scans were analyzed using the OsiriX version 5.6.2 open-source software. The cross-sectional skeletal muscle area (cm^2) was measured by using a standardized approach[1]. The cross-sectional skeletal muscle surface area (cm^2) was measured at the level of the third lumbar vertebra (L3)[2] (Figure 1).

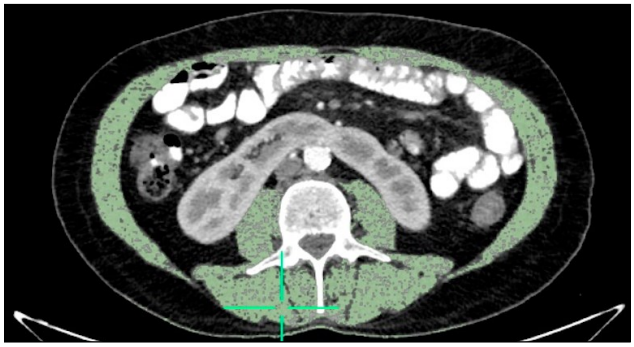

Figure 1. Axial computed tomography image crossing from the level of the third lumbar vertebrae of the gastric cancer patient. Skeletal muscle (green) was measured by using the OsiriX software semi-automatically.

In the axial plane, the section in which both transverse processes were displayed was selected and measurements were performed from this section[4]. L3 skeletal muscles comprise the paraspinal muscles, psoas major, rectus abdominis, and internal and external oblique and transverse abdominal muscles[4] (Figure 1). During muscle area measurement, Hounsfield values varying from -29 to +150 were used in the OsiriX Program[1]. The skeletal muscle index was also acquired by dividing the skeletal muscle area measured by Osirix to height in metric unit (cm^2/m^2)[8]. By conducting descriptive analysis, the cutoff value for sarcopenia was determined to be 34.7±8.5 (mean±SD) in males and 29.3±6.51 (mean±SD) in females, and patients who had below these values were considered sarcopenic.

Markers of systemic inflammation

Systemic inflammatory markers such as hemoglobin, leukocyte, neutrophil, lymphocyte, monocyte, CRP, NLR, and platelet/lymphocyte ratio (PLR) were investigated. In recent studies, NLR and PLR have started to attract more attention as indicators of systemic inflammatory response[9]. Studies demonstrated that these markers can be used as predictable biomarkers in determining the likelihood of advanced-stage disease in cancer patients, the presence of lymphatic metastasis, and the response to treatment and prognosis[9]. A pilot study exposed that PLR is superior to other biomarkers[10].

Statistical analysis

The IBM Statistical Package for the Social Sciences (SPSS ver. 25 for Windows, Chicago, IL, USA) software was used for all statistical analyses. The Kolmogorov-Smirnov test was performed in order to observe the homogeneity and normality among the groups. Descriptive analysis and receiver operating characteristic (ROC) curve were used to calculate the sarcopenia value. A parametric Mann-Whitney U test was executed to determine the differences between sarcopenic and non-sarcopenic patients in terms of albumin, hemoglobin, and lymphocyte levels, as well as differences in postoperative hospital and ICU stay durations. The Youden index calculation was performed to compare the duration of hospital stay and postoperative complications between sarcopenic and non-sarcopenic patients. ROC analysis was conducted to compare inflammatory markers between sarcopenic and non-sarcopenic groups. An independent t-test was used for intergroup comparisons in normally distributed groups. $p<0.05$ was considered a level of significance for the study.

RESULTS

A total of 175 patients with CT images and blood parameters were included, of whom 41 (24.41%) were females and 134 (75.58%) were males. The mean ages were 62.17 years for females and 64.83 years for males, respectively. The calculation of sarcopenia indexes was based on CT images acquired within a maximum of 4 weeks before chemotherapy or surgery.

Based on radiological imaging, 55 patients were deemed inoperable and were considered to have locally advanced or metastatic disease. These inoperable patients were directly referred to chemotherapy. Total gastrectomy on 85 patients, distal gastrectomy on 25 patients, and proximal gastrectomy on 10 patients were carried out (Table 1).

During the investigation of tumor locations, 27 patients had tumor at the cardia, 10 at the fundus, 113 at the corpus (greater and lesser curvature), 15 at the antrum, and 10 at the pylorus (Table 1).

By using the descriptive analysis, the cutoff value for sarcopenia was determined to be 34.7±8.5 (mean±SD) for males and 29.3±6.51 (mean±SD) for females. Patients with values less than the determined cutoff value were considered sarcopenic.

Table 1. Demographic and pathological data of patients with and without sarcopenia.

Characteristics	Sarcopenic	Non-sarcopenic	p-value
Age (years)*	64.96	62.18	0.395[a]
Sex**			
Male	68	66	
Female	20	21	
Height (cm)***	168.50±8.9	170.44±7.5	0.421[a]
Tumor site****			0.354b
Cardia	15	12	
Fundus	5	5	
Corpus	58	55	
Antrum	7	8	
Pylorus	3	7	
Stage****			
1	24	30	0.428b
2	24	17	
3	15	10	
4	25	30	
Type of surgery****			
Total gastrectomy	45	40	0.127
Distal gastrectomy	13	12	
Proximal gastrectomy	5	5	
Inoperable	25	30	

*Mean value, **mean value of age, ***mean±SD, and ****quantity of patients. [a]Independent t-test and [b]Chi-square test.

Accordingly, 88 patients had sarcopenia, comprising 20 females (49%) and 68 males (50.75%).

During the postoperative or chemotherapy period, respiratory complications developed in 62 (36%) of patients, comprising 55 sarcopenic and 7 non-sarcopenic individuals. Cardiac complications developed in 20 (11.4%) patients during the postoperative or post-chemotherapy period, all of whom belonged to the sarcopenic group. Postoperative complications were significantly higher in comparison with non-sarcopenic patients ($p<0.01$).

The means of hospital stay duration were 12.14±3.4 days for sarcopenic and 7.23±2.3 days for non-sarcopenic patients, respectively, while the means of ICU stay duration were 3.11±1.6 days for sarcopenic and 1.48±1.2 days for non-sarcopenic patients, respectively (Table 2). A significant difference occurred in hospital (Figure 2).and ICU stay durations between the two groups ($p<0.01$) (Figure 3).

Table 2. Complications and hospitalization durations of sarcopenic and non-sarcopenic patients.

Outcomes	All patients	Sarcopenia	Non-sarcopenia	p-value
Pulmonary complications	62	55	7	**<0.01**
Cardiac complications	20	20	0	**<0.01**
Duration of hospital stay	9.69	12.14	7.23	**<0.01**
Duration of intensive care stay	2.30	3.11	1.48	**<0.01**

The numbers used in the table correspond to the number of patients in the complication sections and the durations of hospital and intensive care stays. p-value denoted in bold are statistically significant.

Inflammatory parameters like albumin, hemoglobin, platelet, lymphocyte, and neutrophil values were investigated, and NLR and PLR ratios were calculated.

The mean albumin values were 2.64±1.7 g/dL for sarcopenic and 3.08±1.1 g/dL for non-sarcopenic patients, while the mean hemoglobin values were 9.8±3.5 g/dL for sarcopenic and 10.4±4.1 g/dL for non-sarcopenic patients. The mean platelet values were 262.09±113.7 cells/mL for sarcopenic and 268.21±90.06 cells/mL for non-sarcopenic patients, whereas the mean lymphocyte values were 1160.61±990.0 µl for sarcopenic and 1520.90±1030.1 µl for non-sarcopenic patients, while the mean neutrophil values were 4250.34±365.6 µl for sarcopenic and 3380.69±284.1 µl for non-sarcopenic patients, respectively.

By using the Youden test, statistically significant low-grade correlations were observed in sarcopenic patients in terms of albumin, hemoglobin, and lymphocyte ratios in comparison with non-sarcopenic patients (Figure 4) ($p=0.087$). No significant difference was observed between the two groups with reference to neutrophil values, NLR, and PLR.

DISCUSSION

In this study, contrary to many previous studies, the inflammatory markers commonly used to measure NLR and PLR did not indicate significant differences between sarcopenic and non-sarcopenic patients. This could be attributed to the majority of early-stage operable patients in this study, with fewer patients having advanced-stage disease (100 of 172 patients underwent surgery). Supporting this, advanced-stage patients included in the study exhibited significantly higher NLR and PLR values compared with other stages ($p=0.042$). Furthermore, patients with lymph node metastasis also possessed significantly higher NLR and PLR values ($p=0.036$). In sarcopenic patients, other

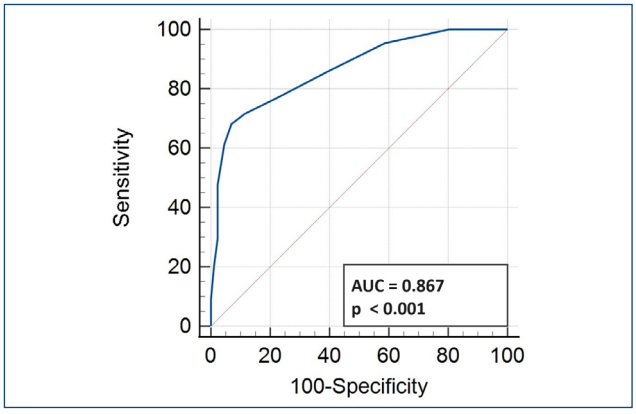

Figure 2. Receiver operating characteristic analysis of postoperative hospital stay.

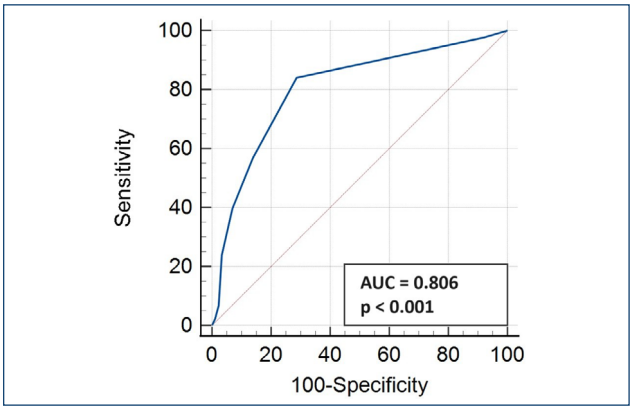

Figure 3. Receiver operating characteristic analysis of postoperative intensive care unit stay.

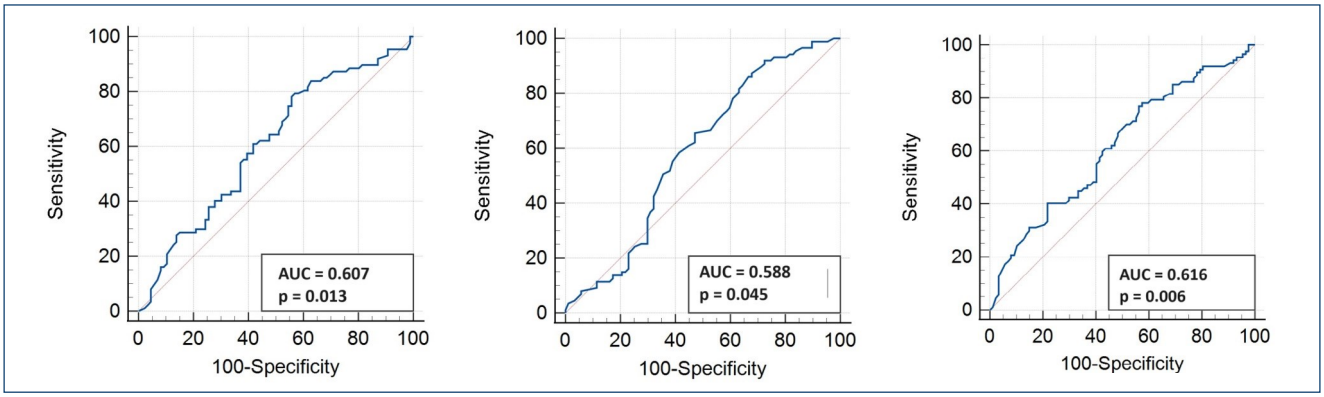

Figure 4. Receiver operating characteristic analysis of albumin, hemoglobin, and lymphocytes, respectively.

inflammatory biomarkers such as albumin, hemoglobin, and lymphocyte values were acquired to be lower compared with the non-sarcopenic patients.

Chronic inflammation plays a significant role in carcinogenesis. This association has been investigated since the 19th century when Virchow discovered the presence of leukocytes in tumor tissues and identified a potential association between tumors and inflammation[11,12].

According to a theory related to cancer cases, approximately one-fourth of cancer cases develop due to chronic inflammation and infection[11]. Neutrophils play a role in both the innate and adaptive immune responses, while monocytes transform into macrophages in the tissue microenvironment to combat tumor cells. Platelets facilitate the migration and extravasation of leukocytes. Lymphocytes can recognize and eliminate tumor cells by influencing their proliferation and thus impacting further disease progression[12].

Previous studies have demonstrated that systemic inflammation is particularly a negative prognostic marker in advanced-stage cancer patients[13]. In this study, lower levels of albumin, hemoglobin, and lymphocyte ratios were obtained in sarcopenic patients, while higher NLR and PLR ratios were noticed in patients with cancer in more advanced stages.

There is no definitive cutoff value defined in the literature for these NLR and PLR[13]. For instance, in a study conducted on patients with metastatic renal cell carcinoma and tumor thrombus who underwent cytoreductive nephrectomy, the patients with NLR<4 had higher survival rates compared with those with NLR>4[14].

Despite previous studies in the literature investigating individual inflammatory markers, postoperative complications, hospitalization durations, and ICU stays in sarcopenic patients and various types of cancers, this study is the pioneer to comprehensively consider all these parameters, particularly in Turkish patients. Additionally, this study has identified mean values for hospitalization durations and ICU stays after treatment in sarcopenic patients, which can serve as a pioneering step in determining a cutoff value with larger quantities of patients in prospective studies.

In sarcopenic patients composed of 50.28% of the total patients in this study, the durations of hospitalization and ICU stay were observed to be significantly higher compared with the non-sarcopenic group.

The skeletal muscle is one of the essential structures responsible for body movement and respiration, and it constitutes the largest protein reservoir in the body[15]. Loss of muscle mass and cachexia lead to protein loss and exercise intolerance, which play a significant role in various diseases, especially in conditions such as cancer, affecting the recovery time, tolerance to treatment, and post-treatment rehabilitation process[15].

Cancer often presents with rapid and aggressive weight loss and deterioration of muscle mass. In cancer patients, muscle mass loss varies depending on the type of cancer and stage of the disease. Muscle mass loss in the body leads to an increase in tumor progression incidence and an increased risk of chemotherapy toxicity, resulting in decreased tolerance to treatment and longer hospital stays[16]. In this study, patients with sarcopenia had longer hospitalization and ICU stays during treatment compared with others.

As an additional observation, sarcopenic patients had a significantly higher incidence of complications during treatment compared with the non-sarcopenic group. Among these complications, respiratory system complications and cardiac complications were prominent. The cause of respiratory complications is attributed to decreased muscle mass and impaired respiratory function, leading to ineffective cough and subsequently resulting in atelectasis and effusion[17]. Alongside the respiratory muscles, cardiac complications also increase due to the involvement of the heart muscles[18]. Furthermore, increased inflammatory stimuli can lead to acute lung injury[18,19].

There are some limitations in this study. The patients were heterogeneous in terms of stages, and due to the heterogeneity in the quantity of early-stage and advanced-stage patients, optimal comparisons of inflammatory markers, in particular, could not be performed. Due to the absence of some laboratory parameters, all inflammatory markers could be surveyed. The pre-treatment weight values of all patients were not available in the system; therefore, a comparison between body mass index and sarcopenia values could not be executed, and the analysis of sarcopenic obesity could not be conducted.

CONCLUSION

The presence of sarcopenia in gastric cancer patients prolongs hospitalization and ICU stay during treatment and increases postoperative complications. As clinicians are aware of the presence of sarcopenia in patients with gastric cancer before starting treatment, they can determine treatment strategies accordingly.

AUTHORS' CONTRIBUTIONS

SGGO: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **TK:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **NKK:** Conceptualization, Data curation, Project administration. **BG:** Data curation. I**HV:** Data curation. **SO:** Data curation.

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ORIGINAL ARTICLE

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The efficacy of liraglutide combined with intragastric balloon on weight loss

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SUMMARY

OBJECTIVE: Intragastric balloon placement is an effective method for weight reduction. The aim of this study was to evaluate the efficacy of combining liraglutide with intragastric balloon.

METHODS: Initially, demographic data of patients such as age, gender, comorbid diseases, adverse events, initial weight, height, body mass index, percent body fat, and waist-hip ratio were collected. Weight, body mass index, percent body fat, and waist-hip ratio were measured in the second, third, fourth, fifth, and sixth months. Then, intragastric balloon was removed and liraglutide was stopped.

RESULTS: A total of 50 patients were included in the study, of whom 28 (56%) were in Group A (intragastric balloon) and 22 (44%) were in Group B (plus liraglutide). Weight change at the time of balloon removal was higher in Group B [median weight change 13.8 (7.8 min to 16.8 max) versus 7.9 (4.8 min to 11.8 max); p<0.01]. When the weight, percent body fat, body mass index, and waist-hip ratio changes were compared according to gender, no significant difference was observed in the groups. Comorbid diseases were hypertension in 7 patients (4 in Group A and 3 in Group B) and diabetes in 9 patients (5 in Group A and 4 in Group B). No statistical significance was found.

CONCLUSION: Liraglutide has benefits in terms of weight, percent body fat, and body mass index reduction when administered with intragastric balloon.

KEYWORDS: Glucagon-like peptide 1. Gastric balloon. Liraglutide. Obesity. Body weight changes.

INTRODUCTION

Intragastric balloon (IGB) is an effective minimally invasive procedure for patients with body mass index (BMI) over 35[1]. Up to 35% success has been reported in obesity control[2]. Minimized gastric volume, increased satiety, and increased gastric emptying time are thought to be related to this weight reduction technique[3,4].

Liraglutide is a glucagon-like peptide-1 receptor agonist (GLP1-RA). Medical treatment has become an alternative method with the development of GLP1-RA. Subcutaneous use of liraglutide has been found to be successful for weight loss in adults and approved for overweight and obese in combination with reduced calorie foods and regular physical activity[5]. Appropriate groups are adults with a BMI≥30 or ≥27 kg/m² with weight-related comorbidities (e.g., hypertension, diabetes, or dyslipidemia)[2]. GLP-1 is an incretin hormone secreted by the L cells of the gastrointestinal tract in response to nutrients digested in the gastrointestinal lumen. GLP1-RA stimulates insulin secretion and reduces glucagon secretion from the pancreas in a glucose-dependent manner. Additionally, it induces a dose-dependent weight loss by decreasing calorie intake through delayed gastric emptying and activation of GLP-1 receptors in the brain[6].

In a small number of studies investigating the efficacy of liraglutide combined with IBG, it has been observed that, when liraglutide is started after balloon removal, it has an additional benefit in terms of efficiency and reduction in body fat[6], the average weight loss is greater after balloon removal and even 6 months later, it does not increase complications[7,8], and it may be effective in preventing weight regain[9]. The effect of combining endoscopic IGB insertion with liraglutide on weight reduction remains unknown despite these reports. In this study, we aimed to evaluate the efficacy of combining the GLP1-RA to endoscopic IGB insertion on weight reduction.

METHODS

This study was retrospectively performed with the data collected between August 2020 and August 2021. The study was approved by the Local Ethics Committee (with decision number 2023/97 on 12.04.2023), and written informed consent was obtained from all patients. All patients were adults aged above

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20 years with a BMI over 27 kg/m² and they were unable to reduce weight with lifestyle modifications and diet. They were presented to the General Surgery Clinic with the requisition of IBG insertion for weight reduction.

Patients who preferred only IBG were classified as Group A and patients who additionally accepted liraglutide as Group B. In Group A (GrA), only IGB (MedSil®, Belgium) was applied. In Group B (GrB), liraglutide (Saxenda, Novo Nordisk, Bagsvaerd, Denmark) was added 0.6 mg/day, subcutaneously for 6 months to the treatment schedule. All IGBs were removed 6 months after the insertion. There was no adverse event causing the early withdrawal of IGB. All patients tolerated both IGB and liraglutide during the study. All IBG insertions and withdrawals were performed by a certified general surgeon.

Demographic data of patients such as age, gender, comorbid diseases, adverse events, initial weight, height, BMI, percent body fat (PBF), and waist-hip ratio (WHR) were collected. Patients' weight, BMI, PBF, and WHR were measured in the second, third, fourth, and fifth months by a dietician and nurse. Finally, in the sixth month, the same parameters were evaluated, IGB was removed with the same physician, and liraglutide was stopped.

Patients were informed about diet and lifestyle modifications. A clear liquid diet was approved in the first week, following the IGB insertion. A full liquid diet in the second week, a soft diet with high protein content in the third week, and a regular high-protein diet with 80% calories of the basal metabolic rate in the fourth week and beyond were applied, respectively. Side effects of liraglutide were noted monthly on visits.

Insertion and removal of intragastric balloon

The MedSil® is designed as a nonadjustable saline-filled balloon with a maximal volume of 700 mL and approved for 6 months. It was applied in the endoscopy unit in the left-lateral position with monitorization. Normal saline was used for inflation under direct visualization endoscopically. Removal of IGB was also carried out in the endoscopy unit. The balloon was deflated in the sixth month by puncturing with an endoscopic needle. All saline was suctioned out and the balloon was extracted from the esophagus by a grasper. Patients may complain of nausea, vomiting, pain, and gastroesophageal reflux disease (GERD) after IGB insertion. Antiemetics and antispasmodics were offered in such cases. A proton pump inhibitor is prescribed routinely once daily with the duration of IGB.

Application of liraglutide

Potential side effects of the drug such as nausea, constipation, headache, and diarrhea were informed to the patients. The initial and maintenance dose was 0.6 mg/day applied subcutaneously on the abdomen, thigh, or upper arm. The drug was started 1 month after IGB insertion and discontinued with removal in the sixth month.

Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics, version 23.0 (SPSS Inc., Chicago, USA). Descriptive statistics were reported as mean±standard deviation and median (minimum to maximum) according to the distribution of variables. The difference between the baseline and sixth month values between the groups was evaluated using the independent samples t-test or the Mann-Whitney U test. The limit of significance was accepted as $p<0.05$.

RESULTS

A total of 50 patients were included in this study. The mean age was 32.5 (26–41) years in GrA and 35.5 (19–55) years in GrB. A total of 28 patients (56%; 19 females and 9 males) were treated with IGB and 22 (44%; 17 females and 5 males) with IGB plus liraglutide, respectively. Comorbid diseases were hypertension in seven patients (four GrA and three GrB) and diabetes in nine (five GrA and four GrB). No statistical significance was found according to the distribution of comorbid diseases in groups. Adverse events of IGB and liraglutide were abdominal pain in four patients (two GrA and two GrB), nausea and vomiting in seven (four GrA and three GrB), and GERD in four (two GrA and two GrB). None of the patient's balloon was removed, with liraglutide being stopped, or excluded because of side effects. There was no statistically significant difference in the distribution of adverse events according to the groups (Table 1).

When the weight, PBF, BMI, and WHR changes of the patients were compared according to the months. The initial and second month PBF values ($p=0.001$ and $p=0.006$) as well as fifth and sixth month BMI values ($p=0.026$ and $p=0.031$) were statistically significant in GrA and GrB, respectively (Table 1).

Patients in GrB lost more weight in the sixth month than GrA. Weight reduction at the time of balloon removal was higher in GrB [median weight change 13.8 (7.8 min to 16.8 max) versus 7.9 (4.8 min to 11.8 max); $p<0.01$]. The median PBF change in Gr A was 9.5 (5 min to 9.5 max), and in GrB, it was 7 (4.5 min to 9.5 max); $p<0.001$. The mean BMI change at the time of balloon removal in GrA was 3.13±0.51, and in GrB, it was 4.9±0.87; $p<0.001$. The median WHR change in GrA was 5 (5 min to 5 max), and in GrB, it was 5 (2 min to 9 max), which was statistically nonsignificant ($p=0.471$) (Table 2). When the changes in the weight, PBF, BMI, and WHR of patients were compared according to gender, no significant difference was observed in groups.

Table 1. Demographic data of patients such as age, gender, comorbidities and adverse events, and baseline and subsequent values of weight, percent body fat, body mass index, and waist-hip ratio measurements.

		Group A (IGB)			Group B (IGB plus liraglutide)			p
Age		32.5			34.5			
Gender		19 females, 9 males 28 (56%)			17 females, 5 males 22 (44%)			
Comorbid diseases	Hypertension	4			3			>0.05
	Diabetes, prediabetes, insulin resistance	5			4			>0.05
Adverse events	Abdominal pain	2			2			>0.05
	Nausea, vomiting	4			3			>0.05
	GERD	2			2			>0.05
		Median	Minimum	Maximum	Median	Minimum	Maximum	p
Weight	Initial weight	78	68	112	82	70	133	0.882
	Weight 2nd month	76.4	66.4	108.4	78.4	65.3	127.0	0.522
	Weight 3rd month	74.8	64.3	107.3	76.3	62.3	124.3	0.449
	Weight 4th month	74.1	63.1	105.1	74.1	60.1	121.1	0.392
	Weight 5th month	71.8	62.8	103.8	72.2	57.8	119.6	0.175
	Weight 6th month	70.2	59.2	100.2	71.1	55.2	116.2	0.148
PBF	Initial PBF	40.5	40.5	40.5	37.6	29.8	42.5	**0.001**
	PBF 2nd month	37.1	37.1	37.1	36.1	27.1	40.1	**0.006**
	PBF 3rd month	34.6	34.6	34.6	34.6	26.6	39.6	0.712
	PBF 4th month	32.9	32.9	32.9	32.9	25.9	38.9	0.712
	PBF 5th month	31.9	31.9	31.9	31.9	25.9	37.9	0.292
	PBF 6th month	31.0	31.0	31.0	31.0	23.0	36.8	0.169
BMI	Initial BMI	30.3	25.8	33.4	29.9	26.0	35.0	0.516
	BMI 2nd month	29	25	32	29	25	33	0.906
	BMI 3rd month	29	24	32	28	24	33	0.455
	BMI 4th month	28	24	31	27	23	32	0.230
	BMI 5th month	28	23	30	27	22	31	**0.026**
	BMI 6th month	27	23	30	26	21	31	**0.031**
WHR	Initial WHR	0.93	0.93	0.93	0.93	0.88	1.11	0.736
	WHR 2nd month	0.92	0.92	0.92	0.92	0.87	1.08	0.712
	WHR 3rd month	0.91	0.91	0.91	0.91	0.87	1.06	0.471
	WHR 4th month	0.89	0.89	0.89	0.89	0.88	1.03	0.049
	WHR 5th month	0.89	0.89	0.89	0.89	0.85	1.02	0.712
	WHR 6th month	0.88	0.88	0.88	0.88	0.84	1.02	1.000

IGB: intragastric balloon; GERD: gastroesophageal reflux disease; PBF: percent body fat; BMI: body mass index; WHR: waist-hip ratio. Bold indicates statistically significant values.

DISCUSSION

Intragastric balloons are recommended to be removed in the sixth month after insertion[10]. The effectivity of IGB is associated with its gastric volume reducing feature. It also diminishes caloric intake and increases the feeling of satiety by delaying gastric emptying. The effectivity of IGB will probably lost and patients desire eating more for satiety with the removal of the balloon. Therefore, measures that can prevent eating and weight regain behavior should be determined and their medical effectiveness should be revealed[3,4]. Liraglutide reduces calorie intake and lowers body weight by causing a delay in gastric emptying[5]. Due to this feature, it was thought that it could be beneficial for weight regain after the removal of the IGB. Weight regain was reported as a major problem after IGB removal up to 35%

Table 2. Change in weight, percent body fat, body mass index, and waist-hip ratio levels between groups.

	Group A (IGB)	Group B (IGB+Liraglutide)	p-value
	Mean±SD Median (min–max)	Mean±SD Median (min–max)	
Weight	7.9 (4.8–11.8)	13.8 (7.8–16.8)	**<0.001**
PBF	9.5 (9.5–9.5)	7 (4.5–9.5)	**<0.001**
BMI	3.13±0.51	4.9±0.87	**<0.001**
WHR	0.5 (0.5–0.5)	0.5 (0.2–0.9)	0.471

PBF: percent body fat; BMI: body mass index; WHR: waist-hip ratio. Descriptive statistics were reported as mean±standard deviation in normally distributed data and median (minimum to maximum) in non-normally distributed data, according to the distribution of variables. Only BMI change was normally distributed, and mean±standard deviation was used. Bold indicates statistically significant values.

as seen after metabolic surgeries[11,12]. The factors that affect weight regain following the IGB removal are not well understood. Hormonal changes, dietary incompatibility, inadequate physical exercises, mental problems, alcohol consumption, and sleep disturbances may cause weight regain[10].

In the first study evaluating liraglutide plus IGB, the mean weight loss was found as 10.2±6.7 kg at the time of IGB removal. After 6 months, the mean weight loss was decreased to 2.7±4.1 kg. This shows that the effectiveness of liraglutide and IGB gradually decreases in the follow-up period[7]. In a nonadjustable IBG study, the weight loss of patients was diminished seriously 5 years after removal (23.9–7.3 kg)[13]. Notably, 78.7% of patients regained weight and have been candidates for metabolic surgeries 3.3 years after IGB removal[14].

The efficacy of liraglutide plus IGB compared with placebo was also studied at different daily doses of 1.2, 1.8, 2.4, or 3.0 mg in randomized controlled trials, and weight loss was found, respectively, as 4.8, 5.5, 6.3, and 7.2 kg after 20 weeks[15]. Another study investigated weight loss following the removal of nonadjustable IGB at 12 months. Weight loss was found to be significantly higher in those using 1.2 mg liraglutide (17.4±3.8 versus 10.7±4.1; p<0.001)[9]. IGB plus 0.6–3 mg/day different doses of liraglutide were evaluated compared with IGB alone. A significant weight loss was found at the sixth month (18.3 versus 22.2 kg; p<0.001) in the combined group[8]. In our study, we did not increase the dose and 0.6 mg liraglutide was used daily throughout the study. No other study combining IGB with low dose liraglutide has been observed in the literature. BMI and weight change at the end of 6 months suggest that a dose as low as 0.6 mg may be effective.

A total of 3,731 nondiabetic patients with BMI≥30 or ≥27 kg/m² with dyslipidemia or hypertension were randomized to receive 3.0 mg/day subcutaneous liraglutide or placebo plus diet and exercise in another study. Weight loss at week 56 was significantly higher with liraglutide compared with placebo. In addition, blood pressure and prevalence of prediabetes were decreased in patients receiving liraglutide, and 76% of patients receiving liraglutide lost more than 5% of their weight[16]. Different daily doses of liraglutide combinations have been applied to prevent weight regain after all weight-reducing treatments, including bariatric surgeries[17-20]. Pharmacotherapy with liraglutide was reported as having lower risk and resulted in significant improvement in hypertension and dyslipidemia[18]. Therefore, daily subcutaneous injection of liraglutide is an alternative option to treat weight gain in eligible patients[18]. Different studies emphasized the beneficial effects of liraglutide on reducing body fat composition and distribution, and improvements in metabolic and cardiovascular tests[21]. In a study examining body fat loss, a significantly greater loss of PBF was found in the liraglutide group compared with the IGB group[6] as in our study. Acute pancreatitis, acute cholecystitis, bowel obstruction, epigastric pain, nausea, and GERD are reported complications of IGB[22]. Similarly, abdominal pain, nausea, vomiting, and GERD were observed in our study.

The meaning and impact of each factor in preventing weight regain are likely different. As patients lose weight, mechanisms in body weight regulation that act to resist weight loss make it more difficult to maintain weight loss and create a higher risk for weight gain. A lifestyle change, including dietary restriction, exercise program, and regular sleep, is strictly recommended after balloon replacement for the effectiveness of the weight loss procedure in obese patients[6,7]. One or two monthly follow-ups with a team including weight loss counselors, personal fitness trainers, dietitians, nurses, and doctors may be beneficial in preventing weight regain as we do. Also, close follow-up protocols may be applied following the IGB removal to prevent weight regain. Mobile applications that give instructions to all patients about exercise, diet, and lifestyle changes can be developed, and mobile communication support groups such as WhatsApp and Telegram can be used more effectively.

The important limitation of this study is that it was retrospective and did not evaluate the weight gain processes after the balloon was removed and liraglutide was stopped. In addition, adaptation processes and lifestyle changes are far from being measurable and remained at the recommendation level. Also, the use of liraglutide was carried out in accordance with the wishes and financial possibilities of the patients and not in accordance with the randomization rules.

More randomized controlled trials with larger numbers of patients with longer follow-ups are needed to evaluate

the effect of liraglutide on weight gain with appropriate duration and dosage, along with metabolic and cardiovascular effects.

CONCLUSION

This study showed that the change in weight, PBF, and BMI is greater in patients receiving additional liraglutide compared with baseline values. There were no significant side effects that required discontinuation of liraglutide. Lifestyle change should also be recommended in all patients as it may positively affect the results of endoscopic IGB placement.

AUTHORS' CONTRIBUTIONS

AY: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration. **SH:** Supervision, Validation, Visualization. **GD:** Software, Writing – original draft, Writing – review & editing. **OK:** Investigation, Resources.

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ORIGINAL ARTICLE

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The evaluation of superoxide dismutase 1 gene insertion/deletion variant in athletes

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SUMMARY

OBJECTIVE: Regular exercise benefits health by increasing the body's antioxidant defenses. However, excessive exercise can produce excessive reactive oxygen species, which can lead to oxidative stress. Superoxide dismutase is the primary enzyme involved in the elimination of reactive oxygen species. This study aimed to determine the relationship between the *SOD1* gene insertion/deletion variant and elite athletes.

METHODS: A total of 305 subjects, including 165 elite athletes from different branches and 140 sedentary individuals, participated in this study. The *SOD1* insertion/deletion variant was genotyped using polymerase chain reaction. The results were evaluated statistically.

RESULTS: There was no statistical significance between the athletes and control groups in terms of *SOD1* insertion/deletion genotype distribution and allele frequency. Then, we evaluated the groups as females and males. There were no female athletes carrying the D/D genotype. The *SOD1* I/I genotype and the I allele were more prevalent in female athletes than in the control group. There was a significant difference in terms of *SOD1* I/I: I/D+D/D in females (p=0.028). *SOD1* genotype and allele distribution did not differ between male athletes and male controls.

CONCLUSION: As far as we know, this is the first study to evaluate the *SOD1* insertion/deletion variant in athletes in Turkey. Our results showed that the *SOD1* I allele was more common in female athletes, but not in male athletes.

KEYWORDS: Sport. Performance. Superoxide dismutase. Variant. PCR.

INTRODUCTION

Physical inactivity is a known risk factor for the development of several diseases, such as obesity, diabetes, and cardiovascular disease. The beneficial effects of regular moderate-intensity exercise are indisputable[1]. Regular physical exercise also improves mental health by positively changing symptoms of depression[2]. In addition, physical exercise facilitates social interaction with positive results for quality of life[2]. Cells constantly produce free radicals and reactive oxygen species (ROS) through metabolic processes. Oxidative stress (OS) results from an imbalance between the production and accumulation of ROS in cells and tissues and the body's ability to detoxify these reactive products. Physical activity increases the formation of free radicals in various ways. Notably, 2–5% of the oxygen used in the mitochondria forms free radicals. As oxidative phosphorylation increases in response to exercise, there will be a concomitant increase in free radicals[3]. Oxidants produced in skeletal muscles are derived from two main molecules: superoxide and nitric oxide.

Superoxide dismutases (SODs) are the most important enzymes that perform antioxidant enzyme defense against ROS and superoxide anion radicals[4]. SOD1 (CuZn-SOD), which is one of the SOD isoenzymes, carries Cu and Zn in their catalytic centers. The other is SOD3 (EC-SOD), which is localized in extracellular elements. SOD1 is found in the cytoplasm, nuclear compartments, and lysosomes of mammalian cells[5]. The *SOD1* gene, which is located on chromosome 21 (region 21q22) in humans, contains four introns and five exons. It is hypothesized that *SOD1* gene mutations may impair antioxidant enzyme activity, resulting in the accumulation of toxic superoxide anions[6]. Numerous genetic polymorphisms in the *SOD1* gene affect regulatory regions, including the promoter region, UTRs, and introns. Several studies have linked *SOD1* variants to an increased risk of diabetes, cardiovascular disease, heroin addiction, breast cancer, and type 1 bipolar disorder[7]. A functional variant called the 50-bp insertion/deletion (I/D) polymorphism (rs 36232792) (1684 bp upstream of the ATG start codon) has been identified in the promoter region of the *SOD1* gene. *SOD1* I/D variant deletion (D) allele changes gene

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expression and results in lower levels of SOD1 mRNA[8]. Based on this information, we aimed to evaluate the *SOD1* I/D variant in elite athletes in this study.

METHODS

Study population

Our research group consisted of 165 athletes who regularly trained at least four times a week (44 females and 121 males, mean age: 22.02±2.87 years) from different sports branches representing the Faculty of Sport Sciences, Samsun. The control group consisted of 140 age- and gender-matched voluntary sedentary individuals (46 females and 94 males, mean age: 22.42±2.91 years) studying at Samsun. The age of all subjects was higher than 18 years, and they were from the Turkish population in the Northern Black Sea region. The demographic data of participants such as age, height, weight, body mass index (BMI), number of daily cigarettes, monthly alcohol consumption, sports branch, family history, disease status, and how many years they have been involved in sports were collected. All subjects submitted informed written consent before enrollment in the study, based on the ethical guidelines of the Declaration of Helsinki, and the Samsun Ethical Committee approved the investigation.

Genotyping

DNA was extracted from all peripheral blood samples using a commercial isolation kit (Zymo Research Kit). The *SOD1* variant was genotyped using a polymerase chain reaction (PCR) method. A total volume of 50 µL was used for the PCR, which included 25 L of Master Mix OneTaq and 1 L of forward primer (10 µM) F:5'-AATTCCTTACCCCTGTTCTA-3', 1 µL reverse primer (10 µM) R:5'-GGCAGATTTCAGTTCATTGT-3', and 2 µL PCR grade dH2O[9]. The PCR program was performed as follows: initial denaturation at 94°C for 5 min, denaturation at 94°C for 20 s, binding of the primer at 54°C for 30 s, elongation at 68°C for 40 s, and final elongation at 68°C for 5 min. The amplified PCR product was separated on a 2% agarose gel at 100 V for 25 min. Genotypes were detected as 247 base pairs (bp) of the D/D genotype, 297 bp of the I/I genotype, and 247 and 297 bp of the I/D genotype, consisting of two bands. To check the results, 10% of the randomly selected samples were reworked, and a 100% match was found.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics, version 21) and the OpenEpi Info software package version (www.openepi.com). The relationship between the demographic and clinical characteristics of the patients was analyzed using the χ^2 test or analysis of variance statistics. Differences in *SOD1* I/D genotype and allele distribution between patient and control groups were evaluated with the chi-square test, and Fisher's exact test was used when needed. OR and 95%CI were also calculated. $p<0.05$ were considered significant.

RESULTS

A total of 305 subjects were evaluated in this study. The mean age of 165 athletes aged between 18 and 30 years was 22.02±2.87. BMD was analyzed as a continuous variable. The baseline clinical and demographic characteristics of patients and controls are reported in Table 1.

Table 1. Demographic characteristics of the subjects.

Characteristics	Athletes (n=165) (%)	Controls (n=140) (%)
Gender		
Female/male, n (%)	44/121 (26.7/73.3)	46/94 (32.9/67.1)
Age (years)		
mean±SD	22.02±2.87	22.42±2.91
min–max	18–30	18–30
Weight (kg)		
40–49	1 (0.6)	0
50–59	23 (13.9)	15 (10.7)
60–69	45 (27.3)	40 (28.6)
70–79	53 (32.1)	40 (28.6)
80–89	23 (13.9)	25 (17.9)
90–99	16 (9.7)	18 (12.9)
100–109	4 (2.4)	2 (1.4)
Height (cm)		
150–159	6 (3.6)	4 (2.9)
160–169	25 (15.2)	19 (13.6)
170–179	75 (45.5)	72 (51.4)
180–189	33 (20)	33 (23.6)
190–200	26 (15.8)	12 (8.6)
BMI		
Lower than 18.5	1 (0.6)	1 (0.7)
18.5 up to 25	133 (80.6)	106 (75.7)
25 up to 30	28 (17)	31 (22.1)
30 and above	3 (1.8)	2 (1.4)

Continue...

Table 1. Continuation.

Characteristics	Athletes (n=165) (%)	Controls (n=140) (%)
Smoking (day)		
0	136 (82.4)	98 (70)
5–9	8 (4.8)	8 (5.7)
10–15	21 (12.7)	34 (24.3)
Number of alcohol drinks per month		
0	147 (89.1)	115 (82.1)
1–4	1 (0.6)	0
5–9	5 (3)	6 (4.3)
10–14	6 (3.6)	13 (9.3)
15–20	6 (3.6)	6 (4.3)
Sports branch		
Football	38 (23.0)	–
Basketball	36 (21.8)	
Volleyball	62 (37.6)	
Wrestle	29 (17.6)	
Training (week)		
2	1 (0.6)	–
3	53 (32.1)	
4	66 (40)	
5	45 (27.3)	
Family history		
Football	102 (61.8)	128 (91.4)
Basketball	15 (9.1)	8 (5.7)
Volleyball	5 (3)	4 (2.9)
Wrestle	8 (4.8)	0
Judo	28 (17)	0
Athleticism	5 (3)	0
Swimming	1 (0.6)	0
Disease		
No chronic disease	146 (88.5)	127 (90.7)
Chronic disease	19 (11.5)	13 (9.3)
Sports time (years)		
1–5	21 (12.7)	–
6–10	99 (60)	
11–20	45 (27.3)	

BMI: body mass index.

Genotyping results

The prevalence of genotypes I/I, I/D, and D/D profiles for the *SOD1* I/D variant was 72.1, 26.1, and 1.8%, respectively, in athletes, and 65.7, 28.6, and 5.7%, respectively, in the control group. There was no statistical significance between the athletes and control groups in terms of *SOD1* I/D genotype distribution and allele frequency (p>0.05). Table 2 represents the *SOD1* I/D genotype distribution and allelic frequency in the groups.

Then, we evaluated the groups as males and females. *SOD1* I/D genotype and allele distribution in female and male groups are shown in Tables 3 and 4. There were no female athletes carrying the D/D genotype. *SOD1* I/I genotype and the I allele were higher in female athletes than in the control group (p=0.055 and p=0.019, respectively). There was a significant difference in terms of SOD1 I/I: I/D+D/D (p=0.028). *SOD1* genotype and allele distribution did not differ between male athletes and male controls (p>0.05).

DISCUSSION

Physical activity can be defined as any bodily movement produced by skeletal muscles that results in energy expenditure[10]. Physical effort and skills constitute the content of "sport," which is a human activity that can be competitive by nature and organization and has the ability to achieve a result that generally requires physical effort and/or physical skill. Oxidant and antioxidant systems are important in order to ensure the structural integrity of cells and tissues and fulfill their normal functions. Fats, proteins, and other cell parts oxidize if free radical levels exceed antioxidant capacity[11]. Free radicals are short-lived and extremely reactive molecules. These have a detrimental effect because of the necessity to create electronic stability[12]. ROS is constantly produced in small quantities in biological systems. However, there is an increase when they are exposed to environmental and physical stress factors[13]. Exercise is one such stressor. It is thought that the increase in oxygen consumption during exercise leads to changes in the oxidant/antioxidant balance[14]. Studies have shown that an increase in oxygen consumption during exercise promotes the massive leaching of free radicals in the mitochondria and subsequently leads to an antioxidant reaction. Moderate exercise can increase antioxidant levels, facilitating an optimal ROS level, while high-intensity exercise can induce ROS generation, providing maximum cellular adaptation[15]. However, there are data showing that regular long-term training induces an antioxidant response to OS. In a study investigating the relationship between OS and excessive exercise or overreach, it was shown to support the possibility of being useful. This physical exercise reduces OS and may be explained by increased antioxidant defense[16]. Studies in sports involving aerobic metabolism, such as running or swimming, have shown an increase in the activity of antioxidant enzymes, such as SOD or MDA, as well as an increase in free radical production[17].

The SOD enzyme activity encoded by the *SOD1* gene is affected by variants in this gene. Differences in gene sequences determine changes in gene expression, which contribute to disease occurrence[18]. Variants have been identified in the *SOD1* gene, mostly affecting the regulatory regions of the gene. The *SOD1* promoter region harbors binding sites for several transcription factors. Sequence differences in these cis-responsive elements affect the expression of various mRNAs[19]. *In vitro*

Table 2. Genotype distribution and allele frequencies of *SOD1* insertion/deletion variant in groups.

SOD1 I/D	Athletes (n=165) (%)	Controls (n=140) (%)	χ^2	OR (95%CI)	p
Genotypes					
I/I	119 (72.1)	92 (65.7)	0.50	1.20 (0.72–2.00)	0.476
I/D	43 (26.1)	40 (28.6)	2.34	2.83 (0.72–14.02)	0.126
D/D	3 (1.8)	8 (5.7)	3.58	3.43 (0.91–16.3)	0.58
I/I+I/D: D/D	162:3	132:8	3.30	3.26 (0.87–15.4)	0.68
I/I: I/D+D/D	119:46	92:48	1.45	1.34 (0.82–2.20)	0.227
Alleles					
I	281 (85.15)	224 (80)	2.821	1.43 (0.93–2.19)	0.093
D	49 (14.85)	56 (20)			

Table 3. *SOD1* insertion/deletion genotype and allele distribution in the female groups.

SOD1 I/D	Female athletes (n=44) (%)	Female controls (n=46) (%)	χ^2	OR (95%CI)	p
Genotypes					
I/I	36 (81.8)	28 (60.9)	3.66	2.54 (0.95–7.12)	**0.055**
I/D	5 (18.2)	16 (34.8)	0.96	–	0.326
D/D	0 (1.8)	2 (4.3)	2.47	–	0.115
I/I+I/D: D/D	44:0	44:2	1.95	–	0.162
I/I: I/D+D/D	36:8	28:18	4.80	2.85 (1.09–7.90)	**0.028**
Alleles					
I	80 (90.90)	72 (78.26)	5.47	2.76 (1.16–7.03)	**0.019**
D	8 (9.10)	20 (21.74)			

Bold indicates statistically significant values.

Table 4. *SOD1* insertion/deletion genotype and allele distribution in the male groups.

SOD1 I/D	Male athletes (n=121) (%)	Male controls (n=94) (%)	χ^2	OR (95%CI)	p
Genotypes					
I/I	83 (68.6)	64 (68.1)	0.14	0.88 (0.47–1.64)	0.707
I/D	35 (28.9)	24 (25.5)	2.13	2.87 (0.64–15.26)	0.143
D/D	3 (2.5)	6 (6.4)	1.83	2.57 (0.61–13.04)	0.175
II+ID: DD	118:3	88:6	2.01	2.67 (0.64–13.38)	0.156
II: ID+DD	83:38	64:30	0.006	1.02 (0.57–1.83)	0.936
Alleles					
I	201 (83.05)	152 (80.85)	0.35	1.16 (0.70–1.90)	0.553
D	41 (16.95)	36 (19.15)			

assays showed that the deletion of *SOD1* 50 bp has been associated with reduced promoter activity and lower mRNA levels due to the loss of two Sp1 binding sites in cells[20]. As ROS interacts highly with DNA, the Ins/Del genetic variant may play an important role in interindividual differences in maintaining genome integrity[21]. In various studies, antioxidant enzyme gene polymorphisms and antioxidant enzymes have become areas of interest as pharmacological targets to reduce ROS production. It provides a strategy to prevent or slow the progression of oxidative damage in these patients.

In this study, we investigated the *SOD1* I/D variant in athletes. As far as we know, there is no association study with the *SOD1* I/D variant in athletes. The results of the first report to analyze this relationship in athletes showed no significant difference between athletes and controls. However, when we evaluated the groups by gender, we found a significant difference between female athletes and female controls. No female athletes with the *SOD1* I/D variant homozygous D/D genotype were found. It is known that the D allele has a deficient activity effect on the SOD1 enzyme. This leads to insufficient cellular protective mechanisms and antioxidant defense capacity. The relationship between sex and OS is important because OS has played a role in many diseases that occur differently in men and women. In one study, OS was shown to be higher in male rats than in female rats[22]. Another study reported that in vivo biomarkers of OS were higher in young men than in women of the same age[23]. A study on athletes showed that women have higher resting antioxidant levels than men[24]. Also, it was observed that OS markers increased similarly in both genders after exercise of similar intensity and duration. In this study, we found that I/I genotypes and the I allele were more common in female athletes compared with sedentary female controls. This may be an indicator of higher SOD levels. Also, we found that there was a significant difference in terms of *SOD1* I/I: I/D+D/D in female groups. The genotype and allele distribution were similar in the male group.

Our study has some limitations. As our sample size was not very large, genotyping according to branches was not possible. In addition, the fact that the blood SOD level was not measured was a limitation.

CONCLUSION

Our results showed that the *SOD* I/D variant genotype and allele distribution were different in female and male athletes. In future studies, it will be necessary to genotype male and female athletes in larger sample groups according to branches.

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ETHICAL ASPECTS

Informed written consent was obtained from all subjects before enrollment in the study, according to the Declaration of Helsinki's ethical guidelines, and the investigation was approved by the Ondokuz Mayıs University Ethical Committee (2022/276).

AUTHORS' CONTRIBUTIONS

AFN: Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft. **ŞÜ:** Conceptualization, Data curation, Investigation, Methodology, Resources, Visualization, Writing – original draft. **SY:** Conceptualization, Formal Analysis, Methodology, Resources, Software, Visualization, Writing – original draft. **ÖMO:** Formal Analysis, Investigation, Resources, Software, Visualization, Writing – original draft. **TA:** Conceptualization, Formal Analysis, Investigation, Methodology, Resources, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Role of cystatin C levels as an inflammatory marker in predicting endometriosis

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SUMMARY

OBJECTIVE: Endometriosis is a common chronic inflammatory disease associated with infertility and pelvic pain. Diagnosis is based on the appearance of endometriotic lesions at the time of surgery. Our study aimed to determine whether cystatin C can be used as a predictor of endometriosis and to investigate its potential role in doing so.

METHODS: The study included 45 patients with endometriosis between the ages of 18 and 40 years whose pathology results were compatible with endometriosis and were operated on, and a control group of 45 healthy women. These two groups were compared in terms of serum cystatin C levels, demographic-clinical characteristics, operation results, and other laboratory values.

RESULTS: The cystatin C and hs-CRP levels of the endometriosis patients were found to be significantly higher than the control subjects ($p<0.005$). Whether the endometriosis disease could be detected for serum cystatin C levels was determined by the receiver operating characteristic analysis and the most appropriate positive cutoff value for cystatin C was found to be 5.14 ng/mL (86.7% sensitivity and 77.8% specificity). In the linear regression analysis, it was observed that the probability of endometriosis increased 2.5 times when cystatin C levels increased above the threshold value of 5.14 ng/mL (OR: 2.5; 95%CI 2.24–2.76).

CONCLUSION: Our study shows that the serum cystatin C levels can be used as a guide for diagnosis in patients with advanced endometriosis. However, more research is needed to prove its reliability and accuracy in order to put it into practice.

KEYWORDS: Endometriosis. Endometrioma. Cystatin C. Diagnosis. Anti-inflammatory agents.

INTRODUCTION

Endometriosis, which is a common chronic inflammatory disease associated with infertility and pelvic pain, is characterized by the presence of endometrium-like glands and tissues outside the uterus[1]. Currently, diagnosis relies on visualizing endometriotic lesions during surgery, as there is no reliable serum marker available[2]. Moreover, the origin of endometriosis is still largely unknown[1]. Therefore, medical history and biochemical markers were investigated together with ultrasonographic methods as an alternative to invasive methods in diagnosis[3]. Although the role of various gene expressions has been demonstrated in recent studies, serum biomarkers (e.g., TNF-α, IL-6, IL-1β, CA-125, and CA 19-9) remain uncertain as suitable candidates for non-invasive methods, even though they may be suitable candidates for non-invasive methods[4]. Furthermore, one study revealed an increased prevalence of endometriosis during the severe acute respiratory syndrome coronavirus 2 pandemic, raising more questions regarding the etiogenesis of endometriosis[5].

Cystatin C is a cysteine protease inhibitor produced by all nucleated cells. It is thought that cystatin C reduces endogenous cysteine protease and neutrophil migration activity in the inflammatory process[6]. Cystatin C is an important marker, especially in demonstrating kidney function and glomerular filtration rate[7]. However, recent studies have revealed the significance of cystatin C in various fields. In addition to studies suggesting that it is an early predictor of cardiovascular diseases[8], some studies show that it can be a good biomarker for cerebrovascular diseases and peripheral vascular diseases[9]. Furthermore, studies have reported that cystatin C shows renal damage in patients with preeclampsia[10]. However, as cystatin C is considered to be a predictor of inflammation, it has been shown to be associated with malignancies. Cystatin C has been shown to be associated with various malignancies, in particular, urogenital malignancies[11]. However, no study in the literature investigates whether it is a suitable biomarker for endometriosis. In this respect, this study is significant in that it is the first of its kind and will make a valuable contribution to the literature.

Hence, the aim of our study was to search for a non-invasive method that could assist in the diagnosis of endometriosis, the etiogenesis of which is still unclear, and to investigate the role of cystatin C in predicting endometriosis.

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METHODS

Our study included 90 women, 45 of whom were volunteer patients between the ages of 18 and 40 years who applied to the city hospital endometriosis polyclinic between January 2022 and June 2022, and the other 45 patients were in the control group who applied to the gynecology polyclinic. Our study was conducted in accordance with the Declaration of Helsinki and in compliance with the country's ethical standards. Ethics committee approval was obtained from the same hospital (21/1046). An informed consent form was signed by all patients. Endometriosis patients were selected using the revised American Fertility Society classification as patients who had undergone surgery for pelvic pain or infertility and whose pathology results were compatible with endometriosis. The control group was selected from healthy women volunteers between the ages of 18 and 40 years without infertility and no additional diseases. Patients who were pregnant and had gynecological comorbidities, active infections, kidney disease, cardiovascular disease, cerebrovascular disease, malignancy, and chronic autoimmune diseases were not included in the study.

The demographic characteristics, obstetric histories, body mass index (BMI) values, ultrasonographic findings, physical examination findings, pathology results, and serum biochemical and hormonal parameters (hemoglobin (Hb), white blood cell (Wbc), neutrophil, lymphocyte, sodium, potassium, AST, ALT, urea, creatinine, hs-CRP, procalcitonin, CA-125, anti-Müllerian hormone (AMH), and cystatin C levels) of each patient were recorded. When calculating BMI, the patients' height and weight were measured, and it was calculated using the formula: BMI=weight (kg)/height $(m)^2$. All these parameters were compared between the endometriosis and control groups.

Cystatin C levels were measured using a commercial ELISA kit (Elabscience, Elabscience Biotechnology Co. Ltd. Wuhan, P.R.C., Catalog No: E-EL-H3643, LOT: ER04688F5606). The measurement range is 0.31–20 ng/mL. Its sensitivity is 0.19 ng/mL, and the intra-assay and inter-assay %CV values are <10%. In the endometriosis group, serum cystatin C levels were taken preoperatively. Blood samples were collected in yellow-capped, vacuum-sealed, plastic gel tubes from both the endometriosis and control groups between 8:00 a.m. and 12:00 p.m. after 12 h of fasting.

Statistical analyses were performed using SPSS version 22. The conformity of the variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses were performed using the mean and standard deviations for normally distributed variables. The means of parametric data determined by Levene's test, which showed normal distribution, were compared using Student's t-test. The Mann-Whitney U test was used to compare the parametric and ordinal data, which were determined not to be normally distributed. The presence of correlation between parametric data was tested using the Pearson test, and the presence of correlation between nonparametric and non-normal distributed data was tested using the Spearman test. Categorical data were compared using chi-square or Fisher's exact test (where values observed in cells did not meet the chi-square test assumptions) as appropriate. Cases with a $p<0.05$ were considered statistically significant. The role of cystatin C in predicting endometriosis was investigated using the ROC curve analysis method.

RESULTS

The comparison of demographic characteristics and biochemical and hormonal parameters between the endometriosis and control groups is shown in Table 1. While there was no significant difference between the groups in terms of mean age and BMI, gravida and parity variables were found to be significantly lower in the endometriosis group. When serum cystatin C levels were compared, a statistically significant difference was found between the endometriosis and control cases ($p<0.001$). Moreover, the hs-CRP ($p=0.002$) and CA-125 ($p<0.001$) values of endometriosis patients were found to be significantly higher than those of the control subjects (Table 1).

According to the ROC curve analysis (Figure 1), the cystatin C level was a discriminating parameter in patients with endometriosis. The area under the curve for cystatin C was 0.92 (0.86–0.98) at 95% confidence interval (Figure 1). The threshold value for cystatin C was 5.14 ng/mL with a sensitivity of 86.7% and a specificity of 77.8%.

In the linear regression analysis, it was observed that the probability of endometriosis increased 2.5 times when cystatin C levels exceeded the 5.14 ng/mL threshold (OR: 2.5; 95%CI 2.24–2.76) (Table 2).

DISCUSSION

This study evaluated the possible association between serum cystatin C levels and endometriosis, and to the best of our knowledge, this is the first study to evaluate the relationship between endometriosis and the proinflammatory marker cystatin C. In our study, serum cystatin C levels were found to be statistically significantly higher in the endometriosis group when compared with the control group ($p<0.001$). Furthermore, the hs-CRP ($p=0.002$) and CA-125 ($p<0.001$) values of endometriosis patients were found to be significantly higher than those of the control subjects. According to the ROC curve analysis, cystatin

C levels were a distinctive parameter in patients with endometriosis. The area under the curve for cystatin C was 0.92 (0.86–0.98) at 95%CI. The threshold value for cystatin C was found to be 5.14 ng/mL, with a sensitivity of 86.7% and a specificity of 77.8%. In the linear regression analysis, it was observed that the probability of endometriosis increased by 2.5 times when cystatin C levels increased above the 5.14 ng/mL threshold (OR: 2.5; 95%CI 2.24–2.76).

As cystatin C is a protease inhibitor that plays a role in inflammatory processes, it has been investigated in many diseases associated with inflammation[7-11], but research in the field of obstetrics and gynecology is limited. In one meta-analysis, it was demonstrated to be a promising biomarker in the detection of preeclampsia[12]. Additionally, another study reported that serum cystatin C levels in late pregnancy were associated with negative birth outcomes[13]. On the contrary, Zhang et al., suggested that serum cystatin C levels are significantly higher in patients with gestational diabetes mellitus (GDM) compared

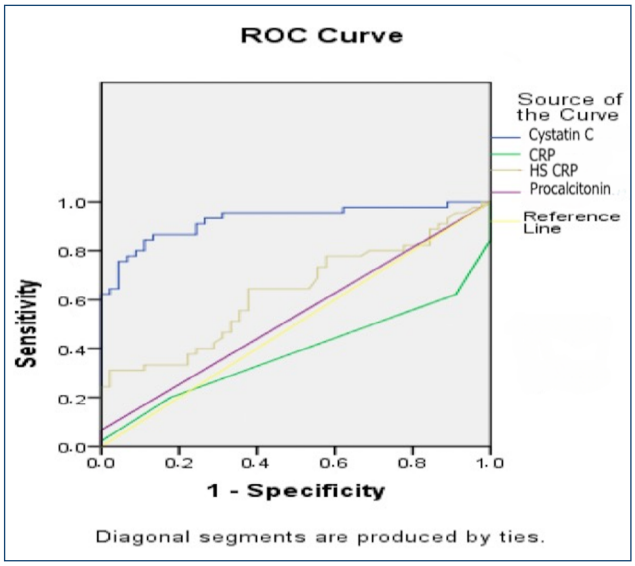

Figure 1. The receiver operating characteristic (AUC: 0.926; p=0.000; 95%CI 0.869–0.983) demonstrates the diagnostic potential of "Cystatin C" and "procalcitonin" as a variable for endometriosis.

Table 1. Comparison of demographic characteristics and biochemical and hormonal parameters in endometriosis and control groups.

	Endometriosis n=45 mean±std	Control n=45 mean±std	p-value
Age (years)	31.67±6.85	31.33±5.43	0.79
Gravida	1.02±1.45	2±0.87	<0.001
Parity	0.73±1.09	1.64±0.645	<0.001
BMI (kg/m^2)	24.66±1.97	24.36±2.10	0.484
Cystatin-C (ng/mL)	10.93±5.79	3.84±2.05	<0.001
HB (g/dL)	12.5±1.17	13.0±1.27	0.057
WBC (×10^9/L)	7.42±1.98	7.33±1.67	0.814
Neutrophil (×10^9/L)	5.64±6.49	4.39±1.37	0.212
Lymphocyte (×10^9/L)	2.02±0.59	2.19±0.55	0.173
N/L	2.99±3.71	2.13±0.99	0.136
Sodium (mEq/L)	139±1.89	138±1.76	0.078
Potassium (mEq/L)	4.97±0.65	4.22±0.26	0.376
AST (U/L)	19.8±6.98	20.48±4.08	0.582
ALT (U/L)	19.8±9.24	17.71±7.1	0.219
Urea (mg/dL)	24.7±5.55	24.3±6.48	0.781
Creatinine (mg/dL)	0.65±0.09	0.66±0.13	0.883
hs-CRP (mg/L)	3.0±3.20	1.35±1.19	0.002
CRP (g/L)	0.26±0.11	0.03±0.005	0.031
Procalcitonin (μg/L)	0.38±0.37	0.03±0.00	0.133
CA-125 (U/mL)	81.5±54.22	11.24±0.00	<0.001
AMH (ng/mL)	3.06±2.01	3.39±1.82	0.421

N/L: neutrophil-lymphocyte ratio.

Table 2. Linear regression analysis and results.

	Endometriosis		
Variables	**Level (ng/mL)**	**OR (95%CI)**	**p**
Cystatin C	5.14	2.5 (2.24–2.76)	<0.001

with the control group[14]. A study reported that increased serum cystatin C levels may be a risk factor for pregnant women with PCOS and GDM[15].

Studies on cystatin C in gynecology in the literature are mostly related to polycystic ovary syndrome (PCOS), and one study stated that cystatin C levels were significantly higher in women with PCOS compared with the control group and could be an important indicator for reducing cardiovascular risks[16]. Moreover, a study conducted in adolescents with PCOS suggested that the risk of PCOS increased 1.556 times when cystatin C increased by one unit and that there was a significant relationship between them[17]. Another study in patients with adolescent PCOS suggested that cystatin C may be a promising indicator in predicting future metabolic risks[18].

Various cytokines and markers have been shown in both the peritoneal cavity and serum in patients with endometriosis[4], but the question of their role in the development of endometriosis and whether they are the cause or the result of endometriosis has not yet been clearly elucidated. In addition, although there is a difference between superficial and deep endometriosis, studies have shown that biomarkers contribute to the diagnosis of both superficial and deep pelvic endometriosis[4]. Although serum CA-125 is the most studied marker, studies have shown that its diagnostic performance is poor[19]. A meta-analysis conducted by Sokolov et al., stated that other markers such as CA 19-9 and CA 72-4 are more valuable in differentiating endometriosis from other pathologies and may help clarify the effect of circulating micro-RNA in the pathology of endometriosis[20]. Furthermore, a study investigating the role of autoantibodies and enzymes in the diagnosis of endometriosis suggested that autoantibodies against tropomyosin 3, α-enolase, and estradiol could be included in the panel of biomarkers for the non-invasive diagnosis of endometriosis[21]. Another study evaluated the hormonal etiologies of endometriosis and showed that, in rat models, gestrinone antagonizes the effects of estrogen on rat peritoneal endometrial implants when given combined estrogen therapy with gestrinone[22].

In their research, Soto et al., stated that numerous potential biomarkers for non-invasive tests for endometriosis, including glycoproteins, inflammatory cytokines, immune molecules, angiogenesis factors, hormones, microRNAs (miRNAs), proteomics, metabolomics, genomics, and microbiomes, have been investigated. However, they explained that the most promising and progressing areas for the non-invasive diagnosis of endometriosis are miRNAs, proteomics, metabolomics, genomics, and microbiome[23]. A study investigating the genetic origin of endometriosis compared the expression of stem cell-related genes in the endometrium, superficial endometriosis, and deep infiltrating endometriosis. It has been revealed that deep and superficial endometriosis tissues have similar stem cell-related genes; however, there are differences in gene expression between them[24]. Despite all these studies, a recent study stated that more confirmatory studies are required to fully establish these markers in the diagnosis, progression, and staging of endometrial lesions[25]. Many markers have been studied and continued to be investigated for the non-invasive diagnosis of endometriosis. The strengths of this study are as follows: this is the first study in the literature showing the relationship between endometriosis and cystatin C and diagnosis of endometriosis was supported by pathological examination in all patients. The limitations of our study are the small number of participants and its non-randomized design.

CONCLUSION

Cystatin C levels seem to be a promising non-invasive indicator in predicting endometriosis associated with inflammation.

AUTHORS' CONTRIBUTIONS

HK: Conceptualization, Writing – original draft. **İH**: Investigation, Writing – review & editing. **MİH**: Data curation, Methodology. **CT**: Data curation, Methodology. **MÇ**: Supervision, Formal Analysis.

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ORIGINAL ARTICLE

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Clinical and serological findings in pregnant women and newborns: patterns of coronavirus disease 2019 placental histopathology

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate the correlation between clinical and serological findings of pregnant women and newborns with patterns of histopathologic changes of the placenta diagnosed with coronavirus disease 2019.

METHODS: A prospective descriptive study was conducted with pregnant women who were positive for SARS-CoV-2 by reverse transcription polymerase chain reaction or serology (IgG and IgM). Clinical analyses were performed using ELISA to detect anti-SARS-CoV-2 IgG and IgA antibodies using the S1 spike protein domain with the Euroimmun kit. Histopathologic analyses of placentas were performed by two expert pathologists.

RESULTS: Maternal SARS-CoV-2 infection was associated with increased neonatal hospital length of stay (p=0.03), increased preterm birth (p=0.04), and Apgar score<7 at 1st min (p=0.00) and 5th min (p=0.02). Pregnant women with positive IgG and/or IgA at delivery had a higher incidence of placental histopathologic changes in addition to a greater likelihood of having an IgG-positive fetus (p<0.0001). Placentas with positive reverse transcription polymerase chain reaction for SARS-CoV-2 had a higher incidence of histopathologic changes such as maternal vascular hypoperfusion changes (p=0.00).

CONCLUSION: Maternal SARS-CoV-2 infection was associated with adverse perinatal outcomes. Pregnant women with positive IgG at delivery had a higher incidence of placental histopathologic changes. Placentas with positive reverse transcription polymerase chain reaction for SARS-CoV-2 had a higher incidence of histopathologic changes such as maternal vascular hypoperfusion.

KEYWORDS: COVID-19. Placenta. Vertical infectious disease transmission. Pathology. Serology.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by SARS-CoV-2, a virus that can cause moderate to severe infections in humans. Since the virus emerged in China, in December 2019, the disease has quickly spread around the world, being declared as a pandemic by the World Health Organization in January 2020[1]. Shortly after the emergence of COVID-19, there have been reports of infections occurring in pregnant women[2], with the majority of patients being asymptomatic or with a mild disease[3].

Physiological, immunological, and mechanical changes during pregnancy increase susceptibility to infections in general, especially when the cardiorespiratory system is affected, and there is a rapid progression to respiratory failure[4,5]. Since this, epidemiological evidence indicates that pregnant women are at greater risk of developing severe disease and mortality from viral infections such as influenza, Ebola, and Lassa fever[6].

Newborns can be indirectly affected by SARS-CoV-2, through the impact of maternal COVID-19 during pregnancy, thus leading to premature childbirth. Vertical transmission is considered rare, and postnatal infections are similarly observed in breastfed and formula-fed infants. Despite the intense research, it is still unclear why neonates primarily present mild symptoms and have lower mortality rates[7].

Studies have already been carried out to assess the susceptibility of the placenta to SARS-CoV-2 infection[8,9]. This virus uses the cell receptor angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease enzyme 2 (TMPRSS2) to enter the cell host. In pregnant women, these receptors are poorly expressed in the placenta, which seemed to decrease the

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chance of vertical transmission of the virus[9], but other studies showed that placental infection is possible[10,11]. Schwartz et al.[12] analyzed 68 placentas of women diagnosed with COVID-19 during pregnancy. It was observed that virus-infected placentas had a significantly different pattern of pathological findings from uninfected placentas, regardless of the state of newborn infection. Placentitis by SARS-CoV-2, defined by the coexistence of three microscopic findings, i.e., "COVID triad"—intervillositis chronic histiocytic disease, increased fibrin, and trophoblast necrosis—were associated with stillbirths and/or neonatal deaths.

The objective of this study was to evaluate the correlation between clinical and serological findings in pregnant women and newborns with placental histopathologic patterns diagnosed with COVID-19.

METHODS

The study was carried out at the Clínica Perinatal, Rio de Janeiro, RJ, Brazil. A prospective descriptive study was carried out through the histopathologic evaluation of pregnant women's placentas diagnosed with COVID-19 in the period from April 2020 to August 2021.

Patients were recruited in the study at two different periods: (1) during pregnancy, after the confirmed diagnosis of COVID-19 and (2) at the moment of delivery, including pregnant women who were positive and verified the result for SARS-CoV-2 in any trimester, through reverse transcription polymerase chain reaction (RT-PCR) or serology (IgG and IgM) in those who did not perform RT-PCR. At the time of delivery, the placenta and samples of maternal and umbilical cord blood were collected to perform IgG and IgA serologies.

Clinical analyses were performed using the ELISA method to detect anti-SARS-CoV-2 IgG and IgA antibodies using the S1 spike protein domain with the Euroimmun kit. Histopathologic analyses of placentas were performed jointly by two expert pathologists in fetal-placental pathology with reference to the criteria of the Consensus of Amsterdam[13].

Maternal data demographics included age, race, gestational age at delivery, comorbidities, and gestational trimester in which SARS-CoV-2 infection occurred. Newborns' outcomes evaluated were small for gestational age (SGA), prematurity, Apgar score<7 at 1st and 5th min, perinatal death, and length of stay of the neonate in intensive care unit (ICU). The evaluation of outcomes included the period from delivery to hospital discharge of the newborn.

To analyze the association between numerical and categorical variables, the Mann-Whitney test was used. Chi-square and Fisher's exact tests were used to analyze the associations between categorical variables. Analyses were performed using the free program R version 3.6.1, accepting the results with a value of $p<0.05$ as significant.

This study is part of another study called Research Network in SARS-CoV-2/COVID-19 in Clínica Perinatal, in progress at IFF/Fiocruz, CAAE 30598020.0.0000.5269, and at the D'Or Institute for Research and Education, CAAE 34268020.53003.5249. In all cases, the patients signed the consent form.

RESULTS

Profile of the studied group

For this study, 152 patients were recruited, of whom 6 were not selected (3 twin pregnancies and 3 fetuses presenting malformations), totaling 146 pregnant women included in the study. No patient was excluded.

Considering the total of 146 patients evaluated in the study, it was observed that 66% (97 patients) were white and the mean age was 34.2 years (ranging from 19 to 44 years). Notably, 75 patients (51.3%) had comorbidities, the most frequently observed being diabetes (type 1, type 2, or gestational) and arterial hypertension (chronic hypertension, preeclampsia, and gestational hypertension). Seven patients (4.7%) were vaccinated against COVID-19 during pregnancy, four were immunized with Pfizer, and three with AstraZeneca.

At delivery, the mean gestational age was 38.4 weeks. Infection by COVID-19 was observed with higher prevalence in the third trimester of pregnancy (54%), and 25% tested positive at delivery, either by RT-PCR or by serology. Cesarean section was performed in 84% of cases, and delivery was preterm in 14.3%.

Placental infection and histopathology

Of the total of patients with positive diagnoses for COVID-19 during pregnancy, only 12 placentas had viral particles detected by the RT-PCR on fresh material. Results showed that there was no significant difference ($p=0.10$) between the gestational age at which the patient contracted the COVID-19 virus and the test result.

The evaluation of the moment when the pregnant woman contracted the disease during pregnancy is not correlated with the finding of the "COVID triad" ($p=0.90$), maternal vascular hypoperfusion ($p=0.67$), inflammation ($p=0.33$), acute fetal vascular hypoperfusion ($p=0.71$), and chronic fetal vascular hypoperfusion ($p=0.62$).

Placentas that presented positive RT-PCR test results for COVID-19 were evaluated to verify if they presented more histopathologic alterations than the placentas negative for

the presence of viral particles. It was observed that the positive RT-PCR test results did not increase the observation of the "COVID triad" (p=0.13), inflammation (p=0.12), acute fetal vascular hypoperfusion (p=0.73), and chronic fetal vascular hypoperfusion (p=0.42), but an association was observed between positive RT-PCR test results and maternal vascular hypoperfusion (p=0.00) (Figure 1).

Perinatal outcomes

It was observed that the presence of maternal comorbidities increased the risk of newborns requiring ICU after delivery (p=0.00).

Of the total of 140 newborns, only 3 were considered SGA, not configuring the relationship between these two variables. Chronic fetal vascular hypoperfusion was associated with increased hospitalization time of newborns (p=0.03), increase in preterm births (p=0.04), as well as Apgar score<7 in 1st (p=0.00) and 5th min (p=0.02).

In this study, two cases of fetal death of pregnant women were observed in the duration of SARS-CoV-2 infection. The first case was 38-year-old, with 38 weeks and 6 days of gestation, and diagnosis of COVID-19 was carried out at the time of delivery through RT-PCR. The placenta showed the presence of viral particles detected by the RT-PCR method in fresh material, with positive immunohistochemistry (Figure 2) and acute fetal vascular hypoperfusion, maternal vascular hypoperfusion, and inflammation. The second case occurred in 41-year-old, with 36 weeks and 3 days of gestation, previously vaccinated with AstraZeneca. COVID-19 diagnosis was performed at the time of delivery through positive results for IgG and IgA and the placenta showed alterations of maternal vascular hypoperfusion and inflammation.

Maternal and fetal serology

Maternal vaccination against COVID-19 did not change the serology of the newborn, for both IgG (p=0.25) and IgA (p=0.0), and this fact may be related to the small number of patients who were immunized during the study.

The serological evaluation of the patients and their newborns showed that when the mother presented reactive serology for IgG, there was a greater risk (p<0.0001) that the fetus was also reactive for the same immunoglobulin. A similar result was observed when evaluating the serology of newborns of IgA-reactive mothers. On the contrary, maternal seropositivity for IgG did not increase the risk (p=1.0) of the fetus being reactive for IgA, i.e., it did not increase the risk of the child being born infected with the disease. Similarly, patients with reactive IgA did not increase the risk (p=1.0) of the fetus being born infected with COVID-19, with reactive IgA.

The evaluation of maternal serologies (IgG and IgA) showed that the reagent result for IgG increased the observation of the "COVID triad" in the placentas (p=0.02) and the IgA reagent result increased the observation of acute fetal vascular hypoperfusion (Figure 3). However, both IgG-reactive serology and IgA did not show a greater risk of observing maternal vascular hypoperfusion (p=0.64 and p=0.39 for IgG and IgA,

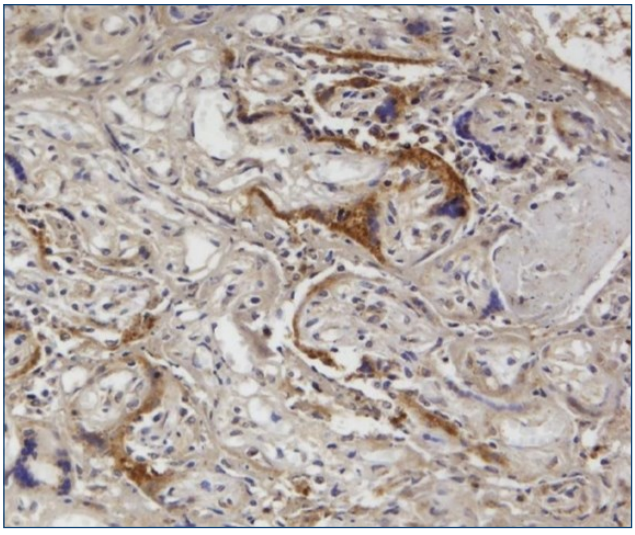

Figure 2. Immunohistochemical staining for SARS-CoV-2 viral particle. Positivity (dark brown) in the cytoplasm of trophoblastic cells: 100× magnification.

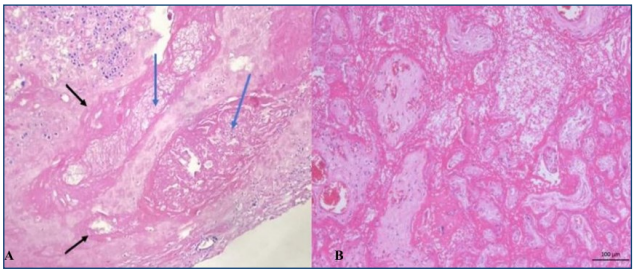

Figure 1. Changes in maternal vascular hypoperfusion. (A) Thrombosis (blue arrow) and fibrinoid necrosis (black arrow) of the decidual vessel. (B) Massive deposition of perivillous fibrin with trophoblast necrosis=area of infarction (hematoxylin-eosin, original 100× magnification).

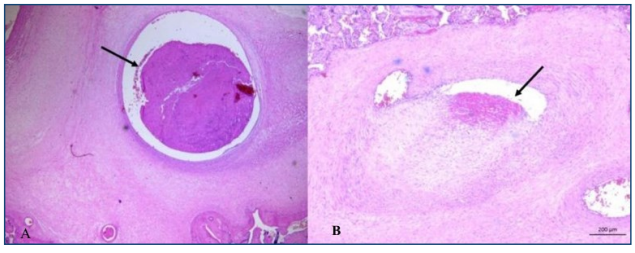

Figure 3. Acute fetal vascular hypoperfusion changes. (A) Occlusive thrombosis of chorionic plaque vessel (hematoxylin-eosin, original 100× magnification). (B) Subintimal fibrinoid degeneration of the villous trunk vessel wall (hematoxylin-eosin, original 100× magnification).

respectively), inflammatory (p=0.20 and p=0.40 for IgG and IgA, respectively), and chronic fetal vascular hypoperfusion (p=0.60 and p=0.14 for IgG and IgA, respectively).

Pregnant women's placentas positive for COVID-19 were also evaluated. It was observed that patients with the "COVID triad" did not have IgG-reactive newborns (p=0.54) or IgA (p=1.0). However, patients with changes in maternal vascular hypoperfusion had newborns with reactive serology for IgG (p=0.04), and patients with alterations in acute fetal vascular hypoperfusion had newborns with reactive serology for IgA (p=0.02).

DISCUSSION

Relationship between neonatal outcomes and histopathologic findings

Studies have described histopathologic evidence of poor fetal and maternal vascular perfusion and thrombosis of vessels maternal decidual veins, as well as occlusive or subocclusive thrombosis of fetal great vessels of the chorionic plaque and villous trunks of the placentas of women with SARS-CoV-2[13,14]. Recently, a study by Mao et al.[15] assessed whether placental hypoxia could facilitate SARS-CoV-2 infection. The authors observed that tissue oxygenation was reduced in regions of the placenta that showed greater fibrin deposition, as in cytotrophoblastic and stroma of the chorionic villi, as well as in the cells of the extravillous trophoblast. The authors also showed that in the regions of greater fibrin deposition, the expression of the ACE2 receptor (receptor used by SARS-CoV-2 to infect the human organism) was also increased. These observations demonstrated that there was a predilection of the COVID-19 virus for regions of the placenta that are experiencing hypoxia. In these regions, ACE2 receptor expression would be increased in trophoblast cells, which would increase the chance of infection of this tissue and, consequently, the fetus.

Menter et al.[16] demonstrated that decidual microvasculopathy, manifested as signs of hypoperfusion of maternal vasculature, was a common finding in placentas of SARS-CoV-2 positive women. In addition, the authors also found a greater inflammatory response, which favors the hypothesis that SARS-CoV-2 can invade the placenta, cause an inflammatory response, and be potentially transmissible to the child, but these data were not observed in this study.

In this study, it was observed that newborns of pregnant women who had comorbidities had an increase in hospitalizations in an ICU after delivery. This result corroborates a meta-analysis that included 435 studies that observed that pregnant women positive for COVID-19 had a greater probability of having neonates admitted to the ICU after delivery when compared with pregnant women not infected by the SARS-CoV-2 virus[17].

Relationship between serological findings at delivery and placental histopathology in pregnant women with coronavirus disease 2019

In this study, we observed that patients with positive serology for IgA detected at the time of delivery presented placentas with acute fetal vascular hypoperfusion alterations disease. Dashraath et al.[6] highlighted that hormonal changes during pregnancy affect the immune response to viral pathogens. In addition, the expression of cytokines such as the interleukins (IL) IL-4 and IL-10, together with a Th2 profile and other mechanisms of immune adaptation, results in less intensity of COVID-19 symptoms in pregnant women compared with non-pregnant women[18].

Mothers with positive serology for IgA were more likely to have newborns with reactive serology for IgG at birth. These results agree with the study carried out by Gao et al.[19] who noted that among the 24 newborns of women with COVID-19, 15 (62.5%) had detectable IgG and 6 (25.0%) had IgM detectable. The RT-PCR test results were all negative. Although the levels of IgG in all 15 IgG-positive newborns have gradually decreased, they decreased more slowly in IgM-positive infants compared with those without detectable IgM. These findings reinforce the possibility of vertical transmission of the disease. Although IgG is transferred passively from the mother to the fetus through the placenta, the duration of maternal IgG passive immunity is not yet fully elucidated.

CONCLUSION

Maternal SARS-CoV-2 infection was significantly associated with adverse perinatal outcomes despite the low positivity for the virus in the placenta. Pregnant women with positive IgG at delivery had a higher incidence of placental histopathologic changes. In placentas with positive RT-PCR for SARS-CoV-2, there was a higher incidence of histopathologic changes such as maternal vascular hypoperfusion.

AUTHORS' CONTRIBUTIONS

RAMS: Conceptualization, Project administration, Supervision, Visualization. **EAP:** Conceptualization, Investigation, Methodology, Validation, Visualization. **TCC:** Data curation, Visualization, Writing – original draft. **LGCV:** Formal Analysis, Visualization. **LMÁ:** Investigation, Visualization. **EAJ:** Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Do myofascial trigger points in masseter muscles affect the symptoms of disc displacement with reduction? A cross-sectional study

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SUMMARY

OBJECTIVE: The aim of this study was to demonstrate the effect of myofascial pain with referral from the trigger points in the masseter muscles on the clinical symptoms and functional limitations of the temporomandibular joint in participants with disc displacement with reduction.

METHODS: This prospective, cross-sectional study recruited participants aged 18–45 years with disc displacement with reduction with/without myofascial pain with referral in the masseter muscles based on the inclusion criteria. Maximum mouth opening and the presence of probable awake bruxism were assessed. The "Graded Chronic Pain Scale version 2.0" and "Jaw Function Limitation Scale-8" were used to evaluate Diagnostic Criteria for Temporomandibular Disorders Axis II. Pain levels were measured using the Visual Analog Scale.

RESULTS: A comparison between the disc displacement with reduction and disc displacement with reduction+myofascial pain with referral groups revealed statistically significant differences in Visual Analog Scale ($p<0.001$), the presence of awake bruxism ($p=0.038$), and Graded Chronic Pain Scale version 2.0 ($p=0.010$). However, no statistically significant difference was observed between the two groups concerning maximum mouth opening and Jaw Function Limitation Scale-8.

CONCLUSION: Participants with both disc displacement with reduction and myofascial pain with referral in the masseter muscle exhibited higher pain intensity, a higher prevalence of awake bruxism, and increased pain-related disability compared to those with disc displacement with reduction alone.

Clinical Trial Registration Number: NCT05187325.

KEYWORDS: Bruxism. Masseter muscle. Temporomandibular joint. Pain. Trigger points.

INTRODUCTION

The temporomandibular joint (TMJ) is a synovial joint containing an articular disc[1]. The functions of the articular disc are to absorb the shocks between the articular surfaces and to separate the articular cavity in the lower and upper divisions[1,2]. Temporomandibular disorder (TMD) is described as a musculoskeletal disease affecting the temporomandibular joints, masticatory muscles, and other surrounding structures[1,3]. Among TMD conditions, disc displacement with reduction (DDwR) denotes an abnormal relationship between the disc and condyle. In cases of DDwR, the disc shifts forward relative to the condyle during mouth closure, reverting to its original position upon mouth opening[4]. Anterior disc displacement gives rise to clicking, popping, or snapping sounds, pain, and TMJ deformities[4-6].

Beyond TMJ problems, muscle and soft tissue-related disorders, including myalgia, local myalgia, myofascial pain, and myofascial pain with a referral (MPwR), also cause pain[5-7]. Referred myofascial pain, a subtype of TMD, is characterized by local or radiating pain in the temporal or masticatory muscles, elicited by palpation or excessive stretching during the examination. Diagnosis is confirmed when pain from the trigger point spreads beyond its boundaries[8].

The Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) are widely accepted as reliable and valid for diagnosing TMD, and in clinical practice, it is recommended for researchers to use them for categorizing TMD sub-diagnoses[9]. It includes a two-axis model. Axis I includes diagnostic criteria for TMD, and Axis II includes the assessment of behavioral and psychosocial factors related to TMD[6,9].

The DC/TMD criteria propose utilizing history-taking, physical examination, and imaging techniques as standard approaches for diagnosing disc displacement disorders. Also, they are classified into four subtypes according to DC/TMD criteria: DDwR, DDwR with intermittent locking, DD without reduction without limited mouth opening, and disc displacement without reduction with limited mouth opening[9]. Pain-related TMD, classified by DC/TMD as myalgia, local myalgia,

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myofascial pain, MPwR, arthralgia, and headache attributed to TMD, may exist in isolation or in combination[6,10]. Notably, the literature lacks investigation into how coexisting TMD sub-diagnoses on symptoms, functions, and clinical findings could substantially inform treatment management strategies.

The aim of this study was to elucidate how the presence of MPwR from masticatory muscles influenced clinical symptoms, pain-related disability, and TMJ function limitation in participants with DDwR. The hypothesis of this study posits that concurrent MPwR with DDwR leads to increased pain levels and restricted jaw functions in patients.

METHODS

This prospective, cross-sectional study was conducted between November 2021 and February 2022. Participants aged 18–45 years with DDwR with or without MPwR in the masseter muscles were recruited from the outpatient clinic based on inclusion criteria. Inclusion criteria encompassed age between 18 and 45 years, diagnosis of DDwR with or without MPwR in the masseter muscles by a specialist using the parameters and criteria of DC/TMD Axis I, and the absence of intermittent locking of the jaw.

The diagnosis of DDwR and MPwR was established through physical examinations in accordance with DC/TMD diagnostic criteria[6]. Participants visiting the outpatient clinic were assessed for clicking, popping, or snapping sounds. Those exhibiting such sounds without intermittent locking were diagnosed with DDwR, obviating the need for imaging. Assessment of TMJ sound was performed by palpation of the TMJ using the index and middle fingers and hearing the sound during mouth opening and closing. In accordance with the literature, the examiner's ear was within 5 cm of the participant's TMJ[11]. Evaluation of MPwR involved palpation of masseter muscles, diagnosing the presence of pain during opening the jaw or pain within 2 s upon palpating the masseter muscle. Participants exhibiting MPwR were included in the DDwR+MPwR group. All evaluations were conducted by the same investigator with a minimum of 7 years experience in TMD assessment.

Exclusion criteria comprised any other TMJ conditions (including rheumatological and neurological diseases, fibromyalgia, serious psychiatric disorders, temporomandibular agenesis, hyperplasia, hypoplasia, dental prosthesis, condylar malignant neoplasm, and continuous medication use like benzodiazepines, antidepressants, and antipsychotics), intermittent locking of the TMJ, ongoing TMD treatment, prior TMJ surgical intervention, occlusal splint usage within the last year, and extensive ongoing dental treatment.

A pre-study power analysis determined the sample size, and recruitment was over once there were 60 participants in each group. Consequently, data from two groups, namely, the DDwR group (n=60) and the DDwR+MPwR group (n=60), were subjected to analysis.

Outcome measures

All participants were evaluated based on the DC/TMD Axis I and II criteria. Within Axis I, the participants were asked about pain, TMJ sounds, and locking of the TMJ during mouth opening and closing. Furthermore, clinical examinations were conducted to ascertain pain location and characteristics, incisal relationships, and mandibular movements. A visual analog scale (VAS) was used to measure the pain levels of the participants. This scale consists of a 10-point Likert scale[12].

Bruxism was diagnosed according to the classification of possible bruxism, probable bruxism, and definite bruxism, as outlined by Lobezzo et al.[13] This study specifically explored probable awake bruxism by assessing participants' symptoms and physical examination findings. The awake bruxism symptoms questionnaire consisted of two questions from the DC/TMD Oral Behavioral Checklist: "Q3-Grind teeth together during waking hours" and "Q4-Clamp your teeth together during waking hours"[14]. In cases where participants answered positively to either question, a physical examination was performed to investigate signs of awake bruxism. This examination encompassed assessing tooth marks on the tongue and cheeks, tooth wear, and masseter hypertrophy. A diagnosis of probable awake bruxism was established for participants who exhibited both positive responses to either of the two symptom questionnaire questions and displayed one of the four physical examination signs[15,16].

For the measurement of anterior maximum mouth opening (MMO), the participants were asked to open their mouths as wide as possible with the distance between the incisors recorded.

Within the Axis II assessment, the "Graded Chronic Pain Scale version 2.0 (GCPS v2.0)" and "Jaw Function Limitation Scale-8 (JFLS-8)" were used. GCPS v2.0 is a valid and reliable instrument that evaluates pain levels and pain-related disability. It consists of three items for pain levels, four items for temporomandibular function, and one item for the number of days of pain. A 1-month version of the scale was used in the present study. According to the results, five grades were determined: Grade 0 (no pain and disability), Grade I (low-intensity pain and without disability), Grade II (high-intensity pain and without disability), Grade III (moderately limiting), and Grade IV (severely limiting)[17].

JFLS-8 assesses global limitations in chewing, verbal and emotional expression, and jaw mobility. Each item consists of a 10-point scale that is evaluated between "no restriction" and "severe restriction"[18].

Data analysis

The Statistical Package for the Social Sciences statistical program (IBM Corp., Armonk, NY, USA) version 21 was used for data analysis. The distribution of the variables was analyzed by the histogram and the Shapiro-Wilk test. Descriptive statistics were presented as the mean (standard deviation) for continuous variables, the median (minimum-maximum) for ordinal variables, and the frequency with percentage for categorical variables. For inter-group analysis, the chi-square test for ordinal variables, the Mann-Whitney U test for nonparametric variables, and the independent t-test for parametric variables were used.

Sample size calculation

The G*Power program (G*Power version 3.1.9, Germany) was used for the calculation of the sample size based on the change in pain intensity. To achieve $\alpha<0.05$ and $\beta=80\%$ based on the VAS scores, as described by Poluha et al.[5] it was determined that at least 47 participants would be required for each group. Assuming a 20% dropout rate, the study required a total of 120 participants to be included.

Ethical approval

The study protocol was approved by the Local Ethical Board (under number KAEK/2021.11.309) in accordance with the Declaration of Helsinki. Verbal and oral consent was obtained from all participants. The Clinicaltrials.gov ID number of the present trial is NCT05187325.

RESULTS

A total of 120 participants diagnosed with DDwR with or without MPwR who applied to the outpatient clinic were recruited in the study according to the inclusion criteria.

The mean age of the population was 30.9±11.4 years. There was no significant difference between the two groups in terms of age, gender, or other demographic characteristics (Table 1).

The mean VAS of the participants was 3.1 (2.5) in the DDwR+MPwR group and 1.6 (2.0) in the DDwR group. GCPS v2.0 was categorized into five grades. In the DDwR+MPwR group, 21.7% of the participants had no pain, 25% of them had low-intensity pain, 30% had high-intensity pain, and 23.3% had moderately limiting pain. In the DDwR group, 36.7% of the participants had no pain, 38.3% of them had low-intensity pain, 18.3% had high-intensity pain, and 6.7% had moderately limiting pain. No participants had severely limiting pain in either group. While 46.7% of the participants had awake bruxism in the DDwR+MPwR group, this rate was 28.3%

Table 1. Demographic characteristics of the participants.

	DDwR+MPwR group (n=60)	DDwR group (n=60)	p-value
Age (years) [mean (SD)]	31.4 (10.8)	30.4 (11.9)	0.385
Gender			
Female/male	46/14	45/15	0.673
Marital status [n (%)]			
Married	21 (35%)	26 (43.3%)	0.705
Unmarried	39 (65%)	34 (56.7%)	
Education [n (%)]			
Primary school	27 (45%)	22 (36.7%)	0.278
High school	20 (33.3%)	22 (36.7%)	
University	13 (21.7%)	16 (26.6%)	
Stress levels of the working environment [n (%)]			
Not working	17 (28.3%)	22 (36.7%)	
Less stressful	22 (36.7%)	21 (35%)	0.265
Moderate stressful	16 (26.7%)	15 (25%)	
Very stressful	5 (8.3%)	2 (3.3%)	

MPwR: myofascial pain with referral; DDwR: disc displacement with reduction; SD: standard deviation.

in the DDwR group. Upon comparison of the DDwR and DDwR+MPwR groups, there were significant differences in terms of VAS (p<0.001), awake bruxism (p=0.038), and GCPS v2.0 (p=0.010) between the two groups (Table 2).

The mean score of JFLS-8 was 4.5 (1.6) in the DDwR+MPwR group and 4.9 (1.8) in the DDwR group. According to the measurement of MMO, it was found to be 38.9 (9.6) mm in the DDwR+MPwR group and 40.3 (6.6) mm in the DDwR group. There was no statistical difference between both groups in terms of MMO and JFLS-8 (Table 2).

DISCUSSION

The present study investigated pain intensity, pain-related disability, and functional limitation in individuals with DDwR with and without MPwR in the masseter muscles. The results indicated that participants with both DDwR and MPWR in the masseter muscle exhibited higher pain intensity, a higher prevalence of awake bruxism, and increased pain-related disability compared to those with DDwR alone.

Pain-related TMDs have been established as a significant cause of hospital admissions[5,19]. The most common cause of chronic musculoskeletal orofacial pain is TMD[20,21]. In this study, both DDwR and MPwR were sources of pain, with DDwR accompanied by MPwR showing an elevation in pain intensity. Similarly, Poluha et al., reported that a patient with DDwR had an increased chance of presenting MPwR as well[5].

The relationship between chronic painful conditions, mental disorders, and temporomandibular disorders has been discussed in the current literature[10,22,23]. A cross-sectional study evaluating the association between TMDs and participants' disability levels, using the GCPS v2.0, did not find a clinical correlation between them[24]. Similarly, DDwR has been linked to amplified jaw disability[25]. In the current study, disability due to chronic pain was found to be higher in participants with DDwR and MPwR than DDwR alone. This could be attributed to the additive effect of pain from trigger points (TrPs), exacerbating the pain caused by DDwR.

Individuals with disc displacement can be either asymptomatic or experience pain, limited jaw movement, and/or joint sounds while opening and/or closing the mouth[26]. Nowak et al., suggested that mandibular movements might be constrained in cases of myofascial pain in masticatory muscles[27]. However, this study found no correlation between the presence or absence of MPwR and limitations of movement or functional restrictions in the TMJ. This might be related to limitations in mandibular movement due to joint disorders. In addition, functional limitations of the TMJ could be linked to the chronic, long-term outcomes of TMDs[28].

It has been reported in the literature that myofascial pain in the masseter muscles is associated with parafunctional activities like bruxism[27]. A study investigating the impact of bruxism on TMD based on DC/TMD criteria reported that awake bruxism is associated with muscle disorders and disc displacement with the reduction subtypes of TMD[16]. According to the

Table 2. Comparison of both groups in terms of clinical symptoms, pain, and limitation of temporomandibular joint function.

	DDwR+MPwR group (n=60)	DDwR group (n=60)	p-value	95%CI of the difference	
				Lower	Upper
VAS (cm) [mean (SD)]	3.1 (2.5)	1.6 (2.0)	<0.001*	0.649	3.348
GCPS v2.0 [n (%)]					
Grade 0: no pain	13 (21.7%)	22 (36.7%)			
Grade I: low-intensity pain	15 (25%)	23 (38.3%)			
Grade II: high-intensity pain	18 (30%)	11 (18.3%)	0.010*	–	–
Grade III: moderately limiting	14 (23.3%)	4 (6.7%)			
Grade IV: severely limiting	–	–			
JFLS-8 [mean (SD)]	4.5 (1.6)	4.9 (1.8)	0.241	-1.024	0.224
Bruxism [n (%)]					
Yes	28 (46.7%)	17 (28.3%)	0.038*	–	–
No	32 (53.3%)	43 (71.7%)			
MMO (mm) [mean (SD)]	38.9 (9.6)	40.3 (6.6)	0.512	-6.179	1.164

MPwR: myofascial pain with referral; DDwR: disc displacement with reduction; SD: standard deviation; VAS: visual analog scale; JFLS-8: Jaw Functional Limitation Scale-8; GCPS v2.0: Graded Chronic Pain Scale version 2.0, Mann-Whitney U test, and chi-square test were used to assess the difference between groups. *p<0.05 is considered statistically significant.

current study results, awake bruxism was found to be more common in the DDwR+MPwR group. This occurrence could be attributed to increased activation of the masseter muscles caused by awake bruxism.

Although MMO can vary by ethnicity, it ranges between 45 and 53 mm in healthy individuals[5]. According to previous studies, restricted mouth opening was defined as less than 40 mm[29,30]. In this study, the mean value of the MMO was 39.6±8.2 mm, and there was no significant difference between the two groups,. which aligned with previous study results. Notably, the study did not distinguish between active and latent TrPs within the masseter muscles. However, according to Xu et al., central sensitization can be induced by stimulation of latent TrPs[31]. Also, Li et al., reported the presence of both nociceptive and non-nociceptive pain sensitivity at latent TrPs[32]. Considering the results of the aforementioned studies, active or latent TrPs might have influenced MMO and other pathological findings, excluding pain.

The strength of the study was to use the DC/TMD criteria that are considered the gold standard for the evaluation of TMD[8]. Furthermore, to the best of the our knowledge, this study uniquely evaluated the additional contribution of MPwR to DDwR. As such, it contributes valuable insights to the literature. Additionally, the study benefited from examinations conducted by a single experienced TMD specialist, mitigating subjective differences during participant evaluations.

Nonetheless, the study's limitations include the diagnosis of DDwR solely through physical examination, omitting the use of imaging methods. Additionally, only possible awake bruxism was evaluated, excluding sleep bruxism. While quantitative tests like polysomnography or electromyography are recommended for a definitive diagnosis of bruxism[13,33,34]. In this study, polysomnographic evaluation was not performed. Possible awake bruxism was investigated based on symptoms and a physical examination. Nevertheless, Lobezzo et al., reported that it would be sufficient to evaluate possible bruxism in studies with large samples[13]. However, in this study, only TrPs in the masseter muscle were examined without addressing referred pain from other muscles in this region. Additionally, pain assessment did not encompass algometric pressure on the muscle, representing a limitation of the study.

CONCLUSION

This study demonstrated that the participants with both DDwR and MPwR in the masseter muscle experienced higher pain intensity, a higher prevalence of awake bruxism, and increased pain-related disability in comparison to those with DDwR alone. On the other hand, the presence or absence of MPwR did not exhibit any association with limitations in mandibular movement or functional restrictions. Well-designed, prospective studies evaluating TMJ with imaging modalities in addition to the physical examination will provide a better understanding of the clinical features of TMD patients.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Kanuni Sultan Suleyman Training and Research Hospital Clinical Research Ethical Board (under number KAEK/2021.11.309) in conformity with the Declaration of Helsinki. All participants were informed about the study verbally and in writing.

AUTHORS' CONTRIBUTIONS

MDK: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **BCK**: Data curation, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Writing – review & editing.

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https://doi.org/10.1590/1806-9282.20230622ERRATUM

In the manuscript "Do myofascial trigger points in masseter muscles affect the symptoms of disc displacement with reduction? A cross-sectional study", https://doi.org/10.1590/1806-9282.20230622, published in the Rev Assoc Med Bras. 2023;69(12):e20230622, on page 3:

Where it reads:

The study protocol was approved by the XXX Ethical Board (under number KAEK/2021.11.309) in accordance with the Declaration of Helsinki.

It should read:

The study protocol was approved by the Local Ethical Board (under number KAEK/2021.11.309) in accordance with the Declaration of Helsinki.

ORIGINAL ARTICLE

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Evaluation of the relationship between toxicity of cyclin-dependent kinase 4/6 inhibitors and body surface area

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SUMMARY

OBJECTIVE: This study aimed to evaluate the relationship between the toxicity of cyclin-dependent kinase 4/6 inhibitors and body mass index and body surface area.

METHODS: A total of 83 patients were included in the study. Patients were divided into 4 groups as 18–24.9, 25–29.9, 30–39.9, and >40 kg/m^2 according to body mass index and into two groups as below and above 1.77 according to body surface area. The relationship between body mass index and body surface area and side effects was evaluated.

RESULTS: No statistically significant difference was found between body mass index groups and side effects. Grade 3 neutropenia was more common in patients on palbociclib with a body surface area≤1.77. In our study, it was revealed that less hematological side effects can be encountered when body surface area is taken into account.

KEYWORDS: Breast cancer. Drug toxicity. Body measures. Toxicity.

INTRODUCTION

Breast cancer is the most common cancer in women and the most common cause of death[1]. In patients with hormone receptor (HR)-positive, HER2-negative metastatic breast cancer, treatment is initiated with a combination of cyclin-dependent kinase (CDK) 4/6 inhibitors plus endocrine therapy if the visceral crisis is not considered[2]. CDK4 and CDK6 form a complex with cyclin D, leading to phosphorylation of retinoblastoma (Rb) and activation of E2F. Rb phosphorylation is prevented by CDK4/6 inhibition. Inactivated E2F prevents the transition from G1 to S phase and decreases cell proliferation[3].

Apart from cell cycle regulation, the cyclin-CDK-Rb-E2F pathway also contributes to important metabolic processes such as lipid synthesis, insulin secretion, and glucose production[4]. Some studies have found an association between CDK4 deficiency with impaired lipogenesis and increased lipolysis[5,6]. Preclinical studies have found that CDK inhibitor therapies increase lipid utilization during a high-fat diet and that CDK4/6 inhibitors may be potential targets in the treatment of obesity[7]. CDK4/6 inhibitors affect body fat and muscle mass.

It is known that weight gain and obesity are associated with a worse prognosis in HR-positive early-stage breast cancer[8,9]. However, there are insufficient data on the effects of BMI in metastatic patients. All metastatic patients start treatment with CDK4/6 inhibitors at the same dose. In our study, we aimed to evaluate the relationship between the toxicity of CDK4/6 inhibitors and body mass index (BMI) and body surface area (BSA).

METHODS

This study included 87 patients with metastatic HR-positive breast cancer who received CDK4/6 inhibitor (ribociclib 1×600 mg or palbociclib 1×125 mg) and endocrine therapy (fulvestrant or aromatase inhibitor) between January 2022 and July 2022 and was followed up in our clinic between January 2022 and July 2022 and used these treatments for at least 3 months. Four patients whose data could not be reached and who were followed up in other centers were excluded from the study. Patients were divided into 4 groups as 18–24.9, 25–29.9, 30–39.9, and >40 kg/m^2 according to BMI and into two groups as below and above 1.77 according to BSA. The study was conducted following the Helsinki Declaration of 1975, revised in 2000. Data use permission and ethics committee approval were obtained from relevant institutions.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (Statistical Package for the Social

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Sciences, IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as n and % for categorical variables and mean±SD and median (IQR) for continuous variables. The drug-related toxicities of the patients were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) guideline. The chi-square test or Fisher's exact test was used to compare categorical variables. $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the patients included in the study was 51.29±12.65 years. BMI, BSA, treatments, mortality, and progression status of the patients are shown in Table 1.

Table 1. Distribution of body mass index, body surface area, and clinical variables of patients.

	n	%
Body mass index, n (%)		
18–24.9	19	22.9
25–29.9	26	31.3
30–39.9	32	38.6
40–60	6	7.2
Body surface area, n (%)		
1.77 or less	43	51.8
1.77 over	40	48.2
Medicines, n (%)		
Ribociclib	62	74.7
Palbociclib	21	25.3
Previous endocrinology treatment, n (%)		
Exist	45	54.2
None	38	45.8
Neoadjuvant or adjuvant treatment, n (%)		
Exist	32	38.6
None	51	61.4
Mortality, n (%)		
Right	73	88.0
Ex	10	12.0
Progression, n (%)		
None	62	75.6
Exist	20	24.4
	Mean±SD	**Median (IQR)**
Age	51.29±12.65	51.00 (19.00)
Body surface area	1.77±0.18	1.77 (0.29)
Follow-up time	67.58±61.44	45.46 (79.73)

Of all patients, 56 (67.5%) had neutropenia and 11 (13.3%) had elevated LFTs. There was no statistically significant difference between BMI groups and side effects ($p>0.05$). When the patients receiving ribociclib or palbociclib were evaluated separately, no statistically significant difference was found between the BMI groups and side effects ($p>0.05$).

As seen in Table 2, no statistically significant difference was found between BSA groups and side effects ($p>0.05$). A statistically significant difference was found only between Grade 1 neutropenia and BSA groups ($p=0.021$). Grade 1 neutropenia was observed more frequently in those with BSA>1.77.

When evaluated separately, no statistically significant difference was found between BSA groups and side effects in ribociclib patients ($p>0.05$).

As observed in Table 3, there was no statistically significant difference between the BSA groups and side effects in palbociclib recipients ($p>0.05$). However, a statistically significant difference was found only between grade 3 neutropenia and BSA groups ($p=0.030$). Grade 3 neutropenia was observed more frequently in patients with a BSA≤1.77.

DISCUSSION

Area under the curve (AUC), the most important pharmacokinetic parameter in anticancer drug exposure, is affected by many factors such as drug dose, age, gender, height, weight, hereditary variations in drug-metabolizing enzymes, and drug clearance. There is a lot of interindividual variation in AUC following a single dose of a drug[10]. To minimize this interindividual variation, the BSA is calculated according to the height and weight of the patient, and the treatment dose is adjusted according to the surface area of the patient when starting chemotherapy for oncology patients. When starting CDK4/6 inhibitors plus endocrine therapy, each patient is given a standard dose of treatment without taking into account the weight and height of the patients.

ASCO's updated guidelines suggest that there is no difference in the toxicity of these targeted agents between underweight and overweight people and that FDA-approved prescribing information should be used in all patients, regardless of obesity status[11].

Studies have shown the superiority of these treatments over placebo plus ET independent of BMI and BSA. However, studies investigating the toxicity of CDK4/6 inhibitors according to BMI and BSA are very limited. In the subgroup analysis in the study in which the safety analysis of MONOLISA 2-3 and 7 was evaluated, it was observed that the patients had similar BMI, so toxicity analysis was not performed according to BMI[12].

Table 2. Comparison of side effects according to body surface area groups.

	Body surface area		p
	≤1.77	>1.77	
Neutropenia, n (%)			
None	14 (32.6)	13 (32.5)	0.995[a]
Exist	29 (67.4)	27 (67.5)	
GR1 neutropenia, n (%)			
None	38 (88.4)	27 (67.5)	**0.021[a]**
Exist	5 (11.6)	13 (32.5)	
GR2 neutropenia, n (%)			
None	43 (100)	40 (100)	–
Exist	–	–	
GR3 neutropenia, n (%)			
None	22 (51.2)	26 (65)	0.202[a]
Exist	21 (48.8)	14 (35)	
GR4 neutropenia, n (%)			
None	40 (93)	40 (100)	0.242[b]
Exist	3 (7)	0 (0)	
LFT, n (%)			
None	35 (81.4)	37 (92.5)	0.136[a]
Exist	8 (18.6)	3 (7.5)	
GR1 LFT, n (%)			
None	42 (97.7)	39 (97.5)	1.000[b]
Exist	1 (2.3)	1 (2.5)	
GR2 LFT, n (%)			
None	41 (95.3)	40 (100)	0.495[b]
Exist	2 (4.7)	0 (0)	
GR3 LFT, n (%)			
None	39 (90.7)	39 (97.5)	0.361[b]
Exist	4 (9.3)	1 (2.5)	
GR4 LFT, n (%)			
None	42 (97.7)	39 (97.5)	1.000[b]
Exist	1 (2.3)	1 (2.5)	
GFR decrease, n (%)			
None	39 (90.7)	37 (92.5)	1.000[b]
Exist	4 (9.3)	3 (7.5)	
Soft tissue infection, n (%)			
None	43 (100)	36 (90)	0.050[b]
Exist	0 (0)	4 (10)	
Other, n (%)			
None	40 (93)	35 (87.5)	0.473[b]
Exist	3 (7)	5 (12.5)	

[a]Pearson's chi-square test; [b]Fisher's exact test; p<0.05 statistically significant. Bold values indicate the cut-off values mentioned in the article.

Table 3. Comparison of side effects according to body surface area groups in palbociclib recipients.

Palbociclib	Body surface area		p
	≤1.77	>1.77	
Neutropenia, n (%)			
None	2 (18.2)	4 (40)	0.361[b]
Exist	9 (81.8)	6 (60)	
GR1 neutropenia, n (%)			
None	11 (100)	7 (70)	0.090[b]
Exist	0 (0)	3 (30)	
GR2 neutropenia, n (%)			
None	11 (100)	10 (100)	–
Exist	–	–	
GR3 neutropenia, n (%)			
None	2 (18.2)	7 (70)	**0.030[b]**
Exist	9 (81.8)	3 (30)	
GR4 neutropenia, n (%)			
None	11 (100)	10 (100)	–
Exist	–	–	
LFT, n (%)			
None	10 (90.9)	9 (90)	1.000[b]
Exist	1 (9.1)	1 (10)	
GR1 LFT, n (%)			
None	11 (100)	9 (90)	0.476[b]
Exist	0 (0)	1 (10)	
GR2 LFT, n (%)			
None	10 (90.9)	10 (100)	1.000[b]
Exist	1 (9.1)	0 (0)	
GR3 LFT, n (%)			
None	11 (100)	10 (100)	–
Exist	–	–	
GR4 LFT, n (%)			
None	11 (100)	10 (100)	–
Exist	–	–	
GFR decrease, n (%)			
None	9 (81.8)	9 (90)	1.000[b]
Exist	2 (18.2)	1 (10)	
Soft tissue infection, n (%)			
None	11 (100)	7 (70)	0.090[b]
Exist	0 (0)	3 (30)	
Other, n (%)			
None	11 (100)	8 (80)	0.214[b]
Exist	0 (0)	2 (20)	

[b]Fisher's exact test; p<0.05 statistically significant. Bold values indicate the cut-off values mentioned in the article.

In the pooled analysis of the MONARCH 2 and 3 studies, patients were divided into four categories according to BMI, and the primary endpoint was PFS and the secondary endpoints were response rate, side effects, and weight loss according to BMI. In this analysis, no difference was found between BMI and PFS, while overweight and obese patients had higher response rates and lower neutropenia[13]. In our study, we did not find any difference between BMI and development of toxicity.

However, as BMI alone is not a good indicator of body fat distribution and sarcopenia and BSA is less affected by body fat distribution and is a better indicator of metabolic mass, we reassessed all patients (palbociclib and ribociclib users) divided into two groups according to BSA. We found that grade 1 neutropenia was more common in patients with BSA>1.77. When we separately evaluated patients on palbociclib, we found more grade 3 neutropenia in those with a BSA≤1.77. A review of the literature shows that neutropenia is more common in patients receiving palbociclib than in those receiving ribociclib, but there are no data on the relationship with BSA[14]. As the patients were generally of similar height, it was the weight of the patients that largely determined the increase in BSA. Neutrophil levels may also increase as an inflammatory marker in overweight patients. This may cause less neutropenia to be observed in these patients. Therefore, we think that we observed more severe neutropenia, especially in patients with lower BSA.

In our study, no difference was observed in terms of impairment in LFT and other toxicities according to BSA and BMI.

We found that CDK4/6 inhibitors can be used with equal safety in all subgroups according to BMI, but it should be taken into consideration that grade 3 neutropenia may be observed more frequently in patients with BSA ≤ 1.77 who are on palbociclib. This study is important because it revealed that less hematological side effects can be encountered when BSA is considered. Other parameters are needed to assess body composition. This idea can be improved with further studies using these parameters and with a larger number of patients.

AUTHORS' CONTRIBUTIONS

ŞYD: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. **EF**: Investigation, Supervision.

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Frailty and its associates in community-dwelling older adults

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SUMMARY

OBJECTIVE: While the literature contains several studies on the frailty assessed during hospitalization and/or outpatient settings and nursing homes, few studies have assessed frailty in community-dwelling older adults. We investigated the prevalence of frailty and associated factors among older adults in a sample of community-dwelling older adults.

METHODS: We included community-dwelling older adults >60 years living in the Fatih District of the Istanbul Province. We conducted the study between November 2014 and May 2015. We collected the data such as age, sex, number of diseases and drugs, functional status, frailty, the presence of geriatric syndromes, common diseases, and quality-of-life assessment. Frailty was evaluated by the FRAIL scale.

RESULTS: A total of 204 adults (mean age: 75.4±7.3 years) were included, of whom 30.4% were robust, 42.6% were pre-frail, and 27% were frail. In multivariate analyses, associated factors of frailty were the number of drugs [odds ratio (OR)=1.240, p=0.036], the presence of cognitive impairment (OR=0.300, p=0.016), and falls (OR=1.984, p=0.048).

CONCLUSION: The present study established the prevalence of frailty in a large district in the largest metropolis in the country through a valid screening method. Our results suggest that clinicians should consider frailty evaluation in patients with multiple drug usage, cognitive impairment, and falls.

KEYWORDS: Community dwelling. Older adults. Frail elderly. Geriatric assessments.

INTRODUCTION

Our country is experiencing the same significant demographic changes worldwide, along with a continuous increase, especially in the older population. It is estimated that one in every six (16%) people will be above 65 years of age by 2050[1]. Frailty is a multidimensional geriatric syndrome that can be defined as a state of increased vulnerability resulting from decreased physiological reserves, multiple system irregularities, and limited capacity to maintain homeostasis[2]. Although frailty is often associated with comorbidities and restrictions on movement, these terms have different meanings[2-6]. The comorbidities that accompany frailty can be caused by frailty but may also be considered a risk factor for frailty and disability[3-9].

While the literature contains several studies on the frailty assessed during hospitalization and/or outpatient clinic visits and nursing homes, few studies assess the community-dwelling older adults in our country[2-5]. However, there is not yet a frailty prevalence study in the most populated metropolitan area of our country, where elderly patients are evaluated with home visits. Istanbul is the 22nd largest metropolitan city in the world and is also located in a region that receives the most significant number of migrations due to our country's industrialization and cultural and historical heritage.

In light of this information, the present study investigated the prevalence of frailty and associated factors among the older adults assessed within the scope of a comprehensive geriatric study in the Fatih District of Istanbul Province.

METHODS

This was a population-based, prospective, cross-sectional study. The sample size was calculated considering the prevalence of frailty in the community with a 10% error margin at a power of 80% and a 95% confidence interval.

The study included community-dwelling older adults aged 61–101 years living in the Fatih District of Istanbul Province between November 2014 and May 2015. We selected the participants by a simple random sampling method among the older adults living at the addresses determined in the Fatih District of Istanbul. Participants aged over 60 years who agreed to participate in the study were included, while participants who had an implant, had edema/major fluid-electrolyte disorders, had

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cognitive impairment without anyone to accompany them, and were illiterate were excluded.

Functional capacity was measured using a six-item KATZ Activities of Daily Living (ADL) Scale and an eight-item LAWTON-BRODY Instrumental Activities of Daily Living Scale (IADL)[10].

Frailty was assessed through the application of the FRAIL scale. Based on the results of the five-item FRAIL scale, fatigue, resistance, ambulation, illnesses, and weight loss were measured[11].

The malnutrition screening was carried out using the Mini Nutritional Assessment-Short Form (MNA-SF)[12].

The cognitive screening was carried out using a Mini-Cog test[13].

The depressive mood was evaluated using the Geriatric Depression Scale-Short Form (GDS-SF)[14].

For the chronic pain assessment, we asked participants if they had pain for more than 6 months and, in the presence of pain, we asked them to give a score between 0 and 10 using the visual analog scale (VAS)[15].

Handgrip strength (HGS) was measured using a Jamar hydraulic hand dynamometer. For HGS, the previously reported cutoffs of 27 and 16 kg for males and females, respectively, were used according to the European Working Group on Sarcopenia in Older People2 (EWSGOP2)[16,17].

Statistical analysis

The normality of continuous data was analyzed with a Kolmogorov-Smirnov test. For the descriptive statistics, continuous variables were expressed as mean±standard deviation, median, and minimum-maximum values, while categorical variables were expressed as number (of subjects) and percentages. The differences between groups were determined by independent samples t-test or Mann-Whitney U test. The chi-square test and Fisher's exact test for 2×2 probability tables are suitable for categorical variables. Multivariate logistic regression analysis with the Enter method was used to determine the independent factors associated with frailty among the factors found to be significant in univariate analyses. Multicollinearity was checked among the selected parameters.

RESULTS

The study involved 204 older adults (94 men and 110 women) with a mean age of 75.4±7.3 years. Of the cases, 30.4% were robust, 42.6% were pre-frail, and 27% were frail. Significant differences between the genders were recorded in the number of diseases and drugs, IADL score, FRAIL score, malnutrition, GDS-SF score, fear of falling, urinary incontinence, chronic pain, and handgrip strength (Table 1).

In univariate analysis, the frailty groups (robust vs. frail) differed significantly in terms of age, sex, number of diseases and drugs, ADL-IADL score, the presence of malnutrition, the risk of malnutrition, cognitive impairment, dementia, depression, fear of falling, falls, urinary incontinence, chronic pain, and probable sarcopenia (Table 2).

A multivariate logistic regression analysis evaluating frailty-associated independent factors [dependent variable: frailty (robust vs. pre-frail+frail)] revealed an association between the frailty and the number of drugs (OR=1.240, p=0.036, CI=1.010–1.500), cognitive impairment (OR=0.300, p=0.016, CI=0.113–0.799), and falls (OR=1.984, p=0.048) (Table 3).

DISCUSSION

There have been several studies examining frailty and potentially associated factors among patients during hospitalization and outpatient clinic visits, while there have been only few studies making extensive assessments of older people living in the community[6,8,18-22]. There have also been studies conducted in our country evaluating the older inpatients and outpatients admitted to family health centers. To the best of our knowledge, to date, no study involving a community screening for frailty, as in the present study, has been conducted[12].

We established that 42.6% of the respondents were pre-frail, while 27% were frail. Çakmur et al., in their population-based study in Kars, a rural area of Turkiye, screened frailty in community-dwelling older adults with the FFI scale[19]. They found the prevalence of frailty to be 7.1% and the prevalence of pre-frailty to be 47.3%. In addition, they found advanced age, lower education level, lower economic level, comorbidities, polypharmacy, diabetes, chronic obstructive pulmonary disease, stomach disease, arthritis, widespread pain, benign prostatic hyperplasia, urinary incontinence, auditory disorder, impaired oral care, caregiver, burden, cognitive dysfunction, depression, and social isolation as factors associated between frailty in univariate analyses in this study[19]. In the study conducted by Jurschik et al., among people aged 75 years and older living in a community in Spain, the frailty prevalence was identified as 9.6% by the Fried Frailty Index (FFI) criteria[21]. In the study by Moreira et al., of the participants aged over 65 years living in a community in Brazil, 9.1% were frail and 47.3% were pre-frail, based on the results of the FRAIL scale[8]. A meta-analysis by Kojima et al., examining five studies in which frailty was assessed using the FFI, involving 11,940 community residents aged 65 years or older in Japan, identified frailty in 7.4% and pre-frailty in 48.1% of the respondents[6]. The study by Collard et al., assessed 21 studies with 61,500 participants using the

Table 1. Comparative data of the study population by sex.

	Male (n=94)	Female (n=110)	Total (n=204)	p-value
Age	74.7±6.6	76±7.8	75.4±7.3	0.190
Number of diseases	3 (0–7)	3 (0–9)	3 (0–9)	0.006*
Number of drugs	3 (0–18)	4 (0–12)	4 (0–18)	0.001*
ADL	18 (6–18)	18 (6–18)	18 (6–18)	0.194
IADL	24 (8–24)	23 (8–24)	24 (8–24)	<0.001*
FRAIL score	1 (0–5)	1 (0–5)	1 (0–5)	0.002*
FRAIL group				0.004*
Robust (n, %)	38 (40.4%)	24 (21.8%)	62 (30.4%)	
Pre-frail (n, %)	39 (41.5%)	48 (43.6%)	87 (42.6%)	
Frail (n, %)	17 (18.1%)	38 (34.5%)	55 (27%)	
Malnutrition (MN+MNR) (n, %)	29 (30.9%)	56 (50.9%)	85 (41.7%)	0.004*
Probable sarcopenia (n, %)	25 (26.6%)	23 (21.7%)	48 (24%)	0.418
Cognitive impairment (n, %)	32 (34%)	33 (31.1%)	65 (32.5%)	0.660
GDS-SF score[x]	2 (0–11)	4 (0–14)	3 (0–14)	<0.001*
Fear of falling (n, %)	17 (18.1%)	49 (45%)	66 (32.5%)	<0.001*
Falls (n, %)	24 (25.5%)	34 (30.3%)	58 (28.1%)	0.470
Urinary incontinence (n, %)	25 (26.6%)	48 (44%)	73 (36%)	0.010*
Faecal incontinence (n, %)	2 (2.1%)	4 (3.7%)	6 (3%)	0.510
Chronic pain (n, %)	32 (34%)	61 (56.5%)	93 (46%)	0.001*
Chronic diseases				
DM (n, %)	29 (30.9%)	33 (30%)	62 (30.4%)	0.890
HT (n, %)	61 (64.9%)	83 (75.5%)	144 (70.6%)	0.100
Dementia (n, %)	17 (18.1%)	15 (13.6%)	32 (15.7%)	0.380

ADL: activities of daily living; IADL: instrumental activities of daily living; BIA: bioimpedance analysis; DM: diabetes mellitus; GDS-SF: geriatric depression scale-short form; HT: hypertension; MN: malnutrition; MNR: malnutrition risk; MNA-SF: mini nutritional assessment-short form. [x]GDS-SF score is between 0 and 15. *Significant p-value.

FFI and reported frailty in 10.7% and pre-frailty in 41.6% of the older community residents[23]. Roche et al., used the FFI in community-dwelling people aged 65 years and older in the United States and identified frailty in 15% and pre-frailty in 45% of the population[7]. The differences in the prevalence of frailty may result from differences in the mean ages of the study groups, the genetic differences between communities, and the differences in sociodemographic characteristics between regions, along with differences in the scales used for the frailty assessment[4,8,9,21]. In Akın et al.'s study, the FRAIL scale was used in those aged 65 years and over who applied to family health centers in Turkiye, and frailty was identified in 10% and pre-frailty in 45.6% of the participants. They also used the FFI scale to screen for frailty. They found frailty at 27.8% and pre-frailty at 34.8% on the FFI scale[5]. Unlike the present study, which involved the community screening of a sample selected by stratification, the study by Akın et al., included patients applying to a family health center (primary care health center). The difference in the prevalence of frailty between the two studies may be due to older people's poorer general health status during home visits, thus not being able to apply to health centers and the higher prevalence of frailty.

Our findings indicated that multiple drug use was associated with frailty. The study by Woo et al., examined frailty and associated factors among community-dwelling residents above 65 years residing in rural and urban areas in China and found polypharmacy to be associated with frailty in both such areas, which was consistent with the findings of our study[22]. The retrospective study by Zheng et al., followed older residents of a community for 1 year and found that older people with polypharmacy became frail within 1 year more frequently[23]. These findings were in line with those of the present study and

Table 2. The associates of frailty (univariate analyses).

	Frail[a] (>3) n: 55 (27%)	Pre-frail[b] (1–2) n: 87 (42.6%)	Robust[c] (0) n: 62 (30.4%)	p-value	Frailty groups
Age	78.2±8.4	75.3± 6.9	72.9 ±5.8	0.001*	(a–c)
Sex (n, %)					(a–c)
Female	38 (69%)	47 (54%)	25 (40%)		
Male	17 (31%)	40 (46%)	37 (60%)	0.004*	
Number of diseases	4 (1–9)	3 (0–7)	2 (0–7)	<0.001*	(a–c), (a–b), (b–c)
Number of drugs	5 (1–18)	4 (0–15)	3 (0–8)	<0.001*	(a–c), (b–c)
ADL	17 (3–18)	18 (1–18)	18 (16–18)	<0.001*	(a–c), (a–b)
IADL	16 (8–24)	24 (10–24)	24 (15–24)	<0.001*	(a–c), (a–b)
Malnutrition (MN+MNR) (n, %)	35 (63%)	35 (40%)	15 (24%)	<0.001*	(a–c), (b–c)
Probable sarcopenia (HGS)	22 (43.1%)	17 (19.5%)	9 (%14.5)	0.001*	(a–b), (a–c)
Cognitive impairment (n, %)	28 (53.8%)	28 (32.6%)	9 (14.5%)	<0.001*	(a–c), (a–b), (b–c)
Depression (GDS-SF) (n, %)	17 (30%)	10 (11%)	1 (1.6%)	<0.001*	(a–c), (b–c)
Fear of falling (n, %)	28 (50%)	28 (32%)	10 (16%)	<0.001*	(a–c)
Falls (n, %)	23 (41%)	25 (28%)	29 (46%)	0.011*	(a–c)
Urinary incontinence (n, %)	32 (58%)	32 (36%)	11 (17%)	<0.001*	(a–c)
Faecal incontinence (n, %)	2 (3.6%)	4 (4.5%)	0 (0%)	0.100	N/A
Chronic pain (n, %)	37 (67%)	39 (44%)	17 (27%)	<0.001	(a–c), (a–b)
Chronic diseases					
DM (n, %)	18 (32%)	27 (29%)	18 (29%)	0.900	N/A
HT (n, %)	43 (78%)	67 (77%)	37 (59%)	0.065	N/A
Dementia (n, %)	15 (27%)	15 (17%)	2 (3.2%)	<0.001*	(a–c), (b–c)

ADL: activities of daily living; DM: diabetes mellitus; GDS-SF: geriatric depression scale-short form; HT: hypertension; IADL: instrumental activities of daily living; MN: malnutrition; MNR: malnutrition risk; MNA-SF: mini nutritional assessment-short form; HGS: handgrip strength. a: Frail, b: pre-frail, and c: robust. a–c: Statistically significant relationship between groups a and c. a–b: Statistically significant relationship between groups a and b. b–c: Statistically significant relationship between groups b and c. *Significant p-value.

Table 3. The associates of frailty (multivariate analyses).

	p-value	OR	95%CI	
			Lower	Upper
Age	0.249	1.039	0.974	1.108
Sex	0.858	0.912	0.330	2.518
Number of drugs	0.036*	1.240	1.010	1.500
IADL	0.232	0.856	0.662	1.105
MNA-SF	0.867	0.927	0.381	2.258
Probable sarcopenia (HGS)	0.726	0.890	0.464	1.706
Cognitive impairment	0.016*	0.300	0.113	0.799
GDS-SF	0.485	0.446	0.046	4.300
Falls	0.048*	1.984	1.005	3.917

CI: confidence interval; GDS-SF: geriatric depression scale-short form; IADL: instrumental activities of daily living; MNA-SF: mini nutritional assessment-short form; HGS: handgrip strength; OR: odds ratio. *Significant p-value.

suggested that a prescribing cascade occurred with older patients due to their focus on different complaints at each admission with no extensive geriatric assessment, leading to polypharmacy. This revealed that a complete examination was required for a comprehensive evaluation of the older population.

In the present study, cognitive impairment was also associated with frailty, which was consistent with the studies of Jurschik et al.[21], Akın et al.[5], Moreira et al.[8], and García et al.[24], all of which demonstrated an association between frailty and cognitive disorder. Furthermore, frailty may lead to cognitive impairment through social isolation, just as cognitive impairment may also lead to frailty. The association between these two factors is two-sided and embedded[5,8,21,24].

Similar to other national studies, the results of our study showed an association between falls and frailty in community-dwelling older adults[18,20-24]. Again, it was conducted in our

society. Akın et al., detected a relationship between falls and frailty, like our study and most studies in the literature[5].

The findings of the present study could serve as a guide for the assessment of frail older adults living in the community, as well as for the development and implementation of intervention strategies and measures for the treatment of frailty in older patients. The strength of the present study lies in its presentation of the results of an extensive geriatric assessment of the older population residing in the community in the Fatih District of Istanbul Province. Furthermore, the fact that the study was conducted in a large district of a metropolitan city like Istanbul is also of importance as it provides insight into frailty in the general population. The patient screening in the present study was based on a stratification method, which enhanced the importance of study findings even further, and the strength of the study is further increased in its analysis of the multiple and variable factors associated with frailty.

There were also some limitations. The exact causes of frailty could not be ascertained due to the cross-sectional design of the study, although major contributing factors were established.

CONCLUSION

The present study established the prevalence of frailty in a large district like Fatih, the largest metropolis in the country, through a valid screening method. The prominent associated factors were multiple drug usage, cognitive impairment, falls, and low quality of life. Nationwide population studies involving multiple centers are required.

ETHICS APPROVAL

We obtained ethical approval from the Istanbul University Istanbul Medical School ethical board (number: 1213 and file number: 2014/1199).

AUTHORS' CONTRIBUTIONS

MEB: Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **NMC:** Conceptualization, Formal Analysis, Investigation, Resources, Writing – original draft. **TE:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MMO:** Data curation, Formal Analysis, Methodology, Supervision, Writing – original draft. CK: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **MAK:** Conceptualization, Data curation, Formal Analysis, Supervision, Writing – original draft, Writing – review & editing. **GB:** Conceptualization, Data curation, Formal Analysis, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effect of ivabradine on ventricular arrhythmias in heart failure patients with reduced ejection fraction

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SUMMARY

BACKGROUND/INTRODUCTION: Heart failure patients with reduced ejection fraction are at high risk for ventricular arrhythmias and sudden cardiac death. Ivabradine, a specific inhibitor of the I_f current in the sinoatrial node, provides heart rate reduction in sinus rhythm and angina control in chronic coronary syndromes.

OBJECTIVE: The effect of ivabradine on ventricular arrhythmias in heart failure patients with reduced ejection fraction patients has not been fully elucidated. The aim of this study was to investigate the effect of ivabradine use on life-threatening arrhythmias and long-term mortality in heart failure patients with reduced ejection fraction patients.

METHODS: In this retrospective study, 1,639 patients with heart failure patients with reduced ejection fraction were included. Patients were divided into two groups: ivabradine users and nonusers. Patients presenting with ventricular tachycardia, the presence of ventricular extrasystole, and ventricular tachycardia in 24-h rhythm monitoring, appropriate implantable cardioverter-defibrillator shocks, and long-term mortality outcomes were evaluated according to ivabradine use.

RESULTS: After adjustment for all possible variables, admission with ventricular tachycardia was three times higher in ivabradine nonusers (95% confidence interval 1.5–10.2). The presence of premature ventricular contractions and ventricular tachycardias in 24-h rhythm Holter monitoring was notably higher in ivabradine nonusers. According to the adjusted model for all variables, 4.1 times more appropriate implantable cardioverter-defibrillator shocks were observed in the ivabradine nonusers than the users (95%CI 1.8–9.6). Long-term mortality did not differ between these groups after adjustment for all covariates.

CONCLUSION: The use of ivabradine reduced the appropriate implantable cardioverter-defibrillator discharge in heart failure patients with reduced ejection fraction patients. Ivabradine has potential in the treatment of ventricular arrhythmias in heart failure patients with reduced ejection fraction patients.

KEYWORDS: Ivabradine. Mortality. Implantable cardioverter defibrillator. Cardiac arrhythmia. Heart failure.

INTRODUCTION

Heart failure (HF) patients, especially those with HF with reduced ejection fraction (HFrEF), are at high risk for ventricular arrhythmias and sudden cardiac death. Both implantable cardioverter-defibrillator (ICD) and cardiac resynchronization therapy with an ICD (CRT-D) have been shown to successfully treat life-threatening ventricular arrhythmias and to reduce cardiac mortality in patients with HFrEF[1,2]. Traditionally, patients with HFrEF who survive from life-threatening ventricular arrhythmias are at increased risk for recurrent lethal arrhythmias in the long-term follow-up. In addition, detection of ventricular arrhythmia in such patients has been reported to be a poor prognostic predictor[3].

Ivabradine, a specific inhibitor of the I_f current in the sinoatrial node, provides a pure heart rate reduction in patients with sinus rhythm[4]. Ivabradine is currently recommended to treat patients with stable angina, HF, as well as inappropriate sinus tachycardia[5-7]. Outcomes of randomized trial on chronic heart failure demonstrated that ivabradine improved the long-term survival and reduced the rate of hospitalization in patients with HFrEF[6]. However, in this study, ventricular arrhythmias were not monitored, and patients with high ventricular arrhythmia burdens were excluded from the trial. Therefore, the effects of ivabradine on ventricular arrhythmias in HFrEF patients have not been fully elucidated. Thus, in this study, we aimed to investigate the effect of ivabradine on life-threatening arrhythmias and long-term mortality in HFrEF patients.

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METHODS

Data collection

In this retrospective study, we reviewed all patients with HFrEF who were admitted to our center between January 2010 and April 2021. The diagnosis of HFrEF was made based on the ICD codes in the hospital electronic database system and a previous transthoracic echocardiographic report demonstrating a left ventricle ejection fraction (LVEF) of ≤40%. In all, 1,639 HFrEF patients were evaluated in this investigation. The electronic database at our institution was employed to gather baseline information, laboratory results, and echocardiographic data. All patients received guideline-directed medical therapy for HFrEF. Transthoracic echocardiography was performed on all patients by a cardiovascular imaging specialist using the Vivid 7 (GE Vingmed Ultrasound AS, Horten, Norway) system. The LVEF was measured using the modified Simpson's method, and left ventricular end-diastolic and end-systolic volumes were evaluated on apical two- and four-chamber views. In addition, patients whose echocardiographic data could not be evaluated accurately and under the age of 18 years were excluded from the study. A 24-h rhythm Holter monitoring was performed in patients with palpitations, presyncope, and unexplained syncope complaints. For the presence of premature ventricular contractions (PVCs), the arrhythmia burden limit was determined to be >10% in 24-h rhythm Holter monitoring[8]. In HFrEF patients who were implanted with ICD or CRT-D, device therapy, such as anti-tachycardia pacing or shock, delivered in response to ventricular tachycardia (VT) or ventricular fibrillation (VF), was considered an appropriate therapy. Inappropriate device therapy was defined as any therapy given in reaction to atrial fibrillation, supraventricular tachycardia, sinus tachycardia, or device malfunction. Data on device therapy were acquired from patients' records and, where applicable, verified with device interrogation records. The regular use of ivabradine was confirmed by the data of the Ministry of Health since ivabradine is required to be used based on the medical reports according to the rules of the current insurance system.

Study outcomes

The primary endpoints of this investigation were long-term all-cause mortality and the occurrence of ventricular tachycardia, the presence of PVC burden >10% on 24-h Holter monitoring, the presence of ventricular tachycardia on 24-h Holter monitoring, and proper ICD shock. The long-term survival status of each patient was determined using the National Death Notification System.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences 20.0 software (IBM SPSS 20, SPSS Inc., Chicago, Illinois, USA). The study population was divided into two groups according to patients' ivabradine use: patients not using ivabradine (n=1363), and patients using ivabradine (n=276). The demographic features and clinical characteristics of the study groups were compared. Kolmogorov-Smirnov test was used for the evaluation of normality. Continuous variables were presented as median and interquartile range or mean and standard deviation compared using the t-test or the Mann-Whitney U test, as appropriate. A $p<0.05$ was considered statistically significant. Categorical variables were presented as numbers and percentages. Analyses of categorical variables were performed by Pearson's chi-square test or the Fisher's exact test. Cox regression models were formed in order to elucidate the effect of ivabradine use on the outcomes. The results of regression analysis were presented as a hazard ratio (HR) with a 95% confidence interval (CI). Two models were used in the Cox regression analysis: model I, unadjusted, and model II, adjusted. Model II was adjusted to baseline demographics and risk factors for admissions, serving as a reference group. The variables co-variated in the model II were age, gender, hypertension, diabetes mellitus, smoking, hyperlipidemia, chronic obstructive pulmonary disease, coronary artery disease, chronic renal failure, HF etiology, LVEF, beta-blockers, angiotensinogen-converting enzyme inhibitors or angiotensinogen receptor blockers, spironolactone, and furosemide.

RESULTS

A total of 1,639 patients with HFrEF [median age: 71 (63–79) years and 946 (57.7%) were males] were included in the study. In total, 276 patients were in the ivabradine group. In terms of baseline features, 1086 (66.3%) patients had ischemic HFrEF, while 553 patients (33.7%) had nonischemic HFrEF. In regard to device therapy, 281 patients had ICD implantation and 105 patients had CRT-D implantation. The mean LVEF was 30% (25.0–35.0). The study population included 91 patients using ivabradine and implanted ICDs, compared to 295 patients with ICDs not using ivabradine. Baseline clinical features are summarized in Table 1.

In terms of arrhythmias, 44 patients who presented with VT on admission were not treated with ivabradine, while 4 patients who developed VT on admission were treated with ivabradine (Table 2). PVCs were observed in 24-h rhythm Holter monitoring in 169 (36.2%) non-ivabradine users, while they were observed in 64 (21.7%) ivabradine users. While VT was detected in 12 (2.6%) patients not using ivabradine in 24-h rhythm Holter monitoring applied to patients, it was detected in 2 (0.7%) patients using ivabradine. The frequency of ICD discharge was significantly higher in patients who were not treated with ivabradine compared with

Table 1. Comparison of demographic, clinical characteristics, laboratory, and echocardiography parameters of patients according to ivabradine usage in patients with heart failure with reduced ejection fraction.

	Overall (n=1639)	Patients not using ivabradine (n=1363)	Patients using ivabradine (n=276)	p-value
Age, years	71 (63–79)	71 (62–79)	74 (65–82)	<0.001
Male gender	946 (57.7%)	797 (58.5%)	149 (54.0%)	0.169
Hypertension	969 (59.1%)	809 (59.4%)	160 (58.0%)	0.670
Diabetes mellitus	596 (36.4%)	486 (35.7%)	110 (39.9%)	0.186
Hyperlipidemia	469 (28.8%)	393 (29.1%)	76 (27.7%)	0.657
Smoking	156 (9.6%)	130 (9.6%)	26 (9.5%)	0.937
Chronic renal failure	408 (25.0%)	345 (25.4%)	63 (22.8%)	0.370
COPD	186 (11.5%)	152 (11.3%)	34 (12.4%)	0.592
Cerebrovascular accident	26 (1.6%)	21 (1.6%)	5 (1.8%)	0.791
Hypothyroidism	63 (3.9%)	51 (3.8%)	12 (4.4%)	0.645
Hyperthyroidism	37 (2.3%)	28 (2.1%)	9 (3.3%)	0.249
Coronary artery disease	1142 (69.7%)	942 (69.1%)	200 (72.5%)	0.269
Heart failure etiology				
Ischemic	1086 (66.3%)	913 (67.0%)	173 (62.7%)	0.168
Nonischemic	553 (33.7%)	450 (33.0%)	103 (37.3%)	0.168
Device types				
ICD	281 (17.2%)	212 (15.6%)	69 (25.1%)	<0.001
CRT-D	105 (6.4%)	83 (6.1%)	22 (8.0%)	0.259
All defibrillators	386 (23.7%)	295 (21.8%)	91 (33.2%)	<0.001
Laboratory values				
Creatinine, mg/dL	1.0 (0.8–1.3)	1.0 (0.8–1.3)	1.0 (0.8–1.3)	0.350
Potassium, mEq/L	4.5 (4.2–4.8)	4.5 (4.2–4.8)	4.4 (4.1–4.7)	0.108
Magnesium, mEq/L	2.1 (1.9–2.3)	2.1 (1.9–2.3)	2.1 (1.9–2.3)	0.330
Calcium, mEq/L	9.3 (8.9–9.6)	9.3 (8.9–9.6)	9.3 (9.0–9.6)	0.252
Echocardiography data				
LVEF, %	30 (25–35)	30 (25–35)	30 (25–35)	0.475
LVEDD, mm	60 (54–68)	60 (54–68)	61 (56–67)	0.114
LVESD, mm	48 (41–56)	48 (41–56)	50 (42–57)	0.154
LAAP, mm	44 (40–49)	44.0 (40–50)	45 (40–48)	0.949
Out-hospital medication				
Beta-blockers	1624 (99.1%)	1350 (99.0%)	274 (99.3%)	0.715
ACEIs or ARBs	1105 (67.4%)	921 (67.6%)	184 (66.7%)	0.770
Spironolactone	1026 (62.6%)	845 (62.0%)	181 (65.6%)	0.262
Furosemide	1516 (92.5%)	1256 (92.1%)	260 (94.2%)	0.238
Follow-up, months				

Continuous variables are presented as median (interquartile range). Nominal variables are presented as frequency (%). COPD: chronic obstructive pulmonary disease; ICD: implantable cardioverter defibrillator; CRT-D: cardiac resynchronization therapy with a pacemaker and an ICD; LVEF: left ventricle ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LAAP: left atrium anteroposterior diameter; ACEIs: angiotensinogen-converting enzyme inhibitors; ARBs: angiotensinogen receptor blockers.

those who were treated (n=64 vs. n=7), respectively. Finally, long-term mortality was observed in 143 (10.5%) non-ivabradine users, while it was observed in 22 (8%) ivabradine users.

According to the model adjusted for all covariates, the risk of VT on admission was observed three times more in non-ivabradine users than in users (Table 3). There was no significant difference between the two groups in terms of long-term mortality. According to the adjusted model for all variables, the presence of PVCs in 24-h rhythm Holter monitoring was 2.4 times higher in patients not using ivabradine than in those using it. All variable-adjusted analyses showed 4.2 times more VT on 24-h rhythm Holter monitoring in non-ivabradine users than in ivabradine users. According to the model adjusted for all variables, approximately 4.1 times more ICD discharges were observed in the group that did not use ivabradine than in the group that used it.

DISCUSSION

The current study has shown that ivabradine reduces appropriate ICD therapy in HFrEF patients. Additionally, the use of ivabradine significantly reduced VT on admission, the presence of PVCs, and VT detection in 24-h rhythm Holter monitoring in HFrEF patients.

Table 2. Distribution of patients' ventricular arrhythmias, appropriate implantable cardioverter defibrillator treatments, and long-term mortality according to ivabradine use.

	Patients not using ivabradine (n=1363)	Patients using ivabradine (n=276)
Admission with ventricular tachycardia	44 (3.2%)	4 (1.4%)
Presence of premature ventricular contractions >5% in 24-h rhythm Holter monitoring	169 (36.2%)	60 (21.7%)
Ventricular tachycardia in 24-h rhythm Holter monitoring	12 (2.6%)	2 (0.7%)
Appropriate ICD shock in follow-up	64 (21.7%)	7 (7.7%)
Long-term mortality	143 (10.5%)	22 (8%)

ICD: implantable cardioverter defibrillator.

Table 3. Multivariate analysis for admission with ventricular tachycardia, presence of premature ventricular contractions >5% in 24-h rhythm Holter monitoring, ventricular tachycardia in 24-h rhythm Holter monitoring, appropriate implantable cardioverter defibrillator shock in follow-up, and long-term mortality by ivabradine usage.

	Patients not using ivabradine	Patients using ivabradine
Admission with ventricular tachycardia, HR (95%CI)		
Model 1: unadjusted	3.6 (1.3–10.2)	1 [Reference]
Model 2: adjusted for all covariates[a]	3.0 (1.5–7.4)	1 [Reference]
Long-term mortality, HR (95%CI)		
Model 1: unadjusted	1.9 (1.2–3.0)	1 [Reference]
Model 2: adjusted for all covariates[a]	1.4 (0.8–2.8)	1 [Reference]
Presence of premature ventricular contractions in 24-h rhythm Holter monitoring, HR (95%CI)		
Model 1: unadjusted	2.9 (2.1–3.9)	1 [Reference]
Model 2: adjusted for all covariates[a]	2.4 (1.1–3.1)	1 [Reference]
Ventricular tachycardia in 24-h rhythm Holter monitoring, HR (95%CI)		
Model 1: unadjusted	6.7 (1.4–30.4)	1 [Reference]
Model 2: adjusted for all covariates[a]	4.2 (1.9–12.1)	1 [Reference]
Appropriate ICD shock in follow-up, HR (95%CI)		
Model 1: unadjusted	4.3 (2.0–9.5)	1 [Reference]
Model 2: adjusted for all covariates[a]	4.1 (1.8–9.6)	1 [Reference]

CI: confidence interval; HR: odds ratio. [a]Adjusted for: age, gender, hypertension, diabetes mellitus, smoking, hyperlipidemia, chronic obstructive pulmonary disease, coronary artery disease, chronic renal failure, heart failure etiology, ejection fraction, beta-blockers, angiotensinogen-converting enzyme inhibitors or angiotensinogen receptor blockers, spironolactone, and furosemide.

The I_f channel, which is one of the most important ionic currents regulating the pacemaker activity in the sinoatrial (SA) node, is a mixed Na–K inward current activated by hyperpolarization. Ivabradine exerts this effect without prolonging QTc or altering conductance, refractoriness, or repolarization time of the atria, atria-ventricle (AV) node, His-Purkinje system, and ventricles[9]. It prolongs diastole by decreasing the diastolic depolarization slope in SA node cells[10]. As a result, by prolonging diastole time, it reduces myocardial oxygen demand and increases myocardial perfusion. Heart rate plays an important role in the pathophysiology of HF, and ivabradine-induced heart rate reduction improves clinical outcomes in selected patient groups[6]. With the use of ivabradine in our patients, heart rate reduction may have improved myocardial perfusion by prolonging diastole and may have prevented ventricular arrhythmias by reducing Ischemia.

The cyclic nucleotide-gated channel 4 current (HCN4), the primary site of action of ivabradine, is highly expressed in the SA node. It is expressed at a low level in normal ventricular myocytes, whereas the expression of HCN channels is increased in ventricular myocytes in HF. I_f currents may be responsible for the abnormal automaticity in the ventricles in HF, and ivabradine may prevent ventricular arrhythmias by blocking the HCN channel[11]. This increased HCN channel expression observed in the ventricles of HF patients may underlie the possible anti-arrhythmic effect of ivabradine observed in our patients.

Ivabradine has also been shown to inhibit $I_f$channels, which are normally found only in the SA node but pathologically expressed in the ventricular myocardium with HF[12]. Heart rate during Ischemia is associated with reperfusion arrhythmias, and a lower heart rate during Ischemia delays Ischemia-induced electrophysiological changes[13]. It has been shown that $I_f$current activity increases the pro-arrhythmogenic potential as a result of prolongation of the ventricular repolarization phase[14]. In conclusion, I_f channel blockers suggest a potential approach to prevent sudden death in HFrEF patients. There is a case report where ivabradine was used in addition to antiarrhythmic drugs and catheter ablation in patients with resistant ventricular arrhythmia[15].

The exact mechanisms of ivabradine's efficacy in the treatment of tachycardias originating from outside the sinus node and due to enhanced automaticity are not yet known. The first possible mechanism is that it inhibits increased automaticity as a result of increased expression of HCN channels in ventricular myocytes. There are case reports suggesting that ivabradine may have potential in the pediatric population for the treatment of a variety of atrial tachycardias in which increased automaticity is considered the primary underlying mechanism[16,17]. The possible anti-arrhythmic effect of ivabradine can be considered as a result of suppressing automaticity, reducing PVC burden, and preventing VT trigger. The second possible explanation is that ivabradine prevents triggered activity-mediated arrhythmias induced by prolonging ventricular repolarization by its inhibiting effect on hERG channels[18].

There are various case reports in the literature regarding the suppression of ventricular arrhythmias. In a study of mice with cardiomyopathy, ivabradine was shown to suppress early PVCs, sustain ventricular arrhythmias, and improve survival[19]. Experimental studies suggest that the antiarrhythmic effects of ivabradine are due to the following mechanisms: [1] it conserves energy by reducing heart rate and prevents electrophysiological effects of Ischemia and [2] it blocks HCN channels that are overexpressed in the ventricles in HF[11].

All these studies show that ivabradine reduces ventricular arrhythmias in HF patients and support our current study results. In our HFrEF patient group, ivabradine may have reduced the ventricular arrhythmias by the abovementioned possible mechanisms. The presence of PVC, appropriate ICD shocks, and ventricular arrhythmias in HFrEF patients is associated with high mortality[20-22]. In the present study, we showed that ivabradine reduces ventricular arrhythmias in HFrEF patients. However, no statistically significant reduction in total mortality was demonstrated. The reason for the statistical insignificance may be that HFrEF patients in our population died because of pump failure. Prospective studies with larger patient populations are needed to demonstrate the effects of ivabradine more clearly on ventricular arrhythmias.

Our study has several limitations. First, our study had a single-center, retrospective design. Second, the optimization of medical treatment for all patients in the follow-up period is unknown. Third, all patients could not be evaluated with 24-h rhythm Holter monitoring. Unfortunately, 24-h rhythm Holter monitoring was applied only to patients with symptoms. Fourth, the optimal medical treatment of patients did not include SGLT-2 inhibitors, which might have affected the results. Finally, the brain natriuretic peptide levels of all patients were not routinely checked during hospitalizations.

CONCLUSION

The use of ivabradine has been shown to reduce appropriate ICD therapy in patients with HFrEF. The use of ivabradine in HFrEF patients may have potential for preventing ventricular arrhythmias.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants or animals performed by any of the authors.

AUTHORS' CONTRIBUTIONS

LP: Conceptualization, Investigation, Writing – original draft. **AÇY**: Data curation, Validation. **OT**: Writing – review & editing. **TÇe**: Methodology. **KK**: Data curation, Software. **SE**: Data curation. **GÇ**: Formal Analysis, Supervision, Visualization. **MİH**: Supervision, Writing – review & editing. **TÇı**: Investigation, Formal Analysis. **AİT**: Project administration, Supervision.

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ORIGINAL ARTICLE

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Serum and urinary angiotensinogen levels as prognostic indicators in acute kidney injury: a prospective study

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SUMMARY

OBJECTIVE: The delayed increase in serum creatinine levels poses challenges in the timely diagnosis of acute kidney injury. This study aimed to investigate the relationship between serum angiotensinogen and urinary angiotensinogen levels and the prognosis of renal function in patients diagnosed with acute kidney injury.

METHODS: A total of 79 newly diagnosed acute kidney injury patients aged 18 years and older were enrolled. Serum angiotensinogen and urinary angiotensinogen levels were measured at the onset of the disease, as well as on the 15th and 30th days of follow-up. After 3 months, renal function was evaluated by measuring serum creatinine levels.

RESULTS: Among the acute kidney injury patients, those in Kidney Disease: Improving Global Outcomes stage 3 exhibited significantly higher urinary angiotensinogen/urine creatinine levels compared with stages 1 and 2 patients at the time of diagnosis ($p<0.05$). Furthermore, a positive correlation was observed between the urinary angiotensinogen/urine creatinine level at the time of diagnosis and the serum creatinine level at the third month ($r=0.408$, $p=0.048$).

CONCLUSION: The findings suggest that urinary angiotensinogen levels can serve as an indicator of the severity of acute kidney injury. Monitoring urinary angiotensinogen levels could potentially contribute to the prognosis assessment and management of acute kidney injury patients.

KEYWORDS: Acute kidney injury. Creatinine. Angiotensinogen.

INTRODUCTION

The diagnosis and staging of acute kidney injury (AKI) are based on the criteria provided by Kidney Disease: Improving Global Outcomes (KDIGO). The parameters used for diagnosing AKI include an increase in serum creatinine (sCr) levels and a decrease in urine output. However, delayed increases in creatinine levels can result in delayed diagnosis of AKI. Furthermore, the severity of the disease may not be accurately reflected by creatinine levels, leading to delays in disease management.

Acute kidney injury occurs in approximately 25–30% of patients hospitalized in intensive care units and 3–7% of general hospitalized patients[1]. While the mortality rate in uncomplicated AKI cases is approximately 5%, it exceeds 40% in intensive care unit patients. AKI often coexists with multiple organ failure rather than isolated organ failure[2].

Many AKI patients regain their kidney function during follow-up, which is evidenced by an increased urine production and a gradual decrease in sCr levels. However, some patients do not fully recover and may develop chronic kidney disease (CKD)[3]. Emerging evidence suggests that timely and effective treatment initiation is crucial for AKI outcomes[4]. Therefore, early identification of AKI severity can facilitate prompt intervention and improve patient outcomes. The current diagnostic markers, such as sCr levels and urine output, have limited prognostic value for potential complications. Insufficient prognostic information regarding AKI is a significant barrier to improving patient outcomes.

Animal studies investigating AKI have demonstrated that the activation of the renin-angiotensin system (RAS) can contribute to the development of AKI. Urinary angiotensinogen (uAGT) levels serve as an indicator of RAS activation and hold promise as a biomarker for assessing AKI progression in patients with acute decompensated heart failure[5]. uAGT has the potential to be a novel biomarker that can aid in determining the intrarenal RAS status and the severity of acute tubular necrosis (ATN).

The objective of this study was to explore the association between serum angiotensinogen (sAGT) or uAGT levels and

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AKI-related complications. Consequently, patients with elevated sAGT and uAGT levels will be closely monitored for the development of potential complications.

METHODS

Study population

A total of 79 AKI patients were included in this study. These patients were recruited from the Internal Medicine Clinic of Gaziosmanpaşa University Hospital, where they were initially diagnosed with AKI and received treatment. Pregnant women, patients who used angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone receptor antagonist drugs at the time of diagnosis, patients with postrenal AKI, and patients with a diagnosis of malignancy, chronic liver disease, multiorgan failure, or sepsis were excluded from the study.

Data collection

At the time of diagnosis, a detailed patient history including age, gender, presence of chronic diseases, medications used, recent exposure to nephrotoxic agents, and history of surgery was recorded. For patients admitted to the intensive care unit, the reasons for hospitalization and Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were documented. Information on the application of hemodialysis, time of death for deceased patients, and discharge time for surviving patients was also recorded.

Biochemical measurements

Blood samples were collected from the patients at the beginning of AKI and on the 15th and 30th days of follow-up. The levels of serum blood urea nitrogen (BUN), sCr, and urine creatinine (uCr) were measured. The renal function of the patients who attended follow-up visits was monitored up to the 90th day.

Ethical considerations

Approval for this study was obtained from the ethics committee of Gaziosmanpaşa University, Faculty of Medicine (19-KAEK-052). Written informed consent was obtained from all patients in accordance with the principles of the Helsinki Declaration, and all relevant documents were recorded.

Biochemical analysis

For the measurement of sAGT levels, the blood samples were centrifuged at 3500 rpm for 10 min. The serum was separated and stored at -80°C in Eppendorf tubes. For uAGT levels, urine samples collected in gel-free tubes were centrifuged at 3500 rpm for 10 min, and the supernatant was transferred to Eppendorf tubes and stored at -80°C. Before the analysis, the frozen samples were thawed at 2–8°C for 8 h. The sAGT and uAGT levels were measured using Human Angiotensinogen kits (Elabscience Biotechnology Co., Wuhan, PRC) and the enzyme-linked immunosorbent assay (ELISA) method in the biochemistry research laboratory of our hospital.

The absorbance values were measured at 450 nm using an ELISA reader (Organon Teknika Reader 230S). Based on the absorbance values, the concentrations were calculated in ng/L using Microsoft Excel. uCr levels were measured using the Jaffé kinetic colorimetric method on the Roche Cobas 6000 device, specifically the Cobas C501 module. According to the uCr values, uAGT levels were corrected and reported as ng/mg.

Statistical analysis

The data were analyzed using the SPSS 19.0 package program for Windows. The Pearson chi-square test was employed to compare the frequency of gender between groups. The Mann-Whitney U test was used to compare the mean age, APACHE II scores, uAGT levels, and sAGT values. According to primary diagnoses, the distribution of biomarkers was evaluated using Kruskal-Wallis analysis. Furthermore, correlation analysis was conducted to assess the relationship between biomarkers in the case and control groups.

Descriptive statistics were used to present the general characteristics of the study groups, and the data were expressed as mean±standard deviation. A $p<0.05$ was considered statistically significant. Repeated-measures analysis of variance (ANOVA) was performed to evaluate the differences in uAGT levels and kidney function at the beginning and on the 15th and 30th days of the study. Statistical significance was determined when $p<0.05$.

Categorical variables were presented as n (%), while continuous variables were reported as mean±standard deviation. Independent sample t-tests or one-way ANOVA was utilized to assess the quantitative differences between groups. Chi-square tests were employed to examine the qualitative differences between groups. Pearson correlation analysis was performed to investigate the relationship between quantitative variables. All statistical calculations were conducted using the IBM SPSS Statistics 19 software (SPSS Inc., an IBM Co., Somers, NY).

RESULTS

The study included a total of 79 AKI patients, with a mean age of 73.8 years (range: 33–96 years). Among them, 35 (44%) were females and 44 (56%) were males. The most common

etiological reason for AKI was cerebrovascular damage (25%), followed by primary AKI (20%), pneumonia (12%), and other causes. The most prevalent comorbidities among the patients were hypertension (58%), coronary artery disease (48%), and diabetes mellitus (30%). During the follow-up period, 58% of the patients died, and hemodialysis was required in 58% of the cases.

In our study, the uAGT/uCr values of the patients were corrected according to uCr. There was no significant relationship between uAGT/uCr and sAGT levels (r=0.063, p=0.579). However, there was a positive correlation between uAGT/uCr and sCr levels (r=0.289, p=0.01). As expected, there was an inverse correlation between sCr and glomerular filtration rate (GFR) (r=-0.74, p≤0.001). Further details can be found in Table 1.

When comparing uAGT/uCr ratios at different stages based on the KDIGO criteria upon admission, the ratios were 9.93±10.82 in stage 1, 5.03±5.04 in stage 2, and 32.15±37.13 in stage 3. As shown in Table 2, stage 3 patients had significantly higher uAGT/uCr values compared with stage 1 and stage 2 patients.

Table 3 examines the relationship between uAGT/uCr values and the development of hemodialysis, mortality, and morbidity. There was no significant correlation observed between uAGT/uCr ratios and the need for hemodialysis, mortality, or morbidity (p>0.05).

Table 1. Correlation between urinary angiotensinogen/urine creatinine and serum angiotensinogen values.

		sAGT	uAGT ng/L	uAGT/uCr	sCr
GFR-MDRD mL/min	r	202	0.063	-0.079	-0.751
	p	0.074	0.580	0.490	<0.001
sAGT	r		0.223	0.063	-0.159
	p		0.048	0.579	0.162
uAGT ng/L	r			0.182	-0.092
	p			0.107	0.421
uAGT/uCr	r				0.289
	p				0.010

Table 2. Serum angiotensinogen and urinary angiotensinogen/urine creatinine ratio by stage of Kidney Disease: Improving Global Outcomes.

	KDIGO		
	Stage 1	Stage 2	Stage 3
sAGT ng/L	143.97±190.15	103.59±88.17	71.04±29.68
uAGT/uCr	9.93±10.82	5.03±5.04	32.15±37.13

A weak positive correlation was found between uAGT/uCr on day 0 and sCr on day 90 in the patients who were followed up (r=0.408). However, no significant correlation was observed between sCr levels on the 90th day and uAGT/uCr on the 15th day of follow-up. There was a strong correlation between uAGT/uCr on the 30th day and sCr on the 90th day, but these values did not reach statistical significance.

DISCUSSION

The diagnostic criteria for AKI are based on a decrease in urine output and an acute increase in sCr levels. However, sCr levels typically rise within 2–3 days due to skeletal muscle release, making interventions based solely on high sCr levels potentially incomplete[6]. An early and accurate diagnosis of AKI is crucial to prevent renal dysfunction and damage[7]. Recognizing the decline in GFR at an early stage is vital, and several biomarkers have shown promising results in providing early detection of AKI[8].

The kidney contains all components of the RAS system. Growing evidence suggests that locally produced angiotensin II (Ang II), which is the principal effector peptide of RAS, may contribute to AKI pathogenesis by upregulating pro-inflammatory and pro-fibrotic cytokines such as TNF and TGF-beta[9]. Ang II expression is observed in the proximal tubule, where it stimulates TGF-beta synthesis[10,11]. Experimental studies have demonstrated increased levels of TGF-beta mRNA and protein in rats following acute ischemic injury[12]. In male Sprague-Dawley rats, renal Ang II levels in the proximal tubule were found to increase 53.5-fold in association with decreased renal perfusion pressure[10].

In a study assessing uAGT and sAGT levels, higher uAGT levels were observed in patients with ATN compared with healthy subjects[13]. This elevation in uAGT levels was found to be correlated with increased intrarenal RAS expression, suggesting that enhanced uAGT synthesis within the kidney

Table 3. Relationship between urinary angiotensinogen/urine creatinine and hemodialysis and mortality.

		uAGT/uCr
Hemodialysis	No	11.56±18.37
	Yes	10.82±14.68
Mortality	Exitus	13.25±19.67
	Alive	8.17±9.02
Day of exitus	0–7	12.27±22.77
	8–14	9.83±10.63
	≥15	13.65±20.2

may contribute to ATN pathogenesis[13]. Another study identified intrarenal angiotensinogen in structures close to the apical membrane of proximal tubular cells, facilitating its secretion into urine[14]. The study results also revealed no association between sAGT levels and intrarenal RAS status, supporting the notion that intrarenal RAS operates independently of circulating RAS[14]. Consistent with the existing literature, our study found no correlation between sAGT levels and uAGT/uCR values, further supporting the idea of independent regulation of intrarenal RAS[14].

In our study, although patients receiving hemodialysis exhibited higher uAGT/uCR values, the difference was not statistically significant. Similarly, no significant difference was observed between uAGT/uCR values and mortality rates. When patients were classified according to the KDIGO stages, no significant correlation was found between uAGT/uCR values and mortality among AKI patients. While higher uAGT/uCR values were observed in patients undergoing hemodialysis and those who died, the lack of statistical significance could be attributed to the small sample size and high standard deviation in our study.

A separate investigation[15] examined uAGT as a potential clinical biomarker for identifying individuals at high risk of CKD. All distributions of 24-h uAGT and uAGT/uCR ratio were found to be higher in CKD patients compared with controls, whereas sAGT levels were similar between the two groups. Notably, uAGT excretion and uAGT/uCR ratio exhibited a strong correlation. These findings suggest that intrarenal RAS may play a significant role in CKD risk, and uAGT levels could aid in stratifying and predicting CKD risk. In our study, a positive correlation was found between uAGT/uCR values obtained at the time of AKI diagnosis and sCr levels on the 90th day. A strong correlation was also observed between uAGT/uCR values on the 30th day and sCr levels on the 90th day, although no statistically significant relationship was found. Analyzing uAGT/uCR values on the 0th, 15th, and 30th days based on the development of CKD during patient follow-up revealed no significant differences between the groups. However, this could be attributed to the limited number of cases included in the study and a high mean standard deviation.

The limitations of this study include the small sample size and high standard deviation, which may have contributed to the lack of statistically significant findings in some analyses. Additionally, the study focused on a specific population and did not explore other potential confounding factors. Furthermore, the study did not assess long-term outcomes or evaluate the predictive value of uAGT/uCR values in terms of disease progression. On the contrary, the study contributes to the existing literature by examining the correlation between uAGT/uCR values and renal function in AKI patients. It also adds to the understanding of the independent regulation of intrarenal RAS. Further research with larger cohorts and comprehensive evaluation of clinical outcomes is necessary to confirm and build upon these findings.

CONCLUSION

The uAGT levels have emerged as potential biomarkers for assessing the intrarenal RAS activity in AKI and CKD. Further research with larger sample sizes is warranted to validate their clinical utility and establish their role in risk stratification and prognosis prediction for renal diseases.

AUTHORS' CONTRIBUTIONS

AA: Project administration, Validation, Writing – review & editing. **AKD:** Project administration, Validation, Writing – review & editing. **ZCÖ:** Project administration, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Effect of pulmonary embolism location on electrocardiological parameters

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SUMMARY

OBJECTIVE: Pulmonary thromboembolism is a disease with high morbidity and mortality. Various changes occur on the electrocardiogram secondary to pulmonary thromboembolism. The objective of this study was to investigate variations in QT dispersion, Tpeak-Tend duration, and Tpeak-Tend/QT ratio in relation to pulmonary thromboembolism localization and their impacts on 30-day mortality.

METHODS: This study was carried out in a tertiary emergency medicine clinic between December 1, 2019 and November 30, 2020. We evaluated correlations between radiological outcomes of patients, QT dispersions, T-wave dispersions, Tpeak-Tend durations, and Tpeak-Tend/QT ratios. We sought statistically significant disparities between these values, considering the presence or localization of pulmonary thromboembolism. The 30-day mortality in pulmonary thromboembolism-diagnosed patients was reassessed.

RESULTS: Electrocardiogramfindings revealed that T-wave dispersion (p<0.001), Tpeak-Tend duration (p=0.034), and Tpeak-Tend/corrected QT ratio (p=0.003) were lower in patients than controls. Conversely, QT dispersion (p=0.005) and corrected QT dispersion (p<0.001) were higher in patients.

CONCLUSION: Electrocardiogram findings such as T-wave dispersion, QT duration, Tpeak-Tend time, and Tpeak-Tend/corrected QT ratio can detect pulmonary thromboembolism. More studies with larger cohorts are required to further understand the role of QT and corrected QT dispersion in pulmonary thromboembolism patient mortality.

KEYWORDS: Pulmonary embolism. Electrocardiography. Morbidity. Radiography.

INTRODUCTION

Pulmonary thromboembolism (PTE), a condition that accounts for approximately 100,000 mortalities per year in the United States, leads to significant morbidity and mortality. It often requires high clinical suspicion for diagnosis due to its non-specific symptoms[1]. Multidetector computed tomographic pulmonary angiography (CTPA) is commonly used for diagnosis, though it cannot predict clinical severity[2].

Electrocardiogram (ECG) changes, such as QT dispersion (QTd), indicative of myocardial repolarization heterogeneity, can occur in PTE. Increased QTd is associated with severe ventricular arrhythmias and sudden cardiac death[3]. Moreover, recent studies have suggested that Tpeak-Tend (Tp-e), a marker of arrhythmogenicity, and Tp-e/QTc ratio increments may influence mortality[4,5]. Due to thrombus-induced pressure changes, especially in the right heart chambers, Tp-e, QTd, and Tp-e/QTc ratios may increase[3,4].

Investigating these changes based on PTE location (main pulmonary artery or other branches) and their impact on mortality may inform patient management, given ECG's accessibility and reproducibility. Thus, our study aims to explore the Tp-e, Tp-e/QT, and QTd variations depending on PTE location and their correlation with 30-day mortality.

METHODS

Our study, conducted in an emergency medicine clinic of a tertiary hospital, received approval from the local Ethics Committee on November 14, 2019. The investigation involved patients over the age of 18 years, who presented to the emergency department between December 1, 2019 and November 30, 2020 with suspected PTE and consequently underwent CTPA. However, patients with ECGs not suitable for examination or those diagnosed with left or right bundle branch block and atrial fibrillation were excluded from the study.

A total of 742 patients with suspected PTE underwent CTPA, of whom 97 were confirmed to have PTE. From this group, 44 patients were further excluded due to unsuitable ECGs, atrial

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fibrillation, and left or right bundle branch block, resulting in a final cohort of 53 patients. To facilitate comparative analysis, we selected a control group of 54 individuals without PTE, matched based on age, gender, and comorbidities (Figure 1).

Upon presentation to the emergency department, written informed consent was obtained from all patients or their legal representatives. We meticulously recorded pertinent patient information, including names, contact information, medical history, and current medications. Standard 12-lead ECGs were obtained from patients presenting with suspected PTE. These ECGs were then digitally scanned, transferred to a computerized environment, and evaluated independently to maintain blindness in the study.

Using the "Windows Photo Viewer" software and the "PixelRuler program," a cardiologist measured the QT interval, T wave, and Tp-e duration for each ECG. In patients diagnosed with PTE, the localization of the thrombi in the right and left pulmonary arteries was individually determined and classified. The relationships between these radiological findings and ECG measurements were subsequently analyzed. Furthermore, the presence or absence of PTE, its localization, and the 30-day mortality of patients diagnosed with PTE were evaluated.

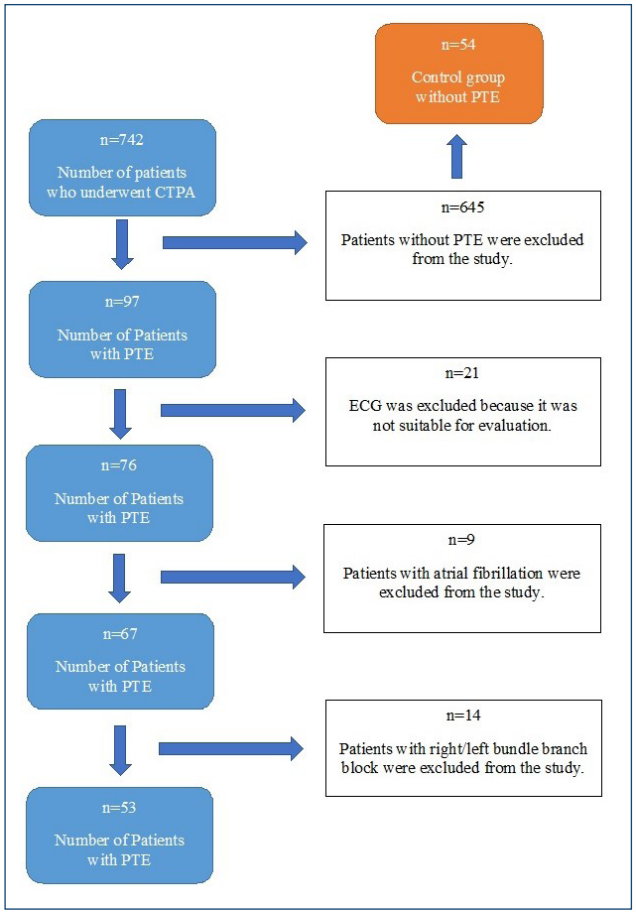

Figure 1. Flowchart of the study design.

By employing statistical analyses, we sought to investigate whether significant differences exist between T-wave dispersion, QTd, corrected QT dispersion (QTcd), Tp-e duration, Tp-e/QT, Tp-e/QTc ratios, and patient mortality. This comprehensive approach allowed us to deeply explore potential correlations between ECG measurements, thrombus localization, and patient outcomes.

Sample size analysis

When the difference between the alpha 0.05 and beta 0.02 groups was considered significant at 32.4%, the sample size was calculated as 54 for each group, with a total of 108 patients with the G Power program[6].

Statistical analysis

All statistical analyses were conducted using the SPSS v21 software (SPSS Inc., Chicago, IL, USA). The normality of quantitative data distribution was assessed using the Kolmogorov-Smirnov test. Quantitative variables were presented as mean±standard deviation or median (range), while categorical variables were presented as frequency (percentage). Quantitative variables following a normal distribution assumption were analyzed using independent samples t-test. For quantitative variables that did not meet the assumption of a normal distribution, the Mann-Whitney U test was used. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. The diagnostic performance of ECG findings in detecting PTE was evaluated using receiver operating characteristic (ROC) curve analysis. Performance measures such as sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were calculated for various cutoff points. Statistical significance was set at a $p<0.05$.

RESULTS

A total of 107 individuals (53 patients and 54 controls) were included in the study. While there were 30 (56.60%) females in the patient group, there were 26 (48.15%) females in the control group (p=0.495). There was no statistically significant difference between the groups in terms of chronic disease. In total, 27 (50.94%) patients had main pulmonary artery involvement, while 26 (49.05%) patients had involvement in other branches. In the patient group, 4 (7.55%) cases were fatal (Table 1).

When the ECG findings were examined, T-wave dispersion, QT wave duration, Tp-e time, and Tp-e/QTc ratio were

Table 1. General characteristics of individuals by groups (qualitative data).

	Patient group (n=53)	Control group (n=54)	p-value
Sex			
Women	30 (56.60%)	26 (48.15%)	0.495[1]
Men	23 (43.40%)	28 (51.85%)	
Hypertension	16 (30.19%)	20 (37.04%)	0.586[1]
Asthma/COPD	11 (20.75%)	6 (11.11%)	0.271[1]
Diabetes mellitus	7 (13.21%)	14 (25.93%)	0.158[1]
History of pulmonary embolism	9 (16.98%)	2 (3.70%)	0.052[1]
Chronic kidney disease	3 (5.66%)	3 (5.56%)	1.000[2]
Chronic arterial disease	9 (16.98%)	15 (27.78%)	0.268[1]
Left pulmonary artery involvement	33 (62.26%)	–	
Subsegmental pulmonary artery	1 (1.89%)	–	N/A
Segmental pulmonary artery	8 (15.09%)	–	
Lobar pulmonary artery	7 (13.21%)	–	
Main pulmonary artery	17 (32.08%)	–	
Right pulmonary artery involvement	43 (81.13%)	–	
Subsegmental pulmonary artery	1 (1.89%)	–	N/A
Segmental pulmonary artery	2 (3.77%)	–	
Lobar pulmonary artery	15 (28.30%)	–	
Main pulmonary artery	25 (47.17%)	–	
Total main pulmonary artery involvement	27(50.94%)	–	N/A
Mortality	**4 (7.55%)**	–	**N/A**

The data are summarized as frequency (percentage). (1) Chi-square test; (2) Fisher's exact test; N/A: test not applicable; COPD: chronic obstructive pulmonary disease. Bold indicates the number of patients with a fatal outcome in the patient group of n=4.

lower in the patient group than in the control group (p<0.001, p=0.001, p=0.034, and p=0.003, respectively). QT and corrected QTd (QTcd) were higher in the patient group than in the control group (p=0.005 and p<0.001, respectively). When ECG findings were analyzed according to pulmonary artery involvement, no statistically significant difference was found between patients with main pulmonary involvement and other patients. When the relationship between ECG findings and mortality was examined, no statistically significant difference was found between patients with mortality and other patients (Table 2).

The performance of ECG findings in the diagnosis of pulmonary embolism was examined. For the T-wave dispersion of 71 cutoff point (values less than this indicate the presence of pulmonary embolism), the sensitivity was 66.04%, the specificity was 79.63%, the correct classification rate was 72.90%, the positive predictive value was 76.09%, and the negative predictive value was 70.49% (AUC=0.733; 95%CI 0.635–0.832; p<0.001). QTd for 58 cutoff point (values above and below this indicate the presence of pulmonary embolism) has a sensitivity of 66.04%, a specificity of 59.26%, a correct classification rate of 62.62%, a positive predictive value of 61.40%, and a negative predictive value of 64.00% (AUC=0.643; 95%CI 0.539–0.747; p=0.011). Corrected QTd was found to have a sensitivity of 64.15%, a specificity of 74.07%, a correct classification rate of 69.16%, a positive predictive value of 70.83%, and a negative predictive value of 67.80% for 75 cutoff point (this or greater values indicate the presence of pulmonary embolism) (AUC=0.744; 95%CI 0.652–0.837; p<0.001). The Tp-e/QTc ratio for 0.20 cutoff point (values less than this indicate the presence of pulmonary embolism) has a sensitivity of 64.15%, a specificity of 61.11%, a correct classification rate of 62.62%, a positive predictive value of 61.82%, and a negative cutoff value of 63.46% (AUC=0.656; 95%CI 0.553–0.759; p=0.005). The performance of corrected QT duration, Tp-e duration, Tp-e/QT ratio, and S1Q3T3 finding in the diagnosis of pulmonary embolism was statistically insignificant.

Table 2. Electrocardiographic findings according to groups, pulmonary artery involvement, and presence of mortality.

		T-wave dispersion (ms)	QT wave duration (ms)	Corrected QT duration (ms)	QT dispersion (ms)	Corrected QT dispersion (ms)	Tpeak-Tend duration (ms)	Tp-e/QT ratio	Tp-e/QTc ratio
Electrocardiographic findings by groups									
mean±std. deviation	Patient (n=53)	71.64±22.78	348.91±34.84	441.36±31.77	66.55±18.15	86.17±25.48	83.17±16.66	0.24±0.05	0.19±0.04
	Control (n=54)	85.31±18.54	376.24±47.24	429.52±31.64	57.70±13.52	67.15±16.92	90.85±20.02	0.24±0.05	0.21±0.04
median (min–max)	Patient (n=53)	64.7 (38.1–139.2)	348.5 (283.3–458)	441 (373–507)	62.2 (38.9–123.5)	82 (45–192)	83.3 (44.4–120.9)	0.24 (0.16–0.34)	0.19 (0.11–0.27)
	Control (n=54)	83.4 (50–130.3)	371.35 (272.7–483,7)	431 (368–490)	55.05 (34.1–88.4)	65 (40–115)	90.55 (51–138)	0.24 (0.12–0.39)	0.21 (0.11–0.30)
p		<0.001 (2)	0.001 (1)	0.056 (1)	0.005 (1)	<0.001 (1)	0.034 (1)	0.714 (1)	0.003 (1)
Electrocardiographic findings according to pulmonary artery involvement									
mean±std. deviation	Main pulmonary artery (n=27)	69.06±17.43	340.28±27.14	439.30±32.78	66.88±20.70	87.89±30.29	79.68±15.68	0.23±0.04	0.18±0.04
	Others (n=26)	74.33±27.36	357.87±39.94	443.50±31.18	66.22±15.48	84.38±19.75	86.80±17.17	0.24±0.05	0.20±0.04
Median (min–max)	Main pulmonary artery (n=27)	64.7 (44.5–106,6)	340 (283.3–391.8)	437 (377–507)	60.9 (40.9–123.5)	80 (52–192)	80 (44.4–111.7)	0.24 (0.16–0.32)	0.19 (0.11–0.25)
	Others (n=26)	69.8 (38.1–139.2)	360.9 (295–458)	445 (373–504)	64.9 (38.9–103.4)	84.5 (45–123)	88.15 (50–120.9)	0.24 (0.16–0.34)	0.20 (0.13–0.27)
p		0.669 (2)	0.066 (1)	0.635 (1)	0.896 (1)	0.621 (1)	0.121 (1)	0.413 (1)	0.177 (1)
Electrocardiographic findings according to the presence of mortality									
mean±std. deviation	Mortality (no) (n=49)	72.20±23.56	349.02±34.86	440.00±29.83	65.73±16.67	84.98±24.37	82.62±15.85	0.24±0.04	0.19±0.04
	Mortality (yes) (n=4)	64.78±6.77	347.63±39.92	458.00±53.37	76.70±33.42	100.75±38.09	89.95±26.97	0.26±0.07	0.19±0.04
Median (min–max)	Mortality (no) (n=49)	64.8 (38.1–139.2)	343.5 (283.3–458)	438 (373–507)	62.2 (38.9–123.5)	82 (45–192)	83.3 (44.4–111.7)	0.23 (0.16–0.34)	0.19 (0.11–0.27)
	Mortality (yes) (n=4)	62.6 (59.4–74.5)	364.35 (288.8–373)	469.5 (389–504)	67.75 (48.7–122.6)	95.5 (63–149)	89.75 (59.4–120.9)	0.27 (0.17–0.33)	0.20 (0.13–0.24)
p(2)		0.661	0.833	0.369	0.661	0.444	0.615	0.405	0.569

(1) Independent samples t-test; (2) Mann-Whitney U test; QTc: corrected QT.

DISCUSSION

Various changes in the ECG are observed in correlation with the severity of PTE[3]. One of the ECG parameters that reflects ventricular repolarization heterogeneity is QTd, which can be affected by several factors[6]. Studies have demonstrated that patients with acute PTE have significantly higher QTd and QTcd compared to control groups, with higher values observed in high-risk PTE patients[7]. This suggests that increased QTd and QTcd may indicate right ventricular failure due to elevated pulmonary artery pressure in acute PTE[8]. Consistent with these findings, our study also revealed significantly higher QTd and QTcd values in PTE patients compared to the control group ($p=0.05$ and $p<0.001$, respectively). Furthermore, we evaluated the sensitivity and specificity of various cutoff points for QTd, indicating its potential as a diagnostic marker. Patient groups were not categorized based on the risk levels in our study, as clinical severity does not always align with CTPA findings[8]. Therefore, we adopted a radiological classification based on PTE involvement sites to ensure an objective evaluation.

To assess the impact of PTE location on ECG parameters, we considered the possibility that involvement of the main pulmonary artery may lead to increased right ventricular workload, resulting in heightened right ventricular tension, heterogeneity, and subsequent elevation of QTd and QTcd. However, no significant difference was observed when comparing major branch involvement with other branch involvement. Several factors could explain this finding. Most patients with major branch involvement in our study had a clinically stable course, suggesting that severe right ventricular afterload may not have been present, thus exerting a limited effect on QTd and QTcd compared to other patients. Additionally, the limited sample size in our study might have affected the statistical

power, potentially influencing the results. In contrast, previous studies have shown a significant association between high QTcd and increased mortality in patients diagnosed with PTE[4]. It has been suggested that a QTcd threshold above 71.5 ms could predict mortality in acute PTE patients, with a sensitivity of 73% and a specificity of 71%[9]. However, our study did not find a significant correlation between QTd, QTcd, and 30-day mortality, likely due to the relatively low number of patients with fatal outcomes compared to the overall study population.

Recent studies have highlighted the importance of Tp-e duration, Tp-e/QT ratio, and Tp-e/QTc ratio as noninvasive indicators of myocardial transmural repolarization[10]. Unlike QTd and QTcd, Tp-e measurements are less influenced by heart rate variations, providing a potentially more accurate assessment of repolarization[10]. In patients with acute PTE, increased Tp-e duration has been associated with adverse outcomes, including higher 30-day mortality[5]. Furthermore, a decrease in Tp-e duration, Tp-e/QT, and Tp-e/QTc ratios has been observed following the thrombolytic therapy in hemodynamically unstable acute PTE patients, indicating improved cardiac function[11].

Contrary to previous studies, our study demonstrated a decrease in Tp-e duration (p=0.034) and Tp-e/QTc ratio (p=0.03) in patients with acute PTE compared to the control group[11]. This unexpected finding may be attributed to differences in the composition of our control group compared to previous studies. Nonetheless, the reported Tp-e duration and Tp-e/QTc ratio values in our study were within a similar range as those in the literature, providing consistency in these measurements. We identified a sensitivity of 64.15% and a specificity of 61.11% for a Tp-e/QTc ratio cutoff point of 0.20, indicating its potential as a diagnostic marker. However, similar to the QTd and QTcd analyses, no significant differences were found in Tp-e duration, Tp-e/QT, and Tp-e/QTc ratios between patients with major branch involvement and those with embolism in other branches. This suggests that the presence of major branch involvement alone may not impose a significant right ventricular load, leading to clinical instability. Nevertheless, due to the limited sample size in our study, further investigations with larger populations are needed to confirm these findings.

Regarding the association between Tp-e duration, Tp-e/QT, and Tp-e/QTc ratios with mortality, our study did not find a significant correlation. This lack of significance can be attributed to the relatively low number of patients who experienced fatal outcomes compared to the overall study population. It is reasonable to assume that with a larger sample size, the relationship between these ECG parameters and mortality could become more apparent.

Finally, our study evaluated T-wave dispersion as an indicator of ventricular heterogeneity within the QT interval. We found a significant difference in T-wave dispersion (p=0.001) between patients diagnosed with acute PTE and the control group. This observation suggests that right ventricular heterogeneity induced by increased right ventricular loading contributes to altered T-wave dispersion in PTE patients. However, no significant differences in T-wave dispersion were found when comparing major branch involvement with other involvements or when considering mortality. Further studies investigating T-wave dispersion in conjunction with clinical severity and PTE involvement sites may yield more comprehensive results. Given the limited literature on T-wave dispersion in PTE, additional studies focusing on this parameter in PTE patients can provide valuable insights.

Limitations

Our study has certain limitations that need to be acknowledged. First, it was conducted at a single center, which may limit the generalizability of our findings to other populations or settings. Additionally, the sample size was relatively small, partly due to the challenges posed by the COVID-19 pandemic, which restricted the study duration and patient recruitment. Consequently, the limited number of patients with fatal outcomes may impact the statistical power of our analysis.

Another potential limitation is the selection of the control group, consisting of patients who underwent CTPA but did not show any evidence of embolism. This approach may introduce selection bias and influence the comparability of the control and PTE groups.

Furthermore, the measurement of QT duration remains a subject of ongoing debate in the scientific community. There are discrepancies and variability in the techniques used to determine the precise end point of the T wave, leading to inconsistent results. Despite utilizing digital measurement methods to improve accuracy, concerns persist regarding the reliability and reproducibility of these techniques, which may influence the interpretation of our study findings[12].

CONCLUSSION

Our study revealed significant alterations in ECG parameters, including QTd, QTcd, Tp-e duration, Tp-e/QT, and Tp-e/QTc ratios, in patients with acute PTE compared to the control group. However, the impact of PTE location on these parameters and their association with mortality was not significant in our study, possibly due to the limited sample size. Future studies with larger populations are warranted to validate

these findings and further elucidate the clinical significance of ECG changes in PTE.

ETHICAL APPROVAL

Our study was approved by the Health Sciences University, Kocaeli Derince Training and Research Hospital Clinical Research Ethics Committee on November 14, 2019 (Decision Number: 2019-117).

AUTHORS' CONTRIBUTIONS

SG: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing – original draft. **EŞ:** Conceptualization, Formal Analysis, Resources. AES: Data curation, Visualization. **SAG:** Data curation, Visualization. **DKÖ:** Data curation, Validation. **HCH:** Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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The concern about the educational quality of online videos on laparoscopic myomectomy

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SUMMARY

OBJECTIVE: The aim of this study was to analyze the surgical content of the 50 most-viewed laparoscopic myomectomy videos on YouTube while evaluating the educational quality and accuracy of the videos.

METHODS: In this cross-sectional study, the keyword "laparoscopic myomectomy" was searched in publicly available content on YouTube, and the videos were sorted by view count using YouTube's advanced search options. Out of the first 66 videos, only 50 were eligible according to our selection criteria. One associate professor of gynecology and one gynecology resident watched these videos independently and evaluated the quality and surgical aspects. Our primary outcome was the scores of the Quality Criteria for Consumer Health Information and Global Quality Score and the features of the surgical technique.

RESULTS: The 50 most-viewed laparoscopic myomectomy videos were uploaded between 2010 and 2021. They had a mean of 66636.6±103772.2 views. According to the Quality Criteria for Consumer Health Information criteria, 78% of the videos were categorized as "poor," 12% of them were "fair," and 10% of them were "very poor." The indication of the surgery was not specified in 27 (54%) of them. The surgeons in 39 (79.6%) of the videos did not use any containment system for the power morcellation, even though it was restricted by the United States Food and Drug Administration. The preoperative and perioperative precautions to minimize blood loss were underemphasized. There was no scientific evidence in 49 (98%) of the videos.

CONCLUSION: Laparoscopic myomectomy videos on YouTube are limited in terms of providing evidence-based and well-organized scientific knowledge.

KEYWORDS: Webcast. Laparoscopy. Uterine myomectomy. Education.

INTRODUCTION

Social media has emerged as an important source of health care-related information for physicians and patients[1]. Among the web-based resources, YouTube, an open-access video-sharing website, is among the three most popular websites, with more than 4 billion videos viewed daily and more than 500 h of video content uploaded every minute[2]. However, the lack of peer review and unconditional acceptance of videos without any elimination process led to inaccurate and misleading information accumulating on YouTube[3].

Uterine fibroid is the most common benign disorder of female genital tract with an estimated incidence of 20–40% in reproductive age[4]. Although the incidence of myomas has been increasing due to the inverse association between myoma risk and parity, approximately one-quarter of women seek treatment due to myoma-related symptoms[5]. Surgical management remains the main therapeutic option. In the last decade, as the minimally invasive approach became more popular, the number of laparoscopic myomectomy procedures has increased until the US Food and Drug Administration (USFDA) issued a statement against laparoscopic power morcellation for myomectomy or hysterectomy without the tissue containment system[6]. Nevertheless, the minimally invasive procedures are associated with better reproductive outcomes and lower perioperative morbidity in suitable patients[7].

The significant increase in the number of laparoscopic myomectomy procedures and the interest of young physicians prompted the sharing of videos. However, the surgical technique and the educational quality of them are disputable. For this reason, we analyzed the 50 most-viewed laparoscopic myomectomy videos on YouTube and described the surgical content of them while evaluating the educational quality and accuracy of the videos using the Quality Criteria for Consumer Health Information (DISCERN) and Global Quality Score (GQS) scales.

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METHODS

Study design

The STROBE guideline was followed. For this observational study, a search was made in publicly available content on YouTube (http://www.youtube.com) by the keyword "laparoscopic myomectomy" on November 15, 2021, and the videos were sorted by the view count using YouTube's advanced search options. The first 66 videos, which are in English, were saved to a playlist. One associate professor of gynecology and one gynecology resident watched these videos independently between November 15, 2021, and November 24, 2021. The inclusion criteria were as follows: (1) narration in English; (2) primary content related to laparoscopic myomectomy; and (3) acceptable audio-visual quality. The exclusion criteria were as follows: (1) narration in languages other than English; (2) poor audio-visual quality; (3) duplicate videos; (4) patient experience videos; and (5) robotic surgery videos. We had no limit on the video length.

Video evaluation process

The following characteristics for each video were noted: upload date, total number of views, total video length in seconds, time passed since upload date, viewer interest parameters such as number of likes, dislikes, and comments, the channel type and the number of subscribers of the channel, the type of content, and the type of visual content and the narrator in the video.

The aspects of surgical technique were recorded as follows: number of layers in wound closure; specimen removal technique; indication of myomectomy; suture material; energy modality of surgical device; number of trocars used; size of the largest myoma; number, type, and position of the fibroid; suturing method; trocar used for suturing; type of serosa incision; usage of uterine manipulator; entrance to the endometrial cavity; duration of operation; presence of scientific evidence; postoperative complications; amount of blood loss; rate of blood transfusion; postoperative discharge day; preoperative management; pathology; any method for adhesion prevention; and the vasopressin injection into the myometrium.

Quality assessment

There is no established standard for evaluating the quality of online surgical videos. We preferred using the DISCERN[8] and GQS scales[9], both of which were used by previous studies to evaluate the quality and reliability of the Internet content. The DISCERN questionnaire has 3 sections with 16 questions and is presented in Supplementary Table 1. Each question has a 5-point scale from no to yes. DISCERN points were categorized as very poor: <27, poor: 27–<39, fair: 39–<51, good: 51–<63, and excellent: 63–75. GQS is a 5-point scale scoring system (Supplementary Table 2)[9]. Also, the scientific evidence was recorded as present or absent[10].

Outcome measures

The primary outcome measures are the DISCERN and GQS scores of the videos and the remarkable features of the surgical technique. Our secondary outcome was the incompatibility between the scores of associate professor and resident.

Ethical implications

As this study does not constitute human participants, ethical approval was waived in accordance with the Institutional Review Board of Istanbul University-Cerrahpaşa, Turkey. The study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of Turkey.

Statistical analysis

The data were analyzed by Statistical Package for Social Sciences (SPSS) 20.0 version. The descriptive statistics were presented as number, percentage, standard deviation, median, minimum and maximum. To assess the popularity of the video, the video power index (VPI) was calculated as [(number of likes/number of likes+number of dislikes)´100]. So, the VPI value was out of 100. The number of views per day for each video was calculated by the formula: [total number of views on the day of viewing/(day of viewing-upload date of the video (days)].

The mean of the DISCERN and GQS scores of both researchers was calculated by the formulas: [DISCERN score of the 1st researcher+DISCERN score of the 2nd researcher)/2] and [GQS score of the 1st researcher+GQS score of the 2nd researcher)/2] for each video. For the evaluation of the correlation between the DISCERN and GQS scores of two researchers, after the data were found to be normally distributed, the Spearmen correlation analysis was used. The level of correlation was assumed as follows: low: the correlation coefficient between 0.10 and 0.29; moderate: the correlation coefficient between 0.30 and 0.49; and high: the correlation coefficient >0.50[11]. The compliance between the researchers was assessed by the Krippendorff alpha (α) value. α>0.80 showed high compliance, α between 0.67 and 0.80 showed moderate compliance, and α<0.67 showed low compliance[12]. The data were evaluated in terms of normality of distribution and parametric tests. Student's t-test and nonparametric Mann-Whitney U tests were used accordingly. $p<0.05$ was accepted as statistically significant.

Table 1. Descriptive characteristics of videos and the aspects of surgical technique.

Variables	n (%)	Variables	n (%)	Variables	n (%)
Channel types	49	The number of fibroid	50	The entrance to the endometrial cavity	49
Physician	39 (79.6)	One	35 (70.0)	No	43 (87.8)
Educational channel	5 (10.2)	Two	7 (14.0)	Yes	6 (12.2)
Medical device firm	1 (2.0)	Three	4 (8.0)	The duration of operation (min)	50
Hospital channel	4 (8.2)	Four	2 (4.0)	Unknown	48 (96.0)
The narrator	50	Five	2 (4.0)	Ninety	1 (2.0)
Physician	44 (88.0)	The type of fibroid	50	Three hundred and sixty	1 (2.0)
Others	6 (12.0)	Intramural	32 (64.0)	Scientific evidence	50
The type of visual content	50	Subserosal	10 (20.0)	Absent	49 (98.0)
Real image	47 (94.0)	Submucosal	1 (2.0)	Present	1 (2.0)
Real image and animation	3 (6.0)	Other	7 (14.0)	Postoperative complications	50
The layers in wound closure	48	The position of fibroid	50	Not specified	48 (96.0)
Single	14 (29.2)	Fundus	21 (42.0)	None	2 (4.0)
Double	21 (43.8)	Posterior	19 (38.0)	Vasopressin injection into myometrium	50
Triple	9 (18.8)	Anterior	8 (16.0)	Yes	34 (68.0)
Quadruple	4 (8.2)	Cervix	1 (2.0)	No	16 (32.0)
Specimen removal technique	49	Other	1 (2.0)	The amount of blood loss (mL)	50
Morcellation without any containment system	39 (79.6)	The technique of myometrial suturing	48	Not specified	48 (96.0)
Morcellation with containment system	3 (6.2)	Continuous non-locking	22 (45.8)	25	1 (2.0)
Removing by mini-laparotomy	1 (2.0)	Single	20 (41.7)	100	1 (2.0)
Not specified	6 (12.2)	Continuous locking	5 (10.4)	The blood transfusion	50
Indication of myomectomy	50	Baseball	1 (2.1)	Not specified	49 (98.0)
Not specified	27 (54.0)	The technique of serosal suturing	48	No	1 (2.0)
Specified	23 (46.0)	Continuous nonlocking	23 (47.9)	Postoperative discharge day	50
The suture material	48	Single	13 (27.1)	Not specified	47 (94.0)
Polyglactin 910	34 (70.8)	Continuous locking	9 (18.8)	The night of surgery	2 (4.0)
Polyglecaprone 25	2 (4.2)	Baseball	3 (6.2)	The first postoperative day	1 (2.0)
V-loc[a]	12 (25.0)	The trocar used for suturing	50	Preoperative management	50
The energy modality of surgical device	49	Ipsilateral	30 (60.0)	Not specified	48 (96.0)
Unipolar	8 (16.4)	Contralateral	20 (40.0)	No	1 (2.0)
Bipolar	1 (2.0)	The type of serosal incision	50	GnRH analog	1 (2.0)
Sinusoidal	29 (59.3)	Horizontal	24 (48.0)	Pathology	50
No energy modality, only scissors	7 (14.3)	Vertical	20 (40.0)	Not specified	49 (98.0)
Sinusoidal and vessel sealing	2 (4.0)	Oblique	6 (12.0)	Benign	1 (2.0)
Bipolar spatula	1 (2.0)	The usage of uterine manipulator	50	Any method for adhesion prevention	49
Not specified	1 (2.0)	Use of LMFI[b], manipulator unknown	26 (52.0)	No	40 (81.6)
The number of the trocars	50	No	9 (18.0)	Yes	9 (18.4)
Three	12 (24.0)	Use of only LMFI[b]	8 (16.0)		
Four	37 (74.0)	Yes	7 (14.0)		
Five	1 (2.0)				

[a]V-loc: barbed absorbable wound device. [b]LMFI: laparoscopic myoma fixation instrument with screw-shaped tip.

Table 2. The Quality Criteria for Consumer Health Information and Global Quality Scores in terms of the researcher degree and the descriptive statistics of the videos in terms of the narrator type.

DISCERN scores (resident)		DISCERN scores (associate professor)			
mean±SD	Median (min–max)	mean±SD	Median (min–max)	r[f], p	Krippendorff α
33.5±6.8	32.5 (16.0–50.0)	32.1±3.8	34.0 (26.0–38.0)	0.441, 0.001	0.475
GQS scores (resident)		**GQS scores (associate professor)**			
mean±SD	Median (min–max)	mean±SD	Median (min–max)	r[f], p	Krippendorff α
3.2±0.9	3.0 (1.0–5.0)	2.5±1.1	3.0 (1.0–4.0)	0.468, 0.001	0.280
Narrator of the video	DISCERN scores[a]		DISCERN categories[b]	n (%)	p[d]
	mean±SD	Median (min–max)			
Physician (n:44)	32.7±4.5	33.0 (22.0–42.5)	Very poor (<27)	4 (9.1)	0.719
			Poor (27–<39)	35 (79.5)	
			Fair (39–<51)	5 (11.4)	
			Very poor (<27)	1 (16.7)	
Others (n:6)	33.4±5.8	32.8 (26.5–43.5)	Poor (27–<39)	4 (66.6)	
			Fair (39–<51)	1 (16.7)	
	GQS scores[c]				
	mean±SD	Median (min–max)	p[e]		
Physician (n:44)	2.8±0.8	3.0 (1.0–4.0)	0.354		
Others (n:6)	3.2±1.0	3.5 (2.0–4.5)			
	Video Power Index (VPI)				
	mean±SD	Median (min–max)	p[e]		
Physician (n:44)	87.7±10.0	90.5 (48.7–98.8)	0.238		
Others (n:6)	67.5±37.6	85.3 (0.0–94.9)			
	The number of views per day				
	mean±SD	Median (min–max)	p[e]		
Physician (n:44)	45.2±85.5	14.5 (2.5–505.7)	0.765		
Others (n:6)	35.9±46.7	21.5 (2.7–127.9)			

DISCERN: Quality Criteria for Consumer Health Information; GQS: global quality scale; SD: standard deviation; min: minimum; max: maximum; %: the percentage in the group of narrator. [a]The mean DISCERN scores of the researchers were calculated by taking the average of the DISCERN scores of the two researchers. [b]Each video was included in the relevant DISCERN category according to the DISCERN scores of the researchers. [c]The mean GQS scores of the researchers were calculated by taking the average of the GQS scores of the two researchers. [d]The significancy test measures the difference between the means of two groups. [e]Mann-Whitney U test. [f]Spearman r correlation coefficient.

RESULTS

The 50 most-viewed laparoscopic myomectomy videos were uploaded between 2010 and 2021. They had a mean of 66636.6±103772.2 views. The videos were uploaded mostly by the physicians (79.6%), and the narrator was a physician in 44 (88.0%) of them. The videos are real surgical videos in 47 (94%) of the cases. In 28 (38%) of the videos, there was no explanation during the video play. The video with the highest view rate (624996 times) was uploaded by a physician from India in 2018. It has also received the maximum number of likes (2200 likes). In that video, a laparoscopic myomectomy was done on a 6 cm intramural myoma located at the anterior wall of the uterus, and the myoma was removed by power morcellation without any containment system. The indication of the surgery was not specified in 27 (54%) of them. The myomectomy procedure consisted of singles in 35 (70%) and intramural myoma in 32 of them (64%). The myomas were located at the fundus of the uterus in 21 (42%) of them. Double-layered wound closure was performed in 21 (43.8%) of the videos. Polyglactin 910 was the preferred suture material in 34 (70.8%) of them. A continuous nonlocking pattern was the technique used in the myometrial suturing of 22 videos (45.8%). There was no scientific evidence in 49 (98%) of the videos. The postoperative complications, amount of

blood loss, blood transfusion rate, and pathology were not specified in 48 (96%), 48 (96%), 49 (98%), and 49 (98%) of the videos, respectively. The rest of the descriptive characteristics of the videos and the aspects of surgical technique are shown in Table 1.

Our two researchers had moderate correlation (r: 0.441, p: 0.001; r: 0.468, and p: 0.001) and low-level compliance (Krippendorff α: 0.475 and Krippendorff α: 0.280) in terms of DISCERN and GQS scores, respectively (Table 2). The mean DISCERN score of all videos was 32.8±4.6 (median: 33.0 and min–max: 22.0–43.5), and the mean GQS score was 2.9±0.9 (mean: 3.0 and min–max: 1.0–4.5). The mean of DISCERN and GQS scores of the videos uploaded by 5 educational channels were 34.2±6.0 (median: 34.0 and min–max: 28.5–43.5) and 3.1±1.1 (median: 3.5 and min–max: 2.0–4.5), respectively. VPI points were calculated as 85.3±16.6 (median: 90.5 and min–max: 0.0–98.8) out of 100.

According to the DISCERN criteria, 78% of the videos were categorized as "poor," while 12% of them were "fair" and 10% of them were "very poor," as shown in Figure 1. There was not any statistically significant difference in DISCERN scores, according to the narrator of the video (p=0.719). Among the videos with narrators who were physicians, 79.5% of them were categorized as "poor," 11.4% of them were "fair," and 9.1% of them were "very poor" (Table 2). While in the group of videos with narrators who were not physicians, 66.6% of the videos were categorized as "poor," 16.7% of them were "fair" and 16.7% of them were "very poor." GQS points, VPI, and the number of views per day were compared, and there was no statistically significant difference between them in terms of narrator type (p>0.05).

DISCUSSION

This study determined the poor quality and reliability of 50 most-viewed laparoscopic myomectomy videos on the most popular video sharing platform, YouTube. The most-viewed laparoscopic myomectomy videos not only lacked scientific evidence but also had generally poor scores according to the DISCERN and GQS scales. Moreover, these scores were moderately correlated between two independent researchers; the associate professor of gynecology gave slightly lower DISCERN and GQS scores to the videos. This emphasizes that the inaccurate content of the videos can be interpreted and filtered by the wisdom of a senior physician, but the junior physicians are at risk of learning nonevidence-based information.

The low educational quality of medical videos on YouTube was shown by many other researchers in other fields[13]. The incompatibility of the laparoscopic myomectomy videos with current surgical guidelines is significantly low. They do not educate the viewer about the patient's history and characteristics, possible treatment options, taking informed consent, preoperative

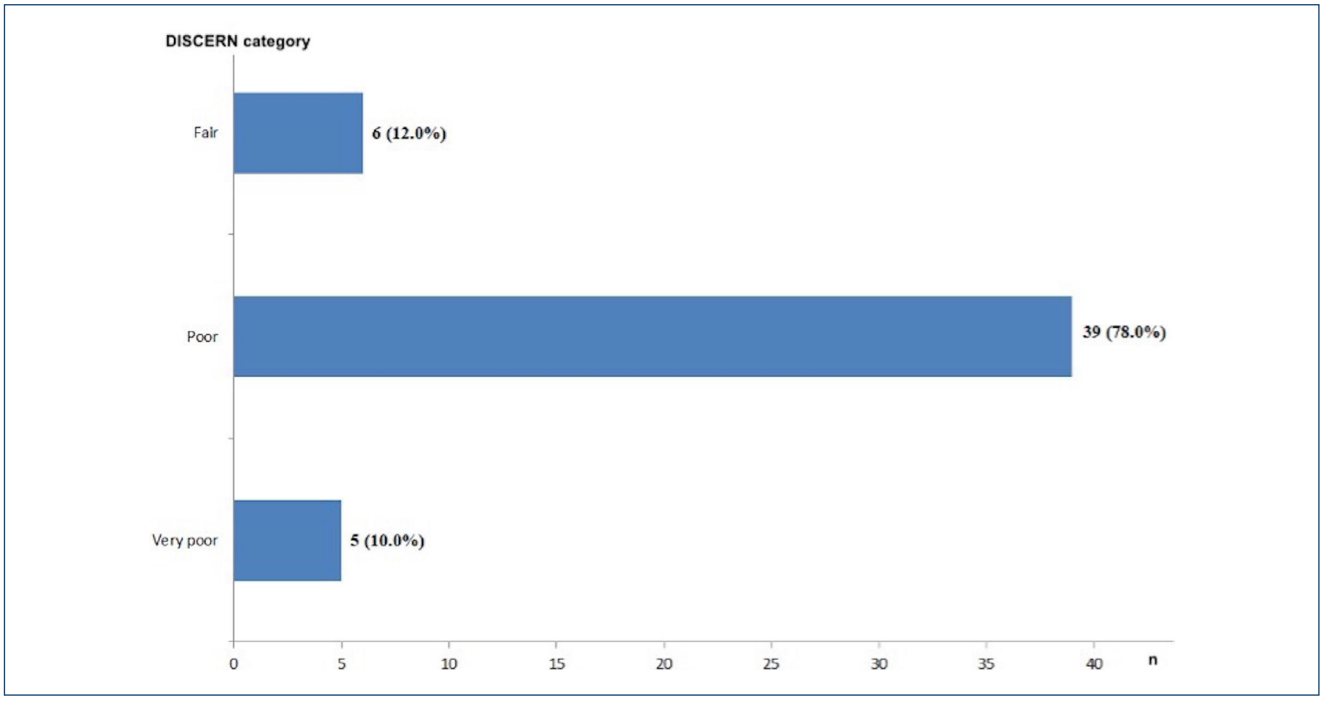

Figure 1. Categorization of the videos according to the Quality Criteria for Consumer Health Information score.

management, surgical setup, etc. In addition to that, the surgical techniques are controversial. The most important concern was the specimen removal technique after the excision of the myoma: power morcellation[7]. Beginning with its warnings in 2014, the FDA finally restricted the use of power morcellation only with a containment system and with the informed consent of the patient in 2020[6]. Even though 32 of the 50 videos were uploaded after 2014, in 39 (79.6%) of them, the surgeons did not use any containment system for the removal without any referral to FDA restriction. The videos about laparoscopic myomectomy should have at least emphasized the preventive role of the containment system against the intraperitoneal spread of the myometrial cells. Intraoperative vasopressin injection, another technique for decreasing intraoperative blood loss, was also underemphasized[14]. Even though preoperative (correction of anemia and GnRHa usage) and perioperative precautions (vasopressin injection, uterine artery ligation, and using barbed suture) are very important for better surgical outcomes, they were not emphasized[15]. There was also inconsistency between the number of suture layers in the myometrium during the wound closure, which determines the risk of uterine rupture in subsequent pregnancies. According to evidence-based medicine, multilayer closure of myometrium (two layers for myometrium and one layer for serosa, with a continuous nonlocking fashion) is recommended[16]; however, no recommendations were made in the videos. Finally, the indication for myomectomy was not specified in 54% of the videos, as were patient histories, which prevented the viewer from learning the correct indications for myomectomy. In this cross-sectional study, we tried to highlight the importance of three major concerns of laparoscopic myomectomy: (1) the risk of uterine rupture in subsequent pregnancies; (2) the malignancy potential of the myoma; and (3) the appropriate indication for myomectomy.

Our study is limited in terms of the sample size since it only included 50 most-viewed "laparoscopic myomectomy" videos on YouTube. Yet, people tend to watch the most-viewed videos on the most popular video-sharing platform. On the other hand, our study is strong in the way that (1) the quality of the videos was investigated using two different scales (DISCERN and GQS), (2) the scorings were independently done by two raters who have different academic degrees, (3) the correlation between the scores of two different raters was analyzed, (4) the surgical aspects were recorded and discussed according to evidence-based medicine, and (5) the safety concerns of the videos were addressed.

CONCLUSION

Laparoscopic myomectomy videos on YouTube are limited in terms of providing evidence-based, well-organized scientific knowledge. An established guideline is necessary to standardize the laparoscopic myomectomy procedure and to facilitate the practice by reducing the learning curve.

AUTHORS' CONTRIBUTIONS

OK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **IBOE:** Conceptualization, Data curation, Formal Analysis, Investigation, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **EB:** Data curation, Formal Analysis, Investigation, Writing – review & editing.

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ORIGINAL ARTICLE

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Use of the laser in the pilonidal sinus alone or in combination with phenol

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SUMMARY

OBJECTIVE: We investigated the effectiveness of combining laser treatment with phenol in the management of pilonidal sinus.

METHODS: We present here a retrospective analysis of patients with pilonidal sinus disease who were treated in the general surgery clinic of the Balikesir University Hospital between October 2019 and February 2022.

RESULTS: Recurrence was observed in three patients (13.6%) in the laser treatment group and one patient (4.8%) in the laser-phenol treatment group after the fourth month. Notably, 22 (91.7%) patients in the laser treatment group and 21 (95.5%) patients in the laser-phenol treatment group had complete healing.

CONCLUSION: Although not statistically significant, the laser-phenol treatment group exhibited a lower recurrence rate and a higher complete healing rate.

KEYWORDS: Lasers. Phenol. Pilonidal cyst. Pilonidal sinus.

INTRODUCTION

Pilonidal sinus disease is a condition that commonly manifests between the ages of 15 and 30 years and has a significant impact on quality of life. It can occur in various locations throughout the body but predominantly in the sacrococcygeal region. The incidence rate is approximately 26 per 100,000. An analysis of the existing literature indicates a male predominance in pilonidal sinus disease, with females accounting for only approximately 21% of total cases[1-3].

The etiology of pilonidal sinus disease involves genetic factors, obesity, prolonged sitting, excessive body hair, and poor hygiene. Patients commonly present with symptoms such as pain, discharge, swelling, and discomfort while sitting. The clinical presentation of the disease can vary from an asymptomatic form to the development of acute abscesses and chronic presentations[4]. The prolonged persistence of pilonidal sinus increases the risk of squamous cell carcinoma within the sinus tracts[5].

Pilonidal cysts are, in fact, not true cysts, as histopathological examination reveals the absence of a definitive epithelial lining. Upon examination of the cyst cavity, the presence of hair, debris, foreign body giant cells, and granulation tissue is frequently observed[6].

The most efficient treatment approach to pilonidal sinus disease remains controversial, although it is accepted that the ideal treatment will be simple with a short operative time and have a low recurrence rate without the need for hospitalization[7]. Various surgical methods have been proposed, such as excision with primary closure, marsupialization, and various flap techniques, as well as non-surgical techniques such as phenol filling of the sinus cavity, endoscopic pilonidal sinus treatment (EPSIT), video-assisted ablation of the pilonidal sinus (VAAPS), and laser treatments[8-12]. Efforts to determine the optimal treatment approach continue by exploring combinations of the aforementioned treatment methods.

This study investigated the effectiveness of combining laser treatment with phenol in the management of pilonidal sinus.

METHODS

We present here a retrospective analysis of patients with pilonidal sinus disease who were treated in the general surgery clinic of the Balikesir University Hospital between October 2019 and February 2022. Patients aged 18 years and above with primary or recurrent pilonidal sinus disease, without abscesses, and without chronic diseases that could affect wound healing were included in the study. The patients were divided into two groups: a laser treatment group (n=24) and a laser-phenol treatment group (n=22). No prophylactic antibiotics were administered prior to the treatment, and all procedures were performed under local anesthesia. Informed consent was obtained from all patients before the procedures.

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Surgical technique

For the cases of pilonidal sinus undergoing laser treatment, the periphery of the sinus pit was first cleansed with povidone-iodine, after which the pilonidal sinus tract was cannulated using a stylet. Any granulation tissue and hair within the cyst were cleared using a curette, and the sinus tract was irrigated with saline solution. After curettage and irrigation, the laser treatment was applied involving the delivery of an average energy of 320 joules using a 1470-nm diode laser (1 pulse: 6 s, 15 watts/1 pulse in 6 s=90 joules) (Figure 1).

For the cases in which the combined laser-phenol treatment was employed, in addition to the previously mentioned laser procedures, crystallized phenol was carefully applied to the sinus pit using a clamp, ensuring minimal damage to the surrounding tissues (Figure 2). A dressing was applied to complete the procedure.

Follow-up examinations were conducted on the postoperative 1st, 7th, and 30th days and in the 3rd, 6th, and 12th months involving an assessment of the patients' wound sites and addressing any issues that may have developed.

The term "complete healing" refers to the total closure of the wound without any indications of infection, regardless of the time since the procedure, while "recurrence" refers to a return of symptoms at least 4 months after the wound has completely healed.

Statistical analysis

Descriptive statistics were used in the analysis of the results of the study. For numeric variables, the mean±standard deviation or median, minimum, and maximum values were presented in tabular form, while categorical variables were expressed as numbers and percentages. The normality of the numeric variables was assessed using Shapiro-Wilk, Kolmogorov-Smirnov, and Anderson-Darling tests.

To analyze the disparities in categorical variables between groups, a Pearson chi-square test was employed for 2×2 tables with expected frequencies of 5 or above, and in instances where the expected frequencies were below 5, Fisher's exact test was used.

For the comparison of numerical variables between the treatment groups, an independent samples t-test was used for variables that followed a normal distribution, whereas a Mann-Whitney U test was employed for variables that did not exhibit a normal distribution.

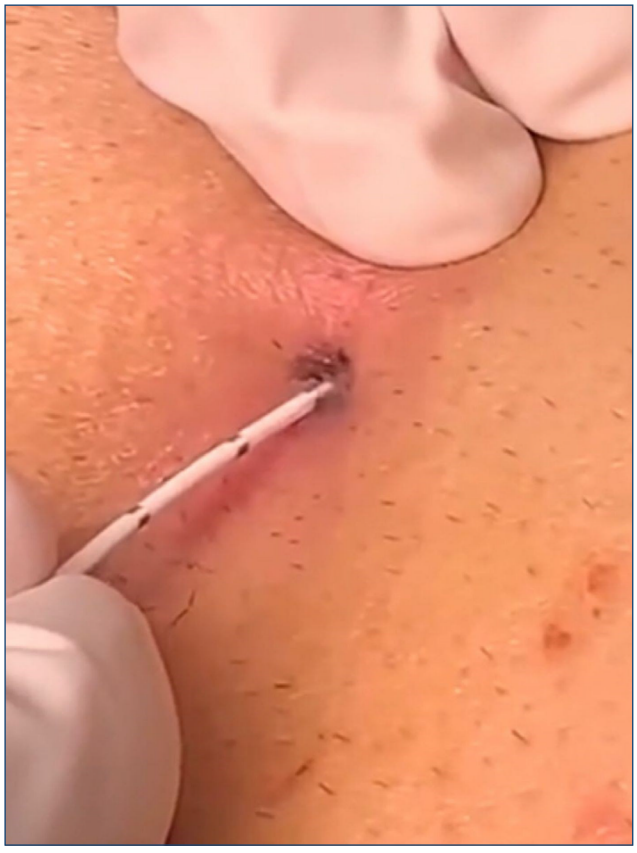

Figure 1. Ablation of sinus tract by a radial diode laser probe.

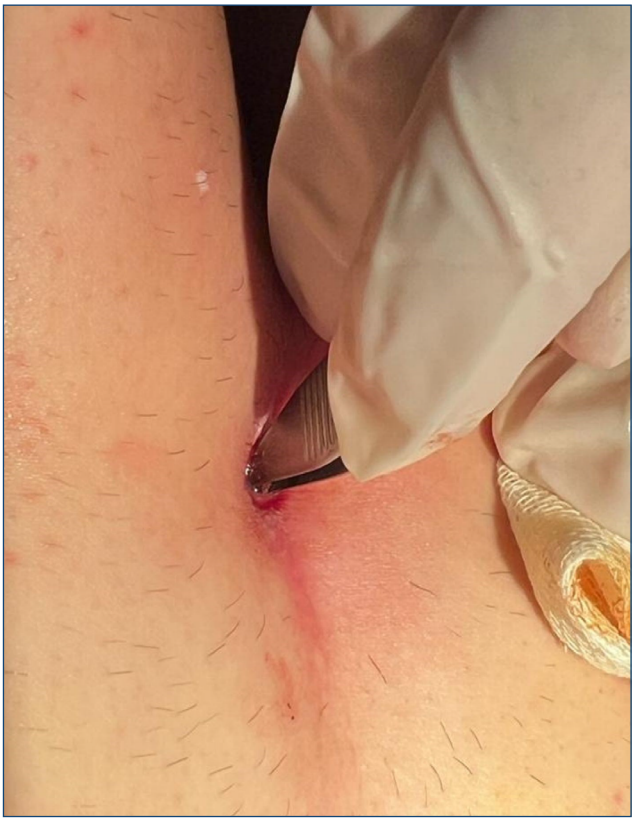

Figure 2. Application of crystallized phenol to the sinus cavity after the laser procedure.

The statistical analyses were performed using two software programs: Jamovi (Version 2.3.18) and JASP (Version 0.16.3.0). The significance level for the analyses was set at 0.05 (p-value).

Ethical aspects

This study was conducted according to the Declaration of Helsinki and approved by the Clinical Studies Ethics Board of Balikesir University (date: 19.04.2023, No. 2023/60).

RESULTS

A total of 46 patients were included in the study, with 24 in the laser treatment group and 22 in the laser-phenol treatment group. No significant differences were observed between the treatment groups in terms of demographic and pilonidal sinus characteristics (p>0.05) (Table 1), and both treatment groups exhibited similar characteristics in terms of the number of pilonidal sinus pits, history of abscess drainage, and duration of hospital stay (p=0.540, p=0.999, and p=0.296, respectively).

On the seventh-day follow-up visit, two patients (8.3%) in the laser treatment group and one patient (4.5%) in the laser-phenol treatment group were identified with wound infections. There was no significant difference observed in the occurrence of wound infection between the two groups (p=0.999) (Table 2).

During the follow-up examinations on and after the 30th day, all patients except those who had previously developed complications in both groups had healed. Notably, 22 (91.7%) patients in the laser treatment group and 21 (95.5%) patients in the laser-phenol treatment group had complete healing. Although the rate of complete healing was higher in the laser-phenol treatment group, the difference was not found to be statistically significant (p=0.999) (Table 3).

Recurrence was observed in three patients (13.6%) in the laser treatment group and one patient (4.8%) in the laser-phenol treatment group after the fourth month. No significant difference was found in the recurrence rates of the two groups (p=0.607) (Table 3).

Table 1. Demographic and clinical characteristics of the patients in the treatment groups.

	Treatment groups		p-value
	Laser (n=24)	Laser+phenol (n=22)	
Age (years)†	29.0±7.7	27.9±5.5	0.552
Gender‡			
Male	19 (79.2)	16 (72.7)	0.869
Female	5 (20.8)	6 (27.3)	
Pit numbers§	2.0 [1.0–3.0]	1.5 [1.0–3.0]	0.540
Abscess drainage history‡			
No	20 (83.3)	18 (81.8)	0.999
Yes	4 (16.7)	4 (18.2)	
Length of hospital stay (days)§	0.0 [0.0–0.0]	0.0 [0.0–1.0]	0.296

†Mean±standard deviation; ‡n (%); §Median [min–max].

Table 2. Comparison of treatment groups in terms of the development of complications at different follow-up times.

Follow-up examination time	Complication	Treatment groups		p-value
		Laser	Laser+phenol	
1st day‡				
	No	24 (100)	22 (100)	NA
	Yes	0 (0)	0 (0)	
7th day‡				
	No	22 (91.7)	21 (95.5)	0.999
	Yes	2 (8.3)	1 (4.5)	
30th day‡				
	No	22 (100)	21 (100)	NA
	Yes	0 (0)	0 (0)	
3rd month‡				
	No	22 (100)	21 (100)	NA
	Yes	0 (0)	0 (0)	
6th month‡				
	No	19 (100)	20 (100)	NA
	Yes	0 (0)	0 (0)	
12th month‡				
	No	19 (100)	20 (100)	NA
	Yes	0 (0)	0 (0)	

‡n (%). NA: not applicable.

Table 3. Comparison of treatment groups in terms of complete healing and recurrence development.

	Treatment groups		p-value
	Laser (n=24)	Laser+phenol (n=22)	
Complete healing‡			
No	2 (8.3)	1 (4.5)	0.999
Yes	22 (91.7)	21 (95.5)	
Recurrence			
No	19 (86.4)	20 (95.2)	0.607
Yes	3 (13.6)	1 (4.8)	

‡n (%).

DISCUSSION

The safe and optimum treatment method for pilonidal sinus remains a subject of debate. While various surgical and minimally invasive treatment approaches are currently available, consensus has yet to be reached on the best treatment option. The ideal treatment method should be simple and short term, require no hospitalization, have a low recurrence rate, and be cost-effective, allowing for a quick return to daily activities and work.

Laser therapy for the treatment of pilonidal sinus disease was first described by Pappas and Christodoulou, who reported a success rate of 90.3% in their prospective study of 237 patients[13]. Recent years have witnessed an increased interest in laser therapy for the treatment of pilonidal sinus disease due to its simplicity and ease of application. A systematic review study reported that laser treatment in patients with pilonidal sinus disease resulted in a complete healing rate of 94.4% and a recurrence rate of 3.8%[14]. In a retrospective study conducted in Belgium in 2017, a success rate of 87.5% (35 out of 40 patients) and a recurrence rate of 2.9% (1 out of 35 patients) were reported[15]. In a retrospective study published by Bonito et al. in 2021, the use of a radial diode laser probe for the treatment of pilonidal sinus yielded a success rate of 84% and a recurrence rate of 9.5%[16]. In a study conducted by Li et al. involving 48 patients, a success rate of 100% was achieved, and a recurrence rate of 2.1% was observed[17].

In this study, a complete healing rate of 91.7% and a recurrence rate of 13.6% were achieved among the patients treated exclusively with laser therapy.

Phenol treatments have long been considered a simple and cost-effective option for the treatment of pilonidal sinus. In a publication investigating the outcomes of a single-session crystallized phenol application for pilonidal sinus disease, a cure rate of 64.5% was reported with no recurrence observed[18]. Another study investigating the efficacy of phenol treatment for pilonidal sinus disease in 76 patients reported a success rate of 67% and a recurrence rate of 2%[19]. In a retrospective study examining 1026 patients with pilonidal sinus, a success rate of 84.3% was observed[20]. In the study conducted by Kaymakcioglu et al. with 143 patients, a recurrence rate of 8.3% was observed following phenol treatment[21].

There have been several studies in the literature investigating the use of different combination treatments involving crystallized phenol on treatment success and recurrence rates. In a study published in 2017, EPSIT used in combination with phenol resulted in zero treatment failure and disease recurrence[22]. Additionally, there have been studies in the literature investigating the use of laser treatments in combination with other methods for the management of pilonidal sinus disease. In a study conducted by Dönmez et al., the results of laser-EPSIT treatments used in combination were compared with those combining electrocautery-phenol-EPSIT, and it was reported that the laser-EPSIT combination achieved a complete healing rate of 92.3% and a recurrence rate of 7.7%[4]. Another study comparing laser treatments with endoscopic treatments for pilonidal sinus disease revealed no significant difference in the complete healing and recurrence rates of the two treatment approaches[23].

In this study, a higher rate of healing (95.5%, compared with 91.7%) and a lower rate of recurrence (4.8%, compared with 13.6%) were observed in the laser-phenol combination group than in the group treated solely with laser therapy, although the differences in outcomes were not found to be statistically significant.

We acknowledge that the limited number of cases included in our study may be considered a limitation, although, to the best of our knowledge, there has been no study to date comparing the efficacy of laser therapy and laser-phenol combinations for the treatment of pilonidal sinus. Consequently, we believe that our study can serve as a preliminary investigation that may stimulate further research in this field.

CONCLUSION

The findings of this study revealed no statistically significant differences in demographic and pilonidal sinus characteristics, and wound infection between the laser and laser-phenol combination treatment groups. Furthermore, although not statistically significant, the laser-phenol combination group exhibited a lower recurrence rate and a higher complete healing rate. Based on the findings of this study, we believe that a series of large-scale studies could be designed to further evaluate the impact of the addition of phenol to a laser treatment regime on the treatment success of pilonidal sinus disease.

ETHICAL ASPECTS

This study was conducted according to the Declaration of Helsinki and approved by the Clinical Studies Ethics Board of Balikesir University (date: 19.04.2023, No. 2023/60).

AUTHORS' CONTRIBUTIONS

EA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources,

Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AGŞ:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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The effect of functional independence levels on sleep and constipation in children with cerebral palsy

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SUMMARY

OBJECTIVE: The study aimed to examine the effect of functional independence levels on sleep behavior and constipation in children with cerebral palsy.

METHODS: This cross-sectional observational single-center study was carried out in a special rehabilitation center in Istanbul. Inclusion criteria were those aged between 4 and 18 years with Gross Motor Function Classification System III-IV-V functional independence levels. Those who had surgery concerning intestinal health, had a chronic infectious bowel disease, had congenital intestinal anomalies, had received botox treatment in the last 6 months, had uncontrolled epileptic seizures, had complained of constipation in the last 6 months, and had cardiopulmonary disease were excluded from the study. The sociodemographic characteristics of the participants and the Gross Motor Function Classification System were recorded. Pediatric Functional Independence Scale (Functional Independence Measure for Children) was used to measure the functional independence level, Pediatric Sleep Questionnaire was used to measure the level of sleep problems, and Constipation Severity Scale was used to measure constipation severity.

RESULTS: A total of 60 children who were diagnosed with cerebral palsy were included. According to Gross Motor Function Classification System, 46.7% of the cases were Level III, 35% were Level IV, and 18.3% were Level V. There was a negative moderate significant correlation between Functional Independence Measure for Children and Pediatric Sleep Questionnaire (r=-0.303; p=0.019) and between Functional Independence Measure for Children and Constipation Severity Scale (r=-0.342; p=0.007).

CONCLUSION: We described that lower functional independence levels were related to worse sleep and constipation symptoms. The results suggest that effective strategies for developing functional independence levels may be beneficial for both sleep and constipation symptoms in the concept of cerebral palsy management.

KEYWORDS: Cerebral palsy. Constipation. Sleep.

INTRODUCTION

Abnormal muscle tone, muscle weakness, constipation, sleep problems, loss of motor control, decreased range of motion, and contractures are common problems in cerebral palsy (CP)[1,2]. Sleep problems are experienced due to improper body position, pain, muscle spasms, pressure sensation, temperature, sweating, and digestive problems. Sleep problems, which may be due to different reasons, affect the physical and emotional states of children with CP and lead to a decrease in their quality of life[3,4]. Obrecht et al., investigated sleep disorders and their relation with impairment in gross motor function and determined that gross motor function impairment level is correlated with maintaining sleep and the need for nocturnal support[5].

One of the comorbidities that affect the quality of life in CP is constipation[6]. Constipation is a common chronic problem in most children with CP. As digestive system problems negatively affect the quality of life, assessment and control of constipation are critically important in patients with CP. Constipation occurs due to uncoordinated muscle contractions, impaired rectal sphincter control, and insufficient fluid intake[6,7]. Although it is thought that the decrease in functionality may be related to constipation and there is no study investigating this relationship, there are studies that aim to increase parasympathetic activity and achieve benefits by applying different intervention methods, similar to the parasympathetic activity mechanism, which is more active at rest after increased physical activity[8-10].

Although there are different studies dealing with sleep or constipation problems in CP[5-11], to the best of our knowledge, there was no study that addresses both together and associates them with functional independence levels. For this reason, the study aimed to examine the effect of functional independence level on sleep behavior and constipation severity in children with CP.

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METHODS

Study design

This cross-sectional observational single-center study was carried out in a special rehabilitation center in Istanbul between September 2021 and April 2022. The study was approved by the (*anonymized*) University Non-Interventional Researches Ethics Committee (2021/015) and was registered in Clinicaltrials.gov (*anonymized*). The method and purpose of the study were explained to the families who participated in the study, and the children of the families who volunteered to be included in the study were assessed. The written informed consent was obtained from all the parents/legal guardians.

Participants

In this study, 60 volunteered children diagnosed with CP, who had attended the rehabilitation center continuously and were followed by a physiotherapist, at the Private Duha Special Education and Rehabilitation Center in Istanbul were included. Inclusion criteria were those aged between 4 and 18 years with Gross Motor Function Classification System (GMFCS) III-IV-V functional independence levels. Those who had surgery concerning intestinal health, had a chronic infectious bowel disease, had congenital intestinal anomalies, had received botox treatment in the last 6 months, had uncontrolled epileptic seizures, had complained of constipation in the last 6 months, and had cardiopulmonary disease that would prevent exercise were excluded from the study.

Assessments

The assessment, including sociodemographic data, functional independence level, sleep problems level, and constipation severity, was performed with an interview technique. The GMFCS was observed and recorded.

The GMFCS for CP is defined as a five-level classification system, evaluating self-initiated movements with particular emphasis on sitting (trunk control) and walking. The primary criterion is that differences in motor function between the levels are clinically significant. The aim was to determine which level best represents the child's current abilities and limitations in motor function. Therefore, it focuses on the child's usual performance in home, school, and community settings. There is less focus on the quality of the movement or its potential for improvement. Level I includes children with less neuromotor impairment and the children walk without restriction. At Level II, children walk with restrictions; at Level III, children walk by holding on to a moving vehicle; at Level IV, children walk with limited self-mobility; and at Level V, children are transported in a manual wheelchair. As a result, as the level increases, the severity of the exposure increases[12]. As constipation and sleep problems are predicted to be more common in children with GMFM III-IV-V neuromotor disorders, it was determined as an inclusion criterion.

The WeeFIM is used to assess the functional independence of pediatric individuals. It consists of a total of 6 subtitles and 18 items: self-care, sphincter control, mobility, locomotion, communication, and social and cognitive domains. It has scores from 1 to 7 according to the supervision/help needed or the use of assistive devices to realize the situation specified in each area. The task is scored as 1, indicating complete dependence, and 7, indicating complete independence, promptness, and safety. As the score increases, there is an increase in the level of independence. A minimum of 18 points and a maximum of 126 points are taken. Notably, 18 points represent full dependency, and 126 points represent complete independence[13,14].

The PSQ assesses sleep-related problems in children aged 2–18 years and has three sections. Part A includes behaviors observed at night and bedtime and consists of 43 questions. Part B includes behaviors observed during the day and possible problems and consists of 23 questions. Finally, part C evaluates attention deficit and hyperactivity and consists of six questions. A high score on the scale indicates the presence of sleep problems[15,16].

The CSS is a concise, easy-to-use, reliable, and valid tool for assessing constipation severity and identifying subtypes of constipation. This scale contains questions about the frequency, intensity, and strain of defecation of individuals and consists of three parts: stool obstruction, large intestine laziness, and pain. The lowest score that can be obtained from the scale is 0 and the highest score is 73. High values indicate the severity of constipation[17,18].

Statistical analysis

It was calculated that the sample size should be 60 cases to have a medium-level correlation (r=0.400) target, 90% power, and 95% confidence level for our study[19].

The IBM Statistical Package for Social Sciences Version 24 (SPSS Inc., Chicago, IL, USA) statistical program was used for data analysis. Descriptive statistics (mean, standard deviation, and percentile) were given for categorical and continuous variables in the study. The normality was determined by the Shapiro-Wilk test. Pearson correlation analysis was used depending on the conformity of the data to the normal distribution. The statistical significance level was accepted as $p<0.05$.

RESULTS

A total of 60 children who were diagnosed with CP were included in the study. According to GMFCS, 46.7% of the cases were Level III, 35% were Level IV, and 18.3% were Level V. Subjects' demographic and clinical characteristics are presented in Table 1.

There was a negative moderate significant correlation between WeeFIM and PSQ and CSS (r=-0.342 to 0.303; p<0.05) (Table 2).

DISCUSSION

This study aimed to determine the relationship between functional independence level, level of sleep problems, and constipation severity in children with CP, and the results revealed that sleep and constipation symptom severities were lower in children with higher functional independence levels.

Related to the motor and neurological symptoms such as spasticity, epilepsy, muscle spasms, and pain, sleep disturbances are frequent in CP. Romeo et al., specified that none of these factors alone were associated with sleep disorders, but the risk of developing abnormal patterns of sleep significantly increased with their presence. Also, they identified that mental retardation and level 5 on the GMFCS were associated with sleep disturbances[20]. In another study, it was observed that higher gross motor function impairment was found to be correlated with disorders of initiating and maintaining sleep[5]. Consistent with the literature, we determined that sleep problems decreased as functional independence levels increased. Increasing functional independence levels encompasses multi-parameter improvements, and since it also includes parameters that are considered risk factors for sleep problems, it is thought that sleep problems also decrease as the functional independence levels increase.

Constipation is a common comorbidity related to both neurological and lifestyle factors in CP. Veugelers et al., determined that the prevalence of constipation was 57%, which was significantly higher among children with severe motor disabilities in their study group[6]. Similarly, in our study, it was found that there is a correlation between functional independence levels and constipation, indicating that constipation symptom severity decreases with the increase in functional independence levels. In CP, a higher functional independence level means a better level of mobility and a less degenerated neural mechanism that allows functionality. Therefore, considering that neural and lifestyle factors are effective in constipation, it is expected that constipation symptoms decrease as the functional independence level increases.

Possible limitations of this study include the fact that the participants were not questioned about their diet and sensory problems and the disparity in the number of participants in each GMFCS group. Studies on constipation and sleep assessments with larger and symmetrical sample sizes will contribute to the management of these problems in CP.

Table 1. Subjects' demographic and clinical characteristic parameters (n=60).

	Mean	(SD)
Age (years)	8.67	3.722
Weight (kg)	32.35	16.362
Height (cm)	127.07	21.541
WeeFIM	74.17	11.28
PSQ	28.63	3.77
CSS	50.17	6.35

WeeFIM: Functional Independence Measure for Children; PSQ: Pediatric Sleep Questionnaire; CSS: Constipation Severity Scale.

Table 2. Correlations between functional independence, sleep, and constipation assessment results.

	WeeFIM		
	r	(95%CI)	p
CSS	-0.342	-1.019 to -0.178	0.007*
PSQ	-0.303	-1.595 to -0.180	0.019*

WeeFIM: Functional Independence Measure for Children; CSS: Constipation Severity Scale; PSQ: Pediatric Sleep Questionnaire; r: Pearson analysis correlation coefficient; *statistically significant: p<0.05.

CONCLUSION

We described the relationship between functional independence and both constipation and sleep symptom severities. Lower functional independence level was related to worse sleep and constipation symptoms. The results suggest that effective strategies for developing functional independence levels may be beneficial for both constipation and sleep symptoms in the concept of CP management.

ETHICAL ASPECTS

The study was approved by the Hasan Kalyoncu University Non-Interventional Research Ethics Committee (2021/015) and complies with the provisions of the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

EIG: Data curation, Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review & editing. **AT**: Formal Analysis, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Ancestry and self-reported race in Brazilian breast cancer women

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SUMMARY

OBJECTIVE: This study aimed to evaluate the association between self-reported race/color and ancestry in Brazilian patients with breast cancer.

METHODS: This was an observational, transversal, epidemiological study, evaluating race and ancestry in 1,127 patients with breast cancer. For genetic ancestry, a 46-AIM-INDEL panel was used. The ancestral profile was evaluated with the Structure v.2.3.3 software. Descriptive statistics were performed. To assess differences between race and ancestry, an analysis of variance with Bonferoni adjustment was used.

RESULTS: The race distribution was 77.7% white, 17.6% brown, 4.1% black, 0.4% yellow, and 0.3% cafuse. The African ancestry proportion was significantly ($p<0.001$) more evident in black [0.63±0.21 (0.17–0.96)], followed by brown [0.25±0.16 (0.02–0.70)], and less frequent in white skin color. The European ancestry proportion was significantly ($p<0.001$) higher in white [0.72±0.17 (0.02–0.97)], followed by brown [0.57±0.19 (0.12–0.92)], yellow [0.27±0.31 (0.12–0.620], and black [0.24±0.19 (0.02–0.72)]. The Asiatic ancestry proportion is significantly ($p<0.001$) higher in yellow [0.48±0.51 (0.04–0.93)]. The Amerindian ancestry proportion frequency was the least frequent in all groups, and cafuse patients did not express differences between all race groups. The brown race group presented differences in African and European ancestry.

CONCLUSION: Although we found many similarities between white European ancestry, black African ancestry, and yellow Asian ancestry, there is great miscegenation between patients. Although they can be labeled as having one race, they do present many ancestral genes that would allow their inclusion in another race group.

KEYWORDS: Breast neoplasms. Epidemiology. Genetic variation. Pathology, molecular.

INTRODUCTION

The human species is considered to have been built over time, occurring at a time when there was a single continent. There were two main theories: one with a specific group of Hominids from Africa and a parallel evolution theory with multiple groups with admixture. Furthermore, our ancient human relatives spread around the globe, co-existing and admixing with other kinds of humans, affecting their DNA distribution. On the European/Asian continent, there were racial separations, i.e., the black race on the African continent, the white race in the North, and the yellow race in the East. The ancient DNA (aDNA) shows a history of humans rich in admixture between modern humans[1-3].

The relationship between ancestry and diseases is complex, involving age of disease onset, its relationship with reproductive capacity, treatment, and mortality rate. Also, external factors influence the main type of population mortality. From the 19th century until the middle of the 20th century, infectious diseases were the main determinants of mortality. The development of medicine and healthcare has led to further aging of the population, making cardiovascular diseases and cancer important factors associated with mortality.

The evolution of clinical and genomic knowledge has allowed us to better understand diseases, identifying subgroups of patients at greater risk for cancer development and specific molecular cancer subtypes[4]. Askenasi descendants are associated with elevated risk of BRCA mutation, breast cancer, and triple-negative tumors[5]. Black race was also associated with triple-negative tumors[6].

Also, the association between race and cancer is somewhat generic, as it involves family groups in which dietary, cultural, income, and education factors are present, influencing diseases, cancer, and the stage of cancer at diagnosis[3,7,8]. The development of ancestry markers has led to a better understanding of these factors, facilitating a better understanding of the relationship among race, ancestry, and patterns associated with neoplasia, i.e., molecular subtypes or cancer in young patients.

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The Brazilian population, due to its origin, is highly mixed, with great variation in the different regions with regard to race and ancestry[6,9]. Evaluating breast cancer, a TP53 mutation was observed in the southern and southeastern regions of Brazil[10]. A previous study evaluated the relationship among age, geographical region, and ancestry and the molecular subtypes of breast cancer[6,11]. Here, negative subtype was reported to be more frequent in white women, whereas triple-negative subtype was more frequent in nonwhite women[12]. Although there is a relationship between ancestry and self-reported race, they do not represent the same condition, justifying a study evaluating these two conditions in breast cancer patients. The objective of this study was to evaluate the self-referred color in relation to genetic ancestry in patients with breast cancer in Brazil.

METHODS

The evaluation of ancestry and Brazilian breast cancer patients was previously reported[11]. Now, we performed a subgroup analysis related to self-referred race/color (SRRC). This study was approved by the local ethics committee under number 1136/2016.

In summary, this was an observational, cross-sectional, epidemiological study. From 1,312 patients in the Biobank, the molecular subtype was identified in 1,282 patients, and DNA extraction was possible in 1,194 patients. Due to the databank and DNA recovery, we used DNA from 21 formalin-fixed paraffin-embedded and 1,194 buff coat samples. The final databank included 1,215 patients. Although DNA was extracted from 1,215 patients, the assessment of genetic ancestry was possible only in 1,127 patients.

We evaluated patients who were diagnosed between April 2000 and June 2018. The main inclusion criteria for the study were as follows: (1) invasive breast carcinoma; (2) female sex; (3) molecular subtype; (4) self-referred ethnicity; (5) born in one of five Brazilian regions; (6) DNA extraction; and (7) the presence of fragments of DNA ≥230 bp, a size that allowed us to evaluate ancestry.

Each patient described its SRRC as defined by Instituto Brasileiro de Geografia e Estatística (IBGE)[13]. We considered five groups of ethnicity/color: white ("branca"), black ("preta"), yellow ("amarela"), brown ("parda"), and indigenous ("indígena"). Briefly, brown ethnicity represents mixed color (white and black). Yellow was considered to refer to an individual who self-declared as being of Japanese, Chinese, or Korean origin, all representing Asian origin. The term Cafuzo represents ancestry admixture of the African with the Indigenous, and based on IBGE information, Cafuzo[13] is considered brown color. Due to its indigenous origin, we considered Cafuzo to have indigenous ancestry.

DNA provided by the Barretos Cancer Hospital Biobank was extracted to evaluate genetic ancestry. For genetic ancestry, a 46-AIM-INDEL panel was used, and the PCR products were subjected to capillary electrophoresis.

Four types of ancestry were considered: European, African, Amerindian, or Asian. The genetic ancestry of the patients was determined using ancestry-informative markers (AIMs), as previously reported[14-17]. Briefly, 46 small insertion-deletion (INDEL) polymorphisms were ascertained to maximize the divergence between four major human population groups: European (EUR), African (AFR), Asian (ASN), and Amerindian (AME). These markers were selected due to their high allele frequency divergence between different ancestral or geographically distant populations, including more than 1,000 individuals from 40 reference populations from the Human Genome Diversity Project (HGDP)-Centre d/Etude du Polymorphisme (CEPH) plus individuals from Angola, Portugal, Taiwan, and indigenous Brazil, which allowed us to establish the ancestral proportions in high admixture individuals and populations, such as the Brazilian population[18,19]. Ancestral profiles were evaluated using the Structure v.2.3.3 software[11].

We performed descriptive statistics [mean±standard deviation (minimum–maximum)] of ancestry contribution in different SRRCs (Table 1), expressed in Figure 1.

Analysis of variance (ANOVA) (Table 1) and Bonferroni's adjustment test (Table 2) were used to assess differences among groups. $p<0.05$ was considered significantly different. IBM SPSS® for Mac® version 20 was used for statistical analyses. STROBE checklist was used in this project[11].

RESULTS

A total of 1,127 patients were evaluated. SRRC was distributed as follows: 77.7% (876) white, 17.6% (198) brown, 4.1% (46) black, 0.4% (4) yellow, and 0.3% (3) cafuse. Evaluating the influence of ancestry in relation to SRRC, we observed (Table 1 and Figure 1) that European ancestry was observed more frequently among women who self-referred as white, brown, and cafuso; African ancestry was more frequent among women who self-referred as black and brown; Asian ancestry was more frequent among women who self-referred as yellow; and Amerindian ancestry was more frequent among women who self-referred as brown.

Genetically, the African ancestry proportion was significantly ($p<0.001$) more evident among women who self-referred as black [0.63±0.21 (0.17–0.96)], followed by brown [0.25±0.16 (0.02–0.70)], and less frequently white. The European ancestry proportion was significantly ($p<0.001$) higher among

Table 1. Percentage of self-referred color in different ancestry.

Ancestry	Mean	Standard deviation	Median	CI (5–95%)	CI (25–75%)	Minimum–maximum	ANOVA p-value
African							
Black	0.63	0.21	0.65	0.57–0.70	0.46–0.85	0.17–0.96	<0.001
Brown	0.25	0.16	0.23	0.23–0.27	0.12–0.37	0.02–0.70	
Yellow	0.15	0.17	0.15	0.01–0.31	0.01–0.31	0.01–0.32	
White	0.11	0.12	0.07	0.11–0.12	0.03–0.17	0.01–0.75	
Cafuse	0.02	0.01	0.02	0.01–0.02	0.01–0.02	0.01–0.03	
European							
Cafuse	0.89	0.06	0.88	0.84–0.88	0.84–0.88	0.84–0.95	<0.001
White	0.72	0.17	0.80	0.74–0.77	0.67–0.88	0.02–0.97	
Brown	0.57	0.19	0.60	0.55–0.60	0.44–0.73	0.12–0.92	
Yellow	0.27	0.31	0.24	0.01–0.58	0.01–0.58	0.12–0.62	
Black	0.24	0.19	0.19	0.18–0.30	0.06–0.36	0.02–0.72	
Asiatic							
Yellow	0.48	0.51	0.49	0.04–0.93	0.04–0.93	0.04–0.93	<0.001
Brown	0.07	0.08	0.05	0.06–0.09	0.03–0.09	0.01–0.86	
Black	0.07	0.06	0.05	0.05–0.09	0.03–0.09	0.01–0.27	
White	0.06	0.10	0.04	0.06–0.07	0.03–0.06	0.01–0.94	
Cafuse	0.05	0.03	0.05	0.02–0.05	0.02–0.05	0.02–0.08	
Amerindian							
Brown	0.10	0.10	0.07	0.84–0.11	0.03–0.08	0.01–0.62	<0.001
Yellow	0.08	0.07	0.05	0.04–0.15	0.04–0.15	0.04–0.18	
White	0.07	0.07	0.04	0.06–0.07	0.03–0.08	0.01–0.56	
Black	0.05	0.04	0.05	0.41–0.65	0.03–0.06	0.01–0.19	
Cafuse	0.04	0.02	0.05	0.03–0.05	0.02–0.05	0.02–0.05	

ANOVA evaluating group differences. CI: confidence interval.

women who self-referred as white [0.72±0.17 (0.02–0.97)], followed by brown [0.57±0.19 (0.12–0.92)], yellow [0.27±0.31 (0.12–0.620)], and black [0.24±0.19 (0.02–0.72)]. The Asian ancestry proportion was significantly (p<0.001) higher among women who self-referred as yellow, with few differences among the other groups. Finally, the Amerindian ancestry proportion frequency was the least frequent among all racial groups, and cafuso individuals did not self-refer as any racial group. Women who self-referred as brown presented differences in African and European ancestry.

DISCUSSION

In the variation in the human genome, there are short tandem repeat sequences and single-nucleotide polymorphisms (SNPs), but there are also sequences of variations in lineage markers and structural variations. In the analysis of human ancestry, genetic polymorphism is a factor of fundamental importance. AIMs represent a genomic sequence. In forensic medicine[20], SNPs are more frequently assessed, but small insertion-deletion (INDEL) polymorphisms are also examined; in the present study, INDELs were used to evaluate ancestry and have been described in previous studies related to the general Brazilian population[9] and cancer[11,15,21].

In the molecular era, it is important to have single, reproductive references using inexpensive classifications. INDEL evaluation needs technology and is associated with representative costs. The same condition occurs in molecular classification of breast cancer, in which immunohistochemistry simplifies breast subtype classification for clinical use[22,23]. SRRC has an association with ancestry, but they are not the same condition, and this study reported this condition in breast cancer patients.

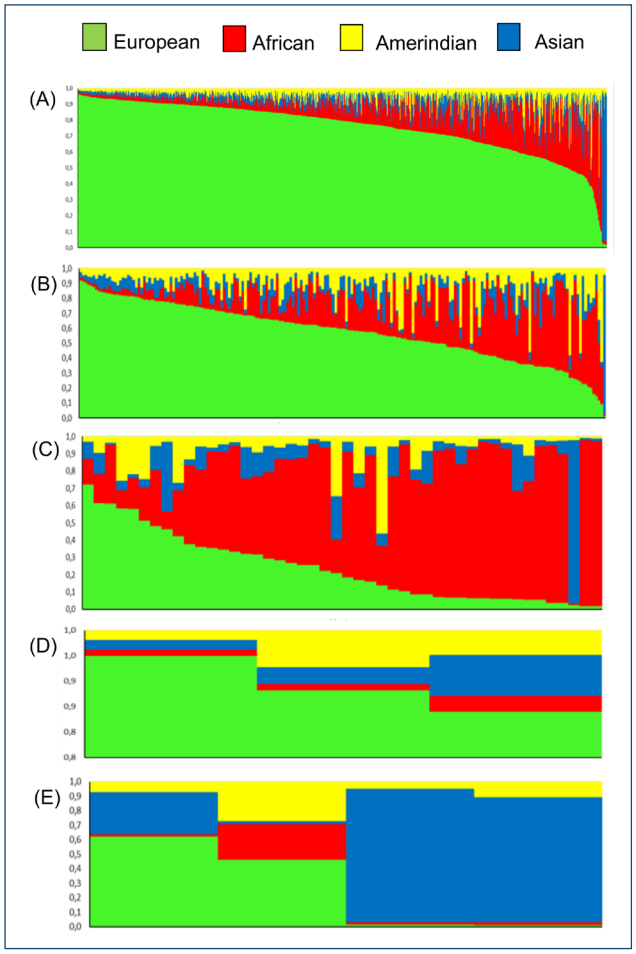

Figure 1. Representation of genetic admixture between ancestry groups in relation to self-reported race/color. (A) White; (B) brown; (C) black; (D) Amerindian; and (E) Asian. Chart line: horizontal: individuals; vertical: genetic ancestry (%).

Table 2. ANOVA with Bonferroni adjustment for correction evaluating multiple comparisons between ancestry and race/color.

Ancestry	White	Brown	Black	Yellow	Cafuse
African					
White	–	<0.001	<0.001	0.966	0.728
Brown	<0.001	–	<0.001	0.628	0.222
Black	<0.001	<0.001	–	<0.001	<0.001
Yellow	0.966	0.628	<0.001	–	0.648
Cafuse	0.728	0.022	<0.001	0.648	–
European					
White	–	<0.001	<0.001	<0.001	0.655
Brown	<0.001	–	<0.001	0.007	0.018
Black	<0.001	<0.001	–	0.993	<0.001
Yellow	<0.001	0.007	0.993	–	<0.001
Cafuse	0.665	0.018	<0.001	<0.001	–
Asiatic					
White	–	0.647	0.986	<0.001	0.999
Brown	0.647	–	1.000	<0.001	0.993
Black	0.986	1.000	–	<0.001	0.996
Yellow	<0.001	<0.001	<0.001	–	<0.001
Cafuse	0.999	0.993	0.996	<0.001	–
Amerindian					
White	–	<0.001	0.770	0.997	0.966
Brown	<0.001	–	0.002	0.984	0.632
Black	0.770	0.002	–	0.965	0.997
Yellow	0.997	0.984	0.965	–	0.954
Cafuse	0.966	0.632	0.997	0.954	–

In Brazil, according to the IBGE, there are four main national races/skin colors. In IBGE surveys, race is self-reported by respondents. The 2022 National Household Sample Survey (PNAD)[24] indicated that the Brazilian population is divided into white (42.8%), brown (45.3%), black (10.6%), and others. In this study, personal ethnicity was self-reported. Miscegenation leads to biogeographic ancestry[25], determining a range of changes and differences in physical structure, such as pigmentation of the skin, hair, and eyes; height; hair type; and nasal and lip formation. Skin color is a difference that is simplistic and subject to potential bias. Thus, using SRRC, we sought to evaluate the relationship between it and ancestry in a subgroup of patients with breast cancer.

The patients used in this study were identified through a database of women with breast cancer, with race being secondary information in the initial study[11]. This study reports the characteristics of population, ancestry variables (reported as continuous or categorical), and main associations about ancestry, geographical region, and molecular subtype. More information can be obtained by evaluating the other article[11] and supplementary file[11], which represent 10 tables and 6 figures. In this article, we performed a subgroup analysis evaluating exclusively the relationship between ancestry and self-reported color in Brazilian women with breast cancer. We repeated some information in the Methods section, but as the objective was different, we took care not to perform plagiarism and opted to show exclusively information associated with the objective of this publication. Repeated information is allowed only in the Methods section and is not considered plagiarism.

We observed that ancestry and race represent different conditions and a great admixture in Brazilian women. Self-reported black patients have high African genetic ancestry (0.63±0.21 SD) and low European genetic ancestry

(0.24±0.19 SD). Self-reported white patients have high European genetic ancestry (0.72±0.17 SD) and low European genetic ancestry (0.11±0.12 SD). Self-reported brown patients have intermediary differences with high European genetic ancestry (0.57±0.19 SD) and intermediary African genetic ancestry (0.25±0.16 SD).

The context of race and the onset of cancer involves many dimensions because the onset of cancer is influenced not only by genetic factors but also by the environment and cultural and dietary habits[8]. Likewise, staging at diagnosis is influenced by factors related to the health system and the availability of individual or public resources, schooling and education regarding the need to perform exams regularly, and attitude toward undergoing exams or seeking health professionals, factors that are associated with advanced staging in more vulnerable populations and that are accentuated in historically disadvantaged races[7]. The relationship between racial disparities in social and historical contexts should increasingly be discussed to better understand the role of racial hierarchy in access to and dependence on public health[26]. The discussion about race is of fundamental importance, as it is associated with prejudices and attitudes related to racial subgroups[27,28]. To minimize some historical differences, in Brazil, the Quota Law[29] was created to determine inclusion in higher education and in public service exams, helping racial subgroups, mainly black, mixed races, and indigenous people.

Evaluating breast cancer in the United States, there is discussion about racial/ethnic disparities, incidence, and mortality rates linked to hereditary factors, risk factors, treatment, and health disparities[30]. The same occurs in Brazil, where differences in mortality may indicate inequities in access to diagnosis and treatment[31], but other pathological factors may be associated[6]. To improve this discussion, we also observed a large percentage of admixture in all races. Based on the data presented here, there are interesting contexts that can be considered in clinical practice. Our data were obtained from patients with breast cancer from the five Brazilian regions, yielding an SRRC distribution of 77.9% white, 17.4% mixed race, 4.1% black, 0.3% yellow, and 0.2% cafuso, percentages that are different from those obtained by the PNAD. In our data, there is potential bias as two regions are numerically underrepresented, reflecting the representativeness bias of the institutional Biobank. We initially sought to increase the sample from these regions by inviting other professors from these regions who could contribute patients, but the response was negative. Despite these limitations, this study investigated SRRC race and ancestry using a convenience sample.

Evaluating the results related to ancestry, something apparently obvious was numerically proven. Regardless of the SRRC, there was significant miscegenation among the groups and among all races. Evaluating European ancestry, a greater proportion of this ancestry was observed among women who self-referred as white, significantly different from that observed among women who self-referred as brown, black, and yellow, and in this sample, cafusos were predominantly of European ancestry. When assessing African ancestry, a high frequency was observed among women who self-referred as black, but a difference was observed among all groups. Asian ancestry was more frequent among women who self-referred as yellow, with a difference among all groups. The frequency of Amerindian ancestry was similar among race groups, with the exception of women who self-referred as yellow.

The mixed race is intermediate between the white and black races. The mixed race had a high rate of European and African ancestry, with equivalent ancestry percentages among women who self-referred as white and black. When evaluating the graphs and Table 1, although 50% percentiles do not overlap, there are women of high African ancestry and low European ancestry with ancestry percentages similar to those for black women; likewise, they present high European ancestry and similarities with African ancestry only in percentiles above 50%. This can be explained by the phenotype, which raises questions about the use of self-reported color for this race, where these individuals can be considered white and/or black depending on skin tone, which may influence results related to race and which may be influenced by the type of evaluator and the place where the individual lives.

Among black women, there was high African ancestry but not 100%; in this group, there is a considerable frequency of European ancestry and a small frequency of yellow and Amerindian races. Women considered to be black have high European miscegenation. Similar to white women, they presented a moderate rate of African miscegenation. The line between color and race is very thin, which should make any derogatory racial discussion unfeasible[27], especially in Brazil, where there is high miscegenation.

As limitations of the study, there was an unequal distribution of participants from all geographical regions in Brazil, and only female patients with breast cancer were included in the study. In ideal conditions, both sexes would be included, with equal distribution among all regions. A convenience sample was used in this study, and therefore future studies with more comprehensive samples are needed. This is one of the first studies to address ancestry and race/color in patients with breast cancer in Brazil and can thus serve as a basis for future comparisons.

CONCLUSION

We observed that there is great miscegenation between patients, and although they can be labeled as having one color, they do present many ancestral genes that would allow their inclusion in another race group. Care should be taken when evaluating race/color, as its conditions do not represent the same ancestry.

AUTHORS' CONTRIBUTIONS

RACV: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **DS'A:** Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **ACL:** Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **RMR:** Conceptualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Fetal vascular malperfusion score is linked with developing preeclampsia in women with gestational diabetes mellitus: a retrospective cohort study

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SUMMARY

OBJECTIVE: Fetal vascular malperfusion is associated with poor perinatal outcomes in women with preeclampsia and gestational diabetes mellitus. The aim of this study was to determine the association between fetal vascular malperfusion score and syncytiotrophoblast basement membrane thickness and clinicopathological variables, such as developing preeclampsia in women with gestational diabetes mellitus.

METHODS: This retrospective cohort study included 65 pregnant participants (34 with gestational diabetes mellitus and 31 controls) between January 2019 and January 2022. Gestational diabetes mellitus was diagnosed as ≥2 of 4 elevated values on a 3-h, 100-g oral glucose tolerance test. The fetal vascular malperfusion score was evaluated by endothelial CD34 positivity in the villous stroma of the placenta. The association between fetal vascular malperfusion score and syncytiotrophoblast basement membrane thickness with clinicopathological variables in women with gestational diabetes mellitus was evaluated.

RESULTS: It was revealed that the gestational diabetes mellitus group had greater fetal vascular malperfusion scores than the control group (gestational diabetes mellitus group fetal vascular malperfusion score: 34.2±9.1 and control group fetal vascular malperfusion score: 26.5±8.7, respectively, p=0.0009). Syncytiotrophoblast basement membrane thickness was correlated with the development of preeclampsia, trophoblast proliferation, and fetal vascular malperfusions (0.3952, p=0.0129; 0.3487, p=0.0211; and 0.4331, p=0.0082, respectively). On the contrary, fetal vascular malperfusions were correlated with the development of preeclampsia, villous edema, and trophoblast proliferation (0.3154, p=0.0343; 0.2922, p=0.4123; and 0.3142, p=0.0355, respectively).

CONCLUSION: The gestational diabetes mellitus group displayed significantly higher fetal vascular malperfusion scores and thickening of the syncytiotrophoblast basement membrane than the control group. There is a correlation between developing preeclampsia and the fetal vascular malperfusion scores and the syncytiotrophoblast basement membrane thickness.

KEYWORDS: Diabetes, gestational. Chorionic villi. Antigens, CD34. Pre-eclampsia.

INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized as impaired glucose tolerance or overt diabetes occurring during pregnancy[1,2]. The prevalence of GDM varies worldwide (2–38%) among racial and ethnic groups, and recently, it has been increasing gradually owing to advanced maternal age and obesity outbreaks[3,4].

The human placenta serves as a temporal organ that might be considered a two-way mirror reflecting the metabolic status of both mother and fetus; therefore, it might be used to denote metabolic dysregulation during pregnancy, such as GDM[5,6]. Hyperglycemia is an essential factor in the formation of histopathological alterations[5]. Maternal hyperglycemia might lead to alterations in the placental structure and function that compromise fetal development, with an increased risk of perinatal morbidity and mortality. The degree to which the maternal plasma level of glucose promotes placental alterations has yet to be unveiled[6].

Recent studies have revealed that GDM is associated with histopathological alterations, including increased placental thickness and weight, perivillous fibrin deposits, villous immaturity and edema, cytotrophoblastic hyperplasia, and thickening of the syncytiotrophoblast basement membrane[7,8]. The villous immaturity leads to an excessive gap between the intervillous space and fetal vasculature that endangers maternal–fetal oxygen transport[9]. The transporting unit in the human placenta is the syncytiotrophoblast membrane, which facilitates glucose transport across the placenta. It is hypothesized that the basement membrane of the syncytiotrophoblast is the rate-limiting step in glucose transport[10].

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The most prominent histopathological characteristic of fetal vascular malperfusion (FVM) is the loss of vasculature in chorionic villi, which can be detected readily with hematoxylin and eosin (H&E) staining at the later stages of FVM. The utilization of CD34 immunostaining for detecting the early stages of FVM has just come into the pathological practice to overcome the inefficiencies of H&E staining[11]. The overidentification of FVM based on CD34 immunostaining in the lobular villous vasculature and endothelium empowers the correlation of FVM with umbilical cord compromise and stillbirth, as well as poor perinatal outcomes in maternal preeclampsia, maternal diabetes mellitus, and intrauterine growth restriction (IUGR)[11-13]. However, it can be appreciated in the normal population in the short term[14]. In light of all the facts mentioned above, identifying FVM has the utmost importance in the histopathogenesis of GDM and its correlation with perinatal outcomes.

This study aimed to determine the association between the FVM score and syncytiotrophoblast basement membrane thickness with clinicopathological variables, such as developing preeclampsia in women with GDM.

METHODS

Ethical statement

This study was held in parallel with the Helsinki Committee's essentials. Ethical approval was obtained from the Ethics Committee of Balikesir University with the approval number 2021-195, and this retrospective cohort study included 65 participants between January 2019 and January 2022.

Study design

Women with singleton pregnancies underwent a two-step approach to detecting GDM, and they were followed until delivery. Women with singleton pregnancies were screened with a 1-h 50-g glucose challenge test (GCT) from the 24th to 28th weeks of pregnancy. Women with positive GCT results (glucose ≥140 mg/dL) proceeded to a diagnostic 3-h, 100-g oral glucose tolerance test (OGTT). Women with negative GCT results were included in the control group. Women with two or more elevated values on a 3-h, 100-g OGTT based on Carpenter and Coustan criteria[1] were included in the GDM group.

Based on these results, 65 age-matched women participated in the study in either the GDM (n=34) or the control group (n=31), and their placental specimens were retrieved after delivery. Women with a history of hypertension, pregestational diabetes, multiple pregnancies, intrauterine infections, and fetal anomalies were excluded from the study.

Immunohistochemistry and fetal vascular malperfusion score evaluation

Standardized tissue preparation protocols were followed during the histopathological examination of the placentas, as in the literature[15]. Afterward, anti-human monoclonal CD34 antibody (anti-CD34 ab, Abcam, Cambridge, MA) was applied to the slides, and the tissue extracts were rinsed again with phosphate-buffered saline (PBS), followed by staining with H&E and periodic acid–Schiff (PAS) to describe the placental alterations.

Villous immaturity is defined as the combination of reduced terminal villous surface area, irregular villous contour, syncytial knots, villous edema, fibrin deposition, trophoblast proliferation, and increased layer thickness. It was evaluated under light microscopy with H&E and PAS staining. The addition of an anti-CD34 antibody, which is primarily used to empower the diagnosis of FVM, is a valuable marker for highlighting the villous vasculature and endothelium (Figure 1)[10]. The pictures of three randomly selected areas of the terminal villi (40×) were analyzed by an image processing system (ImageJ open access program from the National Institute of Health). The pictures were uploaded to the program and then converted into 8-bit images. Afterward, the CD34 staining intensity was evaluated by adding area fractions, which correspond to the FVM scores.

Statistical analysis

Statistical and power analyses of this study were performed with the open-source Jamovi statistical software (version 2.3.21) and G*Power software (version 3.1.9.7). According to the literature, the minimum sample size was calculated as 36 per group based on α error: 0.001, power: 0.95, and effect size d: 1. The distribution and homogeneity of groups were evaluated by skewness, kurtosis, Levene's test, and Kolmogorov-Smirnov test. The variables between the study groups were compared using

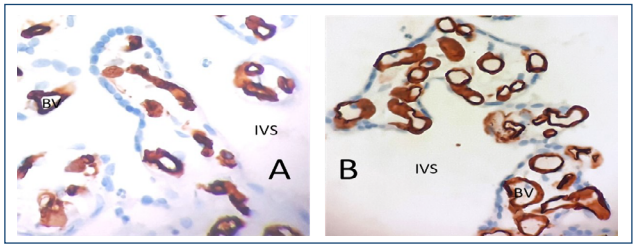

Figure 1. CD34 immunostaining of terminal villi in the control and the gestational diabetes mellitus groups. (A) Weak CD34 (+) immunostaining in the control group (low fetal vascular malperfusion score) (40×). (B) Strong CD34 (+) immunostaining in the gestational diabetes mellitus group (high fetal vascular malperfusion score) (40×). FVM: fetal vascular malperfusion; BV: blood vessel; IVS: intervillous space.

the Mann-Whitney U and chi-squared tests. Spearman's correlation analysis was performed on the variables. Statistical significance was determined as $p<0.05$.

RESULTS

Placental tissues were retrieved from 65 women (n=34 for the GDM group and n=31 for the control group=), and there were no significant differences in the context of age, parity, gestational age, developing preeclampsia, fasting blood glucose level, fetal macrosomia, neonatal hypoglycemia, or fetal birth weight between the study groups. The clinical features of the study groups are summarized in Table 1.

The morphological assessment concluded that there were no differences between the study groups regarding placental weight. The placental tissue of the GDM group displayed significantly higher villous immaturity, trophoblastic cell proliferation, and thickening of the syncytiotrophoblast basement membrane compared with the control group (p=0.0002, p=0.0126, and p=0.0002, respectively) (Table 2).

However, there were no statistical differences between the study groups in the context of villous edema or fibrin thrombus in placental tissue (p= 0.6430 and p= 0.7685, respectively). It was revealed that the GDM group had higher FVM scores than the control group (34.2±9.1 versus 26.5±8.7, respectively, p=0.0009) (Table 2).

Regarding the association between pathological alterations of the placenta and GDM group variables, the syncytiotrophoblast basement membrane thicknesses were correlated with developing preeclampsia, trophoblast proliferation, and FVM scores (ρ=0.395, p=0.01; ρ=0.348, p=0.02; and ρ=0.433, p=0.008, respectively). On the contrary, FVM scores were correlated with developing preeclampsia, villous edema, and trophoblast proliferation (ρ=0.315, p=0.03; ρ=0.292, p=0.41; and ρ=0.314, p=0.03, respectively) (Table 3).

Table 1. The clinical features of the study groups.

Participants' characteristics	GDM (n=34)	Control (n=31)	p-value
Age (years), median	30.5 (24–40)	29 (22–42)	0.47
Parity (n)	1 (0–3)	1 (0–4)	0.91
Fasting blood glucose (mg/dl)	88.5 (68–121)	76 (67–94)	0.09
Gestational age (weeks)	38 weeks+3 days	38 weeks+2 days	0.32
Fetal macrosomia[a], n (%)	6 (17%)	2 (6%)	0.17
Neonatal hypoglycemia[b], n (%)	3 (8%)	0	0.09
Developing preeclampsia[c], n (%)	3 (8%)	0	0.09
Fetal weight (g)	3,390 (2,450–5,150)	3,320 (2,850–4,210)	0.43

SD: standard deviation; GDM: gestational diabetes mellitus. Age and fetal weight are expressed as mean±SD. [a]Fetal macrosomia is defined as birth weight >4,000 g[16]. [b]According to the AAP Neonatal Hypoglycemia Guideline[17]. [c]According to the ACOG Preeclampsia Guideline 2020[18].

Table 2. Pathological features of placentas in the study groups.

Participants' characteristics	GDM (n=34)	Control (n=31)	p-value
Placental weight (g)	538 (365–750)	489 (398–577)	0.23
Umbilical cord insertion			
Central	30	28	0.13
Marginal	4	3	0.18
Villous immaturity	33/34 (97%)	18/31 (58%)	**0.0002*****
Villous edema	7 (21%)	5 (16%)	0.64
Fibrin thrombus	11 (32%)	9 (29%)	0.76
Trophoblast proliferation	30/34 (88%)	19/31 (61%)	**0.01***
Trophoblast BM thickness	27/34 (79%)	10/31 (32%)	**0.0002*****
FVM score	34.2±9.1	26.5±8.7	**0.0009*****

*p<0.05, ***p<0.001. Placental weight and FVM score are expressed as mean±SD. SD: standard deviation; GDM: gestational diabetes mellitus; BM: basement membrane; FVM: fetal vascular malperfusion. Bold indicates statistically significant values.

Table 3. Correlation of fetal vascular malperfusion score and trophoblast basement membrane thickness with gestational diabetes mellitus group variables.

GDM group	FVM score		Trophoblast BM thickness	
Variables	**ρ-coefficient**	**p-value**	**ρ-coefficient**	**p-value**
Fasting blood glucose	0.202	0.09	0.124	0.21
Neonatal hypoglycemia[a]	0.123	0.36	0.115	0.31
Preeclampsia[b]	**0.315**	**0.03***	**0.395**	**0.01***
Fetal weight	0.154	0.12	0.214	0.06
Villous immaturity	0.104	0.19	0.042	0.62
Villous edema	**0.292**	**0.41**	0.151	0.11
Fibrin thrombus	0.185	0.31	0.119	0.30
Trophoblast proliferation	**0.314**	**0.03***	**0.348**	**0.02***
FVM score			**0.433**	**0.008****

*p<0.05, **p<0.01. BM: basement membrane; FVM: fetal vascular malperfusion. [a]According to the AAP Neonatal Hypoglycemia Guideline[16]. [b]According to the ACOG Preeclampsia Guideline 2020[17]. Bold indicates statistically significant values.

DISCUSSION

Gestational diabetes mellitus is one of the most challenging endocrine disorders diagnosed during pregnancy, and it has been related to a considerably high incidence of complications such as fetal macrosomia, preeclampsia, and fetal growth restriction[19]. Even if it stayed under the statistical significance, we noticed that the GDM group had a higher incidence of fetal macrosomia, neonatal hypoglycemia, and developing preeclampsia than the control group.

Gestational diabetes mellitus is associated with the alterations in placental function and villous structure, as correlated with maternal hyperglycemia[5,20]. In line with the literature, we revealed that placental alterations, including increased villous immaturity, cytotrophoblastic hyperplasia, and thickening of the syncytiotrophoblast basement membrane, were more frequent in the GDM group [7,8,21,22]. Moreover, syncytiotrophoblast basement membrane thickness was correlated with the development of preeclampsia and FVM scores. The thickening of the syncytiotrophoblast basement membrane is a frequent histopathological alteration in GDM. It is accompanied by villous immaturity, with diminished total surface area of terminal villi and in number[5,14,23]. These alterations jeopardize maternal–fetal oxygen and nutrient transport and ultimately cause fetal macrosomia, preeclampsia, and intrauterine fetal growth restriction[9].

Fetal vascular malperfusion (formerly known as fetal thrombotic vasculopathy) is a new term and is related to the prominent chronic hypoxic placental injury that can be linked with an increased risk of perinatal morbidity and mortality[24,25]. In this study, FVM scores were correlated with the development of preeclampsia and pathological alterations of the placenta, such as villous edema and trophoblastic hyperplasia, found in the literature[23-25]. Even though the mechanism of FVM is unclear, it has been revealed that maternal hyperglycemia is the main perpetrator in the pathogenesis of endothelial cell injury in fetal vessels via oxidative stress and inflammation, which causes thrombosis and endothelial cell loss in women with GDM[24,25].

The limitations of this study need to be acknowledged. First, the low number of placental tissues could be a barrier to generalizing the study results. Second, the heterogeneity of patients with GDM in pregestational weight, body mass index (BMI), and gestational weight gain might be confounding factors for FVM scores. Third, the retrospective cohort studies provide a level 3 grade of evidence.

CONCLUSION

This study revealed that the GDM group demonstrated significantly higher villous immaturity, trophoblastic hyperplasia, FVM score, and thickening of the syncytiotrophoblast basement membrane. Additionally, syncytiotrophoblast basement membrane thickness and FVM scores were correlated with developing preeclampsia and trophoblast proliferation.

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ETHICAL STATEMENT

This study was held in parallel with the Helsinki Committee's essentials. Ethical approval was obtained from the Ethics Committee of Balikesir University with the approval number 2021-195, and this retrospective cohort study included 65 participants between January 2019 and January 2022.

AUTHORS' CONTRIBUTIONS

SA: Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **GT:** Conceptualization, Validation, Visualization, Writing – original draft. **AY:** Investigation, Methodology, Supervision. **CSU:** Investigation, Validation, Visualization, Writing – original draft. **AU:** Validation, Visualization, Writing – original draft.

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ORIGINAL ARTICLE

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Effect of comfort theory-based nursing care on pain and comfort in women undergoing hysterosalpingography: a randomized controlled trial

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SUMMARY

OBJECTIVE: This study aims to examine the effect of comfort theory-based nursing care on pain and comfort in women undergoing hysterosalpingography.

METHODS: This randomized control trial was conducted on 126 women (42 in each intervention and control group). Virtual reality glasses group (n=42), mobile-assisted education group (n=42), and control group (n=42). The control group received only routine care. Comfort levels were evaluated at the beginning and end of the study using the General Comfort Scale and pain levels evaluated at the beginning and end of the study using the Visual Analog Scale.

RESULTS: The comfort theory-based nursing care (virtual reality glasses and mobile-assisted education group) was effective in increasing women's comfort with painful invasive procedures such as hysterosalpingography and reducing pain.

CONCLUSION: It is recommended that a nurse be present in the hysterosalpingography process, providing nursing care services continuously and introducing this program to working nurses.

Clinical Trial Registration Number: NCT04676932.

KEYWORDS: Comfort. Hysterosalpingography. Nursing care. Pain. Virtual reality.

INTRODUCTION

Hysterosalpingography (HSG), a minimally invasive treatment, is one of the diagnostic methods for infertility[1-4]. HSG is often uncomfortable and leads to discomfort[5]. In addition to pain and discomfort, women may experience other symptoms during HSG, such as nausea, vomiting, fainting, and increase in body temperature. HSG is usually performed in outpatient treatment units, and it is extremely important to apply physical and emotional nursing care that covers the perioperative processes and maintains patient comfort[5-7].

In this study, the authors focus on the effect of comfort theory-based nursing care (CT_bNC) on pain and comfort in women undergoing HSG. Important aspects of this theory are that comfort is holistic and should not be confused with pain as it involves more than physical discomfort or suffering. Valid measures of the effectiveness of holistic interventions are needed to improve nursing practice. With the increased interest in holistic interventions that target responses in the context of human experience (i.e., physical, psychospiritual, social, and environmental), holistic measures that are multidimensional and entail many interrelated parts are essential for understanding effects on an indivisible whole[8] (Table 1). The goal of interventions (e.g., guided imagery, massage therapy, and therapeutic touch) is that many desirable changes will be experienced simultaneously by recipients. These changes might include increased relaxation, positive thinking, well-being, and contentment. Virtual reality glasses (VRG) is one of the imaginary methods used to reduce pain. The reason for using mobile-assisted education (MAEC) is to raise awareness among individuals and clarify the issues they do not understand.

The literature appears that a focal point in studies was additional interventions (video, training and counseling, virtual reality glasses (VRG), listening to music, etc.) during the HSG procedure have a reducing effect on pain or anxiety[2,9-11]. However, pain, privacy concerns, not having information about the procedure, having to face a problem such as infertility, environmental conditions such as sound, smell, and images, and relational dimensions such as not being with a partner or a supportive person during HSG are also characterized by deterioration in comfort[11,12].

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Table 1. Comfort theory-based nursing care.

Comfort	Levels		
Dimensions	**Relief**	**Ease**	**Transcendence**
Physical	-Women stating that the pain that may occur in the perineum area and abdomen due to hysterosalpingography is low on the Visual Analogue Scale -Vital signs being within the normal range -Ensuring effective breathing -Fasting before the procedure -Ensuring elimination before the procedure -Placing the patient in a comfortable position during the procedure -Coping with post-procedure risks (bleeding, nausea, vomiting risk, infection risk, tissue integrity deterioration, etc.) -Ensuring adequate rest	-Less pain due to hysterosalpingography, ensuring that the patient can stay calm and peaceful during the procedure -Providing counseling to the mobile-assisted education group on hysterosalpingography -Allowing the virtual reality glasses group to experience less pain by diverting attention	-Increasing comfort level with nursing care based on comfort theory
Psychospiritual	-Ensuring that women can easily express their pain and fear, -Supporting participation in decisions -Informing patients -Helping women to prepare for the procedure -Showing women proper places to put their clothes -Helping women sit in the proper place where the procedure will be performed -Supporting the woman to sit down -Respecting patient decisions before and during the procedure -Treating the patients diligently before and during the procedure -Allowing patients to worship in accordance with their beliefs during the procedure -Supporting the sense of achievement after the procedure	-Providing counseling on the results of treatment for the mobile-assisted education group and providing relief during the hysterosalpingography procedure	-Increasing comfort level with nursing care based on comfort theory
Environmental	-Ensuring that the ambient temperature does not fall below 20 degrees and does not rise above 27 degrees -Ensuring that noise in the environment is kept to a minimum -Ensuring privacy -Showing nature videos to patients	-Delivery of the training booklet for the mobile-assisted education group and ensuring relief during the hysterosalpingography procedure -Ensuring virtual reality glasses group to watch nature videos and to provide relaxation by diverting their attention from the procedure	-Increasing comfort level with nursing care based on comfort theory
Sociocultural	-Informing patients -Ensuring effective communication	-Delivery of the training booklet for the mobile-assisted education group and ensuring relaxation during the procedure -Providing the necessary information to the virtual reality glasses group before and during the procedure	-Increasing comfort level with nursing care based on comfort theory

There are studies where training and counseling services are provided to women undergoing HSG[2,5,13,14] or VRG are used[9,15]. However, to the best of our knowledge, there are no studies in which CT_bNC or a similar theory is applied during HSG and its effectiveness is evaluated and compared with the use of VRG. Therefore, this is the first randomized controlled trial to use VRG or MAEC intervention with CT_bNC in HSG and to compare the efficacy of these methods.

METHODS

Study design and location

This research was designed as a randomized controlled trial with a parallel group pre-test-post-test design. The study was conducted in accordance with the CONSORT guidelines. The study was registered on clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT04676932). The study was conducted between September 2020 and April 2021 in the obstetric outpatient clinics of a public hospital in northern Turkey.

Sample size and characteristics

Sample size was determined based on a similar study in the literature[16]. Power analysis was performed with an effect size of d=079, a confidence interval of 0.95 (1-β), an alpha error rate of 0.05, and a power of 0.95. Accordingly, the minimum sample size was calculated as 108 participants (36 women in each group). Considering possible data loss, a total of 126 women, 42 in each group, were included in the study. Healthy women aged >18 years who had not been diagnosed with any psychiatric disease, had no

mental and communication problems, not having drug sensitivity or allergies, not having an active genital infection, and having a mobile phone were included in the study. Those who did not meet the inclusion criteria were excluded from the study. Considering possible losses (nausea, vomiting, fainting, and increased body temperature), a total of 126 women (42 in VRG, 42 in MAEC, and 42 in control groups) were included in the study, and the research was completed with the participation of 122 women.

Data collection tools

The introductory information form developed by the researchers in line with the relevant literature consists of 11 items[2,17,18]. The form contains questions related to the sociodemographic characteristics and obstetric histories of women.

Visual Analog Scale

The pain was monitored with a VAS, a 10-cm, one-dimensional measuring tool commonly used to measure the severity of pain. The scale begins with "no pain" (0) and ends with "unbearable pain." A score of 0–4 refers to "no pain," 5–6 refers to "moderate pain," and 7–10 refers to "severe pain"[13].

General Comfort Scale

Kolcaba developed Glasgow Coma Scale (GCS) in 1992 to determine individuals' comfort requirements and evaluate nursing interventions providing and improving patient comfort[19]. It is a 4-point Likert-type scale consisting of 48 items. The questions evaluate the taxonomic structure of comfort with three levels and four dimensions[20]. The score that can be obtained from the scale ranges between 48 and 192.

The total score obtained is the number of scale items. The average value is determined by dividing the total score obtained by the number of scale items and the result is indicated in the 1–4 distribution. Low comfort is expressed with one point, and high comfort with four points. The Cronbach's alpha coefficient of the scale is 0.85. Permission to use the scale has been obtained.

Randomization

Randomization into one of the three study groups was performed with the use of the Excel software (Microsoft, Redmond, Washington) to generate random numbers.

Collection of data

After the consent of all women was obtained and randomization was performed, the sociodemographic and obstetric characteristics and comfort levels were determined via the pre-tests. Routine care in intervention and control groups continued without any interference during the study period.

Intervention groups

In the MAEC intervention group, the day after the pre-test, participants were contacted telephonically. Notably, 48 h before the HSG procedure, the training booklet based on CT, which was created for the HSG procedure, was delivered to the patients via their mobile application, and two participants were provided via e-mail, and kept open for training and counseling. MAEC intervention was continued with women in need through phone calls and text messaging until the day of the process. In total, 180 interviews were conducted with 42 women. The average time for phone calls was 10 min.

The VRG intervention involved the use of VRG during the HSG procedure. Before the process, VRG were introduced to the women and told how to use them. During HSG, each woman was shown the same video with 360° virtual reality, including scenes from nature and a feeling of comfort and peace while making the viewer feel like they were there.

Control group

This group only received routine care in the hospital. This group (n=42) filled out the introductory information form and GCS.

Evaluation of data

Variables were presented as mean, standard deviation, number, and percentage. Shapiro-Wilk test was performed to check whether the data conformed to normal distribution. It was found that the data did not show the normal distribution and non-parametric tests were performed in statistical analysis. Mann-Whitney U test was used for binary categorical variables, and the Kruskal-Wallis H test was used for categorical variables with three or more categories. The signed rank test was performed to compare the pre-test and post-test scores between the groups. Tukey's post-hoc test was performed to find the group causing the difference.

Ethics

Ethical permission (approval number: 41, approval date: 13/02/2019) was obtained from a University Clinical Research Ethics Committee. All participants provided voluntary, informed, written consent prior to being enrolled in the study and could withdraw their consent at any time.

RESULTS

Table 2 shows that the groups are similar, and there is no significant difference between the groups. Participants' age, age during marriage, educational status, employment status, level

Table 2. Descriptive characteristics of women.

Characteristics	Virtual reality glasses (n=42)		Mobile-assisted education (n=40)		Control (n=40)		Statistical analysis
	Number	%	Number	%	Number	%	
Age							
x̄±SD (min–max)	27.4±3.14 (22–36)		27.8±4.59 (19–37)		28.33±4.55 (21–40)		H=0.618 p=0.734
Duration of marriage							
1–2 years	27	64.3	30	75.0	27	67.5	x^2=1.554 p=0.817
3–4 years	8	19.0	6	15.0	6	15.0	
4 years and above	7	16.7	4	10.0	7	17.5	
Education level							
Secondary school	13	30.9	14	35.0	12	30.0	x^2=9.983 p=0.266
High school	7	16.7	8	10.0	10	25.0	
University and above	22	52.4	18	45.0	18	45.0	
Employment in an income-generating job							
Working	23	54.7	18	45.0	22	55.0	x^2=1.051 p=0.591
Non-working	19	45.2	22	55.0	18	45.0	
Receiving hysterosalpingography information							
Yes	32	76.2	22	55.0	29	72.5	x^2=4.776 p=0.092
No	10	23.8	18	45.0	11	27.5	
Body mass index							
Normal	27	64.3	18	45.0	20	50.00	x^2=4.381 p=0.357
Overweight	10	23.8	11	27.5	12	30.0	
Obese	5	11.9	11	27.5	8	20.0	
Menarche age							
x̄±SD (min–max)	13.07±1.26 (11–16)		13.38±1.46 (11–16)		13.17±1.41 (11–16)		H=0.7995 p=0.670
Menstruation pattern							
Regular	39	92.8	33	82.5	34	85.0	x^2=2.114 p=0.288
Irregular	3	7.2	7	17.5	6	15.0	
Dysmenorrhea							
Yes	22	52.4	24	60.0	25	62.5	x^2=0.942 p=0.624
No	20	47.6	16	40.0	15	37.5	
Frequency of menstruation							
x̄±SD (min–max)	28.3±3.43 (24–45)		27.6±3.6 (22–40)		28.4±4.55 (21–40)		H=1.3615 p=0.506
Duration of menstruation							
x̄±SD (min–max)	5.79±0.92 (4–9)		5.62±1.46 (3–9)		5.66±0.99 (4–7)		H=0.5603 p=0.755

H: Kruskal-Wallis H test; p: significance; x̄: mean, SD: standard deviation; min: minimum; max: maximum.

of income, health insurance and family type, and smoking use are similar (p>0.05).

Table 3 shows the comparison of the GCS and VAS scores between and within groups. In the GCS pre-test and post-test measurements, the difference between all three groups and both intervention and control groups was significant (p<0.05). This increase in the GCS scores of the intervention groups was statistically significant (p=0.000, Table 3). The mean GCS score of the control group decreased, and the difference between the intervention and control groups was significant (p=0.000). In the post-test, the statistically significant difference between the MAEC and VRG intervention groups and the MAEC and control groups continued, and there was a statistically significant difference between the VRG and control groups (p=0.000, Table 3).

While no significant difference was found in the mean pain scores during the HSG procedure between the intervention and control groups (p=0.240), it was found that the VAS scores of the women decreased after the procedure regardless of their group and a significant difference was found in VAS scores within all groups (p=0.00, Table 3). After the process, women in the control group defined higher pain levels than women in both intervention groups. The MAEC group had the lowest VAS score during HSG and was also the group with the highest reduction in pain level after HSG. The mean scores of the MAEC group were significantly different from both VRG and control groups. On the contrary, as shown in Table 3, there was a statistically significant difference between VRG and control groups in favor of VRG (H=21.15, p=0.000, Table 3).

DISCUSSION

Researchers of this study suggest that CT_bNC positively affects care outcomes. HSG procedure is characterized by anxiety, pain, and deterioration in comfort for women[2]. In the literature, women rated how uncomfortable the HSG procedure was, and the mean score was found to be 6.36±2.19[21]. With proper nursing care, it is possible and more humane for women to experience less pain and anxiety and have higher comfort during HSG.

Comfort theory-based nursing care has been applied in various research studies[8,16,22-24], and it has been found that comfort has increased in intervention groups receiving theory-based care. According to the results of this study, it was found that both MAEC and VRG caused a significant increase in overall comfort levels in women. In contrast, the comfort level in the control group decreased after the HSG procedure. The highest comfort levels after the procedure were observed in the MAEC group. In Guvenc's study, a 30-min training was given to women before the HSG procedure by the nurse, and the anxiety level of the women decreased in the intervention group[2]. With MAEC, women were allowed to ask questions about the issues they were concerned about regarding HSG, which increased their comfort levels. Some studies emphasize

Table 3. Comfort levels and pain levels of participants according to groups and measurement times (n=122).

Glasgow Coma Scale	Virtual reality glasses (n=42)	Mobile-assisted education (n=40)	Control (n=40)	Test statistic
	x̄±SD (min–max)	x̄±SD (min–max)	x̄±SD (min–max)	
Pre-test	2.55±0.50 (1.5–3.63)[b]	2.99±0.42 (1.73–3.65)[a]	2.65±0.47 (1.25–3.63)[b]	H=22.82 **p=0.000**
Post-test	3.10±0.57 (1.54–3.75)[b]	3.16±0.42 (1.79–3.75)[a]	2.44±0.69 (1.25–3.5)[c]	H=26.80 **p=0.000**
Intra-group statistical analysis				
S / p	−304 / **0.000**	−252 / **0.000**	146 / 0.057	
Pain levels (Visual Analog Scale)	**x̄±SD (min–max)**	**x̄±SD (min–max)**	**x̄±SD (min–max)**	**Test statistic**
During hysterosalpingography	8.24±1.65 (5–10)	7.76±2.10 (4–10)	8.50±1.81 (4–10)	H=2.85 p=0.240
15 min after the procedure	3.00±2.22 (0–8)[b]	2.76±2.50 (0–10)[c]	5.14±2.70 (2–10)[a]	H=21.15 **p=0.000**
Intra-group statistical analysis				
S / p	430.5 / **0.000**	410 / **0.000**	410 / **0.000**	

S: signed rank test; H: Kruskal-Wallis H test; p: significance; x̄: mean, SD: standard deviation; min: minimum; max: maximum; a, b, c: There is no difference between data indicated by the same letter. Statistically significant values are indicated in bold.

that it is essential to provide counseling to reduce anxiety before applying assisted reproductive techniques[2]. The result of this study supports the role of counseling in reducing stress.

Mobile-assisted education aimed to increase comfort and control the pain experienced by providing safety and information. In this study, women identified pain during the procedure that was not significantly different between the intervention and control groups and was higher than the level of pain[21]. In other words, neither MAEC nor VRG affects the pain experienced during the procedure. On the contrary, women in the MAEC group had the lowest level of pain indicated during and after the process. Although the difference between the groups is not significant, this decrease in the level of pain indicated in the MAEC group is most likely due to supporting external well-being-seeking behaviors (education and counseling) according to Kolcaba and the simultaneous control of the environmental factors (light, noise, color, temperature, etc.) that form the external background of the human experience during the procedure. The fact that the post-test pain level indicated in the MAEC group was significantly lower than the VRG and control groups supports this view.

Pain management is a growing healthcare issue all over the world. Swiftly addressing women's need for painkillers after painful procedures will increase patient satisfaction and reduce healthcare costs[25]. VRG reduces pain by distracting individuals from processing signals from pain receptors[26]. It is a non-invasive and low-cost intervention that can be used to cope with pain during outpatient surgical procedures. An increasing body of evidence points to the positive effect of VRG use in acute pain management[14], during various medical procedures such as chemotherapy[27,28], and in wound care[29,30].

In contrast, it has been found that VRG as a distraction method does not affect pain during cystoscopy[31]. In this study, CT_bNC-VRG did not significantly impact the level of pain during HSG but led to a substantial reduction in pain level 15 min after the procedure compared to the control group. Similar to our result, in the Yılmaz Sezer et al.'s study, women in the VR group had lower pain levels during and 15 min after HSG than in the control group[15]. According to this result, companionship (social context of comfort) during the process, regulating the environment, and ensuring and maintaining comfort (environmental context of comfort) via CT_bNC-VRG effectively reduce women's pain levels after HSG.

CONCLUSION

This study showed a significant statistical and clinical improvement in pain and comfort in women undergoing HSG. With CT_bNC, the post-procedural comfort level of women increased and their pain level decreased.

The study's results are also crucial, as they show the positive impact of virtual care technologies on health outcomes and training and counseling. In situations where continuous counseling cannot be provided for HSG, VRG can be used in conjunction with comfort-enhancing measures.

Limitation

Owing to its single-centered nature, the results cannot be generalized to all healthy women.

ETHICS

Permissions were obtained from Ondokuz Mayis University Clinical Research Ethics Committee (approval number: 41, approval date: 13/02/2019).

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AUTHORS' CONTRIBUTIONS

SB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **ÖK:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Software, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Relationship of irisin expression with metabolic alterations and cardiovascular risk in type 2 diabetes mellitus: a preliminary study

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the role of irisin in type 2 diabetes mellitus and its association with metabolic alterations and obesity.

METHODS: A cross-sectional case-control study was conducted on participants treated at Centro Universitário FMABC between August 2018 and July 2019, by comparing a control group (n=14) with type 2 diabetes mellitus patients (n=16). The control group consisted of participants aged above 21 years with no chronic diseases, diabetes, smoking, or illicit drug use. The type 2 diabetes mellitus group included patients aged above 21 years, who were diagnosed with type 2 diabetes for at least 5 years (glycated hemoglobin>7%). Exclusion criteria were not willing to continue, recent hospitalization, and failure to meet inclusion criteria. Biochemical parameters included blood glucose, glycated hemoglobin, plasma irisin levels, and *irisin* gene expression in peripheral blood.

RESULTS: Type 2 diabetes mellitus patients exhibited significantly higher plasma glucose levels [143 (40) vs. 92 (13) mg/dL, *p<0.05] and glycated hemoglobin levels [7.1% (1.6) vs. 5.6% (0.5), *p<0.05] compared to the control group. *Irisin* gene expression in type 2 diabetes mellitus patients was lower 0.02288 (0.08050) than the control group 8.506e-006 (1.412e-005) (p=0.06). Correlation analysis revealed a positive association between *irisin* expression and body mass index in type 2 diabetes mellitus (Rho=0.5221, 95%CI -0.058 to 0.838, p=0.06), while plasma irisin showed a negative correlation with body mass index (Rho=-0.656, 95%CI -0.836 to 0.215, p=0.03). No significant correlations were found between plasma glucose or glycated hemoglobin levels and irisin expression.

CONCLUSION: The data suggests that body mass index directly influences plasma irisin levels and the regulation of irisin gene expression, possibly linking irisin to adiposity changes observed in obesity-related type 2 diabetes mellitus.

KEYWORDS: Diabetes mellitus. FNDC5 protein, human. Obesity. Cardiovascular system.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a syndrome of multiple etiologies, characterized by a lack of and/or resistance to the action of insulin. In other words, there is an inability of this hormone to properly exert its effects. This syndrome is marked by chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism. The genesis of hyperglycemia involves a triad of abnormalities that include increased hepatic glucose production, altered insulin secretion, and action. The severity of these anomalies and their degree of contribution vary and are related to the heterogeneity of metabolic alterations in diabetes[1].

The elevated prevalence of vascular disease in individuals with T2DM is not solely attributed to hyperglycemia but is also influenced by metabolic changes associated with the plurimetabolic syndrome. This syndrome encompasses centrally distributed obesity, hypertension, dyslipidemia, insulin resistance, hyperinsulinemia, increased coagulation factors, platelet adhesion and aggregation, reduced fibrinolysis, and hyperuricemia. The identification of markers indicating the progression of clinical conditions resulting from obesity and insulin resistance, as well as the potential development of cardiovascular disease (CVD), holds significant importance. Irisin, a hormone secreted during skeletal muscle contraction, has emerged as a potential marker. It is released after cleavage of the membrane protein FNDC5 and plays a role in converting white adipose tissue to brown adipose tissue, thereby enhancing insulin sensitivity through the regulation of glucose homeostasis[1-3].

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Several studies have indicated that the expression of the *FNDC5* gene is associated with increased glycosylation of irisin, a process that positively influences the hormone's functionality. Moreover, elevated circulating levels of irisin have been linked to insulin resistance[4]. A study based on the Framingham risk score demonstrated a correlation between higher plasma irisin levels and an increased risk of CVD over a 10-year period[5]. Yilmaz et al. suggested a potential influence of irisin on another crucial cardiovascular risk factor, serum lipid concentration, with findings indicating a correlation between high irisin concentrations and dyslipidemia[6].

The evaluation of irisin gene regulation in peripheral blood samples can elucidate the actions of irisin and its metabolic consequences, particularly in diabetic patients[7]. The objective of this study was to further investigate the relationship between plasma levels of irisin and the gene expression of this hormone, as well as the metabolic alterations present in DM. Additionally, the objective was also to examine whether irisin is involved in the adiposity changes observed in patients with obesity-related T2DM and to evaluate whether there are significant platelet alterations in diabetic patients during this phase of the disease.

METHODS

This cross-sectional case-control study was conducted on participants treated at the specialist outpatient clinic of Centro Universitário FMABC, under the supervision of cardiologist Dr. Neif Murad. The study included a total of 30 participants, who were divided into two groups: the control group, consisting of 14 individuals, and the diabetic group (T2DM), comprising 16 patients.

The control group was formed based on specific inclusion criteria, which required participants to be above 21 years of age, with no history of chronic diseases or diabetes, and no smoking or use of illicit drugs. On the contrary, the T2DM group comprised patients aged above 21 years who had been diagnosed with type 2 diabetes (glycated hemoglobin (HbA1C) >7%) for at least 5 years, were non-carriers of cardiovascular, renal, or hepatic disease, and had no HIV or cancer. To ensure the validity of the study, exclusion criteria were applied. The exclusion criteria were not willing to continue, hospitalization for any reason within the past 30 days, and not meeting the inclusion criteria.

The enrollment of patients for this study took place between August 2018 and July 2019. This time frame was essential for collecting relevant data and ensuring that the study's findings were representative of the specified period.

The collection of personal data and medical history from the participants and verification of all medications used in the treatment of diabetes and its comorbidities took place through an interview. The participants' body weight and height were measured to calculate the body mass index (BMI). Body weight (kg) was divided by height squared (m^2) and the result was expressed in kg/m^2, following the criteria of the World Health Organization (WHO).

Evaluation of fasting plasma glucose levels

Blood samples were obtained by vacuum venipuncture after an 8-h fast to determine the quantification of HbA1C, glucose, and plasma irisin. Values above 140 mg/dL were considered altered, and after three repetitions of the test, they were referred for medical evaluation. The determination of fasting plasma glucose levels was performed by an automated enzymatic-colorimetric method with fluoride serum. Values ≥140 mg/dL were considered altered for this parameter.

Evaluation of glycated hemoglobin levels

Glycated hemoglobin was determined through low-pressure liquid chromatography (LPLC) using the DiaSTAT analyzer - Bio-Rad, which expresses the percentage of total hemoglobin and reflects the glycemic state over the past 8–12 weeks. A 5 mL sample of whole blood with 1 mL of hemolyzed reagent was collected. Values above 7% were considered altered.

Study of irisin gene expression in peripheral blood samples

The total RNA extraction process followed the standard protocol for TRIzol. After total RNA extraction, cDNA synthesis was performed: total RNA samples (initial 1µg) obtained from peripheral blood were converted to cDNA using an SSIII first strand qPCR supermix (Invitrogen, cat no. 11752050), according to the protocol from the manufacturer.

The specific primers for each selected gene were designed using the Primer3 Input 0.4.0 software program. The characteristics of specific primers were (*Ribosomal Protein L13a* (*RPL13a*)): forward – gtggtcgtacgctgtgaag and reverse – acagtgcgccagaaaatgc, amplicon: 126 bp. *Irisin*: forward – gatccagccatcaaggacat and reverse – ttgtccaagctagcatttctga, amplicon: 113 bp.

Evaluation of hematological parameters and plasma irisin

Platelet count (PLT) and mean platelet volume (MPV) were used to evaluate the hematological parameters using the automated flow cytometry method by the XN counter equipment (Roche, Switzerland®).

The hormone irisin was analyzed from centrifuged plasma using an enzyme-linked immunosorbent assay (ELISA) kit (EK-067-29, Phoenix Pharmaceuticals, Burlin). The kit detects specific peptides and related peptides based on the principle of "competitive" ELISA. The kit's immunoplate was pre-coated with a non-specific secondary antibody with blocked binding sites. The secondary antibody bound to the Fc fragment of the primary antibody (peptide antibody), whose Fab fragment was competitively bound by both biotinylated standard peptides and target peptides in the samples. The biotinylated peptide interacted with streptavidin-horseradish peroxidase (SA-HRP), which catalyzed the substrate solution composed of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide to produce a blue-colored solution. The enzyme-substrate reaction was stopped by hydrochloric acid (HCl), and the solution turned yellow. The intensity of yellow color was directly proportional to the amount of biotinylated peptide-SA-HRP complex but inversely proportional to the amount of peptide in standard solutions or samples. A standard concentration curve was established, and the unknown concentration in samples was determined by extrapolation to the curve. Plasma was collected in EDTA tubes on ice, centrifuged at 4°C, 3,000 rpm for 10 min, and then separated into two aliquots (0.5 mL each) to be stored at -20°C until analysis.

Ethical aspects

Participants were enrolled and treated at the Specialist Outpatient Clinic of Centro Universitário FMABC. This work was submitted and approved by the Ethics Committee of Centro Universitário FMABC (process No. 084592-2017, September 28, 2017). Individuals who agreed to participate signed a free and informed consent form (FICF), with thorough explanations about the adopted protocols, following the guidelines, regulations, and relevant ethical principles of the Declaration of Helsinki.

Statistical analysis

In this study, the sample size calculation was performed using GPower®. The input parameters used for the calculation were as follows: the tail of the test was one-tailed, indicating a directional hypothesis, an effect size (p) of 0.50, a significance level (α) of 0.05 (corresponding to a 5% type I error rate), and a desired statistical power (1-β) of 0.80 (equivalent to an 80% probability of detecting a true effect). Based on these inputs, the noncentrality parameter (δ) was determined to be 2.6457. The critical t-value for a one-tailed test at a significance level of 0.05 with 19 degrees of freedom was calculated as 1.7291. To achieve the desired statistical power of 0.80, the total sample size required for the study was determined to be 21 participants. After conducting the study and analyzing the data, the actual statistical power was calculated to be 0.8172279, which indicates that the study had a high likelihood of correctly detecting the hypothesized effect given the sample size and effect size. The calculated sample size of 21 participants ensured that the study had adequate statistical power to detect the anticipated effect size with a reasonable probability of avoiding type II errors. These findings support the validity and reliability of the study's conclusions and demonstrate the importance of carefully considering sample size calculations in research design to optimize the likelihood of obtaining meaningful and conclusive results.

This study employed the Shapiro-Wilk test to assess the normality (>0.05) of the collected data and to distinguish between parametric and non-parametric data. The results were expressed as the mean±standard deviation (SD). These were compared using the Mann-Whitney U test to non-parametric (*irisin* gene expression) data and the Student's t-test to parametric data. Analyses were performed using the GraphPad Prism computer software (GraphPad, version 7.0, USA). For correlation assessments, Spearman tests were performed. The established significance level was 5% (descriptive value of $p<0.05$).

RESULTS

Initially, 25 participants were selected for each group. However, during the screening process, 35% of the participants in the control group did not meet the inclusion criteria due to the presence of associated comorbidities or their unwillingness to continue with the study. In the T2DM group patients, 42% of the enrolled participants did not meet the inclusion criteria. As a result, the final analysis included 16 participants in the control group and 14 participants in the group with diabetes mellitus. These final sample sizes were considered adequate for the statistical analyses and interpretation of the study's findings.

The mean age of the participants in this study was 51±15 years for the control group (n=14) and 64±10 years for the T2DM group (n=16). Regarding the biochemical parameters, the following results were obtained: glucose (control group 92±13, n=14 vs. T2DM 143±40 mg/dL, n=16, $p<0.05$) and HbA1C (control group 5.6±0.5%, n=14 vs. T2DM 7.1±1.6%, n=16, $p<0.05$) (Table 1). Participants with T2DM had increased blood glucose and HbA1C values, as expected. Regarding the results of hematological parameters, the T2DM group had lower MPV values compared to the control group (Table 1). There was no statistical difference in mean corpuscular volume (MCV) between groups.

Table 1 presents the expression profile of irisin in the control and T2DM groups, revealing a noticeable trend toward increased irisin expression in the T2DM group. Furthermore, upon applying the correlation test between plasma irisin quantification and BMI in the T2DM group, a negative correlation was observed. Conversely, there was a trend toward a positive correlation between irisin gene expression and BMI. However, no correlation was found between glucose levels and HbA1C with irisin expression (Table 2). Plasma irisin values were similar between the control and T2DM groups. In the T2DM group, there was no correlation between plasma irisin concentration and *irisin* expression.

DISCUSSION

Among the main findings of this study, it is worth highlighting that there was a trend toward increased gene expression of *irisin* in diabetic patients compared to the control group. A positive correlation was observed between *irisin* expression and BMI in diabetic patients. Plasma irisin values were similar between the control group and T2DM group. There was a negative correlation between BMI and plasma irisin levels.

In this study, the observations revealed a positive modulation between *irisin* expression and BMI in diabetic patients, suggesting a link between irisin and adiposity changes associated with obesity in T2DM. In contrast to prior research, our findings demonstrated a negative correlation between BMI and plasma irisin levels. These results indicate that elevated blood glucose levels in T2DM may initially induce an increase in irisin levels as a compensatory response, but as the disease progresses, reduced sensitivity to irisin's effects may develop, suggesting the presence of irisin resistance in insulin-resistant conditions like metabolic syndrome and T2DM[8].

Reiterating the compensatory role of irisin in the context of insulin resistance, obesity, and T2DM, it can be inferred that this hormone effectively ameliorates some of the metabolic disorders present in early T2DM, in addition to its functions in promoting browning of white adipose tissue, enhancing glucose uptake in skeletal muscle and heart, improving hepatic glucose and lipid metabolism, and stimulating pancreatic β-cell function[9]. The direct relationship between irisin and adiposity, as demonstrated in this study[10], strongly suggests that adipose tissue serves as a secondary inducer of irisin secretion, thereby justifying the increase in gene expression in patients with higher BMI and the observed elevation in plasma irisin concentration.

Increased *irisin* expression, found in some studies, may be related to the initiation of compensatory metabolic processes generated by metabolic stress[11-13] In contrast, Tu et al.,

Table 1. Evaluations of demographic characteristics of the study participants (control group and type 2 diabetes mellitus group), quantifications of biochemical parameters, and irisin gene expression.

Variables	Control group (n=14)	T2DM group (n=16)	p
Age (years, mean±SD)*	51 (15)	64 (10)	**0.008**
Glucose (mg/dL, mean±SD)*	92 (13)	143 (40)	**<0.0001**
HbA1C (%, mean±SD)*	5.6 (0.5)	7.1 (1.6)	**0.002**
Irisin gene expression ($2^{-\Delta Ct}$, mean±SD)#	8.506e-006 (1.412e-005)	0.02288 (0.08050)	0.06
Plasma irisin (mg/dL, mean±SD)*	44.86 (19.83)	49.83 (7.63)	0.44
MCV (mcm^3, mean±SD)*	85.55 (6.91)	89.29 (3.35)	0.06
MPV (mcm^3, mean±SD)*	10.50 (1.18)	7.74 (2.02)	**0.004**

Note: SD: standard deviation; HbA1C: glycated hemoglobin; MCV: mean corpuscular volume; MPV: mean platelet volume. #Mann-Whitney U test. *Student's t-test. Statistically significant values are denoted in bold.

Table 2. Body mass index values and correlation analysis between body mass index values and irisin expression in the peripheral blood

	Control group (14)	T2DM group (16)	p
BMI	26.4 (4.5)	27.6 (4.3)	0.46
Correlation test*			
Irisin expression vs. BMI	Rho (CI) 0.522 (-0.058 to 0.838)		0.06
Plasma irisin vs. BMI	-0.656 (-0.836 to 0.215)		**0.03**

Note: BMI: body mass index; CI: confidence interval; T2DM: type 2 diabetes mellitus. *Spearman correlation test. Statistically significant values are denoted in bold.

and Kałużna et al., demonstrated a reduction in plasma irisin in diabetics, when the low levels of irisin were directly related to cachexia, present in decompensated diabetics[14,15]. AlKhairi et al., demonstrated a significant rise in plasma irisin levels among diabetic individuals, particularly in those with obesity[13]. The authors highlighted that plasma irisin levels may also vary across different ethnicities and genders, underscoring the importance of considering these parameters when quantifying irisin levels. Nevertheless, the precise role of irisin in diabetic individuals requires further investigation, particularly in diverse nutritional contexts.

The regulation of serum irisin levels is influenced by various factors, which have been investigated to understand the metabolic processes in both normal individuals and different pathologies. However, the direct relationship between increased irisin expression and plasma levels of this hormone is complex, as multiple genes are involved in its activation cascade. Additional studies are needed to completely elucidate this metabolic pathway in DM. In an animal model of DM, Varela-Rodríguez et al.[17] observed an upregulation of the *FNDC5* gene in various tissues while protein expression remained unchanged, consistent with the findings of our study. Additionally, the same study established a connection between glycemic metabolism and irisin synthesis and action. Fasting in healthy rodents for 48 h resulted in reduced irisin synthesis, release, and *FNDC5* expression. Conversely, in this study, *FNDC5* expression was decreased in the muscles of diabetic rats, with this reduction directly linked to the animals' nutritional status. In contrast, our study examined *irisin* expression in peripheral blood without changes in nutritional status. Consequently, the regulation of *irisin* gene expression appears to be more influenced by glucose and insulin levels[15,16-22].

In individuals with T2DM, alterations in platelet metabolism, function, and morphology were observed in this study. Increased MPV and MCV indicate changes in thrombopoiesis and suggest micro and macrovascular complications, worsening the disease. However, diabetic patients undergoing drug treatments, particularly those with antiatherogenic effects targeting glycemic and/or hyperlipidemic control, show a decrease in these hematological factors. This hematological profile differs in more severe pathological conditions, such as hemodialysis patients. The direct association between high irisin levels, known for their cardioprotective effects, appears to be lost in disordered metabolic states such as T2DM. Thus, it is suggested that irisin acts as a protective factor for the cardiovascular system only in situations of homeostasis and in healthy individuals.

This study has limitations in terms of the sample size. Due to this reason, we describe the study as preliminary. A new study with the participation of a larger number of volunteers could confirm our findings. It is known that studies with a small sample size may introduce a bias in the interpretation of the data. However, as there are limited studies that have evaluated the gene expression of irisin and its relationship with metabolic alterations in diabetics, we believe that the data presented can guide future research in this area.

CONCLUSION

The data suggest that BMI directly influences plasma irisin levels and the regulation of irisin gene expression, possibly linking irisin to adiposity changes observed in obesity-related T2DM.

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AUTHORS' CONTRIBUTIONS

GLV: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. **VLM:** Conceptualization, Data curation, Writing – review & editing. **MISM:** Conceptualization, Data curation, Writing – review & editing. **FLAF:** Conceptualization, Methodology, Investigation, Validation, Writing – review & editing. **BCAA:** Investigation, Methodology, Validation. **DPS:** Investigation. **CGCA:** Investigation. **MMP:** Investigation. **JFAE:** Investigation. **JRSR:** Investigation.

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ORIGINAL ARTICLE

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Early outcomes of robotic retroperitoneal partial nephrectomy: evaluating surgical success with margin, ischemia, and complication score

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate the minimum number of required cases for successful robotic retroperitoneal partial nephrectomy for an experienced surgeon in transperitoneal robotic surgery.

METHODS: Our prospectively collected clinic database was evaluated retrospectively, and 50 patients who underwent robotic retroperitoneal partial nephrectomy by a single experienced surgeon from January 2019 to February 2023 were included in this study. Demographic and perioperative data and R.E.N.A.L. nephrometry scores were noted. margin, ischemia, and complication score was used to predict surgical success. Receiver operating characteristic curve analysis was used to determine how many cases were required to achieve margin, ischemia, and complication score positivity and to apply the off-clamp technique. Also, the first 25 patients were assigned to Group 1 and the second 25 patients to Group 2, and the data were compared between the groups.

RESULTS: The patients' demographic data and tumor characteristics were similar in the groups. The off-clamp technique and sutureless technique rates in Group 2 were significantly higher than that in Group 1. Margin, ischemia, and complication score positivity was observed in 60% (n=15) of Group 1 and 96% (n=24) of Group 2. At receiver operating characteristic curve analysis, the 25th and later cases were statistically significant in terms of margin, ischemia, and complication score positivity. In terms of performing surgery with the off-clamp technique, the 28th and subsequent cases were statistically significant.

CONCLUSION: A total of 25 or more cases appear to be sufficient to provide optimal surgical results in robotic retroperitoneal partial nephrectomy for an experienced surgeon.

KEYWORDS: Renal. Renal cell carcinoma. Robotic. Retroperitoneal. Partial. Nephrectomy.

INTRODUCTION

Renal cell cancer (RCC) is the third most common urological cancer[1]. Partial nephrectomy (PN) is the principal treatment method of cT1 stage RCC[2]. Robotic PN can be performed using a transperitoneal or retroperitoneal approach. Almost all urologists have become familiar with the transperitoneal method because of its prominent anatomical landmarks, easy trocar placement, and more frequent use in resident training. Although the robotic retroperitoneal approach has advantages, especially in posterior-located tumors, it is not used in many urology clinics due to technical difficulties and it is thought that the learning curve is steep.

The most important goal in PN surgery is to achieve successful oncological results without complications and preserve renal functions (trifecta)[3]. In addition, various scoring systems have been developed to interpret successful PN surgery, such as the margin, ischemia, and complication (MIC) score[4]. The most important factor that affects the success of PN and MIC scores is the experience of the surgeon[5]. The purpose of this study,was to evaluate the minimum number of required cases for successful robotic retroperitoneal partial nephrectomy (RRPN) surgery for an experienced surgeon in transperitoneal robotic surgery.

METHODS

Patients and data collection

Following the institutional clinical research ethics committee approval (no. 22/10, dated 08/12/2022), we reviewed our clinic database which is routinely and prospectively collected.

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Patients with previous histories of ipsilateral kidney surgery, solitary kidney, bilateral kidney tumors, and incomplete data were excluded. A total of 50 patients who underwent RRPN between January 2019 and February 2023 and completed at least 3 months of postoperative follow-up were included in this study. All RRPN procedures were performed by a single experienced surgeon (MA). Demographic, clinical, and perioperative data, R.E.N.A.L. nephrometry and MIC scores, pre- and post-operative third-month laboratory findings, complications, and pathology results were analyzed. These 50 patients were subsequently divided into two groups according to their operation dates. The first 25 patients constituted Group 1 and the second 25 patients constituted Group 2.

R.E.N.A.L. nephrometry scores were calculated as described by Kutikov[6]. Total operation time, console time, renal artery separation time, renography time, amount of bleeding, and Ischemia time in on-clamp PN operations were analyzed. Surgical margin (SM) negativity in pathology reports, Ischemia time less than 20 min, and absence of complication were defined as MIC score positivity (+)[4].

Surgical technique

The Da Vinci Xi (Intuitive Surgical, Sunnyvale, CA, USA) robotic surgery system was used in the operations. All operations were performed with the patient in the 90° angle lateral decubitus position and tumor side up. A standard retroperitoneal radical nephrectomy (RN) four-arm approach port configuration was employed.

The kidney was subsequently released over the psoas muscle. Anatomical landmarks (the ureter and vena cava or aorta) were identified. The renal artery was found and separated with a vessel loop. Subsequently, Gerota's fascia was incised, and renal mass was dissected and enucleated using a robotic bipolar dissector and monopolar scissors. Mass dissection and enucleation were started with the off-clamp technique in all cases. However, the on-clamp technique was applied in cases in which adequate hemostasis could not be achieved with electrocoagulation or suturing during mass enucleation. Renography was performed with two layers of running sutures. A 3-0 monofilament barbed suture (V-Loc™, Medtronic) was used for the deep layer, and a 2-0 polyglactin suture was used for the cortical layer. Cortical layer sutures were secured on the renal capsule using the sliding clip technique (Figure 1).

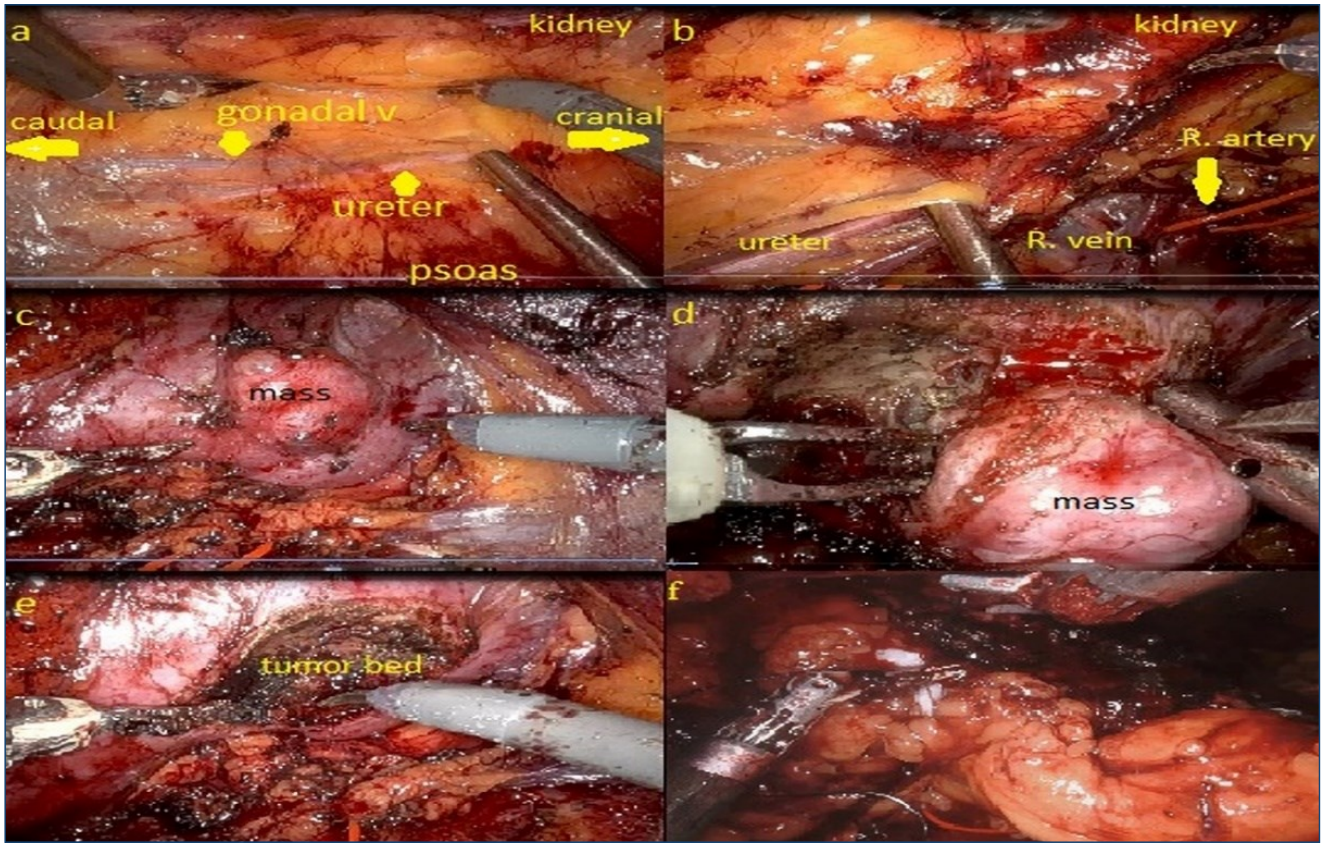

Figure 1. Surgical steps of robotic retroperitoneal partial nephrectomy. (a) Identification of the ureter and gonadal vein in left robotic retroperitoneal partial nephrectomy. (b) Separation of the renal artery and renal vein in left robotic retroperitoneal partial nephrectomy. (c) Incision of Gerota's fascia and identification of the mass in the left kidney. (d) Enucleation and excision of the mass. (e) Tumor bed after excision of the mass. (f) Running suture renography with the sliding clip technique.

Renography was not performed (sutureless surgery) in cases in which complete hemostasis was achieved with bipolar and monopolar energy electrocoagulation, the pelvicalyceal system and vascular structures were preserved, and only a hemostatic patch (Veriset™, Medtronic) was placed on the tumor bed. The operation was terminated with drain insertion.

Statistical analysis

Statistical analyses were performed using SPSS version 15.0 for the Windows software (SPSS, Chicago, IL, USA) program. The Shapiro-Wilk test was performed to determine the normality of distributions for continuous variables. Normally distributed continuous variables were compared using the Student's t-test, and non-normally distributed variables using the Mann–Whitney U test. Pearson's chi-square or Fisher's exact test was applied for categorical data. Normally distributed continuous variables were expressed as mean plus standard deviation (SD), and non-normally distributed variables were expressed as median. Categorical variables were expressed as numbers and percentages. Receiver operating characteristic (ROC) curve analysis was used to determine the number of cases required to provide sufficient surgical experience for achieving MIC score positivity and to apply the off-clamp technique. p-value less than 0.05 were regarded as significant.

RESULTS

Demographic data, tumor characteristics, and R.E.N.A.L. nephrometry scores of patients were similar among the groups (Table 1). There was no significant difference between the groups

Table 1. Patients' tumor characteristics and surgical and functional results.

		Group 1 (n=25)	Group 2 (n=25)	p-value
Age (years)		54.76±14.51	59.64±11.84	
Gender	Female (n, %)	6 (24)	7 (28)	
	Male (n, %)	19 (76)	18 (72)	
BMI (kg/m^2)		27.2±3.62	26.48±3.45	
Tumor side	Right (n, %)	14 (56)	14 (56)	
	Left (n, %)	11 (44)	11 (44)	
Clinic tumor size (cm)		3.65±1.40	3.44±1.21	>0.05
R.E.N.A.L. nephrometry score		6 (4–10)	6 (4–10)	
Mass Location	Anterior (n, %)	3 (12)	6 (24)	
	Posterior (n, %)	22 (88)	19 (76)	
Renal artery separation time (min)		18.52	18.24	
Console time (min)		147.36±55.93	141.16±35.38	
Renography time (min, n)		21.44±1.95 (25)	20.64±2.87 (11)	
Sutureless (n, %)		**0 (0.00)**	**14 (56.0)**	**<0.001**
Off-clamp (n, %)		**12 (48.0)**	**22 (88.0)**	**0.002**
Ischemia time (min, n)		**27.69±9.99** (13)	**17.33±2.5** (3)	**0.051**
Estimated blood loss (mL)		150 (30–500)	120 (30–500)	
Preoperative Hb level (g/dL)		13.93±0.33	14.22±0.36	
Postoperative Hb level (g/dL)		12.97±0.35	12.66±0.35	
Preoperative creatinine level (mg/dL)		0.96±0.03	1.04±0.04	
3rd month creatinine level (mg/dL)		0.98±0.03	1.04±0.04	
Postoperative third month eGFR		80.48±3.20	74.60±3.68	>0.05
Surgical margin positivity (n, %)		5 (20)	1 (4)	
°Malign (n)		23	21	
°°Clear cell		14	14	
°°Papillary		4	4	
°°Oncocytic-chromophobe		5	3	
MIC score positivity (n, %)		**15 (60)**	**24 (96)**	**0.002**

Bold indicates statistically significant values.

in terms of preoperative mean serum creatinine levels, eGFR levels, total operation times, or mean renal artery separation times. However, the mean renography time was significantly shorter in Group 2 (p<0.05). Mean console times tended to become shorter from Group 1 to Group 2 (147.4±56 and 141.2±36 min, respectively).

The off-clamp technique (zero Ischemia) ratios in Group 1 and Group 2 were 48% (n=12) and 88% (n=22), respectively, and significantly higher in Group 2 (p=0.002). In addition, while no sutureless surgery was performed in Group 1, 14 (56%) patients in Group 2 underwent sutureless RRPN (p<0.001). When the sutureless operation patients were excluded, the mean renography time in Group 2 was 20.64±2.87 min and similar to that in Group 1 (Table 1).

Although 14 patients underwent sutureless and clampless RRPN in Group 2, no significant difference was observed between the groups in terms of mean intraoperative blood loss and postoperative Hb levels. Four patients received postoperative single-unit red blood cell suspension transfusion in all patients. Ureteral injury developed in one case in Group 1 as a major complication, and a perioperative ureteroureterostomy was performed. No patient was converted to open surgery. The median time to return of bowel functions (flatulence) was 1 day, and drain removal times and lengths of hospital stay were 3 days in two groups. Prolonged urinary drainage was seen in one patient in Group 2 who underwent sutureless surgery and spontaneously terminated on the 32nd day with D-J stent placement.

There was no statistical difference between the groups in terms of pathological tumor sizes. SM positivity was detected in five cases in Group 1 and one case in Group 2. There was no difference between the groups in terms of pathological benign-malignant tumor rates and malignant subtypes. There was also no significant difference in terms of mean creatinine levels and eGFR averages in the postoperative third month. MIC score positivity reached 60% (n=15) and 96% (n=24) in Groups 1 and 2, respectively, and was significantly higher in Group 2 (p=0.002) (Table 1).

At ROC curve analysis of all RRPN (n=50) operations listed chronologically according to the date of operation, the 28th case and later were statistically significant in terms of off-clamp (zero ischemic) technique surgery (AUC=0.78, 95%CI 0.638–0.924, p=0.001). In terms of MIC score positivity, the 25th case and later were statistically significant at ROC curve analysis (AUC=0.78, 95%CI 0.644–0.918, p=0.005).

The median follow-up period was 13.28 months (3–51.87) for all cases and 30.58 months (7.93–51.57) for the SM-positive cases. Local tumor recurrence was observed in two patients without SM positivity in the first year.

DISCUSSION

The principal therapeutic step in non-metastatic RCC is PN or RN. The positive effects of PN on renal functions and cardiac disease risk have previously been demonstrated in cT1-stage renal tumors[2]. Achieving low complication rates and short hospital stays in surgical treatment is important in terms of patient health and effective use of the healthcare workforce. RRPN provides advantages such as shorter renal artery separation and surgery time, a short bowel function recovery time, and shorter hospital stays compared with the transperitoneal approach, according to the existing medical literature[7]. In addition, RRPN is advantageous in posteriorly located renal tumors and in patients with previous histories of abdominal surgery[8].

The mean renal artery separation time for all patients was approximately 18 min, which is shorter than the results of studies (21–41 min) in the literature[9]. Bowel function recovery time and length of hospital stay were 1 and 3 days, respectively, and consistent with the previous literature[10].

Margin, ischemia, and complication score positivity rates of 55–96.7% have been reported in different studies[11,12]. MIC score positivity was achieved in 39 (78%) cases among all patients in this study. However, the MIC score positivity rate approached 96% in Group 2. In addition, ROC curve analysis showed that the 25th and subsequent cases were significant in terms of providing MIC score positivity and also emphasized the importance of surgeon experience.

The upper limit of renal Ischemia time for maximum preservation of renal functions in the on-clamp technique has been reported at 20–25 min in different studies[13]. On-clamp technique was applied in 32% (n=16) of all cases in this study, and the mean renal Ischemia time was calculated as 25.75±9.91 min. However, the on-clamp technique was applied to only 12% (n=3) of the cases in Group 2.

Greater bleeding may be expected in the off-clamp technique. In this study, although the off-clamp technique rates differed significantly between Groups 1 and 2, no difference in mean blood loss was observed between the groups. This can be attributed to the powerful robotic monopolar and bipolar electrocoagulation in the off-clamp technique surgeries. In addition, ROC curve analysis showed that the 28th and subsequent cases were significant in terms of off-clamp technique surgery.

Sutureless PN studies have appeared in different publications since 2003[14]. These have reported that complete hemostasis can be achieved by means of electrocoagulation and placing hemostatic agents on the tumor bed, with no complications in some PN case series[15]. Sutureless PN is applied with the idea that the renal arcuate arteries can be preserved, renal medulla Ischemia can be minimized, glomerular loss can be reduced, and saturation-caused bleeding may be avoided[16]. Although all the groups in this study registered similar nephrometry scores, the sutureless technique was applied only in Group 2. Sutureless PN is possible with increased surgical experience.

Renal artery separation times were similar in the groups. This was attributed to the fact that the basic steps before separating the renal artery, such as creating an adequate working area by releasing the posterior and superior aspects of the kidney and identifying the ureter and the main vascular structures, were performed respectively in each case. No difference was observed in renography times between the groups, and this shows that, despite an increase in surgical experience, there was no decrease in the time spent by the surgeon on basic safety precautions.

SM positivity was detected in six (12%) cases, a figure that appears to be at the upper limit compared with the previous literature[17]. No recurrence or metastasis was detected in any SM-positive case.

CONCLUSION

Considering the anatomical locations of the urological organs, urologists will inevitably need to perform laparoscopic retroperitoneal surgery. This study suggests that 25 or more operations provide optimal surgical outcomes in RRPN for an experienced surgeon.

AUTHORS' CONTRIBUTIONS

SK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **MA:** Conceptualization, Investigation, Methodology, Project administration, Resources, Software, Writing – review & editing. **MRI:** Data curation, Investigation. **BFB:** Data curation, Investigation. **MS:** Formal Analysis, Investigation, Resources, Visualization, Writing – review & editing. **KY:** Formal Analysis, Investigation, Validation. **MTO:** Formal Analysis, Investigation, Validation.

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ORIGINAL ARTICLE

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The effectiveness of erector spina plane, quadratus lumborum blocks, and intrathecal morphine for analgesia after cesarean: a randomized study

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SUMMARY

OBJECTIVE: This prospective randomized study was conducted at Ataturk University Medical Faculty Hospital, Department of Anesthesia and Reanimation, from June 2022 to May 2023. The aim of this study was to compare the effectiveness of ultrasound-guided erector spinae plane block, quadratus lumborum block, and intrathecal morphine to decrease postoperative pain after cesarean section.

METHODS: Sixty-term pregnant women who were scheduled for elective cesarean sections with spinal anesthesia were included. Patients were randomly divided into three groups (n=20 for each group): Group 1: Patients were administered intrathecal morphine during spinal anesthesia; Group 2: Patients performed bilateral erector spinae plane block postoperatively; and Group 3: Patients performed bilateral quadratus lumborum block postoperatively. In the postpartum care unit, patients received intravenous Patient-Controlled Analgesia. The Patient-Controlled Analgesia devices were set to administer an intravenous bolus of 25 μg fentanyl, with a lockout interval of 10 min. Opioid consumption and maximum pain score in the 24 postoperative hours were recorded.

RESULTS: Patients in Group 1 had a longer time to first analgesic requirement compared to Group 2 (p=0.017). Opioid consumption and resting and moving visual analog score scores in the first 24 h postoperatively were similar between groups.

CONCLUSION: All three methods, including intrathecal morphine, erector spinae plane block, and quadratus lumborum block, are efficacious and comparable in providing postoperative analgesia after cesarean under spinal anesthesia.

KEYWORDS: Anesthesia. Analgesia. Cesarean section. Morphine.

INTRODUCTION

Cesarean delivery is associated with severe postoperative pain[1]. The most important benefits of optimizing postoperative pain control are early mobilization, ease of newborn care, early discharge from the hospital, and better patient satisfaction[2,3]. Combining systemic and regional techniques, a multimodal approach is recommended for postoperative pain management in patients undergoing cesarean surgery[4,5].

Abdominal wall fascial plane blocks under ultrasound guidance have been widely used in pain management after cesarean sections in recent years[6,7]. These techniques allow the deposition of high-volume local anesthetic within a fascial plane. The most commonly used fascial plane blocks are the erector spinae plane (ESP), transversus abdominis plane (TAP), transversalis fascia plane (TFP), and quadratus lumborum (QL) blocks. The erector spinae plane block (ESPB) is a para-spinal regional anesthesia technique that leads to local anesthetic distribution to the interfascial plane between the transverse process and the erector spinae muscles and reveals both somatic and visceral analgesia. It was shown that ESP block provides effective postoperative analgesia in patients undergoing cesarean delivery[8].

Quadratus lumborum block (QLB) has gained popularity as an effective analgesic method in patients undergoing cesarean sections and provides the spreading of the local anesthetic agent into the thoracolumbar fascia. The analgesic efficacy of QLB administration after a cesarean section was first demonstrated by Blanco et al.[9]. They reported a significant reduction in postoperative opioid consumption in patients who injected

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0.125% bupivacaine on the posterolateral border of the QL muscle compared with the control group.

This prospective randomized study aimed to compare the effectiveness of ultrasound-guided ESPB, QLB, and intrathecal morphine to decrease postoperative pain after a cesarean section.

METHODS

After clinical ethics committee approval (protocol number: B.30.2.ATA.0.01.00/2079, date: March 31, 2022), this prospective randomized study was conducted at Ataturk University Medical Faculty Hospital, Department of Anesthesia and Reanimation, from June 2022 to May 2023. After obtaining written consent from the participants, the study was performed on 60 ASA I-II women aged 18–45 years with a term singleton pregnancy who were scheduled for an elective cesarean section because of a previous cesarean section with a Pfannenstiel incision under spinal anesthesia. Patients with complicated pregnancies, body mass index (BMI) >30 kg/m^2, contraindications to regional anesthesia such as bleeding diathesis, additional diseases such as diabetes, the need for emergency cesarean section, and history of allergy to drugs to be used in the study were excluded from the study. Before the operation, patients were informed about the study procedure and visual analog score (VAS). Patients were fasted for 8 h before surgery. A computer-generated table of random numbers and concealed, opaque envelopes were used for randomization. An anesthetist opened the envelopes, and patients were randomly divided into three groups (n=20 for each group): Group 1: Patients were administered intrathecal morphine during spinal anesthesia; Group 2: Patients performed bilateral ESPB block postoperatively; and Group 3: Patients performed bilateral QLB postoperatively.

Intravenous (IV) vascular access was provided to the patient in the operating room using a 20-gauge branule. Routine monitoring consisting of an electrocardiogram (ECG), peripheral oxygen saturation (SpO_2), and noninvasive blood pressure was performed on all participants. All participants received spinal anesthesia with a weight- and height-adjusted 0.5% isobaric bupivacaine dosage regimen and 15 μg of fentanyl solution at L_3-L_4 or L_2-L_3 levels with a 26 G Quincke spinal needle in a sitting position after the skin was prepared sterile. Patients in Group 1 were administered intrathecal morphine (150 μg) in addition to standard spinal anesthetic drugs.

After the spinal anesthesia procedure, the patient was placed in the supine position. It was controlled by the loss of cold sensation in the patients, and the operation was started when the sensory block levels reached T4. IV midazolam was planned for patients with complaints of pain or discomfort during the operation. However, if pain and discomfort persisted, IV fentanyl or ketamine was planned, and these patients were excluded from the study. In all cases, surgery was performed with a Pfannenstiel incision. Blood pressure was measured at 1-min intervals, and hypotension was defined as a decrease in systolic blood pressure below 20% of the basal value. When hypotension occurred, it was treated with norepinephrine or ephedrine (initial dose: 5 μg norepinephrine or 5 mg ephedrine IV) and a rapid infusion of colloids or crystalloids until blood pressure returned to baseline. Bradycardia was defined as a heart rate of 50 beats per minute and was treated with 1 mg of intravenous atropine. After the operation was completed and the skin was closed, while the patients were still lying on the operating table, ultrasound-guided QL and ESP block applications were performed. Patients in Group 2 received bilateral ESP block, and patients in Group 2 received QL block bilaterally by injecting 20 mL of 0.25% isobaric bupivacaine bolus on both sides. All block applications were performed using an aseptic technique, accompanied by ultrasound, by an anesthesiologist with at least 3 years of block application experience.

To perform QLB, the mid-axillary line was detected, and the linear probe (Esaote MyLab30®, CA631 high-frequency probe, United Kingdom) was placed in the transverse axial plane just above the iliac crest. After the QL muscle was confirmed, a 22-gauge, 100-mm needle (Stimuplex®; B. Braun, Melsungen, Germany) was introduced throughout the anterolateral border of the QL muscle. The local anesthetic was injected at the junction of QL with the transversal fascia, and the spread of the anesthetic drug along the lateral side of the quadratus lumborum muscle at the union with the transversal fascia was visualized[10]. The same block procedure was done on the other side.

To perform ESPB, an ultrasonography curvilinear probe was placed at the sagittal plane of the paravertebral region to identify the transverse process corresponding to T9. The local anesthetic was injected in the plane of the erector spinae at the T9 level, and the spread of the anesthetic drug along the long spinal axis was confirmed[11]. This block procedure was performed on both sides.

After surgery, patients with stable clinical status were transferred to the postpartum-care unit. Notably, 1 g of paracetamol and 50 mg of dexketoprofen were administered intravenously to the patients 30 min before the end of the operation. In the postoperative period, all patients were given intravenous 15 mg/kg paracetamol every 6 h and 50 mg dexketoprofen every 12 h for 24 h. An anesthesiologist blinded to group allocation visited the patients and recorded the postoperative data. In the postpartum care unit, patients received intravenous Patient-Controlled Analgesia (PCA). The PCA devices were set to administer an intravenous bolus of 25 μg fentanyl, with

a lockout interval of 10 min. The pain was evaluated using the VAS (0 to 10; 0=no pain and 10=as much pain as possible) during movement (forward-backward movement in bed) and at rest (lying motionless in bed) at 2, 4, 6, 12, and 24 h postoperatively. Opioid consumption at 24 h, the total amount of opioids consumed up to 24 h, and the maximum pain score at 24 h were recorded. The presence of nausea and/or vomiting, shivering, and itching was recorded. In case of nausea and vomiting, 4 mg intravenous ondansetron was administered.

Age, weight, height, BMI, gestational week, parity, operation time, time to the first analgesic requirement, time to the first ambulation, and time to the return of bowel movements were recorded.

Statistical analysis

The sample size calculation was based on the study by Krohg et al.[10] on postoperative opioid consumption between the QL block group and the placebo group in cesarean section surgery. The sample size calculation was performed using the G*Power sample size calculator[11]. An estimated sample size of 18 patients in each study group achieved a power of 80% to detect a 40% reduction in opioid consumption, assuming a type I error of 0.05. A sufficient sample size was thought to be 20 in each group, considering potential dropouts.

The SPSS 20 package program was used for the statistical analysis. Numerical data were expressed as mean and standard deviation, and categorical data were expressed as numbers (n) and percentages (%). Statistical analysis was performed with one-way analysis of variance (ANOVA) if the data conformed to the normal distribution and with the Kruskal-Wallis test if they did not comply with the normal distribution. In group comparisons, analysis of repeated measures was done with ANOVA, and analysis of categorical data was done using a chi-square test and a t-test. Test results were considered statistically significant when $p<0.05$.

RESULTS

Data collection was completed in 60 patients (n=20 in each group; Figure 1). The patients in the three groups were comparable in terms of sociodemographic and surgical characteristics. Ambulation time, time for a bowel movement, and time to T4 level were similar between groups. Patients in Group 1 had a longer time to first analgesic requirement compared to Group 2 (p=0.017). There was no difference between the groups in terms of atropine and ephedrine requirements, frequency of nausea-vomiting, shivering, and itching (Table 1). Opioid consumption in the first 24 h postoperatively was similar between groups (Table 2). There were no significant differences between the groups in terms of mean arterial blood pressure and heart rate values during the operation and postoperative period. The resting and moving VAS scores in the postoperative period were similar between the groups (Table 3).

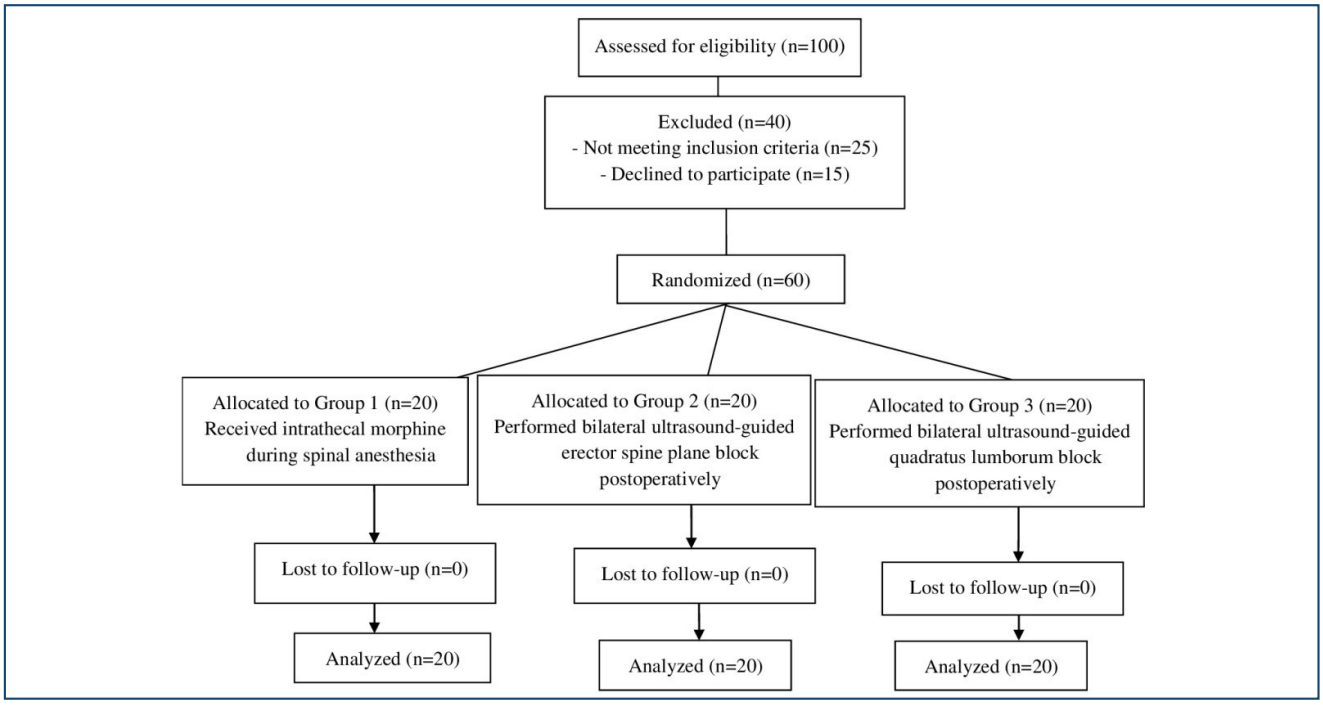

Figure 1. Consort flowchart of study participants.

Table 1. Demographic and anesthetic characteristics of the groups.

	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	p-value
Age (years)	29.70±5.13	30.50±5.31	33.60±6.09	0.071
BMI (kg/m²)	26.69±2.44	27.00±1.36	27.90±1.95	0.141
Operation time (min)	38.75±11.80	38.55±13.44	41.30±9.58	0.711
First analgesic (s)	396.25±291.95[λ]	234.50±137.34	267.05±157.25	0.040*
Ambulation time (s)	400.00±154.28	327.50±95.98	355.90±151.45	0.248
Bowel movement (s)	820.75±325.44	636.00±343.52	817.95±409.29	0.189
Ephedrine requirement, n (%)	13 (65.0)	14 (70.0)	9 (45)	0.233
Atropine requirement, n (%)	2 (10)	3 (15)	1 (5)	0.504
Nausea-vomiting, n (%)	2 (10)	2 (10)	1 (5)	0.804
Shivering, n (%)	2 (10)	1 (5)	0 (0)	0.349
Itching, n (%)	1 (5)	2 (10)	1 (5)	0.153

Group 1: patients were administered intrathecal morphine during spinal anesthesia; Group 2: patients performed bilateral ESP block postoperatively; and Group 3: patients performed bilateral QL block postoperatively. Data were expressed as mean±SD or n (%). Using the ANOVA test, *p<0.05 was considered statistically significant. [λ]p=0.017, compared to Group 2.

Table 2. Postoperative fentanyl consumption (μg) in groups.

	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	p-value
0–2 h	5.50±15.38	13.75±24.96	22.50±36.18	0.145
2–4 h	27.70±42.84	42.00±40.60	34.93±53.39	0.619
4–6 h	32.10±41.94	36.25±34.86	51.09±54.88	0.375
6–12 h	110.10±100.46	101.25±110.16	115.14±122.88	0.924
12–24 h	175.52±171.42	121.00±151.96	231.55±240.96	0.199
During 24 h	370.42±324.19	326.00±311.30	455.45±449.53	0.530

Group 1: patients were administered intrathecal morphine during spinal anesthesia; Group 2: patients performed bilateral ESP block postoperatively; and Group 3: patients performed bilateral QL block postoperatively. Results were presented as mean±SD.

Table 3. Postoperative pain scores for groups at rest and in motion.

	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	p-value
At rest				
2 h	0 (0–40)	10 (0–50)	0 (0–50)	0.090
4 h	0 (0–30)	10 (0–60)	0 (0–70)	0.307
6 h	10 (0–40)	10 (0–40)	10 (0–50)	0.769
12 h	10 (0–50)	10 (0–40)	20 (0–30)	0.537
24 h	10 (0–50)	10 (0–30)	10 (0–30)	0.332
In motion				
2 h	10 (0–50)	20 (0–60)	0 (0–50)	0.117
4 h	10 (0–40)	20 (10–70)	10 (0–80)	0.173
6 h	10 (0–50)	10 (0–50)	20 (0–70)	0.196
12 h	20 (10–50)	30 (0–50)	30 (0–60)	0.545
24 h	20 (10–60)	30 (0–40)	30 (0–50)	0.789

Values are presented as median (min-max). Group 1: patients were administered intrathecal morphine during spinal anesthesia; Group 2: patients performed bilateral ESP block postoperatively; and Group 3: patients performed bilateral QL block postoperatively.

DISCUSSION

This prospective randomized study showed that all three techniques, namely, QLB, ESPB, and intrathecal morphine, were effective in reducing postoperative pain and had comparable analgesic efficacy with respect to postoperative resting and moving VAS scores, hemodynamic parameters, side effects, and postoperative opioid consumption.

There are studies demonstrating the effectiveness of ESP and QLB blocks in the management of postoperative pain following cesarean surgery[9-13]. In agreement with their findings, this present study showed that all three techniques, namely, QLB, ESPB, and intrathecal morphine, effectively reduced the postoperative pain score at all time points from 2 to 24 h during rest and in motion. Similarly, Bakshi et al.[14] reported that QLB and ESPB are effective techniques for providing analgesia after cesarean with similar postoperative pain scores, duration of analgesia, and use of rescue analgesia. In a recent study, Bakshi et al.[14] compared the analgesic efficacy of ultrasound-guided transmuscular quadratus lumborum block (TQLB) and thoracic erector spinae plane block (TESPB) in parturients under cesarean with subarachnoid block. They reported the duration of first-rescue analgesia as 11.90±2.49 h in Group TESPB and 12.56±3.38 h in Group TQLB. In another study, Hamed et al.[15] reported a similar duration (12±2.81 h) for rescue analgesia in parturients who performed ESPB following cesarean surgery under spinal anesthesia. In this present study, the duration of first-rescue analgesia was less (234.50±137.34 s for patients who applied ESPB and 267.05±157.25 s for patients who applied QLB) compared to the above studies[14,15]. In this present study, we included only participants with previous cesarean sections. These participants may have higher pain scores due to associated peritoneal adhesions caused by previous cesarean surgery, which might have influenced the outcome.

The QLB is a deep block and must be performed very carefully by experienced anesthesiologists to avoid complications[5,9]. On the other hand, ESPB is considered a simple and safe block. However, intrathecal administration of morphine is an easier and less invasive procedure than block methods. Moreover, it is easily performed during the spinal anesthesia procedure and does not require additional time. In this present study, we did not observe any serious complications in any patient in any of the three groups. Moreover, the mean time to the first analgesic request in the intrathecal morphine group was longer compared to the ESPB group. We thought that it would be safer to use the intrathecal morphine method instead of the block method, as it takes longer time and has a higher risk of complications.

To the best of our knowledge, no previous studies have compared the analgesic efficacy of all three methods, namely, QLB, ESPB, and intrathecal morphine, for postoperative pain relief after cesarean. There is a limitation to this present study. It would be valuable to create a control group with only standard spinal anesthesia. However, Salama[13] reported that both QLB block and intrathecal morphine provide longer-lasting analgesia with lower postoperative morphine requirements compared to standard spinal anesthesia after cesarean. We demonstrated that QLB and ESPB are equivalent to intrathecal morphine in terms of the consumption of opioids during 24 h postoperatively. The results of this present study also showed that during 24 h after surgery, there were no significant differences in terms of VAS scores at rest or in motion and the incidence of nausea-vomiting and shivering among the three groups. But Salama[13] reported a higher incidence of pruritus and nausea-vomiting in the intrathecal morphine group compared to the control and the QLB groups. We thought that the low morphine-related side effects in our study population may be associated with less postoperative opioid consumption due to routine additional analgesics, including intravenous paracetamol and dexketoprofen.

CONCLUSION

All three methods, namely, intrathecal morphine, ESPB, and QLB, are efficacious and comparable in providing postoperative analgesia after a cesarean section under spinal anesthesia. The intrathecal morphine technique may be recommended due to the longer duration of postoperative analgesia, its relatively low risk of technical complications or failure, and its simple and quick application.

AUTHORS' CONTRIBUTIONS

MA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ANA:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **HO:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review &

editing. **EPTY:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **GNCS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AD:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Use of the Oswestry Disability Index in ankylosing spondylitis

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SUMMARY

OBJECTIVE: The Oswestry Disability Index is considered the gold standard in the evaluation of disability in patients with chronic mechanical back pain. The aim of this study was to assess the applicability of Oswestry Disability Index in patients with ankylosing spondylitis and its relationship with disease assessment parameters for ankylosing spondylitis.

METHODS: A total of 100 patients diagnosed with ankylosing spondylitis were included in the study group. The control group consisted of 50 individuals with nonspecific low back pain. The Oswestry Disability Index and Bath Ankylosing Spondylitis Disease Activity Index were applied to both groups. In addition, the Visual Analog Scale, the Ankylosing Spondylitis Disease Activity Score C-Reactive Protein, the Ankylosing Spondylitis Disease Activity Score-the Erythrocyte Sedimentation Rate, the Bath Ankylosing Spondylitis Functional Index, Bath Ankylosing Spondylitis Metrology Index, and the Ankylosing Spondylitis Quality of Life scales were applied in the study group. the Erythrocyte Sedimentation Rate, C-Reactive Protein levels, and HLA-B27 analysis were noted as laboratory markers in ankylosing spondylitis patients.

RESULTS: The scores of Oswestry Disability Index had a significant correlation with scores of Bath Ankylosing Spondylitis Disease Activity Index in ankylosing spondylitis patients (r=0.543) and in the control group (r=0.401). There was a significant correlation between the scores of Oswestry Disability Index and the Bath Ankylosing Spondylitis Functional Index (r=0.544), Bath Ankylosing Spondylitis Metrology Index (r=0.317), the Ankylosing Spondylitis Quality of Life (r=0.723), the Ankylosing Spondylitis Disease Activity Score-the Erythrocyte Sedimentation Rate (r=0.501), the Ankylosing Spondylitis Disease Activity Score C-Reactive Protein (r=0.530), Visual Analog Scale-Rest (r=0.476), and Visual Analog Scale-Activity (r=0.441) values in patients with ankylosing spondylitis.

CONCLUSION: Evaluation of Oswestry Disability Index in conjunction with Bath Ankylosing Spondylitis Disease Activity Index may warn the physician to interpret high Bath Ankylosing Spondylitis Disease Activity Index scores in the context of mechanical pain. Therefore, the use of Oswestry Disability Index in patients with ankylosing spondylitis will be beneficial.

KEYWORDS: Spondylitis, ankylosing. Axial spondyloarthritis. Back pain. Disability evaluation. Quality of life.

INTRODUCTION

About 80% of people in the general population will have back pain in some form at some point during their lifetimes. The majority of instances of persistent back pain (97%) are reported to have a mechanical character[1]. Inflammatory back pain (IBP) and decreased spinal mobility are the two features of ankylosing spondylitis (AS)[2]. IBP is characterized by back pain that lasts for ≥3 months, develops gradually at the age of <40 years, improves with activity but does not improve with rest, occurs at night, and is accompanied by stiffness in the morning and changes in the findings of several laboratory findings[3]. Mechanical back pain (MBP), which can occur at any age but may be more common in middle-aged, working people, is more frequently caused by an acute injury or damage of an anatomical dysfunction in the lower back[1,4]. Both IBP and MBP have been linked to chronic back pain and both can occur in patients with spondyloarthropathy (SpA)[3]. According to estimates, up to 5% of patients with chronic low back pain (LBP) who visit their primary care provider have AS[2].

The Oswestry Disability Index (ODI), which was created to quantify pain and impairment in patients with chronic LBP, has emerged as the gold standard for determining the degree of disability brought on by MBP. The ODI has not yet been widely utilized for the evaluation and monitoring of AS patients. In general, a more specific AS outcome measure is the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[5]. However, current research indicates that people with mechanical LBP have BASDAI scores comparable to those of patients with AS[6-8]. We aimed to test the applicability of ODI in patients with AS and to determine the correlation of ODI with standard assessment measurements of AS.

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METHODS

A total of 100 patients between the ages of 18 and 65 years who were diagnosed with AS based on modified New York criteria and axial SpA based on the Assessment in SpA International Society (ASAS) classification criteria were enrolled as the patient group. A total of 50 patients with nonspecific LBP, matched in age and gender, were included as the control group in the outpatient clinic of our hospital. Exclusion criteria were a history of lumbar region surgery, peripheral arthritis, the presence of a total hip replacement, and pregnancy.

The BASDAI consists of six questions concerning fatigue, spinal pain, joint pain or swelling, areas of localized tenderness, pain severity, and duration of morning stiffness, and the patients answer the questions on a 10-cm Visual Analog Scale (VAS). Lower scores indicate less active disease[5]. BASDAI's Turkish validity and reliability study was conducted in 2005[9].

A 10-cm VAS was employed to evaluate inflammatory low back discomfort in the last week, and the patients were asked to score between 0 and 10 points (0: no pain and 10: unbearable pain). VAS was questioned on rest and activity[10].

The patient's global evaluation of disease activity, the CRP (mg/L) for the Ankylosing Spondylitis Disease Activity Score-C-reactive protein (ASDAS-CRP), or the ESR (mm/h) for the Ankylosing Spondylitis Disease Activity Score-Erythrocyte Sedimentation Rate (ASDAS-ESR), were used to construct the ASDAS-ESR and ASDAS-CRP, and the scores were then calculated using the responses from questions 2, 3, and 6 on the BASDAI. The disease activity cutoffs for ASDAS are 1.3, which separates "inactive disease" from "moderate disease activity," 2.1, which separates "moderate disease activity" from "high disease activity," and 3.5, which separates "high disease activity" from "very high disease activity[11]."

In both clinical practice and clinical studies, the Bath Ankylosing Spondylitis Functional Index (BASFI) is the measurement that is most frequently utilized[12]. The BASFI index is designed so that the first eight questions concentrate on the functional anatomy of the patient with AS, and the last two questions pertain to global evaluations that assess the patient's functional capacity to manage daily life[13]. A higher score indicates a higher degree of functional limitations.

To reliably assess the axial condition of people with AS, the Bath Ankylosing Spondylitis Metrology Index (BASMI) was developed. The five clinical measures used to determine the BASMI score are the tragus-to-wall distance, lumbar flexion, cervical rotation, lumbar side flexion, and intermalleolar distance. The total score is between 0 and 10. A high score is associated with poor axial mobility[14].

The Ankylosing Spondylitis Quality of Life (ASQoL) questionnaire has 18 items with a binary "Yes/No" response format, each with a score of "1" or "0," respectively. Overall ratings varied from 0 to 18, with a higher number signifying a worse level of life quality[15].

The ODI is among the most frequently used self-reported questionnaires for assessing functional outcomes in individuals with LBP and other spinal diseases. It was designed as a clinical assessment instrument to calculate an individual'slevel of disability. The ODI just takes a few minutes to finish, and it is simple to score (1 min). The ODI is divided into 10 components that measure pain severity, personal care, lifting, walking, sitting, standing, sleeping, participating in social activities, traveling, and altering pain intensity. The ODI produces a final functional score that ranges from 0 to 100 and is decoded as follows: 0–20% as minimal disability without need for therapy; 20–40% as modest disability, requiring conservative therapy; 40–60% as serious disability, requiring further examination; 60–80% as devastating disability, requiring substantial intervention, and>80% as bedridden[16-18].

Statistical analysis

Statistical Package for Social Sciences (version 22) was used to perform the statistical tests (SPSS Inc. Chicago, IL, USA). Descriptive data were presented as mean±standard deviation (SD) for normally distributed data and as median (minimum-maximum) for non-normally distributed data. Categorical data were given as frequency. The comparison of genders was carried out using the Pearson's chi-square test. The fit of the data to the normal distribution was tested with the Kolmogorov-Smirnov test. The independent samples t-test was used to compare normally distributed data, and the Mann-Whitney U test was used to compare non-normally distributed data between independent groups. The Spearman correlation test was used to analyze the association between ODI and measured disease parameters. A correlation coefficient (r) of more than 0.30 and a value of $p<0.05$ were considered statistically significant.

RESULTS

Demographic characteristics and clinical parameters of the patient and the control groups are presented in Table 1. No statistically significant differences were found between groups in terms of gender, age, body mass index (BMI), or ODI scores. BASDAI, ESR, and CRP were statistically significantly higher in the study group ($p<0.05$). The disease duration was 7.5 (1.0–33.0) years in the study group. HLA-B27 was positive in 69% of our study. The disease characteristics of patients are given in Table 2.

Table 1. Demographic, anthropometric, and clinical characteristics of both groups.

	Patients (n=100)	Control (n=50)	p
Gender(female/male) (n)	28/72	17/33	0.45 (χ^2 test)
Age (years), median (min-max)	45.0 (27–64)	43.0 (25–63)	0.527
BMI (kg/m^2), median (min-max)	27.1 (18.1–47.3)	25.6 (21.1–40.2)	0.769·
ODI,median (min-max)	10.0 (0.0–48.0)	6.0 (0.0–30.0)	0.236·
ESR (mm/h), median (min-max)	17.5 (2–107)	8.0 (1–27)	0.0001·
CRP (mg/L), median (min-max)	3.5 (2.0–33.15)	3.0 (0.5–7.0)	0.001·
BASDAI, (mean±SD)	4.1±0.2	1.9±1.9	0.00··

Data were given as median (min-max) or mean±standard deviation (SD). n: number of patients; BMI: body mass index; BASDAI: Bath AS Disease Activity Index; ODI: Oswestry Disability Index; ESR: the Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein. ·Mann-Whitney U test. ··Student's t-test.

Table 2. Disease characteristics of patients.

	Patients (n=100)
BASFI	2.8 (0.0–9.3)
BASMI	2.0 (0.0–9.0)
ASQoL	6.5 (0.0–18)
ASDAS-ESR	2.6 (1.0–5.8)
ASDAS-CRP	2.5 (1.0–4.9)
VAS-R	60.0 (0.0–100.0)
VAS-A	40.0 (0.0–80.0)
Disease duration (years)	7.5 (1.0–33.0)

Data were given as median (min-max). BASFI: the Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index; ASQoL: the Ankylosing Spondylitis Quality of Life; ASDAS-ESR: the Ankylosing Spondylitis Disease Activity Score-the Erythrocyte Sedimentation Rate; ASDAS-CRP: the Ankylosing Spondylitis Disease Activity Score-C-Reactive Protein; VAS-R: Visual Analog Scale-Rest; VAS-A: Visual Analog Scale-Activity.

Table 3. The correlation between Oswestry Disability Index score and Bath Ankylosing Spondylitis Disease Activity Index, Bath Ankylosing Spondylitis Functional Index, Bath Ankylosing Spondylitis Metrology Index, the Ankylosing Spondylitis Quality of Life, the Ankylosing Spondylitis Disease Activity Score-the Erythrocyte Sedimentation Rate, the Ankylosing Spondylitis Disease Activity Score-C Reactive Protein, Visual Analog Scale-Rest, Visual Analog Scale-Activity, and duration of disease.

	Patients (correlation coefficient/p-value)	Controls (correlation coefficient/p-value)
BASDAI	0.543/0.0001	0.401/0.004
BASFI	0.554/0.0001	
BASMI	0.317/0.01	
ASQoL	0.723/0.0001	
ASDAS-ESR	0.501/0.000	
ASDAS-CRP	0.530/0.000	
VAS-R	0.476/0.000	
VAS-A	0.441/0.000	
Disease duration (years)	0.085/0.401	

BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index; ASQoL: the Ankylosing Spondylitis Quality of Life; ASDAS-ESR: the Ankylosing Spondylitis Disease Activity Score-the Erythrocyte Sedimentation Rate; ASDAS-CRP: the Ankylosing Spondylitis Disease Activity Score-C Reactive Protein; VAS-R: Visual Analog Scale-Rest; VAS-A: Visual Analog Scale-Activity.

The correlations between the ODI scores and BASDAI were moderate, with a correlation coefficient of r=0.543 in patients with AS and were weak, with a correlation coefficient of r=0.401 in the control group. Also, there was a significant correlation between ODI scores and BASFI, BASMI, ASQoL, ASDAS-ESR, ASDAS-CRP, and VAS values in patients with AS. There was no correlation found between the duration of the disease and BASDAI and ODI scores in patients with AS (Table 3).

DISCUSSION

Our study has shown that the ODI, a tool frequently used to quantify back pain, correlates quite well with the typical self-reported measures used to evaluate individuals with AS. This score's use in axial SpA was confirmed, and it had a significant correlation with the BASFI and BASDAI scores. The significant correlation of the ODI with the BASDAI and BASFI, when used to measure IBP, shows that it accurately reflects both activity and function domains[2]. We did not come across a study investigating the correlation between ODI and ASDAS-ESR, ASDAS-CRP, ASQoL, and BASMI in the literature. From this perspective, this is the first demonstration.

O'Shea et al.[2] found a strong correlation between the ODI score and BASFI and BASDAI in their study in a group of 49 patients with AS published in 2010. In this study, the correlations between the ODI and the total back pain score, the nocturnal

back pain score, and the patient global assessment scores were considered good. Although our results were very similar, we had a control group differently in our study, and additionally, we used ASDAS-CRP and ASDAS-ESR for disease activity measurement, ASQoL for quality of life (QoL), and BASMI for axial mobility.

In their retrospective case series study, Huang et al.[19] evaluated QoL and its correlation with clinical and radiographic variables in AS patients. In their study, they used SF-36 for health-related QoL. They found that poor QoL was significantly correlated with high disease activity, poor functional status, and decreased mobility in AS. Major predictors for the SF-36 physical function subscale were found to be ODI, BASFI, and BASMI. In our study, we chose to use ASQoL for QoL, and similar to this study, we found a significant correlation between ODI scores and BASFI, BASMI, and ASQoL.

In our study, we found that there is a significant correlation between the scores of ODI and BASFI, BASMI, ASQoL, ASDAS-ESR, ASDAS-CRP, and VAS values in patients with AS. Other medical staff who do not usually follow up with rheumatology patients and who are not familiar with BASDAI and BASFI scores can use ODI to record both activity and function domains in assessing back pain in patients with AS. In the present study, significant correlations were observed between ODI and ASQoL scores. There is also a significant correlation between ODI and BASMI scores in our study. These findings suggest that LBP and spinal immobility affect QoL negatively in patients with AS. It may be practical for active clinics to use only one scale (ODI) to evaluate the physical function, quality of life, and effectiveness of management strategies.

Measurements of AS symptoms and disability are not unique to inflammatory conditions; they also capture mechanical signs and present restrictions[8]. According to recent research, people with mechanical LBP have BASDAI ratings that are comparable to those of patients with AS[6,7]. Even after 40 years of AS, when mechanical symptoms are supposed to become more prominent, BASDAI scores stay comparatively steady. When used alone, BASDAI scores can give patients with long-standing AS a false-positive evaluation of AS activity[8]. Patients may be prescribed biological agents due to false assessments. Acute phase reactants are known to be of limited utility since AS activity measurements and other substitute markers have not yet been identified. For this reason, the use of ODI, ASDAS-ESR, and ASDAS-CRP, based on both clinical and laboratory measurements, can slightly reduce these misconceptions. In our study, we used ASDAS-ESR and ASDAS-CRP in addition to the BASDAI score for measuring disease activity and found a significant correlation with ODI, but we did not find a relationship between the duration of the disease and BASDAI and ODI scores among individuals with AS.

Some limitations of the study were that the patients were not evaluated in terms of concomitant fibromyalgia and neuropathic pain, and we did not divide the patients with chronic LBPs, whom we took as the control group, into specific diagnostic subgroups.

CONCLUSION

Evaluation of ODI with BASDAI may warn the physician to interpret high BASDAI scores in the context of mechanical pain. Medical staff who are not rheumatologists can use ODI during their daily practice to evaluate the physical function, QoL, and effectiveness of management strategies in patients with AS.

ETHICAL APPROVAL

The local ethics committee approved the study protocol with the number 350/2022. The study was performed in accordance with the principles of the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

EA: Conceptualization, Data curation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **LO:** Conceptualization, Data curation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **HC:** Conceptualization, Data curation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. B**TD:** Conceptualization, Data curation, Methodology, Project administration, Validation, Visualization, Writing – review & editing. **SED:** Conceptualization, Data curation, Methodology, Project administration, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Reduced mobility is associated with adverse outcomes after in-hospital cardiac arrest

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SUMMARY

OBJECTIVE: In-hospital cardiac arrest is a critical medical emergency. Knowledge of prognostic factors could assist in cardiopulmonary resuscitation decision-making. Frailty and functional status are emerging risk factors and may play a role in prognostication. The objective was to evaluate the association between reduced mobility and in-hospital cardiac arrest outcomes.

METHODS: This retrospective cohort study included patients over 18 years of age with in-hospital cardiac arrest in Botucatu, Brazil, from April 2018 to December 2021. Exclusion criteria were patients with a do-not-resuscitate order or patients with recurrent in-hospital cardiac arrest. Reduced mobility was defined as the need for a bed bath 48 h before in-hospital cardiac arrest. The outcomes of no return of spontaneous circulation and in-hospital mortality were evaluated.

RESULTS: A total of 387 patients were included in the analysis. The mean age was 65.4±14.8 years; 53.7% were males and 75.4% had reduced mobility. Among the evaluated outcomes, the no return of spontaneous circulation rate was 57.1%, and in-hospital mortality was 94.3%. In multivariate analysis, reduced mobility was associated with no return of spontaneous circulation when adjusted by age, gender, initial shockable rhythm, duration of cardiopulmonary resuscitation, and epinephrine administration. However, in multiple logistic regression, there was no association between reduced mobility and in-hospital mortality.

CONCLUSION: In patients with in-hospital cardiac arrest, reduced mobility is associated with no return of spontaneous circulation. However, there is no relation to in-hospital mortality.

KEYWORDS: Cardiac arrest. Resuscitation. Hospital mortality. Functional status. Rehabilitation.

INTRODUCTION

In-hospital cardiac arrest (IHCA) is a medical emergency with high mortality and an incidence of 1–6 per 1,000 hospital admissions[1,2]. Traditionally, this condition is neglected compared to other cardiovascular conditions, such as myocardial infarction and stroke[3]. However, cumulative evidence points to IHCA as a single clinical entity that deserves special attention[4].

Regarding the prognosis, IHCA presents high mortality rates, reaching 77 to 86%[4,5]. In addition, we must be aware of the sequelae, especially neurological, and important loss of functionality that these patients usually suffer if they survive[6]. Because of this poor prognosis, sometimes the resuscitation of these patients may be considered futile[7]. The knowledge of prognostic factors could assist in cardiopulmonary resuscitation (CPR) decision-making with the patients and their family/caregivers[8].

Frailty and functional status are emerging risk factors for adverse outcomes in cardiorespiratory victims[9,10]. These conditions can be related because frailty is a condition of vulnerability after a stressor event[11], and functional status is the ability to perform daily activities, which is usually compromised in frail patients[12]. Both conditions were chronic markers of an unfavorable prognosis. However, the presence of in-hospital reduced mobility (RM), which reflects acute and chronic functional decline, was not yet evaluated in the IHCA scenario. Therefore, our study aimed to evaluate the association between RM and IHCA outcomes.

METHODS

This study was a subanalysis of a larger unpublished retrospective cohort study approved by the ethics committee of our institution (56979721.9.0000.5411) that evaluated the risk

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factors of IHCA. Considering that the assessment of RM has not been described in IHCA, we used a previous study that evaluated the association between frailty and IHCA to estimate the sample size. The sample size was calculated using the difference of return of spontaneous circulation (ROSC) between frail and nonfrail patients (47.9 and 35.5%, respectively)[8], an α of 5%, and a power of 80%, resulting in a minimum sample of 279 patients.

The inclusion criteria were patients over 18 years of age with IHCA in Botucatu, Brazil, from April 2018 to December 2021. Exclusion criteria were patients with a do-not-resuscitate order or with recurrent IHCA. Our hospital is a University Tertiary Hospital, which usually has severely ill patients hospitalized in the wards.

Demographic, laboratory, and clinical data were collected from the data registry for the rapid response team (RRT) and the electronic medical records. The RRT is a specialized team responsible for the prompt assessment, screening, and treatment of patients with signs of clinical deterioration and IHCA in our hospital. The outcomes of no-ROSC and in-hospital mortality were evaluated.

Recurrent IHCA was defined as a new cardiac arrest during the same hospital stay. The RM was defined as the need for a bed bath 48 h before IHCA. Although there is a controversial definition of RM in the literature, the need for bed baths in our study probably included patients with chronic and acute RM. In our hospital, the nursing staff only performed bed baths when the patient had some mobility difficulty. Shockable rhythms included pulseless ventricular tachycardia and ventricular fibrillation, and in nonshockable rhythms, we included pulseless electrical activity and asystole as IHCA first rhythms. ROSC was defined as the restoration of a pulse for at least 20 min.

All statistical analyses were performed with the SigmaPlot software for Windows v12.0 (Systat Software Inc., San Jose, CA, USA). Data are expressed as percentages, mean values with standard deviation, or medians with 25th and 75th percentiles, where appropriate. Comparisons between two groups for continuous variables were performed using the Student's t-test or the Mann-Whitney U test. Comparisons between two groups for categorical variables were made using the χ^2 test or Fisher's exact test.

We constructed two regression models for each analyzed outcome (no-ROSC or in-hospital mortality). In the first model, the RM was adjusted by clinically relevant variables defined by the literature: age, gender, initial shockable rhythm, time of CPR, and epinephrine administration. In the other model, RM was adjusted with parameters that exhibited significant differences in the univariate analysis for each outcome. The significance level adopted was 5%.

RESULTS

A total of 412 patients with IHCA attended by the RRT were evaluated. However, 25 patients were excluded: 17 due to a do-not-resuscitate order and 8 due to recurrent IHCA. Thus, we included 387 patients in the analyses (Figure 1). The mean age was 65.4±14.8 years; 53.7% were males, and 91.2% of initial cardiac arrest rhythms were nonshockable. Most of the patients, 292 (75.4%), had RM. Among the evaluated outcomes, the no-ROSC rate was 57.1% and in-hospital mortality was 94.3%.

Demographic and clinical data according to ROSC are shown in Table 1. In this analysis, older patients, longer duration of CPR, and RM have been associated with no-ROSC. As shown in Table 1, increased age, duration of CPR, presence of arterial hypertension, higher levels of urea and creatinine, epinephrine administration, and RM were associated with increased in-hospital mortality.

In multiple logistic regression, RM persistence was associated with no-ROSC when adjusted by age, gender, initial shockable rhythm, duration of CPR, and epinephrine administration [odds ratio (OR)=1.999; 95% confidence interval (CI) 1.118–3.575; p=0.020] and also when adjusted for statistically significant variables in univariate analysis such as age and duration of CPR (OR=1.982; 95%CI 1.110–3.539; p=0.021) (Figure 2). We also evaluated the performance of RM to predict no-ROSC. The sensibility was 80.5%, the specificity was 31.3%, the positive predictive values were 61.0%, and the negative predictive values were 54.7%.

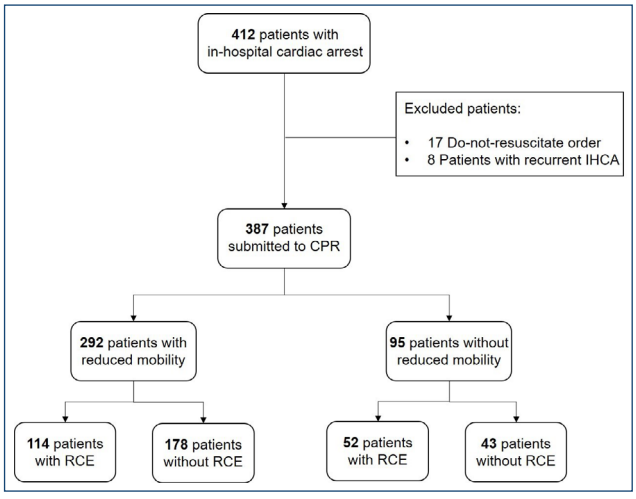

Figure 1. Flow diagram of studied patients with in-hospital cardiac arrest.

Table 1. Baseline characteristics and laboratory data of 387 patients with in-hospital cardiac arrest.

Variable	ROSC		p-value	In-hospital mortality		p-value
	Yes (166)	No (221)		Yes (365)	No (22)	
Age (years)	65.0 (55.0–73.0)	69.0 (58.0–78.0)	0.007	67.0 (57.0–77.0)	53.0 (43.5–68.5)	<0.001
Male gender, n (%)	86 (51.8)	122 (55.5)	0.575	198 (54.2)	10 (45.5)	0.560
Admission category, n (%)						
Medical	81 (48.8)	106 (48.0)	0.953	178 (48.8)	9 (40.9)	0.619
Surgery	85 (51.2)	115 (52.0)		187 (51.2)	13 (59.1)	
Initial rhythm, n (%)						
Shockable (VF, pVT)	18 (10.8)	16 (7.20)	0.290	32 (8.8)	2 (9.1)	0.737
Nonshockable (PEA, asystole)	148 (89.2)	205 (92.8)		333 (91.2)	20 (90.9)	
IHCA initial rhythm, n (%)						
Asystole	41 (24.7)	110 (49.8)	<0.001	151 (41.3)	0 (0)	0.001
PEA	107 (64.5)	95 (42.9)		182 (49.9)	20 (91.0)	
pVT	7 (4.2)	3 (1.4)		9 (2.5)	1 (4.5)	
VF	11 (6.6)	13 (5.9)		23 (6.3)	1 (4.5)	
Duration of CPR (min)	14 (7.0–21.0)	30 (20.0–35.0)	<0.001	24 (15–32)	6 (2–12.5)	<0.001
Medical history, n (%)						
Arterial hypertension	101 (60.8)	145 (65.6)	0.391	238 (65.2)	8 (36.4)	0.012
Diabetes	110 (66.3)	140 (63.3)	0.627	233 (63.8)	17 (77.3)	0.294
Epinephrine, n (%)	162 (97.6)	220 (99.5)	0.218	362 (99.2)	20 (90.9)	0.018
Amiodarone, n (%)	147 (88.5)	203 (91.8)	0.358	35 (9.6)	2 (9.1)	0.767
Reduced mobility, n (%)	114 (68.7)	178 (80.5)	0.010	280 (76.7)	12 (54.5)	0.037
Hemoglobin (g/dL)	12.0 (±2.5)	11.8 (±2.5)	0.605	11.9 (±2.6)	12.2 (±1.9)	0.580
Hematocrit (%)	36.5 (±7.4)	36.2 (±7.4)	0.751	36.3 (±7.5)	36.9 (±.5.2)	0.713
Urea (mg/dL)	54.5 (35.0–95.0)	57.5 (35.7–96.5)	0.240	57.0 (38–96.7)	27.5 (22.2–51.2)	<0.001
Creatinine (mg/dL)	1.0 (0.7–1.7)	1.1 (0.7–2.0)	0.437	1.1 (0.8–1.9)	0.7 (0.6–1.2)	0.005
Sodium (mmol/L)	136 (131–139)	136 (133–139)	0.139	136 (133–139)	135 (131–137)	0.136
Potassium (mmol/L)	4.3 (3.9–4.9)	4.4 (3.9–4.9)	0.643	4.3 (3.9–4.9)	4.3 (3.9–4.9)	0.947

PEA: pulseless electrical activity; pVT: pulseless ventricular tachycardia; VF: ventricular fibrillation; CPR: cardiopulmonary resuscitation; ROSC: return of spontaneous circulation. Data are expressed as the mean±SD, median (25–75%), or percentage.

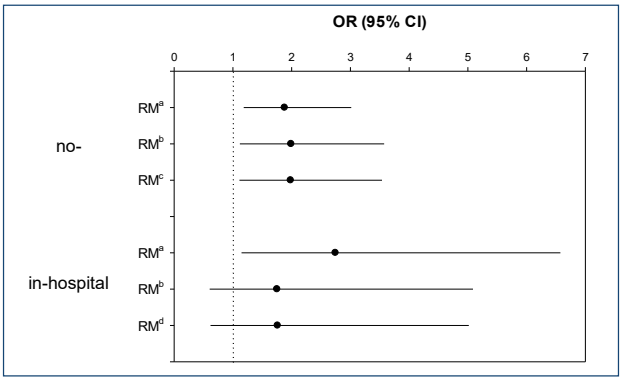

Figure 2. Logistic regression models for the prediction of the return of spontaneous circulation and in-hospital mortality in 387 patients with in-hospital cardiac arrest.

There was no association, in multiple logistic regression, between RM and in-hospital mortality, when adjusted by age, gender, initial shockable rhythm, duration of CPR, and epinephrine administration (OR=1.752; 95%CI 0.604–5.087; p=0.302) or when adjusted by age, time of CPR, arterial hypertension, and epinephrine administration (OR=1.760; 95%CI 0.618–5.016; p=0.290) (Figure 2).

DISCUSSION

The objective of our study was to evaluate the association between RM and IHCA outcomes. We discovered that RM is associated with no-ROSC but not with in-hospital mortality.

IHCA is still a neglected condition compared to out-of-hospital cardiac arrest (OHCA) and other cardiovascular conditions such as myocardial infarction and stroke among others[3]. Although guidelines for IHCA and OHCA are similar[13,14], there are important differences that make IHCA a unique clinical entity. A favorable point to study this condition is that, unlike OHCA, patients are under clinical observation before the event. Despite this observation, mortality is still very high. In our study, mortality was higher than expected by the literature. We believe that the inclusion of COVID-19 patients and the inclusion of patients who had cardiac arrest only in the wards, not in the ICU (intensive care unit), were responsible for this increased mortality. That is, patients in the ICU are under active surveillance and usually receive CPR earlier than in the wards.

Several patient characteristics are associated with IHCA outcomes. A review and meta-analysis that included 23 IHCA studies showed that male sex, increasing age, active malignancy, and chronic kidney disease are among the IHCA prognostic factors[15]. However, all these are nonmodifiable factors, and since patients usually present deterioration signs before cardiac arrest and abnormal vital signs, the search for a possible modifiable factor as RM is an interesting approach[16].

Patients with RM usually have low functional status. Functional status can be viewed as a summary measure of the general impact of health conditions, usually assessed by the ability to perform daily activities, and depending on the degree of impairment, it can result in physical restriction[17,18]. Interestingly, patients with chronic diseases such as frailty usually have functional decline, and both conditions were associated with poor outcomes[18,19]. When assessing RM, we are evaluating both chronic and acute functional decline. In addition, the RM could be the result of previous comorbidities and only a marker of illness severity, or it could be an acute consequence that could be attenuated by interventions.

Regarding being a marker of poor prognosis, RM has a sensitivity of 80.5% to predict no-ROSC. Therefore, its presence is useful clinical information for the health-care team, patients, and families to define care plans and manage hospital resources[8].

RM is also a potentially modifiable factor before cardiac arrest. Although functional decline can be a consequence of varied and complex conditions, evidence points to the importance of early physiotherapy interventions in critical patients[15]. A large review that evaluated more than 80 studies demonstrated that early physiotherapy intervention has a positive effect on functional capacity[20]. Our study did not show an association between RM and in-hospital mortality; however, we believe that this was due to the lower number of patients receiving hospital discharge. Therefore, our study reinforces the importance of physiotherapy protocols for hospitalized patients with initial signs of RM.

Limitations

We must consider some limitations of this study. First, only patients from a single center were evaluated. Second, the retrospective design of the study is restrictive. Despite these limitations, we believe that our study brings important knowledge regarding functional status and IHCA outcomes.

CONCLUSION

In patients with IHCA, RM is associated with no-ROSC. However, there is no relation to in-hospital mortality. These data are among the first to demonstrate that functional decline is associated with the worst outcomes in patients with IHCA.

AUTHORS' CONTRIBUTIONS

TL: Conceptualization, Formal Analysis, Methodology, Writing – original draft. **ELFJ:** Conceptualization, Investigation, Methodology, Writing – original draft. **FAR:** Investigation, Methodology. P**SA:** Investigation, Writing – original draft. B**FP:** Investigation, Writing – original draft. **SARP:** Supervision, Visualization, Writing – review & editing. **LZ:** Supervision, Visualization, Writing – review & editing. **MFM:** Conceptualization, Formal Analysis, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Relationship between cervical spinal cord morphometry and clinical disability in patients with multiple sclerosis

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SUMMARY

OBJECTIVE: Multiple sclerosis is an autoimmune disease that commonly affects the cervical part of the spinal cord. The aim of this study was to evaluate the relationship between cervical spinal cord atrophy and clinical disability in multiple sclerosis patients.

METHODS: We examined the cervical spinal cord area measurements of 64 multiple sclerosis patients and 64 healthy control groups over the images obtained by a T2-weighted magnetic resonance imaging device.

RESULTS: The C2-3, C3-4, C4-5, and C6-7 axial cross-sectional surface area values of the patient group were statistically lower than those of the control group ($p<0.05$). A negative correlation was found between patients' Expanded Disability Status Scale scores and C4-5, C5-6, and C6-7 axial area (axial area $p<0.05$; $r1=-0.472$, $r2=-0.513$, and $r3=-0.415$).

CONCLUSION: When all parameters were evaluated, the data of our control group were found to be higher than the multiple sclerosis groups. There appears to be a significant relationship between patients with cervical spinal cord atrophy and an increase in Expanded Disability Status Scale scores.

KEYWORDS: Atrophy. Cervical cord. Magnetic resonance imaging. Multiple sclerosis.

INTRODUCTION

Multiple sclerosis (MS) is a common chronic inflammatory and demyelinating disease of the brain and spinal cord[1]. The onset of MS can be sudden or insidious[2]. The estimated value is found to be 2.2 million MS patients worldwide[3].

The clinical course of the disease is highly variable and neurological, with a diverse spectrum[2]. The spinal cord is frequently affected pathologically and clinically in MS. Spinal cord atrophy occurs early in the disease and is often progressive[4].

Spinal cord atrophy results from destructive pathological changes in normal-appearing white matter and lesions[5]. Therefore, magnetic resonance imaging (MRI) findings are essential, highly sensitive, and frequently used in imaging lesions[6].

Clinical symptoms such as lower extremity weakness, urinary and fecal sphincter control loss, and progressive motor and sensory disorders may generally be observed in individuals with spinal cord pathology[7,8]. In addition, these focal structural abnormalities in the spinal cord also significantly affect the patient's functional status[9].

Many studies show a clinicopathological correlation between the spinal cord atrophy measurement of the stages of MS and clinical disability[10,11]. Clinical disability levels of patients with MS are evaluated with the Expanded Disability Status Scale (EDSS), which mainly deals with neurological and psychiatric symptoms[12].

In this study, we aimed to evaluate the relationship between cervical spinal cord measurements and EDSS scale results of individuals diagnosed with MS and the degree of clinical disability in the individual.

METHODS

This study was carried out in compliance with the Declaration of Helsinki. Before the inclusion of the patients, ethical approval was obtained from the Local Ethics Committee of the Medical Faculty of Selcuk University (approval number: 2019/189). Our study was carried out at one center.

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Participants

Magnetic resonance (MR) images of 64 MS patients (23 males and 41 females), comprising 38 with Relapsing–Remitting Multiple Sclerosis (RRMS), 16 with Primary Progressive Multiple Sclerosis (PPMS), and 10 with Secondary Progressive Multiple Sclerosis (SPMS), and the control group consisting of 64 healthy individuals (24 males and 40 females), were analyzed retrospectively.

Inclusion criteria for the study were as follows: not having undergone cervical trauma or surgery, having a diagnosis of MS, and having EDSS value, duration, and disease subtypes simultaneously made and obtained by neurologists. For the control group, no systemic disease was diagnosed and no history of demyelinating disease was recorded.

Magnetic resonance imaging technique and clinical evaluation

The examinations of cervical MR were performed in the Radiology Department using a 1.5 T MR device (Siemens, Magnetom Area, Germany). The measurements were performed retrospectively in sagittal and axial planes with T2-weighted (TR/TE: 17/555 ms; slice thickness: 3 mm; field of view (FOV): 270 mm; and matrix: 512×512) (Figure 1).

Statistical analysis

The data were evaluated with the IBM SPSS Statistics Standard Concurrent User V 25 (IBM Corp., Armonk, New York, USA) statistical package program. Descriptive statistics were given as the number of units (n), percentage (%), and mean±standard deviation ($\bar{x}$±sd). The Shapiro-Wilk normality test and Q-Q graphics were used to evaluate the normal distribution of the data for numerical variables. The exact method of the chi-square test was used to compare the groups in terms of gender. A one-way analysis of covariance (ANCOVA) was used, after adjusting the age and gender variables, to compare axial area according to the groups. If there was a statistically significant difference from the ANCOVA, the Bonferroni correction multiple comparison tests were used to identify the different groups. The results of the two-sample independent t-test and one-way analysis of variance (ANOVA) were also given for uncorrected data to see the effects of correction. $p<0.05$ was considered statistically significant. Furthermore, ANOVA was also used to compare the numerical variables according to age groups. The Levene test evaluated the homogeneity of the variances. If a difference was found as a result of the one-way ANOVA, the Tukey's test was used as a multiple comparison test.

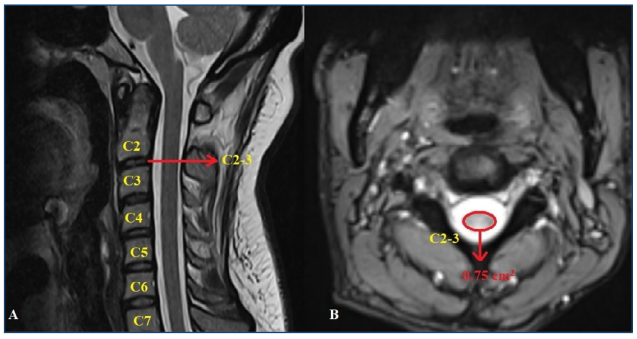

Figure 1. (A) Display of the C2-3 range over a sagittal image (red arrow). (B) Area measurement over the axial section from level C2-3 (red circle).

RESULTS

Demographic characteristics of the participants

A total of 128 participants, 64 in the patient group and 64 in the control group, were included in the study. The patient and control groups were statistically similar in gender ($p=1.000$). The mean age of the patient group and control group was 44.14±10.26 and 34.90±10.90, respectively. The mean age of the patient group was statistically higher than the control group ($p<0.001$). The duration of the disease ranges from 1 to 31 years. Disease groups are similar in terms of gender, age, and disease duration ($p>0.05$). EDSS scores are statistically different according to disease groups ($p=0.012$).

Comparison of axial area values of the groups

There is a difference between the ages of the groups. In this case, axial area values are affected by age and sex variables. These values were corrected for age and sex, and analyses and interpretations were made according to the results of the one-way ANCOVA. The C2-3, C3-4, C4-5, and C6-7 axial area values of the patient group were statistically lower than those of the control group ($p<0.05$) (Table 1).

Magnetic resonance measurements of the cervical spinal cord and differences between groups

The axial area values for all participants, i.e., the patient group and the control group, were compared according to gender variable. The C2-3, C3-4, and C4-5 axial area values of men were statistically higher than those of women in the whole group ($p<0.05$). In the patient group, all axial area values except for axial area C5-6 were statistically higher in men ($p<0.05$). In the control group, the C3-4 and C4-5 axial area values of men were statistically higher than those of women ($p=0.047$ and $p=0.015$). Other axial area values were statistically similar in terms of sex (Table 2).

Comparison of axial area values of the cervical spinal cord by age variable

There was a negative correlation between the ages of the whole group (n=128) and all axial areas measured. There was a negative correlation between the ages of the patient group and the C2-3 axial area (p<0.05; r=-0.378). There was no statistically significant relationship between the age of the control group and the axial area (p>0.05).

Comparison of axial area values of cervical spinal cord according to subtypes of multiple sclerosis

Axial area values are affected by age and sex variables. Thesevalues were corrected for age and sex, and analyses and interpretations were made according to the results of the one-way ANCOVA. Axial area C2-3 values differed statistically according to the groups (p<0.048). PPMS, RRMS, and SPMS values were similar, and the control group values were statistically higher than the patient groups. The group closest to the control group in terms of the mean value was the PPMS group. The C3-4, C4-5, and C6-7 axial area values differed statistically according to the groups (p<0.05). The PPMS, RRMS, and SPMS values were similar, and the control group values were statistically higher than the patient groups. The group closest to the control group in terms of the mean value was the RRMS group (Table 3).

Correlation of Expanded Disability Status Scale scores and duration of disease with cervical spinal cord axial area values

A negative correlation was found between patients' EDSS scores and C4-5, C5-6, and C6-7 axial area (axial area p<0.05; r1=-0.472, r2=-0.513, and r3 =-0.415). The relationship between disease duration and axial area values was not statistically significant (p>0.05). A positive correlation was found between PPMS, RRMS, and SPMS disease group EDSS scores and disease duration (p<0.05; r1=0.522, r2=0.438, and r3=0.711, respectively).

Table 1. Comparison of the axial area values of the groups.

Parameters (axial area)	Groups				Test statistics			
	Patients		HC					
	$\bar{x}$	se	$\bar{x}$	se	t*	p	F**	p
C2-3	0.732	0.012	0.925	0.012	13.701	<0.001	4.070	**0.046**
C3-4	0.720	0.012	0.941	0.012	14.462	<0.001	9.326	**0.003**
C4-5	0.753	0.013	0.968	0.013	13.193	<0.001	4.798	**0.030**
C5-6	0.744	0.012	0.943	0.012	13.709	<0.001	1.006	0.318
C6-7	0.697	0.012	0.881	0.012	12.076	<0.001	5.291	**0.023**

$\bar{x}$ values corrected for age and sex; *independent two-sample t-test; **one-way analysis of covariance; se: standard error. Bold indicates statistically significant p-value.

Table 2. Comparison of axial area values according to gender variable.

Parameters (axial area)	The whole group				Patients				HC			
	M		F		M		F		M		F	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
C2-3	0.849	0.113	0.800	0.148	0.766	0.079	0.681	0.089	0.930	0.076	0.921	0.084
	t=2.145; p=**0.034**				t=3.783; p<**0.001**				t=0.433; p=0.666			
C3-4	0.854	0.134	0.802	0.140	0.747	0.076	0.691	0.095	0.957	0.089	0.916	0.070
	t=2.061; p=**0.041**				t=2.445; p=**0.017**				t=2.025; p=**0.047**			
C4-5	0.889	0.137	0.825	0.145	0.780	0.072	0.716	0.103	0.994	0.096	0.937	0.082
	t=2.458; p=**0.015**				t=2.645; p=**0.010**				t=2.511; p=**0.015**			
C5-6	0.855	0.128	0.818	0.142	0.754	0.077	0.709	0.100	0.951	0.087	0.930	0.076
	t=1.458; p=0.147				t=1.882; p=0.065				t=1.016; p=0.314			
C6-7	0.808	0.120	0.765	0.133	0.720	0.092	0.668	0.100	0.892	0.075	0.866	0.075
	t=1.498; p=0.137				t=2.089; p=**0.041**				t=1.371; p=0.175			

HC: healthy control; sd: standard deviation; F: female; M: male. Bold indicates statistically significant p-value.

Table 3. Comparison of axial area of the groups according to types of disease.

Parameters (axial area)	Groups								Test statistics			
	PPMS		RRMS		SPMS		HC					
	$\bar{X}$	se	$\bar{X}$	se	$\bar{X}$	se	$\bar{X}$	se	F*	p	F**	p
C2-3	0.741[a]	0.022	0.725[a]	0.016	0.714[a]	0.035	0.925[b]	0.012	63.027	<0.001	2.117	**0.048**
C3-4	0.716[a]	0.022	0.726[a]	0.016	0.703[a]	0.035	0.941[b]	0.012	68.619	<0.001	3.455	**0.019**
C4-5	0.743[a]	0.024	0.751[a]	0.017	0.742[a]	0.037	0.968[b]	0.013	57.489	<0.001	2.077	**0.043**
C5-6	0.736	0.022	0.756	0.016	0.703	0.035	0.943	0.012	61.899	<0.001	1.163	0.327
C6-7	0.701[a]	0.022	0.704[a]	0.016	0.621[a]	0.034	0.881[b]	0.012	47.903	<0.001	3.100	**0.003**

HC: healthy control; PPMS: primary progressive multiple sclerosis; RRMS: relapsing–remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis; $\bar{X}$: values adjusted for age and gender. *one-way analysis of variance; **one-way covariance analysis results; and the superscripts "a" and "b" show differences between groups. The groups with the same letters are statistically similar. Bold indicates statistically significant p-value.

In the RRMS group, a negative correlation was found between EDSS scores and axial area C3-4 ($p<0.05$; $r=-0.394$). There was a negative correlation between EDSS scores and C2-3 axial area values in the SPMS group ($p<0.05$; $r=-0.668$).

DISCUSSION

The reduction in the cross-sectional area of the spinal cord is considered an indirect measure of axonal degeneration[13]. MRI predicts spinal cord pathologies in 90% of MS patients[14]. In addition, a significant correlation was reported between cervical cord atrophy and EDSS values[15,16].

In the literature, the measurement of spinal cord segments, area, length, and volume have been examined with different techniques in many studies. In these studies, the subtypes of the disease, cord areas, duration of the disease, and their relationship with the EDSS value were evaluated. Our study compared axial area measurements in MS patients and healthy individuals. In addition, the relationship between atrophy due to disease duration, age, gender, and clinical disability was investigated in patient groups. This study showed that as a result of cord area measurements, cervical spinal cord atrophy in MS patients could be evaluated.

In the study by Rocca et al.[17], the C6-7 axial area measurement results of the RRMS group were lower than those in our study, but the C2-3, C3-4, C4-5, and C5-6 axial area measurements were higher. PPMS and SPMS values were lower than our measurements. The differences might be related to the disease duration and EDSS value.

In a study by Bernitsas et al.[9], the C2 axial area measurements of the PPMS group were lower than those in our study. On the other hand, RRMS group values were found to be higher than in our study. The reason for the difference in the area measurements is due to the mean EDSS value, the difference in the measurement region, the number of samples, or the measurement method.

Cortese et al.[18] measured the C2-3 axial area in a 3-year cohort study of patients with PPMS. While our results were lower than the measurement results at the beginning and the end of the first year, they were higher than those at the end of the third year. This difference is thought to be due to the duration of the disease and the lower mean EDSS compared to our group. It is thought that the fact that our study is a longitudinal study will affect the results. Our measurements were higher when healthy groups were compared in the same study. The differences are due to the mean young age of our sample group.

Biberacher et al.[19] measured the C2-3 axial area of the patient group with Clinical Isolated Syndrome (CIS) and RRMS and detected the lesions in this segment. Our study is similar to the measurement results of the RRMS group. This study reported that the cord area did not differ between patients with and without lesions in CIS and RRMS. Bussas et al.[20] stated that there was no significant difference between the spinal cord areas of MS patients with active lesions. It has been reported in different studies that no relationship was found between spinal cord lesions and spinal cord atrophy[20,21].

It has been shown that the morphometric measurements of the spinal cord, age, gender, height, weight, ethnicity or region of residence, measurement methods, and MRI devices affect the results for MS patients. All parameters in the control group of our study were higher than those in the MS group. There was no statistically significant difference between the axial area measurements of disease duration. There is a negative correlation between the EDSS score of the patient group and the measurement parameters. This condition is thought to be related to cervical spinal cord atrophy, a significant marker of spinal cord pathology.

CONCLUSION

Spinal cord pathology in MS is a complex process. It is thought that clear information will be obtained by knowing the exact etiology and physiopathology of MS and using radiological imaging devices. We aimed to systematically record the data we have obtained as a result of this study and present it for the use of science.

ETHICAL APPROVAL

This study was carried out in compliance with the Declaration of Helsinki. Before the inclusion of the patients, ethical approval was obtained from the Local Ethics Committee of the Medical Faculty of Selcuk University (approval number: 2019/189).

AUTHORS' CONTRIBUTIONS

NGC: Conceptualization, Data curation, Formal Analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **AKK:** Conceptualization, Data curation, Project administration, Writing – review & editing. Z**F:** Data curation, Methodology, Supervision, Writing – review & editing. **HG:** Data curation, Resources. **HC:** Data curation, Resources. **NUD:** Conceptualization, Data curation, Methodology.

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ORIGINAL ARTICLE

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Cytogenetic changes in oral mucosa cells from individuals submitted to oral human immunodeficiency virus pre-exposure prophylaxis use

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate cytogenetic changes in individuals submitted to oral human immunodeficiency virus pre-exposure prophylaxis use through the micronucleus test in oral mucosa.

METHODS: This study consisted of 37 individuals, of whom 17 comprised the pre-exposure prophylaxis group and 20 comprised the control group. A total of 2,000 cells per slide were analyzed for the determination of micronuclei, binucleation, nuclear buds, and cytotoxicity parameters: pyknosis, karyolysis, and karyorrhexis (KR), in a double-blind manner. The repair index was also evaluated in this setting.

RESULTS: In the mutagenicity parameters, the pre-exposure prophylaxis group showed increased frequencies of micronuclei (p=0.0001), binucleation (p=0.001), and nuclear buds (p=0.07). Regarding the cytotoxicity parameters, there was an increase with a statistical difference (p≤0.05) in the karyorrhexis frequency (p=0.001). Additionally, the repair system efficiency decreased in the pre-exposure prophylaxis group.

CONCLUSION: These results indicate that individuals undergoing pre-exposure prophylaxis use have geno- and cytotoxicity in oral mucosal cells.

KEYWORDS: Anti-retroviral agents. DNA damage. Micronucleus tests. Mouth mucosa.

INTRODUCTION

The different existing methods to avoid contamination by the human immunodeficiency virus (HIV) have not yet been enough to eradicate the disease. Since its form of transmission was discovered, through secretions, such as vaginal secretions, sperm, blood, and breast milk, the incessant recommendations before the use of mechanical barrier (condoms), the non-sharing of needles, the decrease of high-risk behaviors, especially for alcohol and drug users, regular testing for HIV, prompt treatment of other sexually transmitted infections, and prevention of transmission by HIV-positive individuals with regular use of antiretroviral therapy (ART) have been part of the strategy to minimize the spread of AIDS[1].

Since 2016, the World Health Organization (WHO) has published a guide of recommendations closely related to AIDS and has oral pre-exposure prophylaxis (PrEP) as part of the combined prevention strategy (biomedical and behavioral) to HIV infection for people at high risk[2]. PrEP consists of the continuous use of antiretroviral drugs in HIV-negative people to reduce the risk of acquiring HIV infection[1]. The medication initially offered included oral tenofovir, either alone or in combination with emtricitabine, both being nucleoside reverse transcriptase inhibitors. In 2021, the use of the vaginal ring with dapivirine was another option for women at risk, and in 2022, the injectable use of long-acting cabotegravir was recently added to the prophylactic medications[3]. Several studies have demonstrated that the treatment with PrEP reduces HIV infection[4,5]. For example, Tsai et al.[6] studied the use of the antiretroviral tenofovir in monkeys (*Macaca fascicularis*) inoculated with HIV and observed that when treated in the first 24 h after infection for 28 days, they did not show viral replication after interruption of treatment. Grant et al.[7], in their study on 2,499 participants from 6 countries, observed a 44% decrease in HIV infection in individuals who made the prophylactic use of tenofovir associated with emtricitabine. In the same year, Abdool Karim et al.[8] observed that the use of 1% tenofovir vaginal gel reduced HIV infection between 39 and 54% in women.

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Genotoxicity assays are widely used to identify the chemical compounds that would be able to induce DNA damage. To evaluate this effect, the micronucleus assay is suitable for this purpose as it is simple and low cost with reproducible results[9]. The assay allows analyzing DNA alterations in exfoliated cells of the oral mucosa in a minimally invasive way, where it is possible to verify nuclear alterations such as the presence of micronuclei (MN), binucleation (BN), and nuclear buds (NB) as indicators of genetic damage. Also, cytotoxicity through the phases of cell death, karyorrhexis (KR), pyknosis (PK), and karyolysis (KL) was evaluated in these individuals. The biological significance of the micronucleus lies in exposure to chemical agents, chronicle diseases, and aging[9].

In this context, this study aims to evaluate possible cytogenetic changes due to the continuous use of PrEP, which are not assessed in routine tests adopted in clinical practice by micronucleus assay. To the best of our knowledge, this approach has not been made so far. Certainly, such data will provide insights for better understanding regarding the safety of PrEP use.

METHODS

Casuistics

The study was approved by the Institutional Human Ethics Committee from the Universidade Federal de São Paulo (UNIFESP), under protocol 0485/2019. All participants received a detailed explanation about the project, and the participants signed an informed consent form.

This study consisted of 37 individuals, of whom 17 comprised the PrEP group and 20 comprised the control group. A single examiner, a dentist, performed the collection, staining, and examination of the unidentified samples. A total of 17 volunteers from the PrEP group aged between 19 and 50 years were recruited under regular monitoring in the Specialized Care Service in the city of Santos, SP, Brazil. Exclusion criteria were the absence of infectious diseases, oral lesions, and exposure to radiographic or tomographic exams in 15 days prior to sample collection. The control group was randomly recruited by a direct approach in public places in the city of Santos, SP, Brazil. Notably, 20 people were recruited, with the exclusion criteria similar to the PrEP group.

Cytogenetic assay

The oral mucosa MN test followed the protocol described by Belien et al.[9]. With the help of a wooden spatula previously moistened with saline solution, a gentle scraping was performed on the inner portion of the jugal mucosa on both sides. The stain used was Feulgen/Fast Green. The correct identification of metanuclear changes was proposed by Bolognesi et al.[10]. For this, the following criteria were established for the correct identification of cytogenetic changes. MN: (1) intact main nucleus and cytoplasm; (2) diameter one-third of the main nucleus; (3) similar stain and texture of the main nucleus; and (4) MN in the same focus as that of the main nucleus. KR: The nucleus may also exhibit extensive fragmentation indicative of advanced nuclear fragmentation. BN: Two main nuclei within a single cell and the nuclei are of similar size and staining intensity. NB: The main nucleus has a sharp constriction forming a bud of nuclear material being attached to the main nucleus by a narrow or wide nucleoplasmic bridge. PK: The nucleus is small and shrunken with a diameter that is approximately one-third of that in a fully differentiated cell being uniformly and intensely stained. KL: They do not have a DNA-containing nucleus or other structures that stain with Feulgen.

The repair index (RI), proposed by Ramirez and Saldanha[11], represented by the formula, RI=(KL+KR)/(MN+NB), was also evaluated in this setting.

Statistical analysis

All data were submitted for normalization using the Kolmogorov-Smirnov test. After that, non-parametric data were confirmed by all data collected in this setting. The nonparametric Mann-Whitney test was used to evaluate the metanuclear alterations and DNA RI between the control and experimental groups. The statistical significance level was set at 5%. The statistical analysis was conducted by the BioStat software (version 5.0, Maringá, Brazil).

RESULTS

All participants in the PrEP group were males and reported eating well, including fruits and vegetables; five participants used vitamin supplements, nine reported using mouthwash, the majority (15 people) reported taking alcoholic beverages, and five were smokers (less than 20 cigarettes/day). The minimum time of treatment with Truvada® was 1 month of continuous use, and the maximum time was 13 months. One participant was diabetic and hypertensive using metformin, and the other was hypertensive using valsartan. In the control group, all volunteers were also males and the age ranged from 20 to 51 years. A total of 5 volunteers were smokers, 10 reported using mouthwash, and none was taking any medication. The demographic characteristics are shown in Table 1.

The PrEP group showed an increase with a statistical difference compared with the control group for all mutagenicity

parameters: the frequency of MN (p=0.001), BN (p=0.001), and NB (p=0.078), according to Table 2. In cytotoxicity parameters, there was a statistical difference in the frequency of KR (p=0.001). In other parameters evaluated in this setting, KL (p=0.57) and PK (p=0.8) did not show significant differences (p>0.05) between groups according to the results presented in Table 3. The RI index is shown in Table 2 and the findings suggest the lower repair capacity in the PrEP group in oral mucosa cells when compared with the control group.

DISCUSSION

According to the UNAIDS (the Joint United Nations Programme on HIV/AIDS) report, it was estimated that more than 1.6 million people worldwide would have received oral PrEP by the year 2021[12]. The goal set for 2025 is 10 million people to use this HIV prophylaxis[12]. Initially concentrated in high-income countries, a substantial increase has been observed in underdeveloped countries in the past 2 years. The rate of HIV infections worldwide has shown a steady decline, but in the last 5 years, this has been associated with the COVID-19 pandemic, as well as with the lack of prevention programs that especially reach the most vulnerable groups of people, as they account for more than half of new infections worldwide[13].

Table 1. General characteristics of study participants.

Parameters	Control group (n=20)	PrEP group (n=17)
Mean age	35.2±9.6	34.6±9.7
Gender	M/20	M/17
Time of treatment	–	6.4±4.2
Tobacco smoking	5	5
Mouthrinse	9	10
Illicit drugs	5	5
Vitamins supplement	4	5
Chronicle diseases	–	3

Table 2. Mean+SD frequency of cytogenetic changes related to mutagenicity in individuals submitted to pre-exposure prophylaxis use.

Groups	MN	BN	NB	DNA repair index
Control	0.35±0.6	0.3±0.5	0.05±0.2	198.5±103.8
PrEP	2.35±1.6*	4.3±2.8*	0.7±0.9*	104.6+75.5*

*p≤0.05; MN: micronucleus; BN: binucleation; NB: nuclear bud.

Table 3. Mean+SD frequency of cytogenetic changes related to cytotoxicity in individuals submitted to pre-exposure prophylaxis use.

Groups	Normal cells	KL	KR	PK
Control	1,664.1±48.3	195.7±56	27.2±6.5	112.5±42.5
PrEP	1,617.7±85	203.2±66.4	48.9±21.2*	122.7±59.5

*p≤0.05; PK: pyknosis; KL: karyolysis; KR: karyorrhexis.

The success of prophylactic treatment is widely documented as lowering the risk of contracting HIV by 90%, provided by good adherence to treatment[13]. Two dosing regimens are proposed, daily and continuous use of one tablet, or on-demand use, which consists of using 2 tablets between 2 and 24 h before exposure, 1 tablet 24 h after the first dose, and 1 more tablet 48 h after the first dose, totaling 4 tablets, with good efficacy[14]. However, some adverse effects such as nausea, headache, flatulence, stool softening/diarrhea, and edema can be reported and can be treated symptomatically.

The association between tenofovir and emtricitabine has been described for PrEP use, but also severe cases depicted by lactic acidosis and hepatomegaly with steatosis and some rare fatalities, especially in women, obese people, and people who take this drug combination for prolonged use[15]. Tenofovir fumarate presents a potential risk of renal toxicity, and its prolonged use can lead to progressive loss of renal function, acute renal failure, and Fanconi syndrome. According to Jotwani et al.[16], subclinical changes in renal tubular function have been observed in people taking PrEP, warranting further study. Tenofovir fumarate is also related to decreased bone mineral density[17,18], although no increase in the number of fractures is documented. According to Havens et al.[19], in a study on 15–22-year-olds, they showed bone loss after continuous PrEP use for 48 weeks, and with its discontinuation, there was partial or complete improvement after 48 months.

Regarding the geno- or cytotoxicity induced by these drugs, the results were largely obtained through experimental studies. Wu et al.[20] observed the genotoxicity of several antiretroviral drugs. Tenofovir was related to the presence of hepatocellular adenomas, carcinomas, and lung adenomas in rats. Emtricitabine showed no changes for genotoxicity and induction of carcinogenesis. Moraes Filho et al.[21,22] used the test for somatic mutation and recombination detection comet assay in *Drosophila melanogaster* and micronucleus assay in bone marrow cells. Tenofovir promoted DNA damage by inducing mutational and/or recombination events, although it did not produce toxic effects.

Recently, Gutiérrez-Sevilla et al.[23] evaluated genomic instability, through the buccal mucosa micronucleus test, of people with HIV on different types of ARTs and also without medication, and increased BN cells and NB were detected in these individuals. However, there are no studies evaluating the cytogenetic changes in HIV-uninfected individuals undergoing PrEP use. In this study, we evaluated HIV-free individuals taking antiretroviral drugs as a preventive measure against HIV

infection (PrEP). Mutagenicity, an irreversible cell damage factor, was evaluated by cell nucleus alteration events such as BN, MN, and NB. For this, we used the micronucleus test in exfoliated cells of the oral mucosa, as this methodology has demonstrated a direct correlation with the micronucleus test in lymphocytes, with the advantage of being a minimally invasive, low-cost method that allows the evaluation of DNA injury. It has versatility and can be employed for various risk factors, such as environmental, nutritional, radioactive, licit, or illicit drug use, among others[24,25]. Our results revealed that all parameters closely related to mutagenicity showed a statistically significant increase compared with the control group. Cytotoxicity was assessed by cell death parameters, in its distinct phases: PK, KR, and KL. KR, a less frequent event to be observed, as it represents a transition between the initial and final phases of cell death, showed a statistically significant increase when compared with the control group, suggesting that the cell damage may be leading to more cell death events, even if not represented by PK and KL. Taken together, these results indicate that PrEP is capable of inducing genotoxicity and apoptosis in oral mucosal cells. The RI also showed a decrease in the PrEP group in buccal mucosal cells. These results are completely new and, therefore, difficult to discuss at the present time. Anyway, we can infer that the ability to repair oral mucosa cells may be reduced in volunteers submitted to PrEP, favoring the processes of genotoxicity and cell death. However, further studies are needed to accurately assess this condition properly.

CONCLUSION

These results indicate that individuals undergoing PrEP use have geno- and cytotoxicity in oral mucosal cells. As PrEP plays a pivotal role in controlling HIV infection, especially in high-risk populations, further studies are needed to elucidate what tissues and organs are more vulnerable to PrEP, in addition to oral mucosa in humans. Certainly, such data will establish correctly and unequivocally the real risks of PrEP use in order to avoid danger to people.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The project was approved by the Research Ethics Committee of the Universidade Federal de São Paulo (UNIFESP), Protocol number #3.461.911.

AUTHORS' CONTRIBUTIONS

MESA: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MMC**: Conceptualization, Writing – original draft, Writing – review & editing. **DAR**: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing. **CMCBM**: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **DVS**: Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Maternal malnutrition during pregnancy among women with sickle cell disease

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SUMMARY

OBJECTIVE: The objective of this study was to compare the nutritional status and dietary intake of pregnant women with sickle cell disease (SS hemoglobinopathy and SC hemoglobinopathy) to healthy controls and report the maternal and perinatal outcomes.

METHODS: This is a prospective, longitudinal cohort study. Pregnant women with a diagnosis of sickle cell disease and control group were recruited in an outpatient clinic of a tertiary care hospital in São Paulo, Brazil. Maternal anthropometric data and dietary intake data were collected at the second and third trimesters.

RESULTS: A total of 49 pregnancies complicated by sickle cell disease were included. Prepregnancy body mass index was significantly lower in the SS hemoglobinopathy group (n=26, median 20.3 kg/m^2) than the SC hemoglobinopathy group (n=23, 22.7 kg/m^2) or control group (n=33, 23.2 kg/m^2, p<0.05). The prepregnancy nutritional status revealed significantly more women classified as underweight in the SS hemoglobinopathy group (15.4%) than in the SC hemoglobinopathy group (4.4%) and control group (1.6%, p=0.009). In the second trimester, maternal protein intake was significantly lower in SS hemoglobinopathy (73.2 g/day) and SC hemoglobinopathy (68.8 g/day) than in the control group (95.7 g/day, p=0.004). In the third trimester, only SS hemoglobinopathy mothers showed dietary intake of protein significantly lower than that of the controls (67.5 g/day vs. 92.8 g/day, p=0.02). Vitamin A and E consumption was also reduced in the third trimester in the SS hemoglobinopathy group (p<0.05).

CONCLUSION: The nutritional status of pregnant women with SS hemoglobinopathy is characterized by a state of undernutrition. The lower protein intake in the second and third trimesters of pregnant women with SS hemoglobinopathy may contribute to this condition. Undernourishment is a serious complication of sickle cell disease, primarily during pregnancy, and it should be addressed during the prenatal period.

KEYWORDS: Nutrition disorders. Sickle cell disease. Pregnancy complication. Anemia.

INTRODUCTION

Sickle cell disease (SCD) refers to a group of hemoglobinopathies caused by inherited single-gene autosomal recessive disorders, which affect the structure of hemoglobin (Hb). Sickle cell anemia, or SS hemoglobinopathy (HbSS), is the result of sickle cell gene homozygosis. Of the other SCD variants, the most common is SC hemoglobinopathy (HbSC). Several studies have reported maternal and fetal complications in pregnant women with SCD: perinatal mortality, preterm labor, fetal growth restriction, preeclampsia, acute painful crises, and urinary and pulmonary infections[1-4].

Medical improvements have allowed women with SCD to reach childbearing age. As this is a multiorgan disease, patients should be monitored for chronic complications and preconceptional counseling has a role in letting them know about the effects of pregnancy[5,6]. Since the late 1980s, undernutrition has been identified as a critical feature of SCD[7]. Growth in children with HbSS is impaired[8], and suboptimal nutritional status has been reported in children and adolescents with SCD[9,10]. Some studies suggest that malnutrition is probably a consequence of increased requirements rather than poor dietary intake[11].

In pregnancy, less is known about the dietary intake of pregnant women with SCD and nutritional adjustments that should be made[12,13]. The aim of this study was to compare the nutritional status and dietary intake of pregnant women with SCD (HbSS and HbSC) to healthy controls and report the maternal and perinatal outcomes.

METHODS

This is a prospective, longitudinal cohort study performed in São Paulo, Brazil. Pregnant participants were recruited from the specialized prenatal care unit in the university hospital from 2010 to 2016. All patients who came to the clinic and met the inclusion criteria were invited to participate in the study. Data were collected from 46 women with a total of 49

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pregnancies complicated by SCD (HbSS and HbSC). A total of 30 healthy pregnant women in the second trimester and 33 in the third trimester were enrolled as controls. They were singleton pregnant women without any obstetrical or clinical complication, and they were matched for gestational age at nutritional interview. This study was approved by the local Human Research Ethics Committee, and informed consent was signed by all participants.

Pregnant women with a diagnosis of SCD (HbSS and HbSC) were invited to participate in the study at their first prenatal appointment at the specialized prenatal care unit. The inclusion criteria were fetus alive at recruitment and diagnosis of HbSS or HbSC.

Maternal dietary intake data were collected by a single research nutritionist. Face-to-face interviews were conducted in the second and third trimesters of pregnancy by using a validated semiquantitative food-frequency questionnaire (FFQ) for the Brazilian population. The FFQ comprises questions about food and beverages, but not about vitamin or mineral supplement use. The tool is composed of 57 food items, including consumption frequency, portion size, preparation method (raw, boiled, or fried), and additions (sugar or salt). For each food item, subjects report serving sizes (i.e., small, medium, or large) and consumption frequencies (nine options). The dietary intake reference period was the previous 6 months. Nutrients were analyzed using the nutrition software Nutrilife©. The reported foods were converted into means of estimated daily intake of total energy, carbohydrates, proteins, total fat, and fibers. All macronutrients were energy-adjusted. The micronutrients analyzed were calcium, iron, zinc, vitamin A, vitamin E, vitamin C, and folates, which included the daily intake. The Brazilian Standard Food Composition Table[14] was used to calculate average daily intakes of total energy, carbohydrates, fat, and fibers. Food intake was reported in grams/day, and micronutrient intakes were reported using the most appropriate unit for each nutrient.

Maternal anthropometric data were measured by trained nurses. Demographic data were collected during the first prenatal visit. Body mass index (BMI) was calculated using weight and height (kg/m^2) and was obtained at baseline using prepregnancy weight and maternal weight at the end of pregnancy (immediately before birth). In late pregnancy, the maternal nutritional status was defined according to the expected BMI for gestational age[15].

Pregnant women with SCD were scheduled for prenatal care every 1-4 weeks, according to the severity of the disease. All patients were known to have SCD at their first appointment; nevertheless, the hemoglobin phenotype of some patients was confirmed by electrophoresis. Hematological data were collected during pregnancy for clinical purposes, and the last evaluation was used for analysis. Data were obtained from the patient's charts and the electronic records of the laboratory. Blood transfusion was performed for symptomatic anemia or worsening anemia with concurrent pain crisis. Prophylactic antenatal transfusions to maintain a certain hemoglobin or hematocrit level during pregnancy were not performed as this is not included in our management protocol. The sickle cell crises mentioned in this study were defined as acute pain episodes requiring hospitalization for analgesia and intravenous hydration. Hypertensive disorders encompassed chronic hypertension and preeclampsia. Pulmonary complications included pneumonia, acute chest syndrome, and pulmonary thromboembolism. Diagnosis of urinary tract infection was based on a positive urine culture routinely screened in each trimester of pregnancy. Other SCD events included persistent proteinuria, aseptic femoral necrosis, and splenic sequestration.

Statistical analysis

Data were analyzed using the Medcalc program, version 11.5.1.0 (Medcalc Software, Belgium). Descriptive statistics were reported as frequency and percentage for categorical data and as means and standard deviation or median and range for continuous variables. The Kruskal-Wallis test was used to compare the medians between the groups. Categorical data were compared using the chi-square test or the Fisher's exact test when appropriate. Statistical significance was set at $p<0.05$.

RESULTS

Table 1 shows the maternal characteristics of the pregnant women enrolled in this study. Prepregnancy BMI was significantly ($p<0.001$) lower in the HbSS group than in the HbSC group or the control group. The prepregnancy nutritional status revealed more women classified as underweight in the HbSS group. Furthermore, the gestational weight gain was significantly lower in HbSS pregnancies. As expected and given the SCD features, when compared with the control group, hemoglobin and hematocrit levels were significantly lower, and white blood cell count was significantly higher. The HbSS group presented a significantly higher platelet count than the HbSC and the control groups.

Maternal dietary intake evaluated by FFQ in the second and third trimesters is presented in Table 2. In the second trimester, the dietary intake of protein was significantly lower in the HbSS and HbSC groups than in the control group. In the third trimester, maternal dietary intake of protein was significantly lower in the HbSS group when compared with the

Table 1. Maternal characteristics of pregnant women complicated by sickle cell disease.

Characteristics	HbSS (n=26)	HbSC (n=23)	Control (n=63)	p
Maternal age, years	25.5 (17–34)	25.0 (19–44)	28.0 (18–39)	0.367
Nulliparous women	16 (61.5)	13 (56.5)	38 (60.3)	0.626
Education				
Elementary school	14 (53.8)	8 (34.8)	26 (41.3)	0.376
High school/college	12 (46.2)	15 (65.2)	37 (58.7)	
Prepregnancy BMI (kg/m^2)	20.3 (16.4–26.3)	22.7 (17.4–27.6)	23.2 (18.0–37.2)	<0.001[a]
Prepregnancy nutritional status				
Underweight	4 (15.4)	1 (4.4)	1 (1.6)	
Eutrophic	20 (76.9)	15 (65.2)	38 (60.3)	0.009
Overweight/obese	2 (7.7)	7 (30.4)	24 (38.1)	
BMI in late pregnancy (kg/m^2)[b]	23.1 (19.1–31.1)	26.1 (22.1–34.8)	28.5 (22.2–41.5)	<0.001[a]
Nutritional status in late pregnancy[b]				
Underweight	15 (60.0)	4 (18.2)	7 (11.1)	
Eutrophic	8 (32.0)	10 (45.5)	26 (41.3)	<0.001
Overweight/obese	2 (8.0)	8 (36.4)	30 (47.6)	
Weight gain during pregnancy, kg[b]	8.0 (–1 to 17.7)	11.9 (3.1–18.5)	13.7 (0.2–28)	<0.001[a]
Hemoglobin electrophoresis				
A2	2.8 (0–5)	0 (0–4.1)	–	
Fetal	11.4 (1–24)	1.3 (0–8.3)	–	
S	83.5 (67.2–96)	50.2 (38.7–83)	–	–
C	0 (0)	46.7 (27.8–53.3)	–	
Hemoglobin, g/dL	7.7 (5.8–9.5)	9.8 (6.5–11.5)	12.2 (11.2–14.7)	<0.001[c]
Hematocrit, %	22.5 (16.7–28.6)	28.2 (19.2–36.4)	36.3 (32.7–41.3)	<0.001[c]
White blood cell count, n.10^3/mL	16.9 (3.5–31.9)	12.4 (4.9–24.2)	9.3 (5.5–14.5)	<0.001[c]
Platelets, n.10^3/mL	322 (36–896)	176 (59–561)	225 (158–357)	0.010[a]

Data are expressed as n (%), mean (SD), or median (min–max). [a]HbSS *vs.* control: p<0.05; HbSS *vs.* HbSC: p<0.05; HbSC *vs.* control: p=NS. [b]25 cases of HbSS and 22 cases of HbSC (one case of miscarriage was excluded in each group). [c]HbSS *vs.* control: p<0.05; HbSS *vs.* HbSC: p<0.05; HbSC *vs.* control: p<0.05.

HbSC group and controls. The maternal micronutrient dietary intake showed no significant differences in the second trimester; however, in the third trimester, the HbSS group presented a significantly lower intake of vitamins A and E than the HbSC and the control groups.

Table 3 shows the maternal complications and perinatal results in the groups. No significant differences were found between the HbSS and the HbSC groups, except for the need of blood transfusions during the prenatal period.

DISCUSSION

This study has demonstrated that the nutritional status of pregnant women with HbSS is characterized by a state of malnutrition associated with adverse maternal and perinatal outcomes. We found few studies addressing the nutritional status of pregnant women with SCD[16] and none investigating maternal dietary intake. In the literature, there were only studies of the nutritional status of children and adults with SCD. Many of the articles reported an association of this disease with malnutrition and growth failure[17].

In children with SCD, accelerated metabolism is triggered by chronic hemolysis, anemia, and vaso-occlusive crises. Even in periods without crises or complications, the demand for protein, energy, minerals, and vitamins increases to fulfill the body's metabolic functions. The same takes place during pregnancy; however, specific dietary recommendations are not clear for women with SCD-complicated pregnancies.

Table 2. Data from nutritional assessment questionnaire applied during the second and third trimesters of pregnant women complicated by sickle cell disease.

Intakes/day	Second trimester				3rd trimester			
	HbSS (n=22)	HbSC (n=21)	Control (n=30)	p	HbSS (n=22)	HbSC (n=20)	Control (n=33)	p
GA at FFQ, weeks	11.1 (2.9)	21.8 (2.8)	21.9 (3.2)	0.202	33.1 (2.3)	33.6 (2.1)	35.0 (3.0)	0.031[a]
Energy, kcal	1,518 (653–2,828)	1,562 (841–2,440)	1,694 (955–3,577)	0.165	1,648 (853–2,721)	1,530 (835–2,357)	1,829 (1,304–3,823)	0.037[b]
Macronutrients								
Carbohydrates								
g	200 (95–440)	223 (102–361)	220 (115–643)	0.403	210 (126–410)	191 (88–350)	228.1 (129.3–464.5)	0.125
% of energy	51.7 (41.1– 73.5)	53.4 (36.9–69.3)	51.3 (40.3–72.0)	0.491	55.8 (40.9–72.5)	50.9 (31.9–61.3)	48.9 (38.4–73.4)	0.097
Proteins								
g	73.2 (36.3–111.3)	68.8 (38.2–113.3)	95.7 (53.7–143)	0.004[c]	67.5 (37.9–127.8)	81.7 (42.6–149.6)	92.8 (51.6–253.3)	0.020[a]
% of energy	18.8 (10.4–31.5)	16.6 (12.6–26.1)	20.0 (11.6–33.4)	0.066	18.4 (10.5–30.9)	23.4 (10.4–29.3)	22.0 (9.3–31.9)	0.010[d]
Fat								
g	40.0 (14.3–133.7)	47.9 (25.9–105.3)	54.9 (26.4–117.5)	0.235	50.4 (17.6–103.2)	51.6 (21.8–71.2)	58.3 (38.4–121.0)	0.090
% of energy	28.0 (15.6–42.6)	27.1 (17.1–41.6)	27.6 (16.4–39.1)	0.861	28.0 (14.8–40.0)	27.8 (20.2–38.9)	28.9 (15.1–36.7)	0.626
Fiber, g	18.0 (9.9–42.3)	23.0 (10.7–33.0)	21.0 (13.0–37.8)	0.094	16.4 (8.4–32.9)	23.3 (10.4–50.6)	18.4 (6.7–36.0)	0.036[e]
Micronutrients								
Calcium, mg	409.9 (91.0–1,247.5)	575.3 (88.1–1,086.9)	747.6 (166–1,208)	0.021[a]	607.2 (91.9–1,974.1)	590.6 (112.0–1,558.4)	734.4 (244–1,726)	0.117
Iron, mg	13.1 (6.7–17.9)	10.1 (6.0–18.4)	12.2 (7.2–25.0)	0.177	10.5 (6.0–25.3)	14.3 (6.2–21.5)	12.7 (6.0–42.2)	0.468
Zinc, mg	10.3 (4.5–25.5)	9.2 (0.8–17.2)	13.0 (4.5–22.0)	0.103	9.4 (3.3–21.6)	13.6 (3.4–25.9)	13.0 (4.0–55.7)	0.063
Vitamin A, µg	538 (58–2,224)	575 (171–1,926)	897 (243–6,330)	0.075	447 (64–3,093)	730 (250–2,441)	940 (232–5,150)	0.005[a]
Vitamin E, mg	5.3 (2.5–17.9)	5.3 (2.1–11.5)	6.7 (2.5–24.7)	0.102	5.6 (1.9–10.9)	9.5 (3.4–14.1)	6.7 (3.0–20.2)	0.007[d]
Vitamin C, mg	96.6 (19.2–936.9)	85.1 (13.5–315.3)	150.6 (32.0–656.6)	0.078	145.2 (40.6–696.7)	108.6 (14.2–291.0)	128.8 (49.6–548.7)	0.417
Folate, µg	93.9 (56.7–162.3)	99.3 (28.8–169.2)	125.6 (51.7–316.8)	0.072	97.6 (35.5–205.0)	130.6 (76.2–213.8)	136.6 (69.9–400.1)	0.053

Data are expressed as mean (SD) or median (min–max). [a]HbSS *vs.* control: p<0.05; HbSS *vs.* HbSC: p=NS; HbSC *vs.* control: p=NS. [b]HbSS *vs.* control: p=NS; HbSS *vs.* HbSC: p=NS; HbSC *vs.* control: p<0.05. [c]HbSS *vs.* control: p<0.05; HbSS *vs.* HbSC: p=NS; HbSC *vs.* control: p<0.05. [d]HbSS *vs.* control: p<0.05; HbSS *vs.* HbSC: p<0.05; HbSC *vs.* control: p=NS. [e]HbSS *vs.* control: p=NS; HbSS *vs.* HbSC: p<0.05; HbSC *vs.* control: p=NS.

Table 3. Maternal complications and perinatal results by a group of pregnant women complicated by sickle cell disease.

	HbSS	HbSC	Control	p
Maternal complication				
Arterial hypertension	6 (23.1)	6 (26.1)	-	0.930
Pain crisis	15 (57.7)	10 (43.5%)	-	0.480
Alloimmunization	10 (38.5)	5 (21.7)	-	0.300
Pulmonary infection	9 (34.6)	2 (8.7)	-	0.070
Antenatal blood transfusion	8 (30.8)	2 (8.7)	-	0.008
Urinary infection	8 (30.8)	4 (17.4)	-	0.451
Blood transfusion at childbirth/postpartum	5 (19.2)	3 (13.0)	-	0.707
Total of deliveries	**n=25**	**n=22**	**n=63**	**P**
Gestational age at delivery, weeks	37.4 (25.6–39.3)	37.9 (25.7–40.1)	39.9 (36.0–41.3)	<0.001[a]
Prematurity (<37 weeks)	10 (40.0)	5 (22.7)	2 (3.2)	<0.001[a]
Delivery mode				
Cesarean	21 (84.0)	18 (81.8)	37 (58.7)	0.024[b]
Vaginal	4 (16.0)	4 (18.2)	26 (41.3)	
Total of newborns	**n=26[e]**	**n=22**	**n=63**	**P**
Birth weight, g	2,220 (292–3,390)	2,865 (378–3,820)	3,220 (2,450–4,520)	<0.001[c]
Birth weight <2.500 g	18 (69.2)	7 (31.8)	1 (1.6)	<0.001[c]
Small-for-gestational age infant	16 (61.5)	6 (27.3)	3 (4.8)	<0.001[c]
1st min Apgar <7	8 (30.8)	3 (13.6)	2 (3.2)	0.001[b]
5th min Apgar <7	1 (3.8)	2 (9.1)	0 (0)	0.071
Perinatal result				
Fetal death	1 (3.8)	1 (4.5)	0	0.029[b]
Neonatal death	2 (7.7)	1 (4.5)	0	
Alive	23 (88.5)	20 (90.9)	63 (100)	

Data are expressed as median (min–max) or n (%). [a]HbSS *vs.* Control: p<0.05; HbSS *vs.* HbSC: p=NS; HbSC *vs.* control: p<0.05. [b]HbSS *vs.* Control: p<0.05; HbSS *vs.* HbSC: p=NS; HbSC *vs.* control: p=NS. [c]HbSS *vs.* Control: p<0.05; HbSS *vs.* HbSC: p<0.05; HbSC *vs.* control: p<0.05. [e]One twin pregnancy.

On comparing the data on maternal weight gain, this study found that the total weight gain of pregnant women with HbSS was below the minimum recommended by the Institutes of Medicine (2009) as well as below that of the HbSC women and the controls. The study by Thame et al.[16] also noted this outcome in pregnant women with SS SCD. This result supported the fact that most of the women began pregnancy with a lower weight than that of the controls. These findings indicate that the condition is due to the disease itself, which further hinders the total weight gain.

In the literature, there are reports on the dietary intake of macro and micronutrients by children, adolescents, and adults with SCD. However, there are no studies conducted with pregnant women. The first study of dietary intake carried out with children showed that an increase in protein intake with the attendant energy rise can improve the clinical status and the growth of children with SCD[18]. Our study found lower protein intake in the second and third trimesters in pregnant women with HbSS and lower energy consumption detected in pregnant women with HbSC in the third trimester. Undernourishment is considered a serious complication of SCD, primarily during pregnancy, and it should be addressed during prenatal care. There is a need for setting new dietary requirements for proteins and energy, particularly for HbSS pregnant women.

Studies show low serum levels of vitamins A, C, and E in HbSS patients, but these studies are not sufficient to establish that supplementation ensures clinical benefits[19]. Vitamin A is relevant in times of rapid proliferation and cell differentiation. Our study found that pregnant women with the SS type

of SCD consume insufficient amounts of vitamin A in the third trimester of pregnancy, for the amounts are lower than the recommended intake and lower than the control group's consumption. Vitamin E is considered a biological antioxidant that maintains the integrity of cell membranes containing polyunsaturated fatty acids[20]. The hypothesis with respect to the HBSS carriers is that vitamin E can protect RBC by inhibiting hemolysis.

The low nutrient intake among pregnant women with SCD indicates a need for more appropriate nutritional support for this particular condition. Not only dietary intake but also socioeconomic factors and lifestyle should be taken into account. Many factors contribute to poor dietary intake and length of hospital stay during pregnancy. Complications such as fever, painful crises, infection, and poor nutrition counseling are unfavorable conditions that negatively influence the nutritional status of this population.

This study has limitations. First of all, this was a single-center study with a limited sample size. More studies are certainly required to define the nutritional needs of women with SCD-complicated pregnancies. Additionally, regional, socioeconomic, and ethnic differences are also potentially confounding variables. Once aware of the complexity of the disease and of the maternal and perinatal complications, nutritionists should outline new preventive strategies and offer nutrition counseling to pregnant women with SCD.

CONCLUSION

The nutritional status of pregnant women with HbSS is characterized by a state of undernutrition. The lower protein intake in the second and third trimesters of pregnant women with HbSS may contribute to this condition. Undernourishment is a serious complication of SCD, primarily during pregnancy, and it should be addressed during the prenatal period. Nutritional care will help minimize adverse outcomes and ensure improvements in maternal–fetal health.

AUTHORS' CONTRIBUTIONS

LVP: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **AMKI:** Conceptualization, Project administration, Writing – original draft, Writing – review & editing. **RMYN:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Determination of the risk of obstructive sleep apnea syndrome in individuals aged 18 years and above

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SUMMARY

OBJECTIVE: This study aimed to increase awareness by determining the risk of obstructive sleep apnea syndrome in individuals aged 18 years and above.
METHODS: The study is a descriptive and cross-sectional study. A total of 633 individuals aged 18 years and above participated in the study. The data were collected online from individuals in the form of describing the demographic characteristics of individuals and with the Berlin survey. The IBM SPSS statistics 26.0 program was used in the analysis of the data.
RESULTS: In this study, 38.9% of individuals were found to be at high risk for obstructive sleep apnea syndrome. A significant relationship was found between the risk of obstructive sleep apnea syndrome and gender, age, body mass index, education level, chronic obstructive pulmonary disease, diabetes, hypertension, presence of cardiovascular diseases, and smoking ($p<0.05$).
CONCLUSION: The results of this study showed that male gender, increasing age, obesity, presence of chronic disease, and smoking increase the risk of obstructive sleep apnea syndrome. Defining the risk of obstructive sleep apnea syndrome, especially in risky groups, will be effective in planning health care, increasing the effectiveness of treatment, and improving the quality of life. It is recommended to include this diagnosis in health care protocols and to expand its use in order to plan and repeat trainings that will emphasize its importance.
KEYWORDS: Care. Obstructive sleep apnea syndrome. Prevalence.

INTRODUCTION

Sleep is a decrease in the sensitivity of individuals to the external environment and is a state of inactivity that can be quickly reversed[1]. Sleep, which covers one-third of human life, is one of the basic human needs. Sleep allows the body to rest and regenerate. A quality sleep is needed to be healthy and spend the day active[2]. Sleep problems, which can be seen in every society, cause individuals to be unable to focus, restlessness, psychological disorders, and problems in interpersonal communication, resulting in a decrease in their quality of life[3]. One of the sleep disorders affecting sleep is obstructive sleep apnea syndrome (OSAS).

Obstructive sleep apnea syndrome is a condition characterized by respiratory arrest due to upper airway obstruction during sleep and thus a decrease in oxygen saturation in the blood. Common symptoms of sleep apnea syndrome are snoring, apnea, and sleepiness during the day[4]. Risk factors of OSAS in studies include obesity, male gender, race, anatomical factors, pregnancy, middle and advanced age, thick neck circumference, genetic factors and diabetes, upper respiratory tract abnormalities, chronic obstructive pulmonary disease (COPD), hypothyroidism, acromegaly, excessive androgen secretion, multiple sclerosis, and amyotrophic lateral sclerosis[5,6]. If OSAS is not treated, it is a cause of morbidity and mortality[5]. Epidemiological studies show that the frequency of OSAS is increasing (2–26%)[7]. When we look at the literature, there are studies that study the prevalence of OSAS in the general population[8-11], but new studies that will raise awareness are needed. This study aimed to increase awareness by determining the risk of OSAS in individuals aged 18 years and above.

METHODS

Study design

This is a descriptive and cross-sectional study.

Sample of the research

The sample of the study consisted of individuals aged 18 years and above in Turkey. A total of 633 individuals who voluntarily accepted to participate in the study were informed about the research and their rights, and their "informed consent" was

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obtained before the research. All the rights of the participants were respected, and attention was paid to the principles of voluntariness and confidentiality.

Data collection method

Data were collected by an online survey method between 15 May and 15 June 2023.

Data collection

While collecting the data, a form describing the demographic characteristics of the individuals and the Berlin questionnaire were used.

Demographic characteristics identification form of individuals The data were created by scanning the literature, and it is an eight item form that includes questions about age, gender, educational status, height, weight, whether there is any additional disease (diabetes, hypertension, COPD, and cardiovascular diseases), smoking, and alcohol use.

Berlin survey

The Berlin questionnaire was created in 1996 at the "Sleep Conference in Primary Care" in Berlin, Germany. This questionnaire is used to determine the risk of OSAS[12], and it consists of 10 questions and 3 categories in total. Each category is evaluated on its own. If the answer to at least two questions in the first two categories is one point, that category is positive. In the third category, if the blood pressure question is answered yes or BMI is >30, this category is positive. OSAS risk is considered high if at least two of the three categories result in a positive result. If only one category is positive or no category is positive, the risk of OSAS is considered low. The validity and reliability study of the Berlin questionnaire was conducted by Acar et al., and the sensitivity of the questionnaire was 87.9%, its specificity was 15.6%, and its positive predictive value was 68.2%[13].

Data analysis

The IBM SPSS Statistics 26.0 program was used for statistical analysis in the study. While evaluating the study data, in addition to descriptive statistical methods (e.g., mean, standard deviation, frequency, and percent), the chi-squared test was used to evaluate the relationships between the variables. The results were evaluated at the 95% confidence interval and the significance level of $p<0.05$.

Ethical approval

Written informed consent for inclusion in the study was obtained from all patients. The study approval was obtained from the local ethics committee (İstanbul Gelişim University Ethics Committee, date and number: 19.04.2023/2023-04-88). The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

The demographic characteristics of the individuals participating in the study are shown in Table 1. It was determined that 65.2% of the participants were women, 51% were university graduates, and the average age was 37.80±12.00 years. While the mean body mass index (BMI) of the individuals was found to be 26.28±4.71, in a situation where whether they had an additional chronic disease or not, the ones with hypertension (8.7%) were determined with the highest rate. It was found that 32.5% of the participants were smokers and 14.8% used alcohol. As a result of the Berlin questionnaire applied to individuals, a high risk of OSAS was found in 246 (38.9%) of 633 individuals (Table 1).

Table 1. Demographic characteristics of individuals and frequency and severity of obstructive sleep apnea syndrome (n=633).

	n	%
Gender		
Male	220	34.8
Female	413	65.2
Age (mean)	37.80±12.00	
Body mass index (BMI) (mean)	26.28±4.71	
Educational status		
Literate	17	2.7
Primary school graduate	51	8.1
Secondary school graduate	41	6.5
High school graduate	201	31.8
Graduate	323	51.0
Diabetes	46	7.3
COPD	6	0.9
Cardiovascular disease	26	4.1
Hypertension	55	8.7
Smokers	206	32.5
Alcohol users	94	14.8
Category 1 (risk of sleep apnea)	166	26.2
Category 2 (risk of sleep apnea)	118	18.6
Category 3 (risk of sleep apnea)	174	27.5
High risk of sleep apnea	246	38.9

Descriptive statistical methods (mean, standard deviation, frequency, and percentage).

The relationship between the demographic characteristics of the participants and the risk of OSAS is shown in Table 2. A significant relationship was found between the individuals' gender, age, BMI, education status, hypertension, cardiovascular disease, diabetes, COPD, smoking status, and OSAS risk ($p<0.05$). Since the mean age was 37.80±12.00 years, the age of 38 years was considered as the limit. In Category 3 of the Berlin survey, BMI of 30 was considered as the limit, since those with BMI 30 and above are at high risk. The risk of OSAS increases as age and BMI increase. For primary school graduates, the risk of hypertension, diabetes, and cardiovascular diseases is higher, meanwhile, for people with COPD and smokers, the risk of OSAS is higher.

Table 2. The relationship between the demographic characteristics of individuals and the risk of obstructive sleep apnea syndrome (n=633).

	Low risk (n)	High risk (n)	Total (n)	p
Gender				
Male	108	112	220	**0.001**
Female	279	134	413	
Age (years)				
≥38	78	168	246	**0.001**
<38	228	159	387	
BMI				
≥30	143	103	246	**0.001**
<30	354	33	387	
Educational status				
Literate	12	5	17	**0.021**
Primary school graduate	22	29	51	
Secondary school graduate	20	21	41	
High school graduate	127	74	201	
Graduate	206	117	323	
Hypertension				
Yes	17	38	55	**0.001**
No	370	208	578	
Diabetes				
Yes	13	33	46	**0.001**
No	374	213	587	
Cardiovascular disease				
Yes	9	17	26	**0.005**
No	378	229	607	
COPD				
Yes	1	5	6	**0.025**
No	386	241	627	
Smoking				
Yes	113	93	206	**0.024**
No	274	153	427	
Alcohol use				
Yes	55	39	94	0.571
No	332	207	539	

Chi-squared test is used. Statistically significant values are indicated in bold.

DISCUSSION

In this study, OSAS was found to be high risk in 38.9% of individuals aged 18 years and above (Table 1). A significant relationship was found between OSAS risk and gender ($p<0.05$). The risk of OSAS in men (50.9%) is higher than in women (32.4%) (Table 2). In the study carried out by Vasu et al., while the incidence of OSAS was between 2 and 26%, it was found to be higher in males (24%) than females (9%)[14]. Karadöl stated in his study that the incidence of OSAS is higher in men than in women[15]. In this study, the risk of OSAS, which is more common in men than in women, is similar to that reported in the literature. However, considering the incidence in the general population, it is observed that the risk of OSAS detected in this study is higher than the results reported in the literature. In this study, it is thought that the presence of chronic disease and high age, which affect the risk of OSAS, affect the results of the study, and hence the risk of OSAS is high in this study compared with the results reported in the literature.

In this study, a significant relationship was found between OSAS risk and age ($p<0.05$). In the age comparison taken by calculating the mean age of the individuals participating in the study (37.80±12.00 years), the risk of OSAS was found to be higher in individuals aged 38 years and above (Table 2). Leppänen et al., and Senaratna et al., found OSAS to be 10 and 17% in the ages of 30–49 and 50–70 years in men, respectively, while it was 3% between the ages of 30 and 49 years and 9% between the ages of 50 and 70 years in women[16,17]. The results of this study are also similar to the results of the literature. With aging, irreversible physical changes occur in the systems of the organism. With aging, sleep problems can be experienced due to the increase in exposure to the environment, movement restrictions, and the increase in chronic diseases.

A BMI of 30 and above is observed as a high risk in OSAS. In this study, a significant relationship was found between OSAS risk and BMI ($p<0.05$). Individuals with BMI ≥30 are

at higher risk for OSAS than those with BMI <30 (Table 2). Ataç et al., found that the risk of OSAS increases with obesity[18]. In the study conducted by Dacal Quintas et al., a higher risk of OSAS was observed in obese patients than in normal-weight patients[19]. The results of this study and the literature are similar to the fact that obese individuals have a higher risk of OSAS than normal-weight individuals. In the study conducted by Schwartz et al., a relationship was determined between central obesity and the width of the neck diameter and OSAS. The increased fat thickness around the neck in obesity causes an increase in airway obstruction and exacerbates obstructive apneas[20].

In the study, a significant relationship was found between the risk of OSAS and the presence of COPD, hypertension, cardiovascular diseases, and diabetes ($p<0.05$). Looking at the literature, in the study conducted by Karakoç et al., the risk of OSAS increased as the incidence of heart diseases, diabetes, COPD, and hypertension increased[21], and in the study conducted by Salepci et al., the frequency of asthma was found to be more common in patients with OSAS than in the normal population[22]. In the study conducted by Bayram et al., the frequency of hypertension in OSAS patients was higher than that in the normal population[23]. The results of the study and the literature show that the presence of COPD, hypertension, diabetes, and cardiovascular diseases further increases the risk of OSAS.

As a result of the study, a significant relationship was found between OSAS risk and smoking status ($p<0.05$). Smoking increases chronic diseases such as cardiovascular diseases and COPD. As a result of a study in the literature, it was stated that the presence of cardiovascular diseases and COPD increases the risk of OSAS[16,17]. Although smoking did not primarily increase the risk of OSAS, it may have increased this risk secondarily.

CONCLUSION

As a result, the risk of OSAS was found to be 38.9% in individuals aged 18 years and above. Sleep patterns and quality greatly affect the life quality of individuals. Therefore, the definition of OSAS in health care becomes an important issue. The results of the literature and the study show that the risk of OSAS is found to be high in male gender, increasing age, smoking, obesity, COPD, cardiovascular diseases, diabetes, and hypertension. The definition of OSAS risk, especially in risky groups, will be effective in planning health care, increasing the effectiveness of treatment, and improving the quality of life. It is recommended to include this diagnosis in health care protocols and to expand its use, in order to plan and repeat trainings that will emphasize its importance.

ETHICS APPROVAL

Written informed consent for inclusion in the study was obtained from all patients. The study approval was obtained from the local ethics committee (İstanbul Gelişim University Ethics Committee, date and number: 19.04.2023/2023-04-88). The study was conducted in accordance with the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

NK: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MK:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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Nonpharmacological treatment of postpartum sexual dysfunction: a systematic review and meta-analysis

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INTRODUCTION

Postpartum is a vulnerable period for women, in which they are faced with many new changes and concerns. The latter period is marked by a series of physiological and psychological changes that directly influence a woman's quality of life and sexual function[1-3]. The prevalence of postpartum sexual dysfunction is high. Studies have indicated that approximately 20–60% of postpartum women experience some type of sexual dysfunction that can last for several months after delivery[4-7].

Such symptoms can be complex and delicate to treat, commonly requiring a combination of different techniques. Especially during the puerperal period, nonpharmacological strategies are necessary to treat sexual dysfunction since there are no clinical studies that demonstrate the safety of pharmacological therapies, especially since there is a risk of passing several drugs to the newborn through breast milk[8]. Nonpharmacological therapies, such as pelvic floor exercises, sex and couples therapy, psychotherapy, lifestyle changes, and the use of vaginal lubricants or moisturizers, are among the most popular treatment options[8-10].

Several clinical trials involving the non-pharmacological treatment of postpartum sexual dysfunction have been published[11,12]. However, despite the importance of treating postpartum sexual dysfunction for women's quality of life, no systematic review has yet attempted to reach a consensus on the optimal nonpharmacological approach.

Therefore, the present systematic review and meta-analysis aimed to determine the effectiveness of different nonpharmacological interventions in the treatment of sexual dysfunction in postpartum women.

METHODS

This systematic review was designed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[13] by the authors ACQA, AKSG, and MNM. The study analyzed data from previously published clinical trials and, thus, did not require ethical approval or patient-informed consent. The protocol for this study was published in an indexed journal[14].

Searches in bibliographic databases were conducted in line with guidelines developed for systematic reviews and meta-analyses under the supervision of an experienced librarian at the Sectorial Library of the Health Sciences Center of the Federal University of Rio Grande do Norte. The following databases were consulted: PubMed, Embase, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, CINAHL, PEDro, and clinical trial.gov, utilizing the Medical Subject Headings (MeSH) "non-pharmacological therapies," "postpartum period," and "sexual dysfunction." The final search was conducted on February 28, 2023.

Study selection

The following inclusion criteria were defined: (1) randomized clinical trial (RCT), (2) women >18 years of age, (3) women with sexual problems that began in the puerperium, and (4) nonpharmacological interventions: REDI Model, EmbaGYN Model, Kegel Model, PLISSIT, Cognitive-Behavioral, Routine Training, Levine's Model, Interactive Postpartum Sexual Health Education Program (IPSHEP), Sexual Health Educational, Women's Postpartum Sexual Health Program (WPSHP).

Other types of studies, such as case reports, narrative reviews, editorials, commentaries, letters, or randomized clinical trials

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that did not meet the inclusion criteria and those with insufficient data to be extracted, were excluded.

The articles retrieved by the search were imported to the Ryyan software for identification and duplicate exclusion. After that, two authors (ACZS and RO) independently reviewed all titles and abstracts, followed by a full text review against the eligibility criteria. Agreement on potential relevance or inconsistencies was reached by consensus or resolved by discussion with a third reviewer (ACAS).

Data extraction

Two authors (ACQA and ACZS) independently extracted the relevant data from the full text of eligible articles after comparing results and resolving any discrepancies, and the data were reviewed by a third reviewer (AKSG).

The data collected included the duration of symptoms and rates of improvement in sexual dysfunction, which was defined as the presence of pain, increased difficulty reaching orgasm, lack of arousal, poor lubrication, and low desire.

Assessment of risk of bias

Two authors (ACAS and RO) independently assessed the risk of bias of the included studies using the Cochrane Collaboration risk of bias tool (RoB2) for RCTs. Bias was considered high, low, or unclear[15]. A third author (MNM) resolved possible inconsistencies between the assessments.

Data synthesis

Review Manager (RevMan) V.5.4.1 was used to perform the meta-analysis. The weighted mean difference (MD) with a 95% confidence interval (CI) was calculated for continuous data to obtain a summary of the overall estimate. Heterogeneity was assessed using the I^2 statistic. A random-effects model was adopted due to the high heterogeneity observed among studies[16].

The quality of the 22 included articles was assessed according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines. Two authors (ACAS and ACQA) independently performed this assessment, and disagreements were decided through discussion with a third author (AKGS)[17,18].

RESULTS

Study selection

The database searches identified a total of 5,390 articles. Of these, 1,604 were excluded for being duplicates, and 3,764 did not meet the inclusion criteria. Therefore, 22 studies that met the eligibility criteria were included in the systematic review. Of these, eight composed the meta-analysis. The study selection is summarized in the PRISMA flowchart (Figure 1).

Study characteristics

The 22 elected articles included 2,227 participants with sexual dysfunction in the postpartum period. Of these articles, 13 were from Iran[19,20,22,24,26,27,32,34,38,40], 4 from Turkey[25,30,31,33], 1 from Taiwan[21], 1 from Norway[23], 1 from India[29], 1 from Germany[39], and 1 from the Russian Federation[28]. All 22 articles were RCTs, and their characteristics are summarized in Table 1.

Risk of bias of the included studies

Of the included articles, the risk of bias assessment showed that eight studies met all items of the RoB2 and were, therefore, classified as "low risk of bias"[19-26]. Meanwhile, 13 were considered to have "some concerns" due to the lack of information regarding intention-to-treat, deviation, or missing outcome data[27-39]. One study was classified as "high risk" due to bias in selecting reported results[40] (Figure 2).

Synthesis of results

A total of 22 studies[19-40] were included in the systematic review, of these 8[19,24,29,31,36,38-40] were included in the meta-analysis, involving a total of 634 patients.

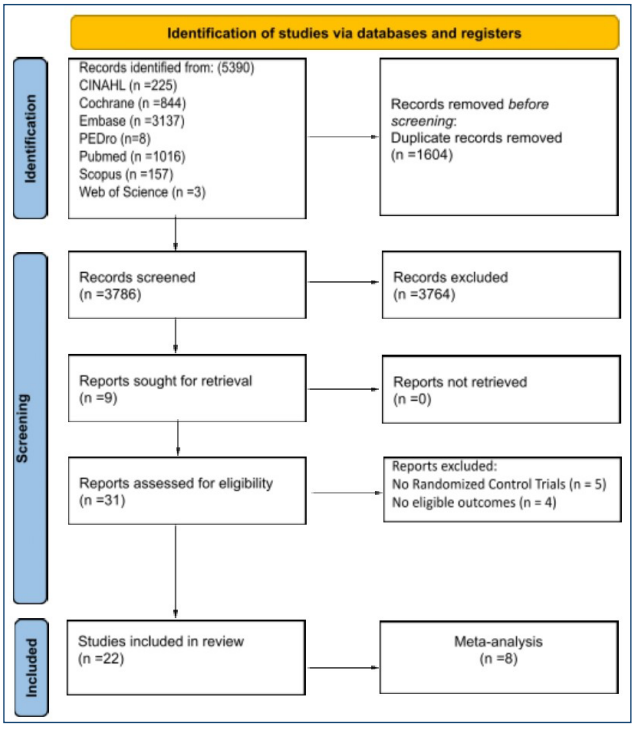

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart study selection.

Table 1. Characteristics of the included studies.

Authors, years	Countries	No. of patients	Mean age	Intervention types	Instrument measures	Follow-up
Aghababaei et al.[27]	Iran	98	30.18	IG: REDI Model. CG: educational package	FSFI and DSDS	4 weeks
Artymuk et al.[28]	Russia	70	29.85	IG: EmbaGYN Model. CG: Kegel Model	FSFI and PFDI-20	4 weeks
Banaei et al.[19]	Iran	87	24.18	IG: PLISSIT. CG: routine consultation	FSFI, EPD, and Larson's scale	4 weeks
Bhat et al.[29]	India	55	29.88	IG: Kegel Model. CG: without intervention	FSFI	6 months
Bokaie et al.[30]	Turkey	79	29.88	IG: Telephone counseling. CG: without intervention	WSFQ and Larsson's scale	5 Weeks
Citak et al.[31]	Turkey	75	22.60	IG: PFME. CG: without intervention	FSFI	7 months
Erfanifar et al.[32]	Iran	84	22.15	IG: Cognitive-behavioral. CG: routine training	FSFI and SEQ	8 weeks
Evcili et al.[33]	Turkey	67	26.40	IG: Levine's Model. CG: without intervention	IFSF, ASEX, and GRISs	6 weeks
Golmakani et al.[20]	Iran	79	25.88	IG: Kegel Model. CG: without intervention	Brink scale and BSEQ	8 weeks
Karimi et al.[34]	Iran	80	30.70	IG: PLISSIT. CG: without intervention	FSFI and DASS-21	2 weeks
Karimi et al.[35]	Iran	80	30.50	IG: PLISSIT and BETTER. CG: without intervention	FSFI and HSSI	4 weeks
Lee et al.[21]	Taiwan	250	31.73	IG: IPSHEP. CG: routine training	PWSS, DAS, and SS	3 months
Malakouti et al.[40]	Iran	68	27.00	IG: PLISSIT. CG: without intervention	FSFI	8 weeks
Modarres et al.[36]	Iran	100	30.30	IG: Kegel Model. CG: without intervention	FSFI	16 weeks
Movahedi et al.[37]	Iran	114	26.59	IG: PFME. CG: without intervention	PDFI-20	16 weeks
Pourkhiz et al.[41]	Iran	84	25.65	IG: PFME. CG: without intervention	FSFI, SQQL-F, and Oxford score	3 months
Schütze et al.[39]	Germany	200	31.94	IG: PFME. CG: without intervention	FSFI, PFQ, and Oxford score	6 weeks
Sheikhi et al.[22]	Iran	94	20.52	IG: Sexual health educational. CG: routine training	FSFI	8 weeks
Kolberg Tennfjord et al.[23]	Norway	175	29.80	IG: PFME. CG: without intervention	ICIQ-VS and ICIQ-FLUTSsex	6 weeks
Torkzahrani et al.[24]	Iran	90	24.18	IG: PLISSIT. CG: without intervention	FSFI	4 weeks
Yörük et al.[25]	Turkey	123	27.30	IG: PLISSIT. CG: without intervention	Arizona scale and SQLQFF	2 months
Zamani et al.[26]	Iran	75	29.45	IG: WPSHP. CG: routine training.	DASS-21 and Larson's scale	8 weeks

IG: intervention group; CG: control group; PFME: pelvic floor muscle exercise; DSDS: Decreased Sexual Desire Scale; FSFI: Female Sexual Function Index; PFDI-20: Pelvic Floor Distress Inventory; EPD: Edinberg Postpartum Depression; WSFQ: Women's Sexual Function Questionnaire; SEQ: Sexual Self-efficacy Questionnaire; IFSF: Index of Female Sexual Function; ASEX: Arizona Sexual Experience Scale; GRISs: Golombok-Rust Inventory of Sexual Satisfaction; BSEQ: Bailes Sexual Self-efficacy Questionnaire; DASS-21: Depression, Anxiety, and Stress Scale-21; HSSI: Hulbert Sexual Self-disclosure Index; PWSS: Postpartum Women's Sexual Self-efficacy; DAS: Diversity of Sexual Activity; SS: Sexual Satisfaction; SQQL-F:Sexual Quality of Life Questionnaire-Female; PFQ: Pelvic Floor Questionnaire; ICIQ-VS: Incontinence Modular Questionnaire—vaginal Symptoms Questionnaire; ICIQ-FLUTSsex: ICIQ Sexual Matters Module; SQLQFF: Sexual Quality of Life Scale-Female Form.

Regarding the sexual dysfunction outcomes evaluated, the articles included in the meta-analysis used the Female Sexual Function Index (FSFI) to estimate improvement. Of the interventions assessed in the meta-analysis, three RCTs performed experimental pelvic floor muscle exercise protocols[31,38,39] and showed no improvement in favor of the intervention group (MD: 4.27; 95%CI 1.23–7.32; I^2: 99%). The three RCTs evaluating the PLISSIT (Permission, Limited Information, Specific Suggestion, and Intensive Therapy)[19,24,40] showed no improvement in favor of the experimental group (MD: 1.56;

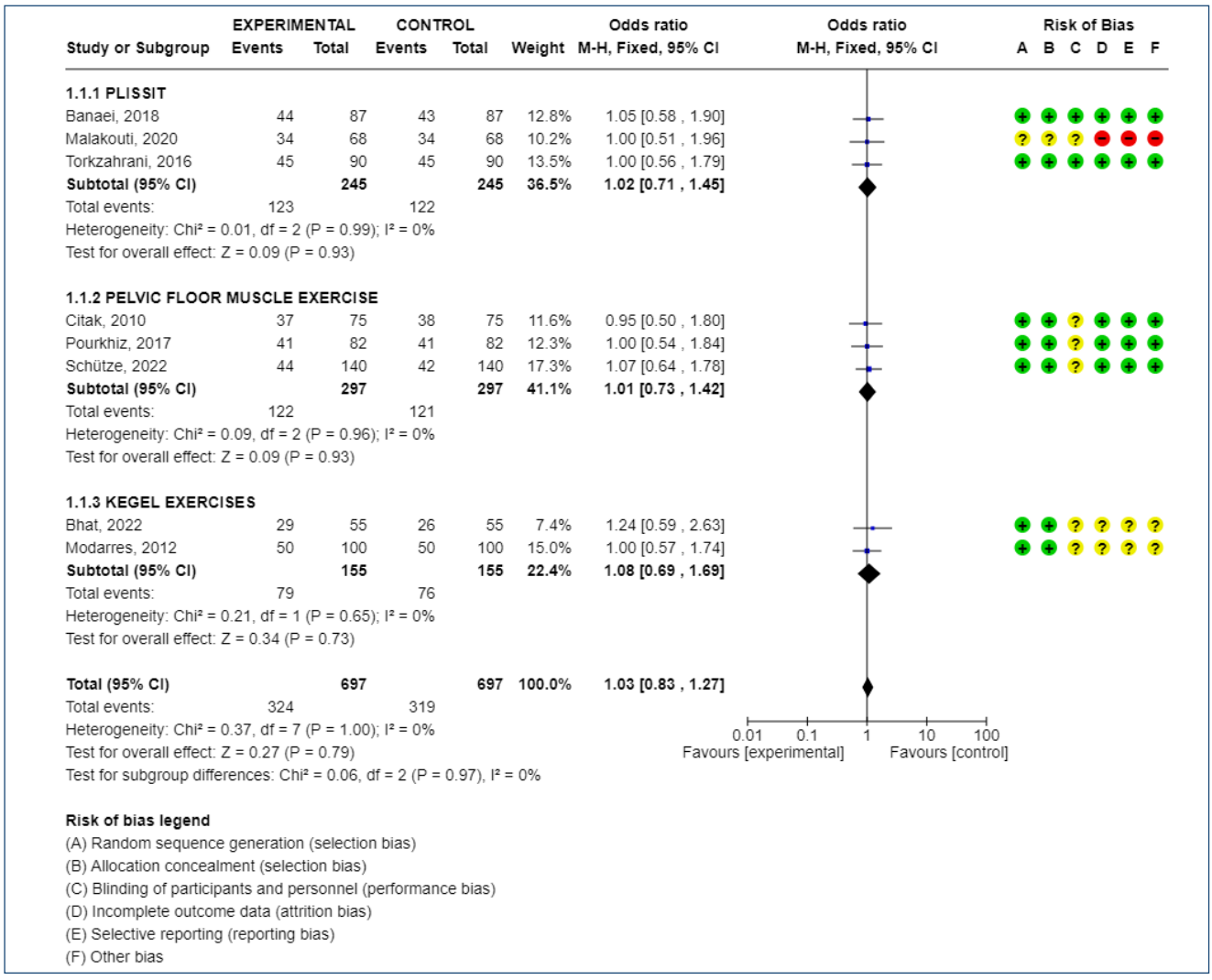

Figure 2. Risk of bias assessment.

95%CI 1.27–1.84; I^2: 0%), and the two RCTs evaluating Kegel exercises[29,36] also showed no improvement in favor of the intervention group (MD: 41.54; 95%CI 33.27–49.80; I^2: 99%).

Assessment of quality

The GRADE rating for the certainty of the evidence for improvement of sexual function outcomes using PLISSIT was considered high, while for Kegel and pelvic floor muscle exercises it was considered very low due to the high inconsistency and imprecision of the data, large confidence interval ranges, and consequently, a high degree of heterogeneity between the studies (Table 2).

DISCUSSION

The current systematic review examined clinical trials of different protocols that investigated the effectiveness of nonpharmacological methods in the treatment of sexual dysfunction in postpartum women. Changes in postpartum sexual life are highly prevalent and of concern as they negatively affect the quality of life of these patients. In this review, we analyzed a wide variety of treatment protocols for postpartum sexual dysfunction.

All interventions studied showed some improvement in the FSFI domains (desire, arousal, lubrication, orgasm, satisfaction, and pain). Concerning the PLISSIT model and Kegel exercises, all studies demonstrated improvement in FSFI domains; however, the latter was not significant in the meta-analysis. Additionally, RCTs involving the PLISSIT intervention show that sexual problems in lactating women have decreased[19,24,25,34,35,40].

Studies using the Kegel exercise technique showed a significant increase in pelvic floor muscle strength after the treatment period, concluding that muscle exercises using the Kegel method increase sexual self-efficacy in postpartum women[20,29,36].

Table 2. Evaluation of the quality of articles according to Grading of Recommendations Assessment, Development and Evaluation.

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	PLISSIT	Placebo	Relative (95%CI)	Absolute (95%CI)		
Sexual function (assessed with: FSFI)												
3	Randomized trials	Not serious	Not serious	Not serious	Not serious	None	123	122	–	Mean 1.56 (1.27 higher to 184 higher)	⊕⊕⊕⊕ High	Critical

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Pelvic Floor Muscle Exercise	Placebo	Relative (95%CI)	Absolute (95%CI)		
Sexual function (assessed with: FSFI)												
3	Randomized trials	Not serious	Very serious[a]	Not serious	Very serious[b]	None	122	121	–	Mean 4.27 higher (1.23 higher to 7.32 higher)	⊕◯◯◯ Very low	Critical

[a]I2: 99%.
[b]95%CI: 1.23–7.32.

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Kegel	Placebo	Relative (95%CI)	Absolute (95%CI)		
Sexual function (assessed with: FSFI)												
2	Randomized trials	Not serious	Very serious[a]	Not serious	Very serious[b]	None	79	76	–	Mean 41.54 higher (33.27 higher to 49.8 higher)	⊕◯◯◯ Very low	Critical

[a]I2: 99%.
[b]95%CI: 33.27–49.80.

An RCT that evaluated electrostimulation on pelvic floor muscles for 4 weeks showed that the technique significantly reduced sexual dysfunction in the treated group[28]. Furthermore, the REDI model showed that the overall FSFI score increased in the treated patients, consequently implying an improvement in the sexual function of this group. However, there was no difference in the orgasm subdomain when comparing the intervention and placebo[27].

In addition, women who received postpartum sexual health education based on the IPSHEP program tended to resume their sex lives earlier but did not differ significantly from those who received routine education[21].

Likewise, Levine's model showed that the intervention group had better sexual function and developed a more satisfactory sexual response than the control group[33]. Regarding the results of the RCT that carried out health counseling by telephone,

there was an improvement in the satisfaction and sexual function of the patients[30].

Concerning the WPSHP, we found higher levels of sexual satisfaction, thus being recommended for women to use this program during postpartum to improve sexual function[26]. Finally, the cognitive-behavioral assessment showed that 8 weeks after the intervention, there was a difference between the two groups, demonstrating that adequate implementation of counseling based on the cognitive-behavioral therapy model improved the sexual function of nulliparous women after childbirth[32].

The results of our meta-analysis regarding pelvic floor muscle exercise differ from data presented in other meta-analyses[7,8]; specifically, no superior effect of this exercise compared to the placebo intervention could be evidenced. Individually, the RCTs that addressed pelvic floor muscle exercises for sexual dysfunction had positive effects on the FSFI global score and subdomains[22,23,31,37-39]. Two previous meta-analyses have evaluated the effectiveness of pelvic floor muscle exercises on postpartum sexual dysfunction: one conducted in Canada involving 15 RCTs[8] and the other in Iran encompassing 12 RCTs[7]. These studies highlighted that pelvic floor muscle training in primiparous or multiparous women can increase sexual function and quality of life in the postpartum period, thus contradicting the outcomes of the present analysis.

The results for the effect of the PLISSIT model and Kegel exercises also showed no superior effect compared to the placebo intervention. However, this is the first meta-analysis performed on these interventions to address this topic. RCTs involving the PLISSIT intervention show that sexual problems in lactating women decreased. Overall, the studies conclude that the use of the PLISSIT model is recommended in health-care settings, promoting improvement in sexual dysfunction[19,24,25,34,35,40].

Studies using the Kegel exercise technique demonstrated a significant increase in pelvic floor muscle strength after the treatment period and concluded that muscle exercises with the Kegel method increase sexual self-efficacy in postpartum women[20,29,36].

Considering the high prevalence of sexual dysfunction among women in the postpartum period[4,9,12], treatment of this condition has been highly valued by the World Health Organization in recent years. The results of our study may be useful for the decision-making of professionals who work directly with these patients—mainly gynecologists and obstetricians—as well as for epidemiologists who discuss public policies aimed at the well-being of this population worldwide.

Our study is the first systematic review aiming to analyze all nonpharmacological treatment options available for sexual dysfunction in postpartum. However, despite the interesting findings, some limitations must be mentioned. Firstly, we identified a risk of bias in some RCTs due to the lack of blinding and incomplete description of the results. Additionally, we detected a high level of heterogeneity among the studies, such as different follow-up times and the use of several measuring tools, compromising the quality of meta-analysis.

CONCLUSION

Our meta-analysis showed that the treatment of postpartum sexual dysfunction using Kegel exercises, pelvic floor muscle training, or PLISSIT did not provide superior effects compared to using the placebo intervention. Considering the impact of this condition on women's quality of life, this study reinforces the need for more RCTs to increase the quality of evidence and guide clinical practice.

AUTHORS' CONTRIBUTIONS

ACQA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft. **ACAS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft. A**KG:** Conceptualization, Formal Analysis, Methodology, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **RO:** Data curation, Software. **ACZS:** Data curation, Software, Validation.

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COMMENTARY

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The 2023 Bethesda system for reporting thyroid cytopathology: novi sub sole, subdivision is no more debatable, in thyroidology

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The 2010 Bethesda system for reporting thyroid cytopathology (TBSRTC) was initially proposed at the National Cancer Institute (NCI) Thyroid Fine Needle Aspiration (FNA) State of the Art and Science Conference held in Bethesda, Maryland, 2007. Afterward, the 2010 TBSRTC, first edition, suggested thyroidologists to utilize a standardized, six-category-based reporting system for thyroid FNA in the States and worldwide by Cibas and Ali, founders of this lexicon, which was published in the 19th volume of *Thyroid*[1]. Wielding TBSRTC has also been endorsed by the 2015 American Thyroid Association (ATA) management guidelines[2] similar to the 2009 ATA guidelines[3], which was the revision of the 2006 ATA guidelines[4], through the management of this delicate endocrine gland[1-9].

On May 28–June 01, 2016, a special 2½ h symposium entitled "The Bethesda System for reporting thyroid cytopathology: past, present, future" was moderated by Ali and Vielh at the 19th International Congress of Cytology, ICC, in Pacifico Yokohama, Japan[10,11]. In addition to this, Pusztaszeri et al.[12] and Ali et al.[10,11] also discussed briefly the consensus of the aforementioned panel, recommendations, proposed modifications, and updates for a second edition of TBSRTC by anticipating its emerging date in early 2018. However, the 2017 TBSRTC, second edition, was then published in the 27th volume of *Thyroid*, by rectifying the implied risk of malignancy (ROM) for each category, remarkable for indeterminate cytology, molecular testing recommendations interpolating explanatory notes in order to state some may represent the newly established non-invasive follicular thyroid neoplasm with papillary-like nuclear features, NIFTP[13].

Mater artium necessitas. After these two successful former editions worldwide, a third edition of this lexicon, the 2023 TBSRTC, has been announced currently again by Ali et al.[14]. Of note, the up-to-date third edition has been published and available online on July 08, 2023 in *Thyroid* in order to shed light on (a) simplifying the six diagnostic categories with a single name for each, adopting the new histologic terminologies according to the 2022 World Health Organization (WHO) Classification of Thyroid Neoplasms: (i) nondiagnostic; (ii) benign, (iii) atypia of undetermined significance (AUS), (iv) follicular neoplasm, (v) suspicious for malignancy, and (vi) malignant; (b) updating and refining each category by implying ROM based on data reported after the second edition; (c) suggesting an average ROM for each category, besides an expected range of risk of carcinoma; (d) subdividing AUS into two subgroups based on the implied ROM and molecular profiling tests; (e) insertion and discussion of pediatric thyroid diseases and ROMs with the management of algorithms; (f) appending two new chapters of expanded use of molecular and ancillary testing in thyroid cytopathology, and clinical perspectives and imaging findings[14].

Herewith, we sincerely appreciate one of the masters of thyroid cytopathology in order to illuminate the challenging issue, noted for this crucial subdivision, in their updated[14] lexicon. To date, assessment for indeterminate cytology, particularly category III, has still been one of the most challenging issues in thyroidology[15-20]. To this end, in February 2021, we emphasized whether or not it is essential to maintain category III as a unique and indivisible category among indeterminate cytology in the 67th volume of *Rev Assoc Med Bras*[21]. Afterward, we published an article in the same volume of *Rev Assoc Med Bras*, about blurred lines for managing thyroid nodules in the era of category III in a possible forthcoming TBSRTC, 3rd edition on October 2021. Of note, we postulated in this publication in the 67th volume of *Rev Assoc Med Bras* that the so-called subdivision demand and so kind of reality in category III, TBSRTC,

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2nd edition as (i) Category IIIA: AUS/FLUS without nuclear atypia (AUS/FLUS wo NA) and (ii) Category IIIB: AUS/FLUS with nuclear atypia (AUS/FLUS w NA)[22], which is very similar to that of Ali et al.[14]. Finally, we have also currently recommended working with subdivisions instead of insisting on a monolithic category III to be able to resolve the issue of the ongoing debate on indeterminate cytology in our epub ahead of the print article in *Ultrasonography* with a submission date of June 08, 2023[23].

Breviter, we have emphasized opting for a subdivision for AUS (formerly AUS/FLUS) and the value of NA in our three works before the updated third edition. NAs have non-negligible clues in these nodules. *E fructu arbor cognoscitur. Bene diagnoscitur bene curatur*. We are deeply grateful to Cibas and Ali, founders and doyennes of this crucial thyroid lexicon invaluably stating "just keep study" instead of "just keep stu(ea)dy" for AUS of TBSRTC, which will be accepted worldwide.

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AUTHORS' CONTRIBUTIONS

IS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **DS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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https://doi.org/10.1590/1806-9282.20230402ERRATUM

In the manuscript "Anatomical features of sella turcica with comprehensive literature review", https://doi.org/10.1590/1806-9282.20230402, published in the Rev Assoc Med Bras. 2023;69(8):e20230402, on page 1:

Where it reads:
Gki onoul Nteli Chatzioglou

It should read:
Gkionoul Nteli Chatzioglou

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Where it reads:

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