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EDITORIAL

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A point of view on hereditary thrombophilia and low-molecular-weight heparin incorporating the management in pregnancy and involving thyroidology

Stefan Dugalić[1], Jovana Todorović[2], Maja Macura[1], Svetlana Vrzić Petronijević[1], Milos Petronijević[1], Miroslava Gojnić[1], Demet Sengul[3*], Ilker Sengul[4,5], Anton Pelikán[6,7], Eduardo Carvalho de Arruda Veiga[8]

INTRODUCTION

Ab initio, the association of hereditary thrombophilia with adverse pregnancy outcomes still remains to be comprehensively understood. Consequently, the demand for screening for hereditary thrombophilia, the introduction of the treatment, the timing of its introduction, and the exact indications are still a matter of debate in most of the clinical studies[1]. Moreover, therapy options for patients with proven hereditary thrombophilia are also a point of discussion. Most physicians use low-molecular-weight heparin (LMWH), but conflicting outcomes remain on whether it improves pregnancy outcomes, hospitalization rates, and quality of life. The resistance index of the uterine artery (RiAu) is a qualified predictor of placental function and vascularization patterns and is therefore involved in pregnancy termination or therapy protocols.

POINT OF VIEW

The first issue that is commonly raised denotes the screening guidelines. Currently, the screening is recommended for pregnant women with a positive history of venous thromboembolism and/or for those having a first-degree relative with a history of high-risk hereditary thrombophilia[1]. There is no screening recommendation for women with a history of fetal loss or adverse pregnancy outcomes. However, studies on the use of anticoagulant therapy, *per se*, among women with hereditary thrombophilia have focused on the prevention of placenta-mediated adverse pregnancy outcomes.

In several studies, published by authors from our center, hereditary thrombophilia was indeed responsible for poor placentation and poor adverse pregnancy outcomes (APO). We have demonstrated that LMWH therapy was negatively associated with RiAu between the 36th and 38th gestational weeks (gw), recently published in *Revista da Associação Médica Brasileira*, Volume 69. Moreover, younger gestational age at delivery, higher D-dimer values, and higher RiAu values were associated with APO, and the LMWH therapy indirectly affected APO via RiAu between the 36th and 38th gw which leads us to believe that previous APO should also be included in the decision-making process for LMWH therapy introduction[2-4]. In addition, the data for that study had been obtained from the hospital's digital database, including comorbid conditions such as thyroid dysfunction. The thyroid hormones such as L-thyroxine (3,5,3',5'-tetraiodothyronine, T4), and L-triiodothyronine (3,5,3'-triiodothyronine, T3) are known to be effective in reproductive functions in humans and animals by regulating the ovarian, uterine, and placental tissues and metabolism in thyroidology[2-7]. Nevertheless, in our previous study, the women with APO in their current pregnancy did not reveal significance compared to the ones without APO in their current pregnancy in terms of thyroid dysfunction[2-5].

In addition, our previous study[3] has proven the requirement for thrombophilia screening when poor vascularization patterns are observed to minimize APO. On the contrary, a recent cohort study and systematic review of the literature[8] has proven similar

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in the setting of recurrent miscarriage without possessing need for thrombophilia screening. Considering this, we believe that thrombophilia screening should be performed when the patients report adverse late pregnancy outcomes in previous pregnancies and recurrent miscarriage as it can be a consequence of poor early placentation. Our viewpoint is that even though there might not be any difference between thrombophilia prevalence between the population of women with regular pregnancy and those with recurrent miscarriage or late APO, the latter might benefit from LMWH introduction and therapy. Specifically, early introduction of the anticoagulant therapy in the early first trimester may have the highest effect on the placentation process and may therefore be able to prevent all the adverse pregnancy outcomes associated with it.

Differences between populations should also be considered since the genetic culprit of inherited thrombophilia is based on gene mutations and polymorphisms. Apart from mutations analyzed in our population, a recent study from India[9] has shown an even greater spectrum of polymorphisms that should be tested in the setting of recurrent pregnancy loss, and similar up-to-date recommendations based on different gene testing studies from Libia[10] and Japan[11]. The prevalence of different hereditary thrombophilia types and especially the differences between the prevalence of homozygous and heterozygous cases might be taken into consideration by decision-making authorities or guidelines in different systems. Factor V Leiden is more common in the population of Northern Europe or Northern European descent and the prothrombin gene mutation is more common in Southern Europe, while MTHFR C677TT mutation is more common among women of European descent, both southern and northern, and of middle east than in Asian populations[12].

Of note, the LMWH therapy has its downsides as well. Although it is proven to have a frequency of adverse effects compared to other anticoagulants and is safe for the fetus, as it does not transfer the placental barrier, it is not without them. The issues with LMWH commonly refer to its expensiveness, the uncomfortable administration, and most worryingly, its association with bleeding. Nonetheless, most women with thrombophilia have stated that they would be willing to take LMWH in future pregnancies to avoid the possibility of pregnancy losses[13].

CONCLUSION

From our point of view, the benefits outweigh the risks and the costs of both screenings for hereditary thrombophilia and treatment of these phenomena. Routine testing scale and treatment modalities with LMWH of inherited thrombophilia in women with previous APO, RPL, and markers of suboptimal placentation (RIAU) are essential. Finally, the tested polymorphisms might be fitted to the population and ethnicity of the mother. This issue merits further investigation. *Several eyes see more than only one.*

AUTHORS' CONTRIBUTIONS

SD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **JT:** Investigation, Methodology, Project administration, Validation, Visualization. **MM:** Methodology, Project administration, Validation, Visualization. **SVP:** Investigation, Methodology, Validation, Visualization. **MP:** Investigation, Project administration, Validation, Visualization. **MG**: Investigation, Methodology, Project administration, Validation, Visualization. **DS:** Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **IS:** Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **AP:** Investigation, Methodology, Validation, Visualization, Writing – review & editing. **ECAV:** Investigation, Methodology, Validation, Visualization, Writing – review & editing.

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LETTER TO THE EDITOR

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Comment on "Evaluation of treatment of the exacerbation of asthma and wheezing in a pediatric emergency department"

Xinmei Zhang[1], Tingting Zhang[1*]

Dear Editor,

A recent study entitled "Evaluation of treatment of the exacerbation of asthma and wheezing in a pediatric emergency department"[1] focused on how asthma exacerbations and wheezing are treated in a pediatric emergency department. The authors examined the treatment strategies used for children with conditions such as bronchodilators, corticosteroids, and other therapies. They observed how these interventions affected respiratory symptoms and overall well-being. However, there are some concerns that need further clarification.

First, the study[1] mainly analyzed the short-term outcomes such as the length of stay in the pediatric emergency unit. While this study provides valuable information about the initial response to treatment, it did not fully capture the long-term effects on patients and their overall prognosis. It is important to consider long-term outcomes[2-4] such as symptom resolution, quality of life over time, and the rate of disease exacerbation. This would give a more comprehensive understanding of the treatment's effectiveness and its impact on the well-being of pediatric patients. Additionally, it would be beneficial to evaluate the patients' prognosis and their response to treatment beyond the acute exacerbation episode. Factors such as disease control, recurrence rates, and functional outcomes should be assessed over a longer period such as 30 days, 6 months, or 1 year. This would provide valuable information about the interventions used in the pediatric emergency department and their long-term efficacy.

Second, a significant finding of this study[1] was the notable difference between the medications used in the pediatric emergency unit and those recommended by treatment guidelines. This raises concerns about the appropriateness and adherence to evidence-based practices in managing asthma exacerbations in children. Therefore, it is important to take into account the level of expertise of the treating physicians, as it may be associated with inconsistent medication usage. This aspect could help healthcare systems identify physicians who may require additional diagnostic and therapeutic training. Inadequate adherence to treatment guidelines and variations in clinical practice among healthcare professionals can affect the quality of care provided to patients. It is crucial to ensure that all physicians involved in managing asthma exacerbations must receive sufficient training and stay updated about the recent evidence-based practices. By assessing the correlation between physician expertise and medication practices, healthcare systems can identify areas where additional training or educational interventions are needed. This would contribute to standardizing care and improving patient outcomes in pediatric asthma management. Furthermore, examining the influence of physician characteristics, such as years of experience, specialty training, and ongoing professional development, could provide insights into the factors contributing to variations in treatment approaches. Identifying specific groups of physicians who may require additional support or training would be beneficial in optimizing care delivery and promoting best practices.

AUTHORS' CONTRIBUTIONS

XZ: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **TZ:** Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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Comments on "Relationship between body composition and PBRM1 mutations in clear cell renal cell carcinoma: a propensity score matching analysis"

André Pontes-Silva[1*], Olga Kovaleva[2], Aida Gadzhiakhmedova[3], Anastasia Luchina[3], Mikhail Sinelnikov[4,5], Roman Maslennikov[6], Asiyat Musaeva[7], Nataliya Zharova[2], Tatyana Zharikova[2], Yury Zharikov[2]

Kidney cancer is one of the leading causes of cancer-related death worldwide and mainly comprises renal cell carcinoma, with an estimated 0.4 million new cases worldwide in 2018[1]. In 2020, Hu et al.[2] carried out a meta-analysis to evaluate the prognostic value of sarcopenia in patients with renal cell carcinoma and observed that patients with sarcopenia had worse overall survival compared with those without sarcopenia in renal cell carcinoma. They[2] concluded that larger, preferably prospective, studies were needed to confirm and update their findings. Recently, in 2023, Demirel and Dilek[3] published, a study entitled "Relationship between body composition and PBRM1 mutations in clear cell renal cell carcinoma: a propensity score matching analysis" in the *Journal of the Brazilian Medical Association*[3], in which they retrospectively examined the relationship between body muscle and adipose tissue composition in clear cell renal cell carcinoma patients with polybromo-1 protein (PBRM1)[4] gene mutation[3]. The study[3] concluded that normal attenuation muscle area is greater in patients with PBMR1 mutation, even after propensity score matching[3]. According to the authors, body composition plays a critical role in understanding the complex effect of PBRM1[3].

This article[3] has the potential to generate new systematic reviews[2] with retrospective designs[3]. As such, to contribute to the authors and journal, we outline a suggestion for novel studies, on body composition and PBRM1 mutations, and calculated the effect sizes for values significant[5] in the outcomes assessment before and after propensity score matching in patients with PBRM1 (Table 1). Our suggestion to researchers and physicians is that thetissues that make up the human body center[6], namely, the abdominal region—subcutaneous adipose tissue (SAT)[7], visceral adipose tissue (VAT)[8]; total adipose tissue (TAT)[9-11], intramuscular adipose tissue (IMAT)[12-14], low attenuation muscle area (LAMA)[15], normal attenuation muscle area (NAMA)[16,17], and total abdominal muscle area (TAMA)[18,19]—should be analyzed based on the patients' stature (mean±SD). This is necessary because it is possible to observe patients with the same stature[20], however, with different areas (cm^2) in the abdomen (Figure 1)[21].

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Table 1. Evaluation of age and body composition parameters before and after propensity score matching in patients with PBRM1 mutated and not mutated-unknown mutation status.

Variables	Before matching (n=291)				After matching (n=152)			
	PBRM1 mutation (+)	PBRM1 mutation (-)	p-value	d-value	PBRM1 mutation (+)	PBRM1 mutation (-)	p-value	d-value
	Mean±SD	Mean±SD			Mean±SD	Mean±SD		
Age (years)	60.1±11.1	60.3±12.7	>0.05	n/a	59.9±11.1	59.8±13.4	>0.05	n/a
SAT (cm^2)	231.2 ±125.8	226.2±116.8	>0.05	n/a	232.4±126.2	225.4±115.1	>0.05	n/a
VAT (cm^2)	229.4±119.3	212.3±115.8	>0.05	n/a	230.4±119.8	219.4±120.7	>0.05	n/a
TAT (cm^2)	460.6±214.2	438.6±192.2	>0.05	n/a	462.9±214.6	444.8±200.6	>0.05	n/a
IMAT (cm^2)	27.2±14.6	29.6±15.2	>0.05	n/a	27.3±14.7	29.7±17.7	>0.05	n/a
LAMA (cm^2)	58.2±24.2	55.8±22.3	>0.05	n/a	58.1±24.4	57.2±26.2	>0.05	n/a
NAMA (cm^2)	104.2±38.7	88.9±35.6	**<0.05***	**0.4'**	104.3±38.9	90.9±37.3	**<0.05***	**0.3'**
TAMA (cm^2)	189.6±40.9	174.3±40.8	**<0.05***	**0.3'**	189.7±41.2	177.8±42.1	>0.05	n/a

Table and sample based on the study by Demirel and Dilek[3]. SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; TAT: total adipose tissue; IMAT: intramuscular adipose tissue; LAMA: low attenuation muscle area; NAMA: normal attenuation muscle area; TAMA: total abdominal muscle area. *Significant values (p <0.05). 'Effect Size. n/a: not applicable.

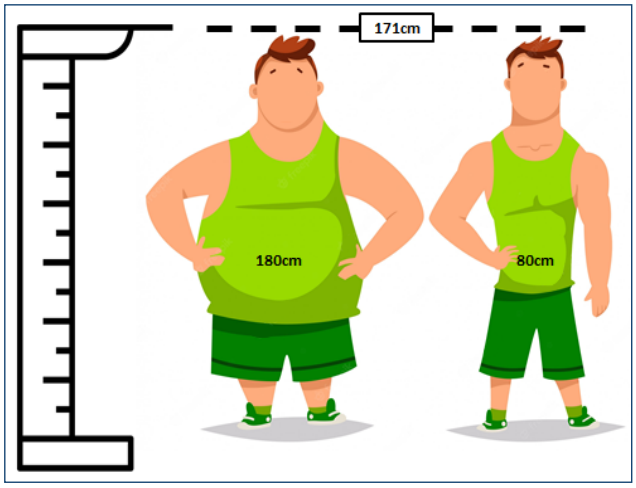

Figure 1. Patients with the same stature but with different areas in the abdomen.

AUTHORS' CONTRIBUTIONS

OK: Validation, Visualization, Writing – original draft, Writing – review & editing. **AG:** Validation, Visualization, Writing – original draft, Writing – review & editing. **AL:** Validation, Visualization, Writing – original draft, Writing – review & editing. **MS:** Validation, Visualization, Writing – original draft, Writing – review & editing. **RM:** Validation, Visualization, Writing – original draft, Writing – review & editing. **AM:** Validation, Visualization, Writing – original draft, Writing – review & editing. **NZ:** Validation, Visualization, Writing – original draft, Writing – review & editing. **TZ:** Validation, Visualization, Writing – original draft, Writing – review & editing. **YZ:** Validation, Visualization, Writing – original draft, Writing – review & editing. **APS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Collagen content and C-X-C motif chemokine ligand 12 expression in neoplastic breast stroma

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SUMMARY

OBJECTIVE: This study aimed to evaluate the expression of C-X-C motif chemokine ligand 12 and its C-X-C chemokine receptor type 4, and the tumor-stroma ratio using collagen stromal content of breast cancer samples, correlating it with clinicopathological data.

METHODS: Through a retrospective cohort study, samples were obtained from female patients, over 18 years of age, with the disease in stages 1–4, who underwent mastectomy or lumpectomy. The biopsies were provided by the Oncology sector of the Hospital das Clínicas of Universidade Federal de Pernambuco, Recife city, in 2011–2014, including samples of invasive ductal carcinoma, ductal carcinoma in situ, or benign changes (fibroadenoma and hypertrophy), which were analyzed between 2020 and 2022 by immunohistochemistry for the expression of stromal cell characteristics. Collagen content was tested by Gomori staining and digital analysis of images.

RESULTS: Absence of stromal expression of C-X-C motif chemokine ligand 12 was associated with longer disease-free survival (disease-free survival=0.481), and expression of C-X-C chemokine receptor type 4 was associated with lower disease-free survival. An association was observed between clinicopathological variables and stromal expression of chemokines, that is, an association of stromal C-X-C motif chemokine ligand 12 with histological grade, angiolymphatic invasion, and an association between C-X-C chemokine receptor type 4 expression and histological grade. Analyses of digital pixels images of collagen and tumor cells showed a lower percentage of collagen in the invasive ductal carcinoma samples (39%), unlike samples without neoplasms (78%).

CONCLUSION: Low expression of C-X-C motif chemokine ligand 12 may be associated with a worse prognosis for breast cancer. Collagen staining analyzed using digital images represents an opportunity for clinical application and is indicative of the prognosis of the tumor microenvironment in breast carcinoma.

KEYWORDS: Breast carcinoma *in situ*. Collagen type I. Chemokine CXCL12.

INTRODUCTION

Breast cancer is the main malignant neoplasia among women, and there is still a necessity for a better understanding of this disease[1]. In recent years, the tumoral microenvironment has been considered indispensable for elucidating both the cellular transformation process and the cancer cells spread through the human body[2]. Carcinogenesis of breast cancer is assumed as a progression from hyperproliferation, followed by an evolution to ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC), and finally tumoral metastasis[3].

Tumoral microenvironment refers to a set of different cellular and physical-chemical factors including immune cells, blood vessels, and extracellular matrix (ECM) that interfere with many disease aspects and therapeutic results[4,5]. ECM components take part in cancer development, and special attention has been devoted to tumor-stroma ratio (TSR) as a significant prognosis indicator. Because of this, the measuring of TSR has been used in the evaluation of diverse types of malignant neoplasms[6,7]. The first use of the TSR for breast cancer prognosis was described by Kruijf et al.[8]. Since then, other studies pointed out a significant association between an increase in TSR and worse prognoses. The most abundant constituent of EMC is collagen that can be involved in breast cancer progression by supporting the cancer cell migration or influencing cell differentiation and proliferation[9].

Cytokines participate in cell movement control, adhesion, and proliferation during embryogenesis and tissue repairment

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after damage. These peptides and their receptors are highly expressed in tumor cells affecting their proliferation, survival, and invasiveness[10]. An example is the C-X-C motif chemokine ligand 12 (CXCL12)/stromal-derived factor 1 cytokine that functions by C-X-C chemokine receptor type 4 (CXCR4) and C-X-C chemokine receptor type 7 receptors. When overexpressed in cancer, they can promote greater cell multiplication, increase their survival, and contribute to their invasiveness in adjacent tissues[11,12].

Therefore, the objective of this study was to measure TSR by specific collagen staining and to evaluate the expression of CXCL12 and CXCR4 by immunohistochemistry (IHC) in breast cancer biopsies, correlating it with clinicopathological data.

METHODS

Sample's obtainment

The study was approved by the Ethics Committee (protocol number 2.701.211, on June 8, 2018) following Resolution CNS 466/12 which regulates research involving human beings in Brazil. All participants who agreed to participate in the research signed a free and informed commitment term. The sample size was determined according to the demand of the hospital sector, including only samples of women, aged 18 years or older, with a confirmed diagnosis of cancer in stages 1–4, and surgery performed by lumpectomy or mastectomy was included in the study. Patients with unavailable slides and biopsy blocks that did not show a representative tumor in the residual sample were excluded from the study.

Biopsies from 43 diagnosed with breast cancer or with benign alterations (hypertrophy and fibroadenoma) were obtained from 2011 to 2014 in the Oncology Sector, Hospital das Clínicas – Universidade Federal de Pernambuco, Recife City, Brazil. The samples diagnoses were confirmed by an anatomopathologist under optical microscopy (100× magnification) as ICD, DCIS, fibroadenoma, and hypertrophy.

After selection, the samples were analyzed by IHC to determine the expression of estrogen and progesterone receptors, cytokine CXCL12 content, and its respective receptor CXCR4, and the content of collagen was studied by specific stain followed by digital analysis.

Immunohistochemistry

The IHC analysis with anti-ER, anti-RP, anti-CXCL12, and anti-CXCR4 antibodies (Boster Biological Technology) followed the protocol described by Santos et al.[13], with revelation by the streptavidin-biotin-peroxidase complex technique (Kit LSAB+peroxidase, K0690, DAKO; kit Liquid DAB+Substrate, K3468, DAKO). Positive controls were used as indicated by the respective antibody manufacturers, and the antibody was replaced by PBS for negative controls.

Collagen staining and tumor-stroma ratio measuring

Slices with ICD, CIDS, fibroadenoma, and hypertrophy were stained with Gomori's Trichrome according to the manufacturing kit (Leica Biosystems). To quantify collagen and stroma percentual calculation, five interest regions (regions that must contain neoplastic cells) were manually selected per slide, with a field of 0.145 mm^2. A micrograph at 100× magnification was acquired for each IR using a Panthera Series L optical photomicroscope (MOTIC, San Antonio, United States). For micrograph analysis, the standard color thresholding method was used in the ImageJ software (ImageJ 1.53c, National Institutes of Health, United States) with the following parameters: Tone 145–195, Saturation 20–255, and Brightness 0–200 for collagen quantification, while, for measuring the area of tumor cells or normal glands red and pink tones by excluding the Tone ranging between 15 and 220, the parameters for Saturation and Brightness were 20–255 and 0–200, respectively. A total of 40 micrographs were analyzed by two independent observers, and a correlation coefficient was calculated using the SPSS software (Version 20.0. Armonk, New York, USA). The percentage of the area marked in blue or pink/red corresponding to each slide was obtained, and the ratio between the percentage of the pink/red tone area and the area occupied by the blue tone was used to represent the TSR in cases of ICD and stroma gland in the groups without malignancy.

Variables and statistical analysis

Data were collected from the medical records of patients for age, tumor size, lymph node involvement, histological subtype, and ER and PR expressions and analyzed for the CXCL12 and CXCR4 expressions using a logistic regression model and probability analysis, with the following logit model: $A(x'\beta)-\varepsilon x'\beta(1+\varepsilon x'\beta)$, which allows evaluating the multiplicative effect of a single variable on the others, through the Stata statistical package, version 13. Disease-free survival (DFS) was analyzed using the Kaplan-Meier method, and the curves were compared using log rank between CXCL12 positive and negative and CXCR4 positive and negative patients. Statistical significance was considered when $p<0.05$ for two-tailed tests.

RESULTS

Anatomopathological analysis showed the following diagnosis: 30 (69%) patients with IDC, 3 (7%) with CDIS, 6 (13%) with hypertrophy, and 4 (9%) cases of fibroadenoma. Of note, 21 (48%) patients were >50 years old, 20 (46%) patients were <50 years old, and 2 patients without age information. Most IDC (60.6%) presented tumor size ≥2 cm. The same percentage showed axillary lymph node involvement. The most prevalent histological grades were II and III (42% for both). There is no information about tumoral size, lymph node commitment, and histological grades for benign alterations of fibroadenoma and hypertrophy samples (n=10). In relation to the IHC results, 44% of patients showed positivity for ER and 33% for PR, while, concerning the cytokine evaluation, 29% of patients were positive for CXCL12 expression and 25% of patients were positive for CXCR4 (Figure 1). According to the logistic analysis, the absence of stromal CXCL12 expression was associated with a higher DFS 0.481 (95%CI 0.08–2.72), without statistical significance (p=0.408). CXCR4 expression was related to a higher DFS with OR=1.7 (95%CI 0.18–16.42), also without statistical significance (p=0.643). These results were limited by the small sample amount. There was an association between clinicopathological variables, histological grade, angiolymphatic invasion, and chemotherapy with stromal expression of CXCL12 and between CXCR4 and histological grade (Table 1).

Cellular CXCL12 expression did not show significant association with any variable. No expression of stromal CXCL12 was associated with high histological grades. After robustness test, there was confirmation of the result for grades 1–3. For CXCR4, no expression was influenced by histological grade 1 only. However, the stromal CXCL12 positivity was correlated with angiolymphatic invasion and influenced by chemotherapy, according to Table 1.

The collagen content and the ratio average were calculated for each group of patients (Table 2). The TSR results indicate a consistent relationship for the percentage of collagen area, especially in the groups with and without malignancy, being remarkably reduced in patients with IDC (39%) considering the markedly high value (78%) in the benign cases. TSR showed no significant association with prognosis, adjuvant chemotherapy, or TNM staging.

DISCUSSION

According to our results, the sample mainly consisted of patients with ICD, as typically found in the literature[14], most of whom were diagnosed after menopause[15]. Furthermore, tumor size corresponds to the diameter of the primary tumor and is an important negative prognostic factor. In our study, most patients had a size greater than 2 cm, which reveals late diagnosis, increasing the chance of metastasis, treatment ineffectiveness, and probability of recurrence[16]. In view of this, as tumor size is fully correlated with lymph node involvement, most patients presented metastasis.

The release of growth factors that function as chemoattractant occurs in tumoral microenvironment which modulates the cell behavior through positive feedback, including the cytokine production[17]. In our study, the lowest expression of the chemokines CXCL12 and its receptor CXCR4 was observed in larger tumors and with lymph node metastases. In addition, high histological grades significantly correlated with lower expression of CXCL12. According to this result, we realized that, for breast cancer, the lower expression of these chemokines can worsen the prognosis for patients, as it increases the malignancy of the tumor[18].

Collagen is the main component of the cellular matrix, and its increased deposition favors greater tumor aggressiveness and the occurrence of metastasis[19,20]. Due to its importance in pathological processes, several methods can be used for its evaluation[21,22]. Here, we used Trichrome Gomori staining and the collagen content served to calculate the TSR. Through the TSR analysis, it is possible to perform a risk stratification and predict the prognosis and the highest value in the quantity parameters[23]. In addition, the uniformity of collagen fibers is associated with the worst prognosis in IDCs. Our data revealed that collagen deposition in tumor lesions occurs because tumors with a high incidence of metastases may have less adhesion between cells. This result infers that tumor behavior depends not only on genetic characteristics but also on the ECM and collagen framework[24]. Lower collagen deposition in the ECM and the increase in TSR in the IDC can favor a more aggressive behavior of neoplastic cells, with an increase in the chances of metastases and higher histological grade. The TSR assessment is a simple, reproducible, and predictor variable for the diagnosis of malignancy and can be incorporated into the pathological assessment given the reduced cost[25].

The main limitations of the study are related to data from retrospective studies and the limited sample size, which made it impossible to perform some more robust statistical analyses. In addition, the reduced sample size may have caused the non-observance of statistically significant results in relation to collagen content and TSR with clinicopathological variables, which is one of the main limitations of the study. However, it was possible to demonstrate how to calculate TSR by specific collagen staining using the color threshold method, which is an important prognostic factor.

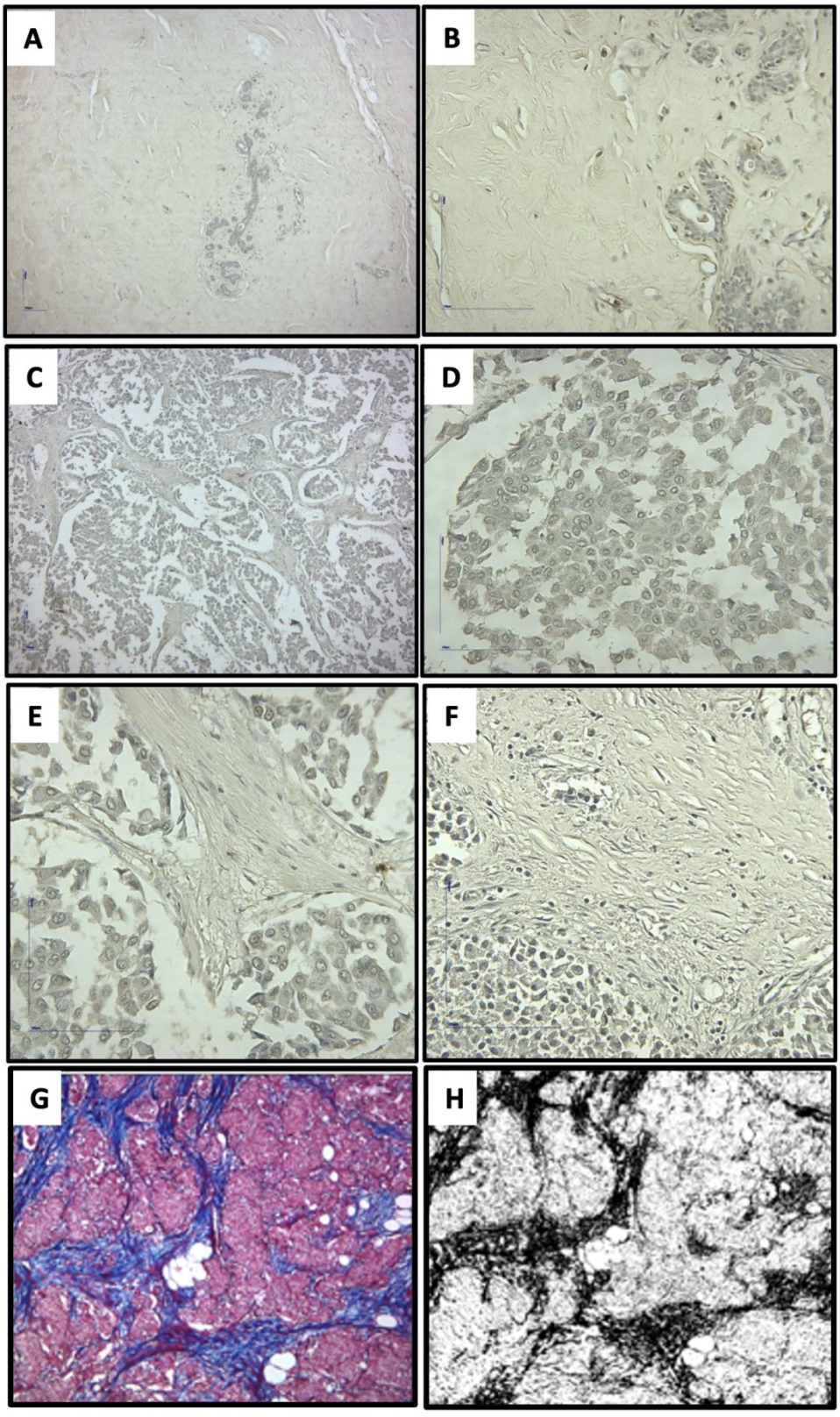

Figure 1. Immunohistochemistry staining pattern of breast cancer for C-X-C motif chemokine ligand 12 and collagen stain. (A,B) Normal breast tissue (100× and 400×, respectively) showing stroma and cellular positivity. (C–F) Invasive ductal carcinoma staining (100×) showing the cytoplasmatic and stromal positivity (400×). (G) Micrograph of the region of interest from tissues stained by Gomori, 100× magnification. (H) Image obtained for stroma quantification by collagen labeling.

Table 1. Correlation between cellular and stromal expression of C-X-C motif chemokine ligand 12 and C-X-C chemokine receptor type 4 with clinicopathological variables by the logit method, demonstrating an association between stromal C-X-C motif chemokine ligand 12 expression and histological grade, amphiolymphatic invasion, and chemotherapy, and an association between C-X-C chemokine receptor type 4 expression and grade histological.

Variables	Cellular CXCL12		Stromal CXCL12		p	CXCR 4
	p	(95%CI)	p	(95%CI)		(95%CI)
Tumoral size	0.45	(-6.01 to 2.69)	0.75	(-3.38 to 4.64)	0.14	(-3.97 to 2.78)
Lymph node commitiment	0.22	(-7.65 to 1.76)	0.25	(-4.38 to 16.44)		
Histological grade	0.45	(-5.35 to 2.40)	<0.001	(-28.58 to -10.13)	0.003	(-6.52 to -1.37)
Inflammation	0.95	(-3.59 to 3.40)	0.11	(-1.28 to 11.19)	0.62	(-3.79 to -2.26)
Angiolymphatic invasion	0.14	(-0.97 to 6.53)	<0.001	(28.33 to 52.98)	0.26	(-1.0 to 3.70)
Histological subtype	0.08	(-0.13 to 1.82)	0.67	(-9.47 to 1.46)	0.99	(-1.17 to 1.19)
Chemotherapy	0.33	(-5.87 to 1.99)	<0.001	(-25.56 to -14.34)		

CXCR4: C-X-C chemokine receptor type 4; CXCL12: C-X-C motif chemokine ligand 12.

Table 2. Analysis of collagen and tumor/normal cells areas in the indicated groups.

	Invasive ductal carcinoma	Hypertrophy	Fibroadenoma
	Average±standard deviation		
Percentage of collagen area (%)	39.029±13.442	78.929±20.924	59.378±17.034
Percentage of tumor cells or normal cells (%)	43.463±9.2	10.234±3.394	25.535±17.121
Tumor-stroma ratio	1.263±0.899	7.802±0.542	3.519±2.545

CONCLUSION

In our study, the immunohistochemical evaluation of CXCL12 and its CXCR4 receptor was correlated with clinical pathological data in breast cancer, indicating that the low expression of both chemokines promotes worse tumor biology. However, further studies are needed to better understand the functioning of these chemokines in the breast tumor microenvironment. The collagen content that was used to calculate the TSR through digital image analysis represents an excellent opportunity for clinical application and may be indicative of the prognosis of the tumor microenvironment in breast carcinoma.

AUTHORS' CONTRIBUTIONS

CJSF: Investigation, Formal Analysis, Writing – original draft. **IQSC**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology. **WJBCN**: Formal Analysis, Methodology, Writing – original draft. **SMVA**: Project administration, Resources, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

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Does total knee arthroplasty affect pelvic movements? A prospective comparative study

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SUMMARY

OBJECTIVE: Pathology in any segment of the spine-pelvis-lower extremity may impair the global postural balance, leading to compensatory alterations in other parts. The aim of this study was to compare the pelvic movements of patients suffering from knee osteoarthritis with patients who underwent total knee arthroplasty and healthy controls.

METHODS: This study was performed at the Department of Orthopedics and Traumatology Clinic of a Cankiri State Hospital between April 2021 and February 2022. This study included 84 participants. Of them, 31 patients who underwent total knee arthroplasty between 2018 and 2020 years were selected as the total knee arthroplasty group, while 28 patients with knee osteoarthritis were selected as the knee osteoarthritis group. In the control group, there were 25 healthy individuals. Exclusion criteria from the study included any kind of neurological disease, an inability to walk a distance of 100 m unassisted, or a history of surgery to the lower limb. Pelvic movements (i.e., tilt, rotation, and obliquity) and gait parameters (i.e., "gait velocity," "cadence," and "stride length") were assessed using a wireless tri-axial accelerometer.

RESULTS: Total knee arthroplasty and control groups had decreased minimum anterior tilt of the pelvis, decreased maximum anterior tilt, and decreased oblique range of the pelvis compared with the knee osteoarthritis group. In comparison with the control group, gait velocity and length of stride during gait were remarkably lower in both knee osteoarthritis and total knee arthroplasty groups.

CONCLUSION: In this study, total knee arthroplasty was found to affect pelvic movements. It was thought that total knee arthroplasty changed these variables, probably owing to the frontal and sagittal plane alignment correction through surgery.

KEYWORDS: Knee replacement arthroplasty. Knee osteoarthritis. Pelvic examinations.

INTRODUCTION

Knee osteoarthritis (KOA), which is a chronic and degenerative joint disease, leads to impaired mobility and pain, affecting a substantial number of individuals globally[1]. Total knee arthroplasty (TKA) is the gold standard that alleviates pain, improves functions, and restores tibiofemoral joint alignment in patients with KOA[2,3].

Biomechanically, the human body has a multi-segmental structure to execute main coactions between the adjacent segments. The interaction that happens between segments may be of utmost importance for asymptomatic musculoskeletal function. The primary role of the pelvis located in the body center is to connect the upper torso to the lower limbs[4]. Any segment of the spine-pelvis-lower extremity that has pathology may impair the global postural balance, leading to compensatory alterations in other parts of the body[5].

Severe KOA can dramatically influence the sagittal alignment of the spine-pelvis-lower extremity. To the best of our knowledge, few studies in the literature evaluated pelvic movements in patients with KOA undergoing TKA. This study aimed to compare the pelvic movements of patients suffering from KOA with patients who underwent TKA and age-matched healthy controls. In this study, we tested the hypothesis that patients suffering from KOA could walk with an anterior tilt of the pelvis, and following TKA, pelvic movements would be similar to those of healthy individuals.

METHODS

This single-centered and prospective study was performed at the Department of Orthopedics and Traumatology Clinic of a Cankırı State Hospital between April 2021 and February 2022. A total of 84 participants took part in this study. After

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the approval by the institutional Ethics Committee (Approval Date: 02.03.2021 and Approval Number: 2021-274), participants who underwent bilateral TKA due to osteoarthritis and had bilateral KOA, and age- and sex-matched asymptomatic healthy individuals were included.

A total of 31 patients who underwent bilateral TKA performed by an experienced orthopedic surgeon were selected as the TKA group. The patients were recruited if they underwent knee replacement 1 year, but no longer than 3 years prior to their inclusion. All TKA patients used posterior stabilized implants. A cemented total knee prosthesis (Tipmed, Turkey) was implanted using a standard medial parapatellar approach. Exclusion criteria were as follows: (1) spinal surgery pre- and post-TKA, (2) morphological changes in the vertebral body because of osteoporotic compression or traumatic fractures, (3) pain in low back and hip during walking, (4) history of a central nervous system illness, (5) severe heart or lung disease, (6) rheumatoid arthritis, and (7) inability to walk independently.

A total of 28 patients with KOA who required surgery were selected as the KOA group. All of the participants in the KOA group had bilateral KOA. The Kellgren-Lawrence (KL) radiographic grade from basic X-ray images was used to verify progression in patients with KOA. The KL grade was found to be III or IV in patients with KOA. An experienced orthopedic surgeon diagnosed all participants. In this study, the knee OA subjects had OA changes not only in the tibia but also in the femur, and only medial OA was examined.

The control group who had neither diseases nor gait disorders consisted of 25 age- and sex-matched healthy volunteers. Healthy participants were included in the study on the condition that they had not had any lower limb pain or back pain for the last 6 months.

Exclusion criteria from the study included any kind of neurological disease, cardiovascular disorder, an inability to walk a distance of 100 m unassisted, or a history of surgery to the lower limb.

Before the study, the researcher informed all participants about the nature of the study and obtained written informed consent from the participants. The study was carried out pursuant to the principles of the Declaration of Helsinki.

The demographic and epidemiological characteristics such as age, weight, height, and body mass index of all participants were registered.

Outcome measures

Pelvic movements

Pelvic movements (i.e., tilt, rotation, and obliquity) and gait parameters (i.e., gait velocity, cadence, and stride length) were measured, while participants walked freely along a 10 m walkway by using a wireless tri-axial accelerometer (G-Walk, BTS Bioengineering S.p.A., Italy) that was attached to the fifth lumbar vertebra and tightened with VelcroTM.

Gait velocity is the distance traveled by the body in a given time period. Stride length is the distance between the ground contact point of one heel and the ground contact point of the same heel. Cadence is the number of steps in a given time. It is usually calculated as the number of steps per minute. To assess the pelvic movement and gait parameters, the participants stood still in an orthostatic standing position, which continued for a few seconds until the stabilization of the G-Walk device ended. The participants received instructions on how to walk on a 10 m track, whose boundaries were labeled for an accurate analysis. The participants were allowed to walk with non-heeled casual shoes on firm surfaces. The participants kept a totally straight line while moving at their usual pace. They did a successful trial by completing the 10 m track and returning to the initial point[6,7].

The accelerometer data were wirelessly transferred by a Bluetooth system and analyzed with BTS G-studio software (BTS Bioengineering S.p.A., Italy) on a computer. The weight of the accelerometer was 37 g, with dimensions of 70×40×18 mm. The frequency of the accelerometer was from 4 to 1,000 Hz and sensor fusion was 200 Hz. The pelvic movement which has included all three planes was analyzed by using the BTS G-studio software program. G-walk is a reliable device for evaluating gait in healthy adults. The assessment of pelvic angles had moderate test–retest reliability (ICC: 0.463-0.659)[7,8].

Statistical analysis

In the *post-hoc* power analysis for the study, the effect size (f), calculated considering the pelvic movement values, was 0.446. When a total of 84 participants were involved, we calculated that the power of the study was 0.95 and α value of 0.05 (G*Power 3.0.10 system, Franz Faul, Universität Kiel, Germany)[9].

Statistical analysis was carried out by using the SPSS v. 22.0 software (IBM Corp., Armonk, NY, USA). The variables were assessed using analytical (i.e., Kolmogorov-Smirnov/Shapiro-Wilk test) and visual methods (i.e., histograms and probability plots) to ascertain whether or not normal distribution was ensured. Where applicable, descriptive statistics were provided as mean±standard deviation (SD), median (min-max), or number and frequency. As the demographic information and gait metrics were not normally distributed, the Kruskal-Wallis tests were used to compare these metrics between the groups. The Mann-Whitney U test was used to analyze the importance of pairwise differences by using the Bonferroni correction to measure multiple comparisons. As the pelvic movements

were normally distributed, one-way analysis of variance was benefited in the comparison of these parameters between the groups. The homogeneity of the variances was tested by using the Levene test. Pairwise *post-hoc* tests were conducted using the Tamhane-T2 test in the case of an overall significance. A p-value of <0.05 was deemed statistically significant.

RESULTS

Characteristics of participants

Initially, 110 participants were identified from the recorded data. After applying the inclusion and exclusion criteria, 11 (10%) patients who underwent bilateral TKA and unable to walk independently, 9 (8.1%) patients with KOA who had a history of surgery to the lower limb, and 6 (5.4%) healthy participants who had any lower limb pain or back pain for the last 6 months were excluded. Thus, a total of 84 participants were eligible for this study. The attributes of the participants are shown in Table 1. The groups showed no significant differences in terms of age, body weight, or body mass index (p>0.05).

Pelvic movements

The maximum and minimum anterior tilts and obliquity range of motion (ROM) of the pelvis differed significantly by the groups. According to the pairwise comparison results, this difference was due to the KOA group.

Minimum and maximum anterior tilts and obliquity ROM of the pelvis were significantly larger in the KOA group than control (p=0.002, p<0.001, and p<0.001, respectively) and TKA (p=0.008, p<0.001, and p=0.006, respectively) groups.

The groups showed no significant differences regarding the rotation ROM (p>0.05). Pelvic angles for the TKA, KOA, and control groups are shown in Table 2.

Table 1. Demographic and epidemiological data for the total knee arthroplasty, knee osteoarthritis, and control groups.

Variable	TKA (n=31)		KOA (n=28)		Control (n=25)		p-value*
	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	
Age (years)	67 (50–76)	65.39 (6.76)	64 (54–75)	63.86 (6.71)	64 (53–73)	62.48 (5.36)	0.187
Height (cm)	158 (151–176)	159.97 (6.35)	165 (152–178)	163.43 (7.33)	175 (150–161)	162.36 (6.17)	0.129
Weight (kg)	88 (67–115)	88.11 (13.22)	87 (66–117)	89.60 (12.05)	83 (67–114)	85.65 (11.75)	0.465
BMI (kg/m^2)	35 (24–49)	34.69 (5.76)	33 (24–47)	33.79 (5.90)	31 (25–46)	32.54 (5.18)	0.326
Sex (male: female)	6:25		6:22		7:18		0.734

TKA: total knee arthroplasty; KOA: knee osteoarthritis; SD: standard deviation; BMI: body mass index. *Kruskal-Wallis test (p<0.05).

Table 2. Pelvic Angles for the total knee arthroplasty, knee osteoarthritis, and control groups.

Pelvic angles	TKA (n=31)		KOA (n=28)		CONTROL (n=25)		p-value*
	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	
Anterior tilt max (°)	13 (7–18)	12.09 (2.9)	15 (10–20)	15.1 (2.5)	11 (5–19)	11.5 (4.3)	**0.002*** KOA-Control **0.008*** KOA-TKA
Anterior tilt min (°)	8 (4–14)	8.2 (2.5)	11 (6–15)	10.9 (2.4)	7 (2–11)	6.7 (3.5)	**0.000*** KOA-Control **0.000*** KOA-TKA
Obliquity range of motion (°)	8 (3–10)	7.3 (1.4)	9 (5–12)	8.8 (2.0)	7 (3–11)	6.2 (2.0)	**0.000*** KOA-Control **0.006*** KOA-TKA
Rotation range of motion (°)	8 (5–11)	7.8 (1.6)	8 (3–11)	7.6 (1.7)	8 (4–14)	8.0 (2.0)	0.726

TKA: total knee arthroplasty; KOA: knee osteoarthritis; SD: standard deviation. *One-way ANOVA (p<0.05), post-hoc Tukey's test (p<0.017). Statistically significant p-values are given in bold.

Gait parameters

There were meaningful differences between the groups relating to gait velocity and stride length ($p<0.05$). According to the pairwise comparison results, the difference in speed and stride length was caused by the difference control group. When compared with the control group, gait velocity and stride length during walking were lower in both KOA and TKA groups at a significant level. There were no meaningful differences in cadence between the groups ($p>0.05$). The gait parameters for the TKA, KOA, and control groups are shown in Table 3.

DISCUSSION

In this study, patients with KOA had significantly larger anterior pelvic tilt than the asymptomatic controls. The anterior pelvic tilt decreased in patients with TKA compared with the KOA group, but not as much as the control group. This study has introduced outcomes supporting our theory that TKA could lead to changes in pelvic movements. To the best of our knowledge, this is the first study to evaluate pelvic movements (i.e., rotation, tilt, and obliquity) in patients following TKA.

A previous study asserted that, in the simulation of a 30° unilateral knee flexion contracture in healthy females by the use of a knee brace, an increase was observed in the anterior inclination of the pelvis and trunk in the course of walking; however, this was not detected at a 15° of contracture in knee flexion[10]. It is possible that the patients who had severe KOA were susceptible to anterior pelvic tilt, as seen in the participants of this study. Kuwahara et al.[11], similar to our study, reported that patients with KOA could walk along with larger anterior tilts of the pelvis and trunk prior to the operation when compared with controls. However, after 1 year of surgery, no significant differences in the anterior tilt of the pelvis were observed between pre- and post-TKA in the KOA patients. All body joints are functionally structured with the purpose of minimizing energy use depending on the gravity line in the capacity of being a center during standing[12]. Murata et al.[13] showed significantly reduced lumbar lordosis in patients whose knee extension limitation was found to be over 5°, and gonarthrosis could indirectly lead to lumbar spine-related symptoms. The authors defined this fact as "knee-spine syndrome." In our study, the limited knee joint extension patients with KOA gave rise to a posterior displacement in the center of gravity, suggesting that an induced anterior pelvic tilt might be a compensatory approach. As the extension limitation disappeared after TKA, the anterior pelvic tilt may have decreased.

Although frontal plane kinematics has been discussed in limited research, evidence suggests that TKA changes these variables, probably because of frontal plane alignment correction through the operation. The main goal of TKA is to enhance the tibiofemoral loading environment, specifically by decreasing malalignment in the frontal plane which usually occurs with KOA[14]. The existence of extreme varus-valgus movement in patients with KOA may have caused abnormal knee joint loading, leading to increased oblique movements of the pelvis[14]. This study indicates that the obliquity ROM of the pelvis was significantly larger in the KOA group, although this obliquity was decreased after TKA.

There are several studies showing that 1 year following TKA knee kinematics is still different in comparison with healthy joints. In general, compared with the control individuals, the knee flexion range in patients with TKA is smaller and extension of the knee during walking is decreased, and stride length and cadence are reduced[15,16]. Although our main objective in our study was to evaluate the pelvic movements, we also examined the walking speed of the participants

Table 3. Gait parameters for the total knee arthroplasty, knee osteoarthritis, and control groups.

Gait parameters	TKA (n=31)		KOA (n=28)		Control (n=25)		p-value*
	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	
Gait velocity (m/s)	0.93 (0.55–1.30)	0.91 (0.19)	0.93 (0.64–1.14)	0.90 (0.14)	1.07 (0.61–1.48)	1.05 (0.23)	**0.005*** KOA-Control **0.014*** TKA-Control
Cadence (steps/min)	102 (79–126)	103.86 (10.25)	106 (87–125)	105.25 (9.32)	107.04 (91–135)	109.93 (10.10)	0.070
Stride length (m)	1.04 (0.78–1.43)	1.06 (0.16)	1.03 (0.71–1.42)	1.03 (0.15)	1.21 (0.77–1.56)	1.17 (0.22)	**0.007*** KOA-Control **0.016*** TKA-Control

TKA: total knee arthroplasty; KOA: knee osteoarthritis; SD: standard deviation. *Kruskal-Wallis test ($p<0.05$), Mann-Whitney U test with Bonferroni correction ($p<0.017$). Statistically significant p-values are given in bold.

due to the relationship between pelvic movements and gait speed. A previous study showed that the ROM of the pelvis and lumbar region during gait was greater at high velocity than at the preferred velocity[17]. Nonetheless, there was no significant change in the walking speed of the KOA and TKA groups; therefore, it was unlikely for speed to be a confusing variable in this study. Moreover, even after TKA an average of 1.9 years, the stride length and walking speed were significantly lower than the control group. This may be the result of patients still having differences in knee kinematics 1.9 years after surgery and ongoing pain symptoms. We believe that improved comprehension of both deteriorations and functional limitations after operation may be helpful for clinicians to tailor rehabilitation programs. Also, rehabilitation following TKA takes a long time, which should be reminded to the patients.

Nonetheless, there are some limitations to this study. First, ROMs were not measured during the study. Second, because there was neither pain nor deformity in the control subjects' knee joint in the process of gait, the presence of radiological knee OA was unable to be confirmed. Depending on the ages of the controls, there might be inconsiderable degeneration in their knee joints. However, we employed them in the control group because they nearly had a normal knee function.

In spite of the limitations of this study, as far as we know, not many studies have discussed the movements of pelvic on a large population suffering from severe KOA and following TKA, which is the main strength of this study.

CONCLUSION

In conclusion, patients with severe KOA showed a more anterior pelvic tilt compared with asymptomatic controls. In patients with TKA, the anterior pelvic tilt decreased, but not as much as in the control group. Furthermore, the KOA group had significantly greater obliquity ROM of the pelvis; however, following TKA, this obliquity was decreased. Further studies may focus on changes in sagittal alignment and low back pain in individuals with KOA before and after TKA.

AUTHORS' CONTRIBUTIONS

EAP: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **YP**: Conceptualization, Formal Analysis, Investigation. **GMK**: Formal Analysis, Investigation, Writing – review & editing. **ME**: Formal Analysis, Investigation, Writing – review & editing. **NK**: Methodology, Supervision, Writing – review & editing. **NAG**: Conceptualization, Methodology, Supervision.

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ORIGINAL ARTICLE

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Serum levels and gestational curve of adiponectin and leptin during adolescent pregnancy

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SUMMARY

OBJECTIVE: This study aimed to develop a curve of weekly serum levels of adiponectin and leptin among pregnant adolescents. In addition, pre-gestational body mass index and weight gain were assessed and correlated with the serum concentration of these molecules.

METHODS: This was a prospective cohort study, including only pregnant adolescents with eutrophic pre-gestational body mass index who were weekly followed during the evolution of gestation. The serum concentrations of adipokines were determined using commercial ELISA kits and were correlated to pre-gestational body mass index and pregnancy weight gain. A total of 157 pregnant women participated in this study.

RESULTS: Adiponectin levels showed a significant decrease among the trimesters (p=0.0004). However, we did not observe significant differences among its levels when compared weekly, neither of which was between adiponectin concentration and pre-gestational body mass index or weight gain (p=0.36 and p=0.10, respectively). In contrast, we detected a significant increase in weekly serum leptin levels (p<0.0001), positively correlated to both pre-gestational body mass index and weight gain (p=0.003 and p=0.0007, respectively).

CONCLUSION: These adipokines present a different profile throughout adolescent pregnancy.

KEYWORDS: Adiponectin. Adipokines. Adolescent. Leptin. Pregnancy. Body mass index.

INTRODUCTION

Adipokines are involved in diverse processes. Serum adiponectin levels may vary according to sex, and it is higher in women[1]. This difference is not observed between adolescents.

In adult pregnancy, it is established that the serum adiponectin concentration decreases with the advancement and installation of insulin resistance (IR), returning to the pregravid concentration after delivery. Studies indicate that there is a negative correlation between serum levels and gestational age[2-4]. Contrarily, there is a significant increase in plasma leptin levels with pregnancy progress[4,5] which seems to play a crucial role in regulating placental growth, nutrient transfer, angiogenesis, pulmonary maturation, and trophoblast invasion[5,6]. Impaired levels of adipokines have been associated with obstetric pathologies such as preeclampsia, intrauterine growth retardation (IUGR), gestational diabetes, and preterm birth[7-10]. Few studies have evaluated adipokines in adolescent pregnancy[11-13]. Noreña et al.[14] reported that, in teenagers' pregnancy, increased serum leptin levels are positively associated with IUGR.

It is now recognized that leptin, in addition to being an important mediator of energy balance, acts in the control of fertility and growth[4,5]. Leptin serum concentrations are greater in pregnant women than in non-pregnant women. Substantial increases in leptin levels occur early in pregnancy, before any significant increase in body weight, suggesting that factors other than adiposity are involved in the control of serum leptin levels[10-13]. Leptin concentrations peak in the second trimester of pregnancy and remain high until delivery[6-8]. The proposed physiological role of leptin during pregnancy is not yet known.

Adolescents appear to be at increased risk for adverse pregnancy outcomes, such as premature birth, low birth weight, preeclampsia, maternal death, and perinatal death[15,16]. In addition, it is known that pregnancy and childbirth complications are the main causes of mortality among adolescents in developing countries[17]. Despite the investigations carried out, it remains unclear whether these changes are associated with biological or sociodemographic factors. Therefore, this study aimed to evaluate weekly serum levels of adiponectin and leptin and investigate whether these adipokines' levels are related to pre-gestational body mass index (BMI) and total weight gain throughout adolescent pregnancy.

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METHODS

This prospective cohort study was approved by the Research Ethics Committee of the Federal University of São Paulo (UNIFESP) under the consubstantiated opinion nº 1514/1. All adolescents attended in the Adolescent Prenatal Sector of the UNIFESP from February 2013 to March 2018 were included in the research after reading, understanding, and signing the informed consent form. As well as the respective guardians signed confirming their consent to participate in the study. The patients were followed during gestational evolution; every 15 days, their nutrition status was evaluated through weight, height, and BMI. Blood samples were collected throughout pregnancy (from 9 to 39th gestational weeks), once in every trimester. In this study, pregnant women were between 12 and 20 years of age and presented eutrophic pre-gestational BMI (18.5-24.9 kg/m²). Exclusion criteria were multiple gestations, use of corticosteroids, antibiotics, immunosuppressants, and anti-inflammatories, and adolescents with systemic disorders such as hypertension, diabetes mellitus, systemic lupus erythematosus, rheumatoid arthritis, rheumatic fever, and asthma. In addition, patients who developed obstetric intercurrence with pregnancy progress were excluded. On the first prenatal visit, BMI was calculated based on the pre-gestational weight and height reported by the pregnant girl; after all, the weight gain of pregnant women usually changes in a short period of time. The nutritional diagnosis was established according to the Institute of Medicine (2009)[18]. Gestational weight gain was determined by the difference between the pre-gestational weight and the patient's weight on her last pre-delivery visit.

According to the Ministry of Health, it is considered the first trimester of pregnancy from 0 to 14 weeks, the second trimester from 14 to 26 weeks, and the third and last trimester and pregnancy from 27 to 40 weeks (2016)[19].

A volume of 8 mL peripheral blood was collected (after 12 h fasting) in tubes with spray-coated silica and polymer gel for serum separation (BD Diagnostics, Franklin Lakes, NJ, USA). After clot retraction, the sample was centrifuged, and the resultant serum was aliquoted and stored at -80°C until its assessment. We used commercial kits for all tests, and they were performed at the same time.

For serum adipokine levels, ELISA commercial kits were used – Human Adiponectin, Human Leptin Duoset (R&D Systems®, USA). Adiponectin and leptin sensitivity were 0.06 ng/mL and 32.25 pg/mL, respectively. Intraassay and interassay coefficients of variation (%) for adiponectin and leptin were 3.5 and 6.5 and 3.2 and 4.4%, respectively.

The normality tests such as skewness and kurtosis, Kolmogorov-Smirnov, and Shapiro-Wilk were applied to evaluate the quantitative variables. For the analysis of variance (ANOVA) between the groups, the repeated-measures ANOVA test was applied to measure parametric distributions, and the Friedman test was used to analyze the non-parametric ones, followed by Durbin-Watson post-tests or Dunn-Bonferroni post-test, respectively. For the analysis of categorical variables, the chi-square test was adopted. The Pearson test was applied to calculate the correlation coefficients. The level of significance was set at $p<0.05$. Statistical analyses were performed using the standard software GraphPad Prism, v6.0 for Windows (SPSS Inc., Chicago, IL, USA). No studies have been found allowing the calculation of sample size; therefore, we proposed to carry out a study that serves as the basis for future research. Thus, the totality of patients who suited the established parameters was included in the study period.

RESULTS

The study included 157 healthy pregnant adolescents, comprising 471 blood samples collected between the 9 and 39th gestational weeks. Table 1 shows the main characteristics of the participants. Pregnant women were between 12 and 20 years of age, with a mean age of 16.51 years (standard deviation 1.76).

Serum adiponectin levels exhibited significant differences with the evolution of pregnancy, characterized by a drop in concentration ($p=0.0004$; Friedman test). The data are presented in Table 2 and Figure 1A.

We did not observe a correlation between pre-gestational BMI, pregnancy weight gain, and serum adiponectin levels ($p=0.36$ and $p=0.10$, respectively). In addition, there were no statistical differences in serum adiponectin levels between the adolescents' gestational weeks (Dunn-Bonferroni post-test). Conversely, we detected an increase in serum leptin levels with pregnancy progress ($p<0.0001$; repeated-measures ANOVA). The results are described in Figure 1B. We also observed significant differences regarding serum leptin levels between gestational weeks (Durbin-Watson post-test), particularly after the second trimester.

Finally, we identified a positive correlation between pre-gestational BMI and pregnancy weight gain with serum leptin levels ($p=0.003$ and $p=0.0007$, respectively).

DISCUSSION

This research demonstrated the weekly changes in serum adiponectin and leptin levels throughout gestation (from the 9 to 39th gestational weeks). Similar to previous studies, we observed significant differences in adipokines concentrations between

pregnancy trimesters[7,20,21]. These adipokines present different profiles comparing weekly levels. Adiponectin concentration did not show relevant variations between weeks; neither was related to pre-pregnancy BMI and weight gain; contrarily, leptin exhibits significant changes considering week values; moreover, it is positively correlated with the evaluated variables.

Different studies have shown that these two adipokines exert different effects and present an opposite pattern during healthy pregnancy progress. Adiponectin seems to exhibit anti-inflammatory properties, whereas leptin plays an inflammatory role[2-4,20].

Evidence supports that, in adult pregnancies, adiponectin is negatively correlated with gestational age[20]. Similar results were observed in this study with adolescents.

Some authors have reported that plasma adiponectin concentrations decrease gradually with the advancement of pregnancy due to fat tissue increase[3]. However, these values return to normal soon after delivery[3]. Studies have revealed that, in

Table 1. Adiponectin serum concentrations (ng/mL) during the gestational weeks of adolescents.

Gestational week	n	Minimum	25% Interquartile range	Medium	75% Interquartile range	Maximum
9th	16	1,726	2,047	3,473	4,331	6,282
10th	16	1,141	2,507	3,733	5,267	7,295
11th	15	1,350	2,596	3,786	6,389	7,575
12th	15	1,058	2,891	3,677	5,263	6,924
13th	18	2,392	2,958	3,578	4,442	8,879
14th	16	1,507	1,570	3,149	3,823	5,690
15th	15	1,258	1,913	2,635	6,579	14,284
16th	15	1,001	1,514	2,569	6,250	10,943
17th	15	1,205	2,447	3,735	5,690	8,377
18th	15	1,205	2,476	3,781	4,483	6,579
19th	15	1,575	2,964	3,835	7,362	9,697
20th	15	1,322	1,599	2,998	3,459	5,960
21th	15	1,526	2,224	3,520	5,784	10,943
22th	15	1,579	2,152	3,369	4,954	9,697
23th	15	1,569	1,632	2,363	5,236	5,471
24th	15	1,233	3,349	4,183	6,940	8,520
25th	15	1,511	2,569	3,120	4,250	6,199
26th	15	1,540	1,789	2,286	4,445	5,391
27th	15	1,278	2,157	3,292	3,697	5,541
28th	15	1,353	2,458	3,520	4,723	8,588
29th	15	1,036	1,599	2,362	3,261	5,367
30th	15	1,169	1,838	2,372	3,336	4,445
31th	15	1,247	1,831	2,534	3,687	7,287
32th	15	1,007	1,951	2,326	3,372	6,352
33th	15	1,252	1,568	2,160	2,470	5,037
34th	15	1,158	1,336	2,564	3,569	5,348
35th	15	1,506	1,800	2,963	3,185	22,103
36th	15	1,621	1,883	2,610	4,789	9,056
37th	15	1,036	1,659	1,931	3,466	9,754
38th	15	1,509	1,943	2,964	3,233	5,031
39th	15	1,504	1,863	2,275	3,103	3,589

Friedman test, p=0.0004.

Table 2. Serum leptin concentrations (ng/mL) during the gestational weeks of adolescents.

Gestational Week	n	Minimum	Maximum	Mean	Standard deviation
9th	16	9.6	59.75	24.29	14.83
10th	16	9.57	45.9	22.11	10.09
11th	15	11.29	98.01	38.46	30.29
12th	15	5.83	95.65	40.10	32.56
13th	18	11.48	103.3	29.91	22.45
14th	16	5.59	54.48	26.12	15.27
15th	15	2.15	65.16	24.51	16.51
16th	15	5.29	82.23	32.91	22.24
17th	15	7.74	43.74	18.47	10.69
18th	15	7.73	38.06	19.54	8.86
19th	15	8.84	55.34	28.1	11.16
20th	15	11.56	96.91	38.42	23,87
21th	15	12.85	54.45	28.34	11.01
22th	15	12.15	54.46	24.37	11.97
23th	15	7.18	55.45	33.53	15.64
24th	15	3.42	46.22	20.01	13.26
25th	15	4.76	61.51	21.84	17,05
26th	15	11.7	48.05	31.00	12.96
27th	15	4.47	51.06	26.15	13.98
28th	15	1.31	40.67	24.89	11.11
29th	15	11.05	69.42	31.59	16.25
30th	15	4.67	87.76	39.67	23.52
31th	15	11.88	83.12	40.42	23.78
32th	15	4.49	77.36	39.57	22.15
33th	15	11.04	160.2	39.30	39.22
34th	15	7.56	68.42	30.62	17.49
35th	15	12.38	85.75	45.52	21.32
36th	15	2.87	77.51	42.39	22.37
37th	15	14.17	79.61	40.15	16.93
38th	15	19.63	85.75	49.93	22.51
39th	15	11.07	85.5	34.75	20.57

Repeated-measures ANOVA, p<0.0001.

healthy women, serum adiponectin concentration may be altered since the first trimester[21].

The literature suggests that, unlike other hormones secreted by adipose tissue, serum adiponectin levels decrease as adiposity increases, correlating inversely with obesity, IR, and metabolic syndrome[21].

Regarding leptin, our study showed the opposite result. We detected a significant increase as pregnancy trimesters advanced, which was also observed comparing values of gestational weeks. The literature indicates that plasma leptin concentrations increase during pregnancy in adults, especially when comparing the three trimesters of gestation with the postpartum decline[21,22].

Different investigators have reported that, throughout gestation, median adipokine levels differed significantly according to pre-gestational BMI. Changes in serum adipokines concentrations in overweight/obese seem to be different from those in lean and eutrophic groups[23,24]. Therefore, we selected only eutrophic adolescents. However, it is important to note that the accuracy of BMI to diagnose obesity is limited[25].

Our results are consistent with data obtained in adult pregnancy studies, where a positive correlation was observed between maternal plasma leptin concentration and anthropometric data at the beginning and the end of gestation. This association suggests that body weight and, probably, adiposity gain are critical factors for the increase in circulating leptin levels[7,21].

Serum adipokines levels have been extensively investigated during pregnancy with the primary purpose of elucidating a possible relationship to obstetric and perinatal intercurrences such as preeclampsia, gestational diabetes, IUGR, and low birth weight[21,22].

The opposite effects of adiponectin and leptin are well-known. Several studies focused on evaluating these serum adipokines levels, in both adult and adolescent pregnancies, showing significant differences between the gestational trimesters[7,21,22]. However, this is the first study addressing serum adiponectin and leptin

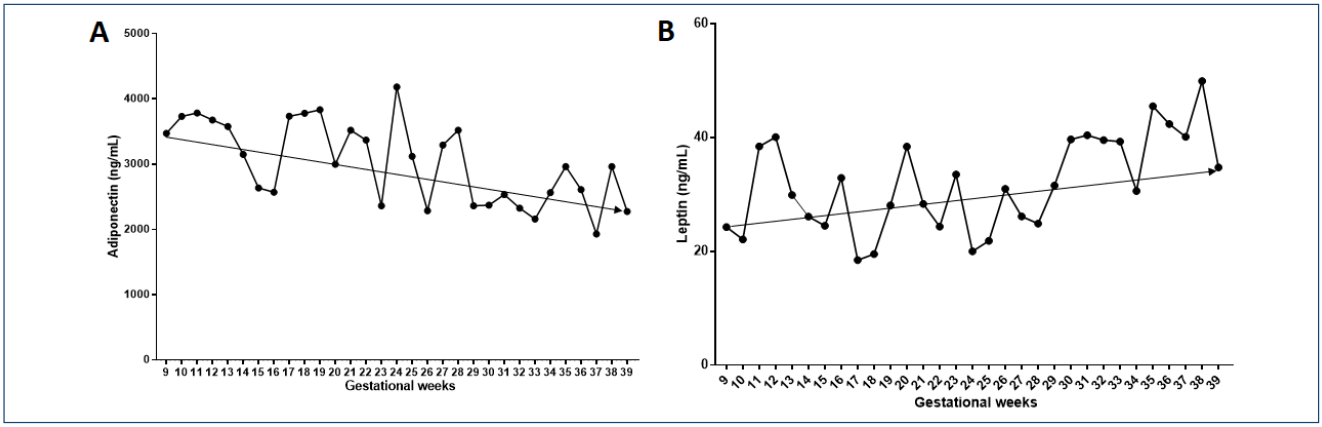

Figure 1. Serum adiponectin (A) and leptin (B) levels (ng/mL) during gestational weeks of adolescents.

levels weekly, from the 9 to 39th gestational weeks. The observed weekly differences in serum leptin levels seem to be associated with the development of considerable maternal-fetal interface changes. Thus, samples collected at the same trimester of pregnancy, although in different weeks, may explain at least part of the existing contradictions between the studies. Furthermore, in the early third trimester, leptin concentration may be correlated with weight gain. However, this difference may not be detected later.

This study limitation is that we analyzed only peripheral blood samples. In addition, we only assessed the complete form of the adiponectin molecule. Specific forms (high or low molecular weight form) evaluation could lead to different results (i.e., significant differences between weeks or association with pre-pregnancy BMI or weight gain). Further research is required to confirm these findings, and it is crucial checking whether similar gestational curves of adipokines are observed in adult pregnant women.

CONCLUSION

Adiponectin levels showed a significant decrease when comparing the three trimesters, whereas there were no significant level changes when compared weekly, and there was no correlation between adiponectin concentration and pre-gestational BMI or weight gain. In contrast, we detected a significant increase in weekly serum leptin levels, positively correlated to both pre-gestational BMI and weight gain.

AUTHOR'S CONTRIBUTIONS

CAFG: Conceptualization, Project administration, Validation, Visualization. **IB**: Data curation, Visualization, Writing – original draft. **MDF**: Data curation, Visualization. **KPTP:** Formal Analysis, Visualization. **EAJ**: Visualization, Writing – review & editing. **TFL**: Investigation. **SD:** Methodology, Supervision, Validation, Visualization.

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ORIGINAL ARTICLE

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Survivin expression as a prognostic marker for breast cancer

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SUMMARY

OBJECTIVE: Due to the speed of development observed in breast cancer, several studies aimed at discovering new biomarkers have been carried out in order to arrive at an early diagnosis. As survivin plays a fundamental role in the evasion of apoptosis in tumor cells, the aim of this study was to verify the expression profile of the survivin gene in paraffin-embedded breast tumor samples and associate it with the clinical characteristics of the patients.
METHODS: This is a cross-sectional study, for which 100 tumor samples were obtained from cancer patients treated throughout the year 2019 at Instituto de Mama do Cariri (Juazeiro do Norte, in the state of Ceará). This study included women over 30 years old who had confirmed breast cancer through anatomopathological examination but excluded those with non-neoplastic breast comorbidities, other neoplasms, or chronic diseases. Survivin gene expression was assessed by quantitative polymerase chain reaction.
RESULTS: The expression of survivin is associated with the lack of expression of estrogen (p=0.027) and progesterone (p>0.0005) receptors. It means that survivin expression is higher in patients in which labeling was absent for estrogen receptor and progesterone receptor.
CONCLUSION: Our data reinforce that survivin expression is higher in estrogen receptor-patients, thus representing an additional prognostic tool.
KEYWORDS: Survivin. Breast neoplasm. Prognosis. Paraffin embedding. Hormone receptors.

INTRODUCTION

Cancer is highly prevalent worldwide, with breast cancer being the second most common in women[1]. Due to the speed of development observed in breast cancer, several studies aimed at discovering new biomarkers have been carried out in order to arrive at an early diagnosis and quickly initiate treatment, thus increasing the chances of achieving a cure for the patient.

Currently, survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, has been the subject of several studies involving cancer[2]. This 16.5 kDa protein, encoded by the *BIRC5* gene, was cloned for the first time in 1997 by Ambrosini et al.[3] and is detected in many cases of malignant tumors[4], but not in healthy tissue samples, therefore directly relating its presence to oncogenesis[2,4,5].

Survivin participates in several biological pathways[4-6], but one of the main pathways in which this protein is expressed is in escape from apoptosis. Apoptosis, i.e., programmed cell death, can be initiated by extrinsic and intrinsic cell expression pathways[7]; in the extrinsic pathway, an external agent (i.e., radiotherapy, chemotherapy, and so on) stimulates p53, an apoptosis-linked transcription factor that regulates a number of other pro-apoptotic genes that lead to cell death[2]. Survivin acts directly to inactivate the apoptosis cascade. Thus, p53 acts antagonistically to the anti-apoptotic action of surviving[2,4,6]; in this way, research is being carried out to evaluate how the inactivation of survivin would be an effective form of treatment in patients with metastatic cancer[2]. In cells that are being signaled for apoptosis, survivin is more expressed and released into the cytosol, preventing the activation of caspases, and thus, inhibiting apoptosis[2,5].

The intrinsic pathway, in turn, is initiated by signals from mitochondria, a cytoplasmic organelle in eukaryotic cells. After intrinsic stimulation, cytochrome-C and Smac/DIABLO produced in the mitochondria form the apoptosome that activates caspase-9[7]. Then caspase-9 activates other caspases that induce cellular apoptosis. In mammals, IAP proteins inhibit apoptosis by indirectly inhibiting caspases[7].

As survivin plays a fundamental role in the evasion of apoptosis in tumor cells, the aim of this study was to verify the expression profile of the survivin gene in paraffin-embedded breast tumor samples and associate it with the clinical characteristics of the patients.

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METHODS

Patients

This is a cross-sectional study, for which tumor samples were obtained. The samples were collected. A total of 100 breast cancer patients from Instituto de Mama do Cariri (Juazeiro do Norte, in the state of Ceará) were included in this study throughout the year 2019. This study was approved by the Centro Universitário FMABC Research Ethics Committee under research protocol no. 346712/14 of August 2013. A free and informed consent form was signed by each individual included in this study. All experiments were performed in accordance with the Declaration of Helsinki. Women over 30 years of age with breast cancer confirmed by anatomopathological examination were included. Women with other non-neoplastic breast comorbidities or with other neoplasms or chronic diseases such as human immunodeficiency virus, hepatitis, and diabetes were excluded from this study.

The parameters studied were age, staging, and the presence of prognostic markers [i.e., human epidermal growth factor receptor-2 (HER2), Ki-67, estrogen receptor (ER), and progesterone receptor (PR)] in addition to survivin gene expression.

Tumor samples

Breast tumor biopsies obtained from the patients were fixed in 10% buffered formalin, embedded in paraffin (FFPE), and sliced into 10 µm sections using a microtome for histopathological, immunohistochemical, and molecular analyses.

Immunohistochemistry

In slides with paraffin material, the percentage of Ki-67, ER, PR, and HER2 labeling was analyzed to determine the tumor type of each patient.

Survivin gene expression

A total of five 10 µm sections of tumor samples from each patient were subjected to RNA extraction using the RNeasy® FFPE Kit (Qiagen, cat. no. 73504, Hilden, Germany), following the manufacturer's recommendations. RNA concentration was measured by spectrophotometry (NanoVue Plus – GE Health Care, Buckinghamshire, UK).

Complementary DNA synthesis was performed using 100 ng of RNA with the QuantiNova Reverse Transcription Kit (Qiagen, cat. no. 205413, Hilden, Germany), according to the manufacturer's recommendations. Specific primers for target and endogenous genes (GAPDH – glyceraldehyde-3-phosphate dehydrogenase) were designed using the Primer3 Input 0.4.0 software; surviving F-CAGATTTGAATCGCGGGACCC and surviving R-CCAAGTCTGGCTCGTTCTCAG generated an amplicon of 187 bp; GAPDH F-GACCACAGTCCATGCCATGA and GAPDH R-CAGCTCAGGGATGACCTTGC generated an amplicon of 148 bp. Amplifications were performed on an Applied Biosystems 7500 Real-Time PCR Systems thermocycler (Applied Biosystems, Foster City, USA), using the 1× Quantitec SYBR Green PCR fluorophore kit (Qiagen, Cat No. 204143, Hilden, Germany), and 0.2 µM of each specific primer, in a final volume of 15 µL per sample. The thermal conditions were an initial phase with hot start at 95°C for 10 min, followed by 45 cycles of 95°C for 10 s and 60°C for 25 s.

The expression of the target gene for each patient was determined by the $2^{-\Delta^{Cq}}$ [8] method and associated with the characteristics, clinical variables, and anatomopathological results of the patients.

Statistical analysis

Absolute and relative values were used to describe the qualitative variables. Quantitative analyses were performed using the Shapiro-Wilk test, with a significance of $p<0.05$, using medians and the 25th and 75th percentiles. The Kruskal-Wallis and Mann-Whitney tests were also used to study the association between diagnostic variables and the expression of survivin. Spearman's test was also used to observe the correlation between survivin and Ki-67. For all analyses, a confidence level of 95% was used. The program utilized was Stata, version 11.0.

Among the quantitative variables, the markers such as Ki-67, HER2, ERs, and PRs were evaluated and quantified by immunohistochemistry technique. Age and staging were also quantitatively assessed, and the obtained values were expressed as mean±standard deviation, median, maximum, and minimum values. The criteria to include variables involved factors such as theoretical relevance, statistical significance, correlation with the outcome of interest, and adjustment for potential confounders. In addition, the criteria were adjusted to avoid possible confounders. It is common to use stepwise selection (i.e., forward, backward, and stepwise) or statistical significance criteria (e.g., $p<0.05$) approaches for the inclusion or exclusion of variables in the logistic regression model.

RESULTS

The clinical characteristics of the patients included in this study are described in Table 1. Of the 100 samples submitted to gene amplification, adequate quality for molecular analysis was obtained only in 89% of the samples (89 out of 100 samples). Of these, expression of survivin was absent in 13.5% of the samples (n=12); the other 77 samples (86.5%) had detectable expression levels.

Table 1. Clinical characteristics of the patients.

Variables	n	%
Staging*		
IA	15	15.5
IIA	34	35.1
IIB	32	33.0
IIIA	5	5.1
IIIB	11	11.3
HER2		
Negative	62	62.0
+	20	20.0
++	6	6.0
+++	12	12.0
ER*		
Negative	18	18.2
Positive	81	81.8
PR		
Negative	21	21.0
Positive	79	79.0
Chemotherapy		
No	4	4.0
Yes	96	96.0
	Median	**p.25–p.75**
Age	56.0	49.0–68.0
Survivin	1.6	0.008–23.6
Ki-67 (%)	5.0	0.0–20.0

HER2: human epidermal growth factor receptor 2; ER: estrogen receptor; PR: progesterone receptor *without information for some samples.

Table 2. Association between survivin expression values and clinical characteristics of the patients.

Variables	Survivin Median (95%CI)	p[a]
Staging		
0	136.2 (0.0; 272.3)	0.469
IA	3.7 (0.14; 145.0)	
IIA	0.1 (0.01; 7.2)	
IIB	1.4 (0.2; 4.1)	
IIIA	0.5 (0.0; 30.1)	
IIIB	23.6 (0.0; 182.6)	
HER2		
Negative	1.6 (0.1; 10.5)	0.835
+	0.2 (0.02; 4.4)	
++	3.8 (0.0; 25.1)	
+++	2.5 (0.2; 8.2)	
	Median (95%CI)	**p[b]**
ER		
Negative	18.0 (0.5; 134.4)	0.027
Positive	0.8 (0.06; 2.8)	
PR		
Negative	26.5 (2.9; 139.4)	0.0005
Positive	0.2 (0.04; 2.1)	
Chemotherapy		
No	136.2 (0.0; 1528.5)	0.623
Yes	1.6 (0.2; 3.9)	

ER: estrogen receptor; PR: progesterone receptor. [a]Kruskal-Wallis. [b]Mann-Whitney.

The association between survivin expression values and the clinical characteristics of patients is described in Table 2. As the expression of survivin is associated with the expression of ER and PR (higher in patients in which labeling was absent for ER and PR), samples were divided according to the tumor subtype in which survivin expression was analyzed (Figure 1). As can be seen, patients with triple negative tumors have the highest expression of survivin, while those who are positive for hormone receptors and HER2 have the lowest expression of this marker.

There was no correlation between survivin expression and patient age or between survivin and Ki-67 marker status.

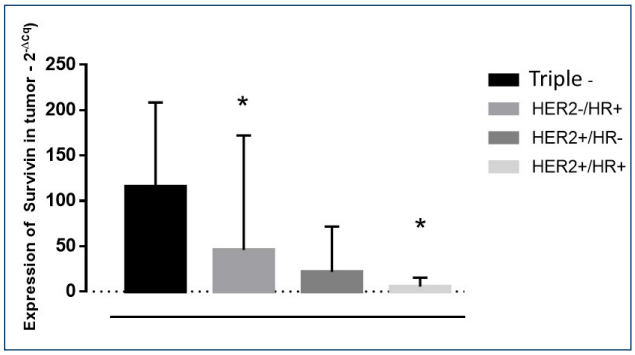

Figure 1. Representation of survivin gene expression in breast cancer tumor subtypes: triple- (n=7), HER2-/HR+ (n=36), HER2+/HR- (n=10), and triple+ (n=21). One-way ANOVA test. *p<0.05.

DISCUSSION

In this study, the expression of survivin in the tumor of patients with breast cancer without hormone markers is greater than that found in patients whose tumors express hormone receptors. Survivin is an apoptosis-inhibiting protein whose expression is associated with a poor prognosis in breast cancer patients[5]. In these patients, staging is very important to inform the mastologist on the correct therapeutic approach in order for the treatment to be as effective as possible[9]. Increased cytoplasmic expression of survivin has already been correlated with the stage and histological grade of the tumor and the development of metastasis[10]. Although, in this study, an association was not found between the expression of survivin and the staging of the patients, it was possible to establish an association between the increased expression of survivin and the absence of a hormonal biomarker (i.e., ERs, PRs, and the HER2 protein) in paraffinized tumor samples. According to our data, patients with triple-negative tumors have a significantly higher expression of survivin compared with that found in other subtypes, and the lowest expression of this gene was found in triple-positive tumors. Thus, by separating patients into groups (HER2-/HR- or Triple N, HER2-/HR+, HER2+/HR, HER2+/HR+, or Triple+, where HR stands for hormone receptor), it was possible to clearly visualize the difference in expression levels between these groups.

Triple-negative breast cancer (TNBC) are a tumor subtype that does not express HER2 or ERs and PRs; as they do not respond to hormonal or anti-HER2 therapies, TNBC tend to be more aggressive and develop metastases[11]. In addition to the lack of a therapeutic target, this tumor subtype lacks an adequate prognostic marker[12]. According to the authors, survivin and epidermal growth factor receptor expression evaluated by immunohistochemistry were considered of prognostic value for TNBC. Likewise, survivin expression together with zinc finger of the cerebellum 1 also showed prognostic potential[13]; in this case, the expression of these was evaluated in frozen tumor samples using quantitative polymerase chain reaction (qPCR) and in paraffin-embedded samples by immunohistochemistry. Our results show that the isolated expression of survivin obtained by qPCR can be considered a prognostic marker for all breast tumor subtypes, and that this evaluation can be performed on stored paraffin-embedded samples.

In breast cancer, HER2 is overexpressed in 15–30% of tumors[14]; HER2 is a trans-membrane tyrosine kinase receptor that participates in the control of cell proliferation and tumorigenesis[15]. As this protein has the function of suppressing apoptosis and promoting tumor growth, its expression has been used as a prognostic biomarker[16]. Increased expression of HER2 induces the activation of the PI3K/Akt pathway which, in turn, leads to increased surviving expression[17]. However, although HER2 expression is accompanied by that of survivin, our results indicate that the triple-negative tumor samples had increased survivin expression compared with the triple-positive ones.

Breast cancer is a hormone-dependent tumor, and the analysis of hormone receptors, such as progesterone and estrogen, is a widely accepted prognostic marker for predicting treatment response[18]. According to the authors, survivin expression in invasive ductal carcinoma tumors is negatively correlated with PR and is independent of ER. Our data show that lower expression of survivin was indeed obtained in the samples with hormone expression. Elevated levels of progesterone have an antiproliferative effect on breast cancer[19], having, therefore, contrary action to survivin, a fact that may explain this negative correlation between these biomarkers.

The absence of ER expression indicates a worse prognosis for the patient. Thus, additional markers that can complement this assessment and indicate a more effective treatment are of great importance. Our data reinforce that, in ER– patients, increased survivin expression is present; therefore, this protein has great potential as a therapeutic target, and as such, it has been widely tested in different carcinomas by several groups[20,21].

This study has some limitations: the study of gene expression in paraffin material, even using adequate methodology for this purpose, may have loss of information associated with the quality of the isolated genetic material. In addition, the beginning of the pandemic that occurred shortly after the collection of the material did not allow us to follow up the patients in order to associate the data obtained with clinical outcomes. Even so, the data described here demonstrate that survivin mRNA expression in samples is higher in negative hormone-receptor tumors. Thus, the evaluation of surviving mRNA expression in tumors immersed in paraffin can be considered a tool to aid prognosis, as this profile is different for breast cancer subtypes.

AUTHORS' CONTRIBUTIONS

PQ: Conceptualization, Investigation, Writing – review & editing. **RQ:** Conceptualization, Investigation, Writing – review & editing. **MMP:** Methodology, Formal Analysis, Writing – review & editing. **GLV:** Methodology, Formal Analysis, Writing – review & editing. **BCAA:** Validation, Writing – original draft, Writing – review & editing. **ECP:** Validation, Writing – original draft, Writing – review & editing. **FLAF:** Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Surgical outcome of spinal schwannoma and neurofibroma

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the outcome and surgical complications in patients with spinal schwannoma or neurofibroma surgically treated at the Hospital das Clínicas of the State University of Campinas.

METHODS: This was a retrospective cohort study, using medical records of patients operated between 2011 and 2021. The sample distribution was verified using the Kolmogorov-Smirnov test. The dynamics between qualitative variables were assessed using Fisher's exact test. We used means analysis to assess patient improvement based on Frankel scores.

RESULTS: A total of 16 patients were evaluated, of whom 56.25% (9) were men and 43.75% (7) were women. There were 13 (81.25%) patients with schwannomas and 3 (18.75%) with neurofibromas. Patients with deficits had neurological improvement, such as walking or with at least Frankel D or E after surgery. Laminectomy, performed in 8 patients (50%), and laminoplasty, used in 9 patients (56.25%), were the main techniques.

CONCLUSION: The surgical approach was proved to be an effective and safe alternative to the treatment of these tumors, with neurological improvement and minor surgical complications.

KEYWORDS: Quality of life. Spinal cord compression. Neurofibroma.

INTRODUCTION

Spinal tumors can be classified according to their relationship with the spinal cord in intradural and extramedullary, intradural and intramedullary, or extradural[1]. The intradural and extramedullary tumors correspond to about 40% of the cases of spinal tumors, and the main histological representatives of this group are the schwannomas and neurofibromas, both of these tumors originated from the peripheral nerve sheath, besides meningiomas[1-4]. Patients with intradural and extramedullary tumors generally present with axial pain and neurological symptoms due to progressive compression of the spinal cord, and the main therapeutic modality is surgical resection, given the high efficacy and safety of the method[5-9]. The resection when performed with a wide exposure of the tumor and with the proper surgical precaution proves to be curative for patients. Surgery has immediate and lasting efficacy, although, in isolated cases, other treatment modalities may play a role, such as radiosurgery, in selected situations[5].

Nerve sheath tumors, schwannomas and neurofibromas, are the most common forms of intradural and extramedullary tumors. Schwannomas are the most common, followed by neurofibromas and ganglioneuromas[1,10]. About 35-45% of patients affected by these tumors suffer from neurofibromatosis (NF), with NF1 related to neurofibromas and NF2 related to schwannomas[1]. We emphasize that both can still be found singly or sporadically, especially schwannoma, unrelated to NF. Schwannomas are neoplasms derived from Schwann cells, and most of these tumors originate from the dorsal roots and less frequently originate from the ventral roots, where surgical access is more difficult, as, in general, the standard exposure of the spine is performed by approaches to the posterior midline of the spine[2,5]. These tumors are usually associated with radicular pain as well as sensory deficits. Neurofibromas, on the contrary, are commonly involved with the cervical spine, which can make surgical resection difficult, especially when plexiform and involving multiple segments, typical of type 1 NF[3]. Thus, it is clear the importance of evaluating the treatment of these tumors, as, despite being generally benign, they can cause severe neurological sequelae and great impact on quality of life due to spinal cord comprehension. The identification and treatment of these neoplasms provide great gain for the patient's quality of life, and the treatment is generally

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curative. Considering the lack of national studies evaluating surgery in our country, the aim of this study was to evaluate the role of surgical treatment in the management of these tumors in a Brazilian tertiary clinical hospital.

METHODS

Patient selection

We performed a retrospective cohort study. Data collection was carried out at the Clinical Hospital of the State University of Campinas. It included all patients with spinal schwannoma or neurofibroma operated on by the neurosurgery team between 2011 and 2021 who met the inclusion criteria. The inclusion criteria were patients with spinal schwannoma or neurofibroma who were surgically treated at the institution, performed by the same surgeon (AFJ). The exclusion criteria were patients who had not undergone surgical treatment and whose data were either missing or not available for analysis. The project was submitted and approved by the Medical Ethics Committee of UNICAMP (CAAE: 48731721.0.0000.5404). Importantly, the confidentiality of these patients was maintained, and their identities were preserved. The is a retrospective study, so there is no risk to the patients involved in this study.

Data collection and definition of some indicators

The clinical data of patients were retrospectively collected. Neurological status was evaluated before and after surgery using the Frankel classification for spinal cord injury. Histological data, as well as information about surgical treatment were collected. The patients included in the study were followed up until the last follow-up visit before data analysis. The medical records were requested and evaluated, and after applying the inclusion and exclusion criteria, the patients included in the study were added to an Excel spreadsheet together with the information of interest to the project. Using the data obtained, a descriptive statistical analysis was performed in order to answer the objectives proposed by the study. The patient characteristics that were selected for the study were length of hospitalization, occurrence of complications, and postoperative functions, and the results were analyzed.

Statistical analysis

After data collection, the results were analyzed. The data obtained were then processed with Excel. To verify the normality of the quantitative variables, the Kolmogorov-Smirnov tests were applied for the age of the patients, whereas the qualitative variables will be presented in absolute and relative values. The evaluation of the association between variables was done using the chi-square test. Furthermore, mean improvement analysis was performed to evaluate the improvement of neurological status between Frankel scores before and after surgery.

RESULTS

The analysis included 16 patients after applying the inclusion and exclusion criteria; 56.25% (9) were men and 43.75% (7) were women, so it was not possible to identify a preference for a specific gender. The mean age of the population was 44 years, with 41.5 years for males and 47.3 years for females. We used the Kolmogorov-Smirnov test (age analysis), aiming to verify if there is a normal distribution of the data, and from this, we could identify some biases. The overall age range was 20-71 years, 25-67 years in women and 20-71 years in men. Regarding tumors, there were 13 patients with schwannomas (81.25%) and 3 with neurofibromas (18.75%). All patients who were affected by neurofibromas had the diagnosis of type 1 NF (100%). We did not observe recurrences in our sample during the study follow-up. In one patient, it was necessary to divide the surgical procedure into four surgical steps–two for tumor removal and two for deformity correction. This strategy was used due to the high complexity of the procedure, in which it was necessary to perform an intra- and extradural resection with the release of the vertebral artery. One year after the removal of the tumor, due to an iatrogenic kyphosis, it was necessary to perform a cervical arthrodesis between C3 and C6 in this patient.

The tumors presented an uneven distribution as to location, 7 (43.75%) were ventrally located, 6 (37.5%) were lateral to the spinal cord, and 5 (31.25%) were dorsal; among all the tumors, one had ventral and dorsal location simultaneously and another had lateral and dorsal location. No correlation was found between the location of the tumors and the clinical recovery of the patient.

Regarding the correlation of tumor location with the main complaint, we noticed a tendency for more cranial tumors to have motor deficits as the main complaint, as we can see in Figure 1. To test this correlation, Fisher's test was applied to the sample. The p-value for our sample was 0.023601, which means that, considering that the distribution is random, the chance of this scenario to occur is 2.36%. Considering an alpha of 0.05, we have to reject the null hypothesis, which predicted an absence of a relationship between the location and the main complaint of the patient, so there is an association between the location of the tumor and the patient's main complaint. The main symptom found in the patients was very severe pain, and the information about irradiation was compromised because many medical records did not describe whether or not there was irradiation of pain.

The analysis of the average improvement was made, and each patient had an average increase of 0.8125 on the Frankel scale, a mode of increase of 1 on the scale, with most moving from D to E. When analyzing the data, there was a trend of improvement on the Frankel scale. In Table 1, a comparison between Frankel score in the preoperative and postoperative stages is presented, indicating that the distribution is altered through the surgical intervention. The mode was that the patients had an improvement of 1°, with the majority moving to grade E on the scale, as we can see by comparing Figures 2 and 3. It was identified that 14 (87.5%) of patients had spinal cord compression by the tumor, of which two had a worse score on the Frankel scale, being classified as B. Interestingly, even patients with severe spinal cord compression (Frankel B) showed significant improvement with surgery, and 11 (68.75%) patients had an improvement of at least 1° in the Frankel score.

The main techniques used were laminectomy and laminoplasty. Laminoplasty was used as one of the surgical techniques of approach in 9 (56.25%) patients, whereas laminectomy was done in 8 (50%) patients and 1 patient underwent both techniques. Laminectomy had to be associated with other techniques in 7 (87.5%) patients, such as facetectomy, concomitant arthrodesis, and duroplasty, among others. Arthrodesis was performed in 3 patients (18.75%) together with laminectomy, and 1 patient had to undergo circumferential arthrodesis 1 year after tumor removal due to iatrogenic kyphosis. Laminoplasty was associated with two other procedures (i.e., laminectomy and flavectomy) in 2 (22.22%) of the patients. In figure 4 we can see an example of the surgery procedure with demonstrative images.

Two patients had to undergo drainage of the thorax due to postoperative bleeding of intrathoracic tumor removal. There were also two cases of thoracic bleedings in which the patients required a chest drain during the initial postoperative period. One of these patients, during return visits, complained of chest pain, which was controlled with medication. One of the patients operated on for neurofibroma, during the return visit, complained of leg pain; the pain was coming from new neurofibromas in the leg region. The patient had the diagnosis of NF1. There was a case of surgical wound infection, treated with ciprofloxacin and with resolution without the need for further surgery. There was also one patient with micturition disorder during the post-surgery period who was referred to the urology service to investigate the origin of the problem. Regarding pain, two patients remained with chronic pain in the postoperative period and required medication for pain of neuropathic origin. Thus, despite having complications (5 of

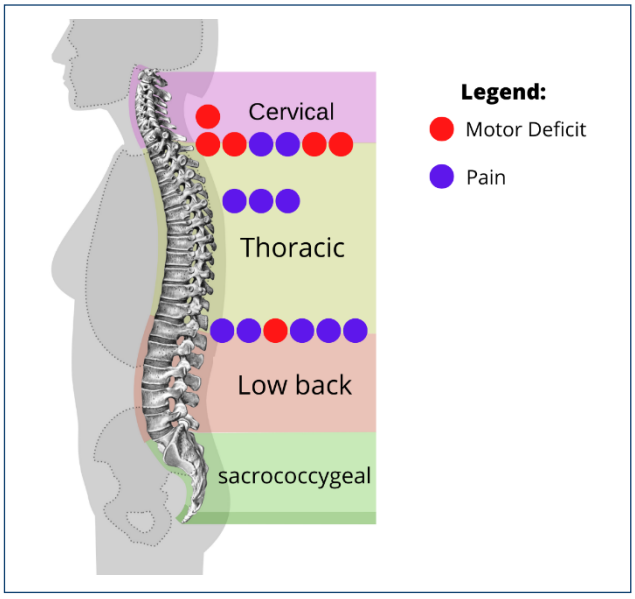

Figure 1. Presentation of symptoms.

Table 1. Comparative improvement of Frankel grade before surgery and postoperative at the final follow-up.

	A	B	C	D	E
Preoperative Frankel	0	2	0	12	2
Postoperative Frankel	0	0	0	5	11

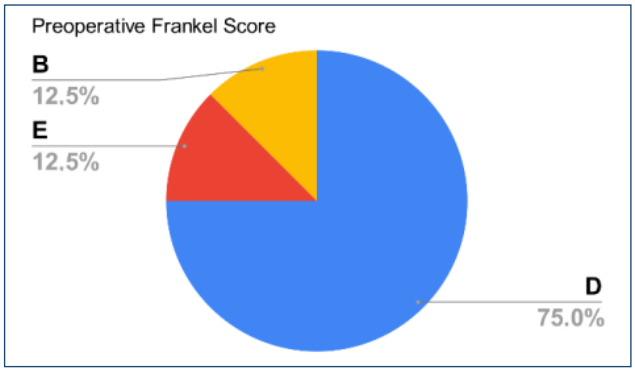

Figure 2. Preoperative Frankel score.

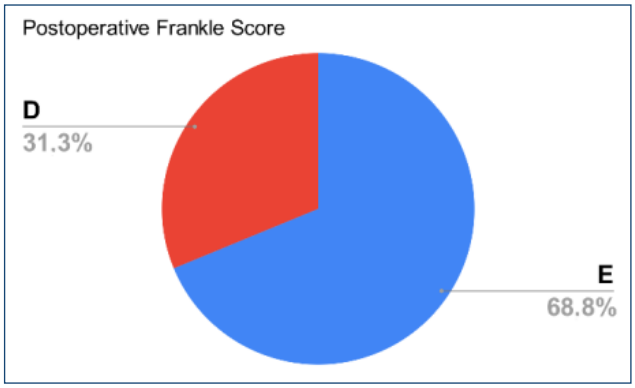

Figure 3. Postoperative Frankel score at the last follow-up.

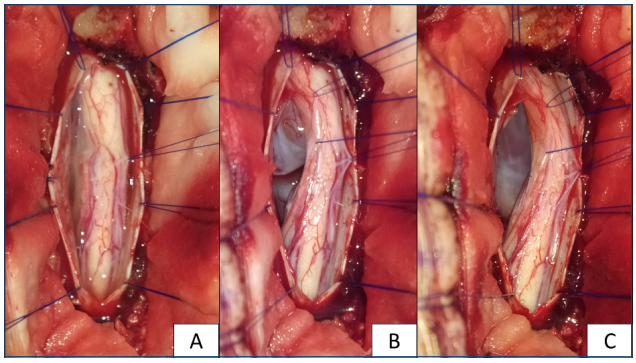

Figure 4. Patient with thoracic ventral schwannoma. (A) Initial exposure after dural opening. The tumor cannot be seen initially. (B) After sutures in the dentate ligament, the spinal cord is rotated to the contralateral side, with good tumor visualization. (C) Surgical image after total tumor removal without any additional injury to the spinal cord.

the 16 patients) such as bleeding, infection, fistula, difficulty to urinate, and chronic pain, no patient had serious sequelae from the surgery. Thus, joining this to the benefits that were brought to the patients, we classified the surgical approach as an effective and safe alternative for intradural tumors.

DISCUSSION

In this study, we present a series of 16 patients who underwent surgical treatment of tumors derived from the neural sheath of peripheral nerves in a single center. There was a significant neurological improvement in all patients with deficits, and at the end of the study, all were walking and with at least Frankel D (preserved strength in four limbs and able to ambulate). Among those found in the sample, we observed a higher prevalence of schwannomas when compared with neurofibromas, with no gender preference. This is probably due to the high rate of patients with sporadic schwannomas (not associated with NF2) in the studied region. Among the patients with neurofibromas, all had NF1, demonstrating the correlation between the appearance of tumors and this underlying genetic disease. The main symptoms found were motor deficit and pain, and a correlation between tumor height and the main complaint was demonstrated. More cranial tumors have a higher incidence of motor complaints.

As for the prognosis, it was noticed that, in general, the surgical approach provided clinical improvement to the patients, with a reduction of motor deficit and pain. Most patients were classified as E on the Frankel scale after surgery. Regarding the approach and techniques, we would have to increase the number of patients to identify the prognosis of the patients approached via the ventral approach. The main techniques used were laminectomy and laminoplasty. In two cases, a late reapproach was necessary because of kyphotic deformity after laminectomy and because of residual tumor growth (partial resection).

Limitations

This was a retrospective study with limited statistical power, rather than a prospective cohort study. Besides, the number of patients enrolled in this study was not enough. Anyway, the benefits of surgery were clearly demonstrated.

CONCLUSION

The surgical results were considered good because there were neurological improvements, no major complications, deaths, or neurological worsening in this series. Schwannoma-type tumors not related to NF predominated in our series. No patient had severe neurological sequelae. The surgical treatment at our institution was safe and effective, with acceptable complication rates and few neurological sequelae.

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AUTHORS' CONTRIBUTIONS

JVCT: Data curation. **AFJ:** Formal Analysis, Project administration, Supervision, Writing – review & editing. **ACFF:** Conceptualization, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **CMBB:** Writing – original draft. **GBM:** Data curation.

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ORIGINAL ARTICLE

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Sexual function according to infiltration of endometriosis of the rectovaginal septum: a cross-sectional study

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SUMMARY

OBJECTIVE: The aim of this study was to associate the degree of infiltration of rectovaginal septum endometriosis with dyspareunia and sexual function.
METHODS: A cross-sectional study was carried out with 127 women followed up at a tertiary hospital from March 2021 to March 2022. The women's sociodemographic and clinical conditions and dyspareunia were evaluated. The sexual function was evaluated by the Female Sexual Function Index.
RESULTS: A total of 53 women with type I, 37 with type II, and 37 with type III rectovaginal septum endometriosis were evaluated. The women had a mean age of 38.76±6.63 years and a mean body mass index of 27.62±5.11 kg/m^2. The mean time of diagnosis of endometriosis was 6.94±4.98 years. On average, the study participants engaged in sexual activity/intercourse 1.88±1.25 times per week. There was no difference between the dyspareunia score (p=0.822) and sexual function (p=0.174) according to the types of rectovaginal septum endometriosis. Overall, 93.7% of the women with endometriosis had sexual dysfunction. There was no correlation between the degree of rectovaginal septum endometriosis infiltration with dyspareunia (r=0.05; p=0.55) or sexual function (r=0.07; p=0.39).
CONCLUSION: Women with endometriosis have impaired sexual function, regardless of the degree of endometriosis infiltration.
KEYWORDS: Endometriosis. Dyspareunia. Physiological sexual disorders.

INTRODUCTION

Endometriosis is an inflammatory, estrogen-dependent disease defined by the presence of tissue that resembles the endometrial gland and/or stroma outside the uterus, predominantly, but not exclusively, in the female pelvis. Deep endometriosis is defined as the subperitoneal infiltration of ³5 mm endometrial implants, leading to the formation of endometriotic nodules[1].

Transvaginal ultrasound (TVUS) can classify deep rectovaginal septum endometriosis into three types: type I: pelvic area with a typical or atypical lesion surrounded by scar tissue, in the form of a cone, whose depth is diagnosed when surgically removed; type II: lesion formed by retraction of the rectum involving a typical lesion; and type III: endometriotic nodule infiltrating the rectovaginal septum[2].

Dyspareunia, which is a disabling condition that severely affects women physically and psychologically, is one of the most common symptoms of deep endometriosis[3]. Deep infiltrative endometriosis is also related to reduced quality of life and sexual function[4]. Several studies have investigated the relationship between different pain symptoms and the specific anatomic location of endometriotic lesions[5,6]. The presence of vaginal lesions is frequently associated with severe dyspareunia[5], as is the number of previous surgical procedures for treating endometriosis, the extent of the lesions, the obliteration of the rectouterine excavation, and the degree of peritoneal infiltration, among others[6,7]. Despite this, it is not yet known whether the degree of infiltration of rectovaginal septum endometriosis correlates with dyspareunia and sexual dysfunction. Thus, this study aimed to assess whether there is a correlation between the degree of endometriotic infiltration and dyspareunia and sexual function.

METHODS

A cross-sectional study with 127 women was carried out at the Endometriosis Outpatient Clinic of a tertiary hospital from March 2021 to March 2022. Eligible participants were between 18 and 45 years old, diagnosed with endometriosis in the rectovaginal septum (diagnosis performed by TVUS with bowel preparation[8]), and sexually active. Women with cognitive deficits that impeded the understanding of questionnaires were excluded.

The analyzed variables included age, parity, education (i.e., elementary school–up to 9 years of schooling, high school–up to 12 years of schooling, and higher education–more than 12 years of schooling), low income (<R$291

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per capita), middle class (R$291 per capita <family income <R$1,019 per capita), and upper class (>R$1,019 per capita), professional activity (e.g., unemployed, retired, student, and housewife), marital status (with or without a partner), body mass index (BMI: calculated as weight in kilograms divided by the square of height in meters), sexual activity frequency (i.e., times per week), number of sexual partners, time of diagnosis (months), treatment (hormone therapy–estrogen or progestogen alone or none), treatment time (months), number of lesions, and lesion size.

Endometriosis in the rectovaginal septum was evaluated by TVUS and classified into three types: type I: pelvic area with a typical or atypical lesion surrounded by scar tissue, in the form of a cone, diagnosing its depth when surgically removed; type II: lesion formed by retraction of the rectum involving a typical lesion; and type III: endometriotic nodule infiltrating the rectovaginal septum[2,9]. The ultrasound was performed by one investigator who had more than 10 years of experience using the Toshiba X (Aryan, Spain) appliance, providing a description of the injuries in millimeters.

Sexual function was assessed using the Female Sexual Function Index (FSFI). This questionnaire has 19 questions grouped into six domains: desire, arousal, lubrication, orgasm, satisfaction, and pain. Each domain receives a score from 0 to 6, except for desire and satisfaction, with minimum scores of 1.2 and 0.8, respectively. The final score is the sum of all specific domain scores and can range from 2 to 36. Sexual dysfunction is characterized by a score £26.55[10].

To assess dyspareunia, the scale that classifies it into scores was used: 0=absence of pain, 1=mild pain that does not require interrupting the sexual activity, 2=moderate pain that does not require interrupting sexual activity, and 3=severe pain that requires interrupting sexual activity[11].

All women signed an informed consent form before participating in the study. The Research Ethics Committee of the institution approved the study (number: 4.490.734).

Statistical analysis

For the calculation of the sample size for the purpose of evaluating the correlation between the degree of endometriosis infiltration in the rectovaginal septum and the level of dyspareunia and the sexual function score (FSFI) in women with endometriosis, the alpha significance level or type I error and the power of the sample were fixed at 5% (α=0.05) and 80% (β=0.20), respectively, and various correlation estimate values were used. From the results, it was estimated that a sample of 124 women would be representative to assess a minimum correlation of 0.25 between the parameters of interest.

The variables were described as frequency, mean, and standard deviation. The chi-square and Fisher's exact tests were used to detect associations between categorical variables. The Kruskal-Wallis test was used to detect the association between continuous variables. Spearman's correlation coefficient was used to analyze the relationships between numerical variables. A probability value (p-value) of <0.05 was considered statistically significant. The SAS version 9.04 software (SAS Inc., Cary, NC) was used for all statistical analyses.

RESULTS

Of the 127 women, 53 (41.8%) women with type I rectovaginal septum endometriosis, 37 (29.1%) women with type II, and 37 (29.1%) women with type III were evaluated. Women with type I rectovaginal septum endometriosis had a mean age of 38.0±7.0 years, those with type II had a mean age of 39.1±6.3 years, and those with type III had a mean age of 39.4±6.3 years (p=0.38). The mean BMI was 27.6±5.1 kg/m^2 with no difference between groups (p=0.07). The mean time of diagnosis of endometriosis was 6.9±4.9 years, and the women engaged in sexual activity 1.8±1.2 times per week on average, with no difference between the groups (p=0.11 and p=0.56, respectively). Of the women who did not use hormone therapy, 13.2% had type I, 27.0% had type II, and 8.1% had type III endometriosis (p=0.10). Women with type I rectovaginal septum endometriosis had fewer partners and fewer lesions than women in the other groups (p=0.005 and p<0.001, respectively) (Table 1). Of the 127 women with endometriosis of the rectovaginal septum, 62% had a lesion in the rectosigmoid and 12% had an ovarian endometrioma (data not shown).

It was observed that 18.8, 13.5, and 18.9% of women with types I, II, and III rectovaginal septum endometriosis, respectively, did not have dyspareunia. In contrast, 16.9, 21.6, and 21.6% of women with types I, II, and III rectovaginal septum endometriosis had severe dyspareunia with the need to interrupt sexual intercourse. There was no difference between types of dyspareunia (p=0.94), mean dyspareunia score (p=0.82), and sexual function (p=0.17) among women according to the types of rectovaginal septum endometriosis. Most women in the study presented sexual dysfunction, with 90.5, 94.5, and 97.3% of women with types I, II, and III endometriosis, respectively, having a total FSFI score lower than 26.5 (p=0.53) (Table 2).

There was no correlation between the degree of rectovaginal septum endometriotic infiltration with dyspareunia (p=0.55) or with sexual function (p=0.39). Additionally, there was no correlation between dyspareunia and sexual function. However, we observed a significant correlation between dyspareunia and the FSFI's pain domain (p<0.0001) (Table 3).

Table 1. Clinical and sociodemographic characteristics of women with rectovaginal septum endometriosis (n=127).

	Type I (n=53) mean±SD/n (%)	Type II (n=37) mean±SD/n (%)	Type III (n=37) mean±SD/n (%)	p-value
Age (years)	38.0±7.0	39.1±6.3	39.4±6.3	0.38*
Parity	1.2±1.5	0.9±1.0	1.0±1.2	0.85*
Education				
<9 years	14 (26.4)	4 (16.2)	11 (29.7)	0.47**
9-12 years	19 (35.8)	18 (48.6)	11 (29.7)	
≥12 years	20 (37.7)	13 (35.1)	15 (40.5)	
Income >R$1,500.00	22 (41.4)	16 (43.2)	16 (43.2)	0.89**
Employed	36 (67.9)	26 (70.2)	29 (78.3)	0.39***
With partner	40 (75.4)	28 (75.7)	29 (80.5)	0.83**
BMI (kg/m^2)	27.8±5.1	26.2±5.1	28.6±4.7	0.07*
Frequency of sexual activity/week	1.7±1.0	2.0±1.3	1.9±1.3	0.56*
Number of partners	0.8±0.3	1.2±1.4	1.0±0.1	0.005* (1≠2,3)
Time since diagnosis (months)	75.1±55.3	104.2±72.2	80.8±48.4	0.11*
Treatment	46 (86.7)	27 (72.9)	34 (91.9)	0.10***
Time of treatment (months)	31.5±30.8	24.8±33.4	36.1±41.0	0.22*
Number of lesions	3.0±1.3	3.8±1.8	4.3±1.4	<0.001* (1≠3)
Size of rectovaginal septum lesion (mm)	41.8±45.2	39.5±6.1	44.4±10.9	0.001* (1≠3)

SD: standard deviation; BMI: body mass index. *Kruskal-Wallis test; **Chi-square test; ***Fisher's exact test.

Table 2. Sexual function and dyspareunia in women with rectovaginal septum endometriosis (n=127).

	Type I (n=53) mean±SD/n (%)	Type II (n=37) mean±SD/n (%)	Type III (n=37) mean±SD/n (%)	p-value
CPP	35 (66.0)	21 (56.7)	19 (51.3)	0.35**
Dyschezia	17 (32.08)	6 (16.2)	9 (24.3)	0.23**
Dysuria	9 (16.98)	4 (10.8)	7 (18.9)	0.60**
Dyspareunia	1.51 ± 0.99	1.6±0.9	1.6±1.0	0.82*
0	10 (18.87)	5 (13.5)	7 (18.9)	0.94**
1	15 (28.30)	12 (32.4)	8 (21.6)	
2	19 (35.85)	12 (32.4)	14 (37.8)	
3	9 (16.98)	8 (21.6)	9 (21.6)	
Desire	3.9±1.1	4.1±1.1	3.9±0.9	0.65*
Arousal	2.9±1.5	3.4±1.0	3.1±1.6	0.35*
Lubrication	3.2±1.6	3.8±0.9	3.3±1.5	0.23*
Orgasm	3.0±1.6	3.5±1.0	3.0±1.5	0.67*
Satisfaction	2.9±1.2	2.8±1.2	3.0±1.2	0.79*
Pain	2.6±1.9	3.4±1.7	3.0±1.9	0.24*
FSFI score	19.1±6.0	21.4±3.8	19.4±5.7	0.17*
FSFI ≤26.5	48 (90.5)	35 (94.5)	36 (97.3)	0.53***

SD: standard derivation; CPP: chronic pelvic pain; FSFI: female sexual function index. *Kruskal-Wallis test; **Chi-square test; ***Fisher's exact test.

Table 3. Correlation between rectovaginal septum endometriosis and dyspareunia and sexual function (n=127).

	Dyspareunia	FSFI total	Desire	Arousal	Lubrication	Orgasm	Satisfaction	Pain
Rectovaginal septum								
R	0.053	0.075	-0.019	0.080	0.087	0.011	-0.008	0.091
P	0.55	0.39	0.83	0.40	0.36	0.90	0.92	0.33
Dyspareunia								
R	-	-0.066	0.260	0.192	-0.068	0.048	0.285	-0.618
P	-	0.46	0.005	0.04	0.47	0.61	0.002	<0.0001

FSFI: female sexual function index; R: spearman's correlation coefficient.

DISCUSSION

Our study showed that women were 39 years old on average, had one partner, engaged in sexual activity twice a week, were diagnosed with endometriosis for 7 years, and were on hormone treatment for at least 2 years. Approximately 20% of the women did not exhibit dyspareunia, but all women had sexual dysfunction. There was no correlation between the degree of rectovaginal septum endometriotic infiltration with dyspareunia and sexual function.

Dyspareunia is observed in most women with endometriosis undergoing surgery and those prescribed hormonal therapies. More than half of women with endometriosis experience dyspareunia throughout their sexual lives, especially those with involvement of the uterosacral ligaments[4].

In our study, approximately 80% of women had dyspareunia, with 30% mild, 35% moderate, and 20% severe. In the literature, according to a previous study, 67% of women with endometriosis of the rectovaginal septum experienced dyspareunia, with 28% mild, 60% moderate, and 12% severe[12], showing results similar to those in this study.

Overall, women with rectovaginal endometriosis had worse sexual functioning than those without endometriosis. However, when compared with women with endometriosis in other sites (peritoneal or ovarian), no differences are observed particularly in sexual function and the frequency and severity of dyspareunia. This result suggests that the relationship between endometriosis and sexual dysfunction is more complex than can be explained by the anatomical distribution of lesions[12].

Dyspareunia is often associated with less sexual activity/intercourse, feelings of fear because of coital pain, and feelings of guilt toward the partner. It also correlates with a lower level of desire/arousal and a lower number of orgasms. Furthermore, dyspareunia is significantly correlated with sexual dysfunction and/or sexual distress[13]. Our study found no correlation between dyspareunia and the frequency of sexual activity and sexual function.

Unlike our study, which did not show an association between the degree of infiltration and sexual function, another study showed that women with deep endometriosis with involvement of the rectovaginal septum have more significant impairment in their sexual function when there is partial or total infiltration of the septum. Notably, that study was carried out with women before their surgical treatment and, therefore, without hormone therapy[4].

Studies have demonstrated that women with untreated endometriosis presented altered sexual function, as evidenced by lower scores in all the FSFI domains and severe dyspareunia[14,15]. In our study, most women were treated, which may explain the low dyspareunia scores, although the majority had sexual dysfunction. A previous study from our group showed that long-term hormonal treatment attenuates dyspareunia and improves sexual function but does not restore them to normal values[16].

Although the literature on the influence of cognitive and emotional factors on sexual function and pain perception has increased in recent years, the presence of some aspects, such as beliefs, automatic thoughts, and emotions regarding sexuality, remains unexplored in women with endometriosis. Despite this, these factors play a vital role in developing and maintaining all sexual dysfunctions[17].

This study has some limitations, such as the absence of a control group without endometriosis, and the small sample size, which does not allow a correlation analysis to be carried out with the isolated groups (especially with group 3). Also, the fact that it is a cross-sectional study allows conclusions of cause and effect.

Sexuality is a complex and multidimensional phenomenon influenced by three main factors: physical, psychological, and social well-being[18]. Thus, female sexual function cannot be related only to pain during sexual intercourse and the location of endometriosis. Indeed, aspects such as marital distress, anxiety, and depression certainly influence sexuality and should be evaluated by a multidisciplinary team.

CONCLUSION

Women with endometriosis have impaired sexual function regardless of the degree of infiltration of endometriosis into the rectovaginal septum.

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AUTHORS' CONTRIBUTIONS

DAY: Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Writing – review & editing. **CLBP:** Writing – review & editing. **GKS:** Conceptualization, Data curation, Writing – original draft.

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ORIGINAL ARTICLE

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Investigation of the effects of upper extremity home exercises on grip strength, range of motion, activity performance, and functionality in individuals with systemic sclerosis: a randomized controlled trial

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SUMMARY

OBJECTIVE: This study aimed to investigate the effects of upper extremity home exercises on grip strength, range of motion, activity performance, and functionality in individuals with systemic sclerosis and to compare with patient education.

METHODS: A total of 46 individuals with systemic sclerosis (55.52±11.54 years) were included. Individuals were randomly assigned into intervention (n=23) and control (n=23) groups. Dynamometer, goniometer, Canadian Occupational Performance Measurement, Disabilities of the Arm, Shoulder, and Hand, Score for Assessment and Quantification of Chronic Rheumatic Affections of the Hands, and Duruoz Hand Index were used for evaluation.

RESULTS: Post-treatment, in terms of delta (Δ) values, hand grip and pinch strengths (p: 0.000-0.016), active (p: 0.000-0.032) and passive (p: 0.000-0.043) total range of motions, Canadian Occupational Performance Measurement performance and satisfaction, Disabilities of the Arm, Shoulder, Score for Assessment and Quantification of Chronic Rheumatic Affections of the Hands, and Duruoz Hand Index (p: 0.000) were in favor of the intervention group.

CONCLUSION: Upper extremity home exercises increase grip strength, range of motion, activity performance, and functionality in patients with systemic sclerosis. We recommend that rehabilitation programs include not only hand exercises but also upper extremity exercises.

KEYWORDS: Systemic sclerosis. Upper extremity. Exercises.

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease characterized by fibrosis, causing musculoskeletal-related disorders. Fibrosis and edema of the skin affect hand function by decreasing grip strength and range of motion (ROM). Therefore, patients with SSc have difficulties in using their upper extremities in a useful manner[1].

Exercises play a key role in the initial stages of rehabilitation of SSc. Literature has focused on hand rehabilitation. To prevent hand disorders due to SSc, hand exercises should be started in the early period and should be a part of daily life[2]. In this case, it is important to emphasize the role of home exercises to improve patients' capacity to manage the disease[1]. Stretching, mobility exercises, and isometric and isotonic strengthening exercises were used[3-5].

Moreover, hand and upper extremity is one of the areas where individuals have more problems that affect their work ability[6]. However, studies on upper extremity rehabilitation were limited. More randomized controlled studies were needed to standardize protocols[1]. In addition, previous studies did not investigate the effects of a detailed upper extremity exercise program on shoulder ROM, activity performance, and functionality in SSc. To the best of our knowledge, this was the first randomized controlled trial investigating the effects of upper extremity home exercises on shoulder ROM and activity performance in SSc. This research was conducted to investigate the effects of upper extremity home exercises on grip strength, ROM, activity performance, and functionality in individuals with SSc, to compare with patient education, and to contribute to the standardization of upper extremity exercise protocols in SSc. We hypothesized that home exercises might show more improvement than patient education in terms of grip strenghts, ROM, activity performance, and functionality in individuals with SSc.

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METHODS

Procedures and study design

The design of the study was a randomized controlled trial. Clinical Research Ethics Committee of Health Sciences University Antalya Training and Research Hospital Ethics Committee approved the study (Protocol no: 2021-085). The clinical trial registration number is NCT050080738.

Participants

Individuals diagnosed with SSc followed by a rheumatologist in the Rheumatology Outpatient Clinic of Antalya Training and Research Hospital were included in the study. Informed consent according to the Declaration of Helsinki was obtained. Individuals with SSc who were included in the study were randomized into intervention (n=28) and control (n=27) groups after initial evaluation with the statistical program according to age and gender.

The inclusion criteria were as follows: being diagnosed with SSc according to 2013 ACR/EULAR criteria[7], over 18 years old, having upper extremity/hand involvement, and agreeing to participate. Exclusion criteria were as follows: being diagnosed with an additional rheumatic or any non-rheumatic disease, having a deformity preventing exercises, presence of an active digital ulcer, being involved in another rehabilitation program, and cognitive impairment.

Outcome measures

Demographic and health-related information was recorded.

Hand grip strength and pinch strengths (i.e., lateral, triple, and fingertip) were measured with Jamar hydraulic hand dynamometer (Sammons Preston, USA) and pinch meter (Pinchmeter-Sammons Preston, USA) for both hands and were recorded in kilograms[8].

Active and passive ROMs for both upper extremities at appropriate positions were measured using a universal and finger goniometer. Total active and passive ROMs were calculated for right and left shoulders, elbow and forearm, wrist, and fingers[9].

Activity performance and satisfaction were assessed using Canadian Occupational Performance Measurement (COPM). Individuals rate their performance (COPM-P) and satisfaction (COPM-S) on a scale of 1-10. Then, the average scores are taken for each category[10]. Increasing scores of COPM indicate an individual's own perception of activity performance and more satisfaction with this performance.

Functionality was evaluated using Disabilities of the Arm, Shoulder, and Hand (DASH), Score for Assessment and Quantification of Chronic Rheumatic Affections of the Hands (SACRAH), and Duruoz Hand Index (DHI). Lower scores indicate better status.

DASH is a questionnaire evaluating disability, activity limitations, leisure time activities, and limitation of participation owing to upper extremity injury[11]. All questions are scored with a 5-point Likert system (1: no difficulty, 5: not able to do at all) (0: no disability, 100: maximum disability).

SACRAH contains 23 visual analog scales of 100 mm determining the status of individuals with rheumatic diseases of hand[12]. The average score is calculated for each category. The overall average for the three category scores is then taken. The overall score ranges from 0 to 100.

DHI is a functional assessment scale specifically for rheumatoid hand[13]. Difficulty of individuals in trying to perform activities without any assistive devices is scored with a 6-point Likert scale (0: perform without any difficulty; 5: completely impossible). The total score ranges from 0 to 90.

All assessments were made at baseline and at the end of 8 weeks.

Protocols

Intervention group: upper extremity home exercises

Individuals performed upper extremity home exercises including stretching and strengthening for 5 days a week for 8 weeks[1,3,4,14]. Exercises were performed from distal to proximal (from fingers to shoulders), first stretching (10 s×10 repetitions), and then strengthening (2 sets×10 repetitions) for each part. Individuals could take rests during exercises. Adherence was checked regularly by phone. An exercise diary and brochure were given to increase adherence.

Control group: patient education

Patient education includes information in the following areas: principles of joint protection, energy conservation techniques, pain and pain control, maintaining body function, organizing activity and rest periods, and posture[15].

Statistical analysis

The G-Power version 3.1.7 (University of Kiel, Kiel, Germany) power analysis was performed to determine sample size. Based on reference study[4] with a medium effect size (d=0.74), with a confidence interval of 95% and a power analysis of 80%, at least 46 (23 for each group) patients applied to the rheumatology outpatient clinic.

Statistical analyses were performed using the SPSS version 22 (IBM SPSS Statistics; IBM Corporation, Armonk, NY, USA) software. Kolmogorov-Smirnov test was used to evaluate normality. In-group comparisons were evaluated

with paired-samples T-test or Wilcoxon signed-rank test. Independent-samples T-test or Mann-Whitney U test was used to compare the groups. The statistical significance level was assumed as $p<0.05$.

RESULTS

A total of 46 individuals completed the study with an 83.6% response rate (Figure 1). The rate of exercise compliance was 87.1% for the intervention group. Wrist pain during the first week of exercise was reported (n=1). There was no statistically significant difference between groups in terms of demographic and health-related variables ($p>0.05$) (Table 1). Individuals mostly reported activities in the field of self-care by COPM. Most frequently reported activities were cooking (45.7%), up-down stairs (30.4%), bathing (28.3%), and dusting (28.3%), respectively.

There was no significant difference ($p>0.05$) between groups except for passive total ROM of the right fingers and COPM scores in the pre-treatment values (Table 2).

When pre-treatment and post-treatment intra-group evaluation results were examined, there was a statistically significant difference in all parameters in the intervention group except for right-hand lateral grip strength ($p<0.05$); in the control group, there was a significant difference in pinch strengths for both hands, active total ROM of left shoulder, right and left wrist and fingers, passive total ROM of left shoulder and wrist, right and left fingers, DASH, SACRAH, and DHI scores ($p<0.05$) (Table 3).

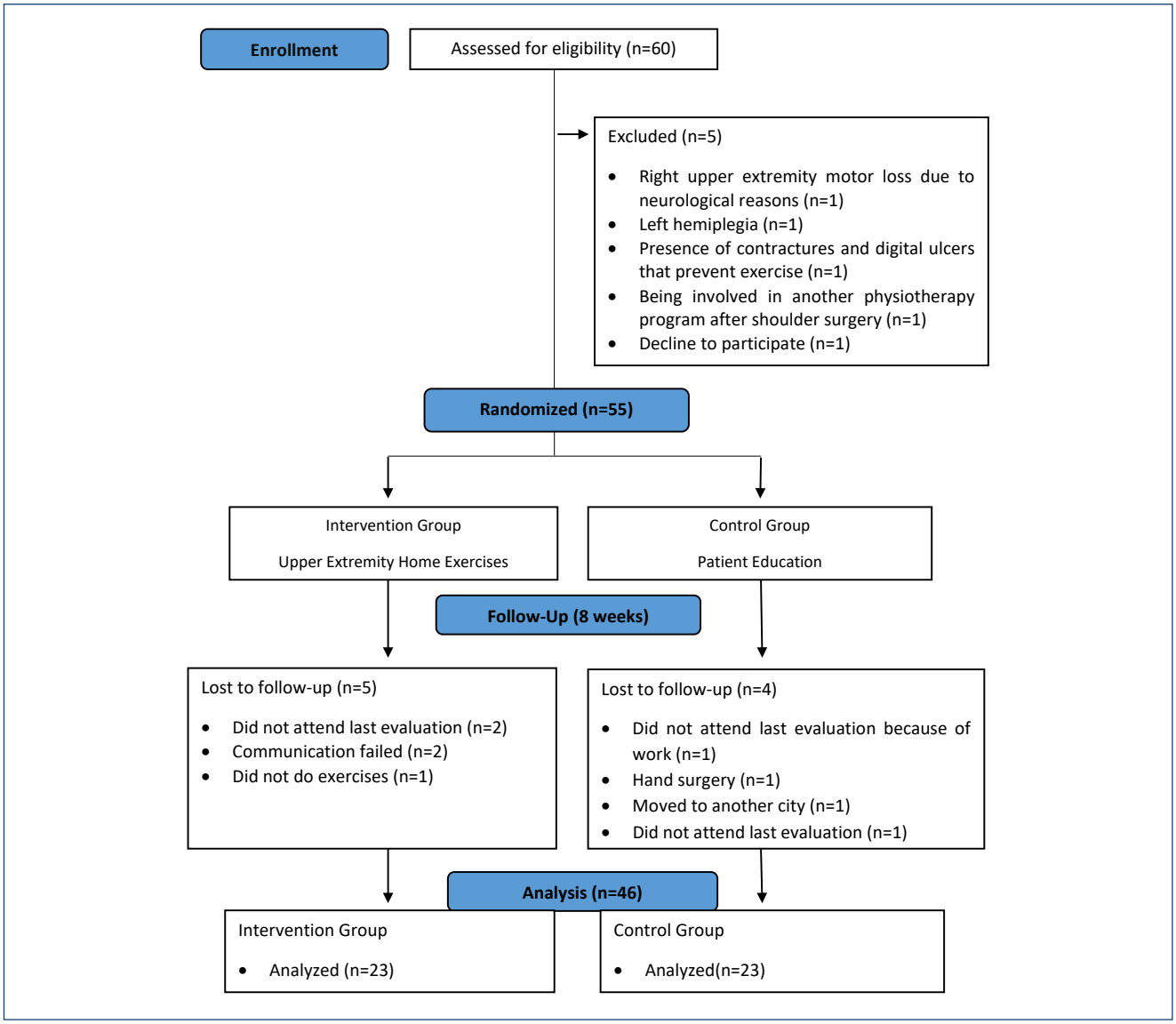

Figure 1. CONSORT flow diagram.

Table 1. Comparison of groups in terms of demographic and health-related variables at baseline.

	Intervention Mean±SD	Control Mean±SD	Intervention n (%)	Control n (%)	Z[b] t[a]	p*
Gender					0.000[b]	1.000
Female			21 (91.3)	21 (91.3)		
Male			2 (8.7)	2 (8.7)		
Age (years)	53.43±11.95	57.60±10.97	-	-	0.583[a]	0.224
BMI (kg/m^2)	27.72±2.98	27.04±3.53	-	-	0.494[a]	0.488
Smoking (years)	3.26±10.83	2.47±8.37			-1.380[b]	0.167
Yes			2 (8.7)	6 (26.9)		
No			21 (91.3)	17 (73.1)		
Alcohol (years)	2.17±10.42	0.00±0.00			-1.000[b]	0.317
Yes			1 (4.3)	0 (0)		
No			22 (95.7)	23 (100)		
Disease duration (years)	6.04±3.19	7.60±5.20	-	-	-1.202[b]	0.229
Dominant hand	-	-			-0.591[b]	0.555
R			22 (95.7)	21 (91.3)		
L			1 (4.3)	2 (8.7)		
Morning stiffness	-	-			-0.933[b]	0.351
Yes			14 (60.9)	17 (73.1)		
No			9 (39.1)	6 (26.9)		
Stiffness duration (min)	20.76±15.39	42.52±69.88	-	-	-0.550[b]	0.582
ESR (mm/s)	7.80±4.99	11.66±12.27	-	-	-0.354[b]	0.723
CRP (mg/L)	2.55±2.89	3.05±2.91	-	-	-0.975[b]	0.330
RF (IU/mL)	13.91±9.93	11.40±6.59	-	-	-0.933[b]	0.351
Employment status					-0.014[b]	0.989
Not working			17 (73.9)	16 (69.6)		
Employee			6 (26.1)	7 (30.4)		

SD: standard deviation; R: right; L: left; BMI: body mass index; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor. [a]Independent-samples T-test. [b]Mann-Whitney U test. *p<0.05.

Delta (Δ) values were calculated to examine the difference between the groups after treatment. In terms of D values, hand grip and pinch strengths (p: 0.000-0.016), active (p: 0.000-0.032) and passive (p: 0.000-0.043) total ROMs, COPM performance and satisfaction, DASH, SACRAH, and DHI (p: 0.000) were in favor of the intervention group (Table 2).

DISCUSSION

In this study, it was concluded that upper extremity stretching and strengthening exercises applied at home were effective in improving hand grip and pinch strengths, upper extremity active and passive total ROM, activity performance, and functionality of individuals with SSc.

Rehabilitation approaches for the hand/upper extremity in scleroderma primarily aim to improve grip strength, mobility, and function[3,4]. To manage the disease and reduce the financial burden on health sources, rehabilitation interventions in SSc are arranged in a way that individuals can apply on his/her own and become a part of their lives[2]. In this context, our home exercise program consisted of stretching and strengthening exercises involving the entire upper extremity. Exercise duration, frequency, intensity, and repetitions were similar to the literature[1,3-5,14,16].

In this study, pre-treatment, patient education group was superior in terms of some values. This could be explained by the presence of clinical subtypes of SSc affecting individuals at different levels and the use of self-reported assessment tools.

Table 2. Comparison of the groups pre-treatment and comparison of Δ values between groups in terms of grip strength, total range of motion, Canadian Occupational Performance Measure, Disabilities of the Arm, Shoulder and Hand, Score for Assessment and Quantification of Chronic Rheumatic Affections of the Hands, and Duruoz Hand Index post-treatment.

		Pre-treatment				Post-treatment (Δ values)			
		Intervention Mean±SD	Control Mean±SD	Z[b] F[a]	p	Intervention Δ (Mean±SD)	Control Δ (Mean±SD)	Z[b] t[a]	p
Hand grip strength	R	22.11±7.93	24.38±9.01	1.488[a]	0.369	-2.31±4.69	0.57±2.95	0.484[a]	0.016*
	L	20.77±8.35	23.66±11.03	1.048[a]	0.323	-2.78±4.60	0.36±2.94	2.458[a]	0.008*
Lateral pinch strength	R	6.57±1.73	7.14±2.27	1.391[a]	0.344	-0.49±1.33	0.76±0.85	1.980[a]	0.000*
	L	6.49±1.83	6.75±2.15	0.282[a]	0.663	-0.63±1.08	0.65±0.74	2.283[a]	0.000*
Triple pinch strength	R	5.17±1.74	6.07±2.21	1.787[a]	0.129	-0.97±1.12	0.82±1.01	0.089[a]	0.000*
	L	5.05±1.80	5.80±2.25	1.278[a]	0.220	-0.89±1.17	0.80±1.11	0.078[a]	0.000*
Fingertip pinch strength	R	4.54±1.48	5.07±1.50	0.039[a]	0.231	-0.97±1.32	0.61±0.97	4.956[a]	0.000*
	L	4.33±1.42	4.96±1.63	1.323[a]	0.171	-0.68±1.09	1.01±1.02	0.546[a]	0.000*
Shoulder active total ROM	R	549.78±44.55	576.30±38.76	-2.146[b]	0.032	-34.34±31.99	3.69±12.35	-4.933[b]	0.000*
	L	554.78±47.42	576.52±36.25	-1.496[b]	0.135	-35.65±31.16	12.17±14.68	-5.456[b]	0.000*
Elbow-forearm active total ROM	R	309.56±16.91	317.73±9.45	-1.108[b]	0.268	-7.17±10.09	-0.08±2.37	-2.717[b]	0.007*
	L	309.56±15.66	317.39±9.87	-1.480[b]	0.139	-6.95±11.84	0.43±4.74	-2.151[b]	0.032*
Wrist active total ROM	R	165.00±37.92	171.43±25.54	-0.341[b]	0.733	-24.13±23.04	8.60±15.01	-4.906[b]	0.000*
	L	172.39±38.34	175.86±27.66	-1.165[b]	0.869	-18.69±21.70	17.82±16.22	-5.470[b]	0.000*
Fingers active total ROM	R	1234.08±131.79	1295.65±107.71	1.065[a]	0.090	-142.43±100.50	46.56±39.58	14.549[a]	0.000*
	L	1255.47±137.75	1299.26±104.65	0.677[a]	0.231	-120.39±78.81	43.08±53.72	1.735[a]	0.000*
Shoulder passive total ROM	R	567.82±37.68	584.26±33.11	-1.081[b]	0.280	-25.21±28.46	0.34±6.25	-4.527[b]	0.000*
	L	571.52±38.91	584.95±29.57	2.649[a]	0.194	-28.69±26.50	4.30±8.13	21.984[a]	0.000*
Elbow-forearm passive total ROM	R	314.91±11.40	320.65±7.27	-1.567[b]	0.117	-4.47±6.45	-1.00±2.74	-2.021[b]	0.043*
	L	314.95±10.81	321.30±7.10	-1.935[b]	0.053	-4.39±7.35	0.43±2.57	-2.971[b]	0.003*
Wrist passive total ROM	R	182.00±32.95	185.95±21.73	-0.176[b]	0.860	-19.52±19.38	2.34±9.46	-4.579[b]	0.000*
	L	187.52±32.28	188.69±23.94	-0.121[b]	0.903	-16.82±15.61	6.47±9.18	-5.554[b]	0.000*
Fingers passive total ROM	R	1317.69±114.10	1387.78±97.34	0.702[a]	0.030*	-117.30±81.97	17.82±33.68	10.614[a]	0.000*
	L	1332.60±123.34	1390.17±86.20	0.943[a]	0.073	-105.43±72.29	16.30±34.52	5.937[a]	0.000*
COPM-performance		7.21±1.64	8.29±1.63	0.261[a]	0.031*	-1.09±1.25	0.31±0.85	1.123[a]	0.000*
COPM-satisfaction		7.13±1.69	8.30±1.65	0.095[a]	0.022*	-1.24±1.34	0.32±0.85	1.991[a]	0.000*
DASH		32.57±18.49	22.23±18.23	0.028[a]	0.063	15.47±12.93	-6.14±8.35	4.522[a]	0.000*
SACRAH		22.05±18.36	17.78±17.59	-0.802[b]	0.422	9.71±10.21	-8.05±10.46	-5.218[b]	0.000*
DHI		10.00±10.84	7.82±11.07	-1.528[b]	0.126	5.04±5.91	-3.60±5.07	-5.090[b]	0.000*

SD: standard deviation; R: right; L: left; ROM: range of motion; COPM: Canadian Occupational Performance Measure; DASH: Disabilities of the Arm, Shoulder and Hand; SACRAH: Score for Assessment and Quantification of Chronic Rheumatic Affections of the Hands; DHI: Duruoz Hand Index. [a]Independent-samples T-test. [b]Mann-Whitney U test. *p<0.05.

In the literature, no change or decrease in grip strength was observed when no exercise was applied or when exercise duration and/or frequency were lower[1,2,3,9]. Some studies showed that grip strengths increased[3,5,16], while in the study of Murphy et al., grip strength decreased after 8 weeks; lateral grip strength did not change[9]. In this study, grip strengths increased after exercise, similar to other studies[3,5,16]. We thought that exercises are necessary to protect and maintain hand grip and pinch strengths in SSc. Stretching and strengthening exercises should be applied at appropriate frequency and time for the upper extremity.

Table 3. Comparison of the groups in terms of grip strength, total range of motion, Canadian Occupational Performance Measure, Disabilities of the Arm, Shoulder and Hand, Score for Assessment and Quantification of Chronic Rheumatic Affections of the Hands, and Duruoz Hand Index before and after treatment.

		Intervention group				Control group			
		Pre-treatment Mean±SD	Post-treatment Mean±SD	Z[d] t[c]	p	Pre-treatment Mean±SD	Post-treatment Mean±SD	Z[d] t[c]	p
Hand grip strength	R:	22.11±7.93	24.43±8.77	-2.369[c]	0.027*	24.38±9.01	23.80±9.18	0.939[c]	0.358
	L:	20.77±8.35	23.56±7.55	-2.902[c]	0.008*	23.66±11.03	23.30±9.91	0.589[c]	0.562
Lateral pinch strength	R:	6.57±1.73	7.07±1.82	-1.770[c]	0.091	7.14±2.27	6.38±2.23	4.302[c]	0.000*
	L:	6.49±1.83	7.13±1.64	-2.808[c]	0.010*	6.75±2.15	6.10±1.97	4.168[c]	0.000*
Triple pinch strength	R:	5.17±1.74	6.14±1.82	-4.157[c]	0.000*	6.07±2.21	5.25±1.89	3.871[c]	0.001*
	L:	5.05±1.80	5.94±1.49	-3.625[c]	0.000*	5.80±2.25	4.99±1.81	3.461[c]	0.002*
Fingertip pinch strength	R:	4.54±1.48	5.51±1.73	-3.521[c]	0.002*	5.07±1.50	4.46±1.44	3.009[c]	0.006*
	L:	4.33±1.42	5.01±1.34	-2.986[c]	0.007*	4.96±1.63	3.95±1.31	4.764[c]	0.000*
Shoulder active total ROM	R:	549.78±44.55	584.13±26.57	-3.998[d]	0.000*	576.30±38.76	572.60±37.68	-1.205[d]	0.228
	L:	554.78±47.42	590.43±25.71	-4.112[d]	0.000*	576.52±36.25	564.34±38.91	-3.149[d]	0.002*
Elbow-forearm active total ROM	R:	309.56±16.91	316.73±10.51	-2.810[d]	0.005*	317.73±9.45	317.82±8.63	-0.378[d]	0.705
	L:	309.56±15.66	316.52±9.70	-2.689[d]	0.007*	317.39±9.87	316.95±9.50	-0.465[d]	0.642
Wrist active total ROM	R:	165.00±37.92	189.13±26.31	-4.071[d]	0.000*	171.43±25.54	162.82±22.40	-2.421[d]	0.015*
	L:	172.39±38.34	191.08±29.50	-3.687[d]	0.000*	175.86±27.66	158.04±25.61	-4.001[d]	0.000*
Fingers active total ROM	R:	1234.08±131.79	1376.52±99.76	-6.79[c]	0.000*	1295.65±107.71	1249.08±93.98	5.64[c]	0.000*
	L:	1255.47±137.75	1375.86±106.66	-7.32[c]	0.000*	1299.26±104.65	1256.17±84.65	3.84[c]	0.001*
Shoulder passive total ROM	R:	567.82±37.68	593.04±23.14	-3.920[d]	0.000*	584.26±33.11	583.91±32.08	-0.122[d]	0.903
	L:	571.52±38.91	600.21±21.76	-5.191[c]	0.000*	584.95±29.57	580.65±29.43	2.538[c]	0.019*
Elbow-forearm passive total ROM	R:	314.91±11.40	319.39±8.31	-2.814[d]	0.005*	320.65±7.27	321.65±5.88	-1.667[d]	0.096
	L:	314.95±10.81	319.34±8.16	-2.677[d]	0.007*	321.30±7.10	320.86±7.33	-0.816[d]	0.414
Wrist passive total ROM	R:	182.00±32.95	201.52±23.37	-3.976[d]	0.000*	185.95±21.73	183.60±19.10	-1.143[d]	0.253
	L:	187.52±32.28	204.34±24.55	-4.028[d]	0.000*	188.69±23.94	182.21±22.69	-2.968[d]	0.003*
Fingers passive total ROM	R:	1317.69±114.10	1435.00±88.62	-6.86[c]	0.000*	1387.78±97.34	1369.95±91.60	2.53[c]	0.019*
	L:	1332.60±123.34	1438.04±97.17	-6.99[c]	0.000*	1390.17±86.20	1373.86±76.55	2.26[c]	0.012*
COPM-performance		7.21±1.64	8.30±1.23	-4.170[c]	0.000*	8.29±1.63	7.98±1.55	1.753[c]	0.094
COPM-satisfaction		7.13±1.69	8.38±1.26	-4.463[c]	0.000*	8.30±1.65	7.98±1.57	1.802[c]	0.085
DASH		32.57±18.49	17.10±15.89	5.734[c]	0.000*	22.23±18.23	28.37±22.94	-3.528[c]	0.002*
SACRAH		22.05±18.36	12.34±15.19	-4.107[d]	0.000*	17.78±17.59	25.83±20.76	-3.319[d]	0.001*
DHI		10.00±10.84	4.95±8.13	-3.627[d]	0.000*	7.82±11.07	11.43±12.38	-3.051[d]	0.002*

SD: standard deviation; R: right; L: left; ROM: range of motion; COPM: Canadian Occupational Performance Measure; DASH: Disabilities of the Arm, Shoulder and Hand; SACRAH: Score for Assessment and Quantification of Chronic Rheumatic Affections of the Hands; DHI: Duruoz Hand Index. [c]Paired samples T-test. [d]Wilcoxon signed-rank test. *p<0.05.

There was no study evaluating shoulder ROM. In a study, total active ROM for fingers increased for both hands, but the difference was found only for the left hand; wrist and elbow flexion did not change[9]. In the study of Mancuso and Poole, the total ROM of fingers improved clinically[14]. In the study of Piga et al., finger ROM increased in the dominant hand for both groups[4]. In this study, ROM of the shoulders, elbows, forearms, wrists, and fingers increased in total in the whole upper extremity after exercise.

COPM has been used as an assessment tool in various rheumatic disorders and conditions with upper extremity

involvement[15]. In the study of Sandqvist et al., performance and satisfaction scores were found to be 4 and 3, according to COPM, and individuals had most difficulties in the area of household chores and work[2]. In another study, activities related to nutrition and personal care were reported as the most difficult activities, while indoor mobility and transfers were the easiest[17]. In this study, compared with others, individuals' activity performance and satisfaction were higher before and after treatment[15,18,19]. This may be due to psychological and sociocultural factors, and advances in treatment that affected performance and satisfaction scores[18]. Stefanantoni et al. reported that COPM scores increased after hand exercises besides occupational therapy[19]. In this study, individuals reported activities in the field of self-care frequently: in general, cooking (45.7%), up-down stairs (30.4%), bathing (28.3%), and dusting (28.3%). In this respect, our results were similar to others evaluating difficulties in ADL in SSc[17,18]. Besides, activity performance and satisfaction improved after upper extremity home exercises.

It was reported that the most important factor restricting functionality in SSc is hand impairment[6]. In the study by Murphy et al., upper extremity function increased after 8 weeks[9]. In the study by Waszczykowski et al., upper extremity and hand function decreased after the first month but increased in the 6-month period compared with the beginning. The group doing home exercises for only 30 min showed improvement after 1 month, but no difference was found[16]. In this study, similar to other studies showing positive effects of upper extremity/hand exercises on functionality in SSc, functionality improved after upper extremity home exercises according to DASH, SACRAH, and DHI.

The strength of our study is that it also included upper extremity exercises, unlike others that included only hand exercises and were not comprehensive[4,16,19]. Another importance of the study is that, because this study was conducted during the COVID-19 pandemic period and due to the chronic nature of SSc, the long-term rehabilitation needs of individuals were met with confidence due to home exercises. One of the strengths of the study was the use of objective assessment tools such as dynamometer. In addition, individuals were able to stay in touch with the physiotherapist. Thus, coping strategies were supported in every sense, and they were better adapted to the exercises.

The study has several limitations. First, it did not have a follow-up period to determine the persistence of effects of exercises. Second, measurement evaluating edema, vascular function, or skin condition was not performed. Therefore, we cannot make a definite conclusion about the effect of exercise on the mechanism. Finally, cardiopulmonary parameters were not monitored during exercise. However, for individuals at risk of cardiopulmonary disease, monitoring them during upper extremity exercises is recommended[20].

CONCLUSION

Grip strength, active and passive total ROM, activity performance, and functionality improved after upper extremity home exercises. We recommend that rehabilitation programs include not only hand exercises but also routine upper extremity exercises. More well-designed randomized controlled studies are needed to standardize protocols for total upper extremity in SSc.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Scapular kinesiotaping improves upper extremity functionality in healthy active subjects

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the effect of scapular kinesiotaping and sham-taping applications on upper extremity functionality in healthy active subjects.

METHODS: In total, 60 participants were randomly divided into two groups: scapular kinesiotaping group (n=30) and sham-taping group (n=30). While scapular kinesiotaping was applied to the kinesiotaping group, scapular rigid taping was applied to the sham-taping group. At the end of the third day of the taping application, the individuals were re-evaluated.

RESULTS: Participants in the scapular kinesiotaping group showed improvement in upper extremity functionality and quality of life after taping ($p<0.05$). In the sham-taping group, there was no statistically significant difference after taping ($p>0.05$).

CONCLUSION: Scapular kinesiotaping is effective in improving upper extremity functionality in healthy active subjects.

KEYWORDS: Kinesiotape. Upper extremities. Health related quality of life.

INTRODUCTION

Taping has been used for years in the prevention and treatment of sports injuries as it provides joint and muscle support during movement and is also known to improve proprioception in the prevention of acute and chronic injuries[1]. It is known that kinesiotaping (KT) improves local circulation, reduces edema, facilitates muscles, and improves joint functions by stimulating sensory mechanisms[2].

Scapula is the most important structure connecting the upper extremity and the axial skeleton[3]. Abnormal movement pattern in the scapula indicates shoulder pathologies[4]. Due to the close relationship between scapula and glenohumeral joint functions, it is necessary to focus on scapular control and performance in the rehabilitation of shoulder problems[5]. One of the recommended rehabilitation methods to facilitate scapula control is taping[6]. Although it is known that KT improves functional alignment and local muscle control, the mechanism is still unclear. In a study conducted on athletes, it was stated that ankle taping slows down the speed of inversion movement by activating the cutaneous receptors and fibularis muscle, thus reducing the risk of injury[7]. Similarly, KT application may have a proprioceptive and even mechanical effect on the shoulder joint by stimulating the cutaneous receptors in the upper extremity[8]. Although few studies have mentioned the positive effects of scapular banding on the dynamic position of the scapula, they have not been able to explain its mechanism of action on scapular kinematics[9]. Besides, studies conducted in recent years show that KT has a short-term positive effect on pain and shoulder joint range of motion, and tape applications can affect muscle activation levels[6].

To the best of our knowledge, there is no study in the literature investigating the effect of scapular KT on upper extremity functionality. Therefore, the aim of this study was to determine the effect of scapular KT on upper extremity functionality.

METHODS

Study design

This study was designed as a randomized controlled study. The participants were randomly assigned to the KT group or the control group (sham-taping (ST) group). All assessments were evaluated by a researcher who was blind to the groups.

Participants

A total of 60 healthy volunteers participated in this study. The inclusion criteria for the study group were that participants had (1) to be between the ages of 18 and 50 years[2]; no major musculoskeletal injuries in the upper extremity and shoulder girdle (i.e., osteoarthritis, rheumatoid arthritis, frozen shoulder,

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lateral epicondylitis, etc.)[3]; to involve in exercising at least 3 days a week; and[4] to be healthy and volunteers to be included in the study. Potential participants were excluded if they had[1] any systematic disease[2]; any pathology at the cervical region; or[3] surgery in the upper extremity and neck region. The research was approved by the ethics committee of Clinical Researches of XXX (Decision number: 2022-18/165).

Measurements

Purdue-Pegboard test

Purdue-Pegboard test (PPT) was used to assess dexterity. It consists of PPT nails, washers, and a perforated board assembly. The board has two parallel rows of 25 holes on each side. Nails and washers are located in the spaces reserved above the board[10].

Quick - Disabilities of the Arm, Shoulder, and Hand

Quick - Disabilities of the Arm, Shoulder, and Hand (Q-DASH) was used to measure the functionality of the upper extremity. The questionnaire consists of 11 items addressing the level of difficulty in performing daily activities and the participant's ability to work[11].

Upper Extremity Functional Index

Upper Extremity Functional Index (UEFI) evaluates the upper extremity function of the participant. UEFI consists of 15 items. Each answer is scored from 0 to 4 on a Likert scale. A higher total scores indicate better upper extremity function[12].

Short Form 36

Short Form 36 (SF-36) was used to assess the health status and quality of life. It consists of 36 questions that address areas such as mental health, general perception of health, physical functioning, role limitations, energy and vitality, social functioning, and bodily pain. Higher scores represent better quality of life and health status[13].

Interventions

Scapular kinesiotaping application

Scapular kinesiotaping application was performed by the same physical therapist on the KT group. After cleaning the skin, three strips of red KT were applied on the trapezius muscle. Scapular KT was applied on the three parts of the trapezius muscle. The kinesiotape was applied as type I without any tension (paper-off tension). The basic kinesiotaping method was used for the upper part of the trapezius muscle. The kinesiotape was applied from the insertion to the origin. The base of the kinesiotape was applied to the lateral one-third of the clavicle. The patient was then asked to rotate his head toward the opposite shoulder, and the tape was attached to the tensioned skin just below the hairline. The origo-insertion method was used for the middle and lower trapezius muscles. The processus spinosus of C6-T3 was applied for the middle trapezius, and the processus spinosus of T4-T12 was applied for the lower trapezius muscle.

Sham taping Application

ST was applied to the control group without applying any tension or method. Rigid (non-elastic) tape was applied on the three parts of the trapezius muscle: upper part, middle part, and lower part. All of them were applied from the insertion to the origin[14].

Sample size

A sample size of 26 participants was chosen for each group based on a power calculation of the outcome of Purdue Pegboard[15] to be published separately. We expected few drop-outs and, therefore, aimed 30 participants in each group and a total of 60 participants.

Statistical analysis

While the paired sample t-test is used to examine the change in the values determined by measurement of the KT and ST groups separately over time, the independent-sample t-test was used to compare the baseline data of both groups. Two-way mixed-design repeated-measures ANOVA was used to evaluate the change in the variables determined by the measurement over time and the group-time interactions of the groups.

RESULTS

A total of 66 volunteers applied for the study, and 60 satisfied the inclusion criteria. The volunteers' distributions were n=30 for the KT group and n=30 for the ST group after randomization. The flowchart of the study is shown in Figure 1.

Of the 66 healthy active subjects assessed for eligibility, 6 did not meet the inclusion criteria. Finally, the study was completed with 30 participants in the KT group and 30 participants in the ST group. The sociodemographic data of the participants in both the KT and ST groups were similar ($p>0.05$) (Table 1).

A comparison of pre-taping and post-taping findings showed that participants in the KT group had significant improvements in PPT, UEFI, Q-DASH, and some parameters of Quality of Life (SF-36) (Table 2).

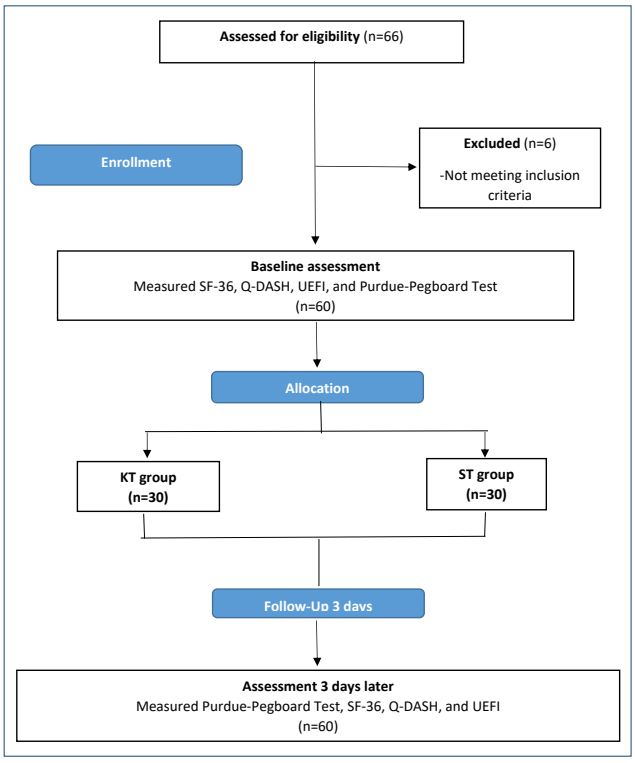

Figure 1. Study flowchart.

DISCUSSION

This is the first study to investigate the effect of scapular KT and ST on upper extremity functionality and quality of life in healthy active subjects. The findings of our study revealed that scapular KT caused significant improvements in upper extremity functionality and quality of life.

A previous study found that scapular taping in overhead athletes resulted in an improvement in scapular dyskinesia and pectoralis major length[16]. In addition, in a randomized controlled study comparing the effect of scapular rigid taping and scapular KT in patients with shoulder impingement syndrome, it was found that KT application reduced pain and had positive effects on scapular kinematics by increasing scapular retraction[17]. In another study, Van Herzeele et al. stated that scapular taping caused an increase in posterior tilt and scapular upward rotation of the scapula in asymptomatic female handball players and suggested that taping caused a mechanical effect on scapular movement[9]. Hsu et al. stated that applying taping to the lower trapezius muscle with shoulder impingement syndrome resulted in positive improvements in scapular movement and muscle performance[6]. The findings of our study are also consistent with the results reported in the literature.

Table 1. Baseline clinical and demographic characteristics of groups.

	Kinesiotape group	Sham tape group	p
Kadın n (%)–Erkek (n) %	(28) 93.3–(2) 6.7	(23) 23.3–(7) 76.7	0.071[a]
Age, mean±SD (min–max)	31.16±8.26 (22–50)	30.36±8.45 (25–54)	0.712[b]
Height, mean±SD (min–max)	165.86±7.20 (155–185)	169.63±9.95 (155–189)	0.990[b]
Weight, mean±SD (min–max)	66.13±10.42 (49–90)	68.86±16.40 (43–107)	0.444[b]
BMI, mean±SD (min–max)	24.11±3.37 (18.36–32.27)	23.69±4.16 (17.47–31.22)	0.663[b]
Dominant side right (n) %	(30) 100	(28) 93.3	0.150[a]
Purdue Pegboard			
Dominant hand	15.13±1.71	16.10±2.23	0.065[b]
Non-dominant hand	12.96±2.34	13.43±1.99	0.409[b]
Both hands	9.63±2.09	10.06±2.03	0.419[b]
Right+left+both hands	37.73±5.43	39.10±5.51	0.338[b]
Assembly	29.20±6.31	32.80±8.09	0.060[b]
Upper Extremity Functional Index	75.51±14.41	89.64±13.76	0.073[b]
Quick DASH	18.62±15.40	14.54±19.73	0.375[b]
SF36 (Quality of Life)			
Physical functioning	75.16±22.83	80.00±18.24	0.369[b]
Role limitations (physical)	61.66±35.19	70.66±30.78	0.296[b]
Role limitations (emotional)	61.03±41.19	67.77±41.54	0.531[b]
Energy and vitality	53.50±18.24	54.06±19.26	0.907[b]
Mental health	58.66±20.91	60.53±16.39	0.702[b]
Social functioning	61.36±25.63	71.66±20.48	0.091[b]
Bodily pain	63.30±22.55	69.66±23.49	0.289[b]
General perception of health	53.16±16.32	61.66±19.57	0.073[b]

SD: standard deviation; BMI: body mass index; min–max: minimum–maximum. [a]Chi-square test; [b]independent sample t-test.

Table 2. Baseline, post-intervention, and change scores for the Purdue-Pegboard test, Upper Extremity Functional Index, Quick - Disabilities of the Arm, Shoulder, and Hand, and Short Form 36.

	KT group			ST group			p^b (ES)	
	Pre-tape	Post-tape	p^a	Pre-tape	Post-tape	p^a	Time	Group×Time
Purdue Pegboard								
Dominant Hand	15.13±1.71	17.73±1.83	<0.001*	16.10±2.23	16.30±1.93	0.565	<0.001 (0.340)*	<0.001 (0.275)*
Non-dominant Hand	12.96±2.34	15.43±1.63	<0.001*	13.43±1.99	14.03±2.02	0.608	<0.001 (0.399)*	<0.001 (0.197)*
Both Hands	9.63±2.09	12.20±1.24	<0.001*	10.06±2.03	11.00±2.22	0.902	<0.001 (0.338)*	<0.001 (0.350)*
Right+Left+Both Hands	37.73±5.43	45.36±3.50	<0.001*	39.10±5.51	42.40±4.92	0.115	<0.001 (0.475)*	<0.001 (0.328)*
Assembly	29.20±6.31	38.00±6.10	<0.001*	32.80±8.09	32.93±9.43	0.882	<0.001 (0.330)*	<0.001 (0.317)*
Upper Extremity Functional Index	75.51±14.41	90.96±12.41	<0.001*	89.64±13.76	90.84±13.76	0.139	<0.001 (0.372)*	<0.001 (0.303)*
QuickDASH	18.62±15.40	9.22±9.43	<0.001*	14.54±19.73	13.15±16.96	0.504	0.001 (0.176)*	0.011 (0.106)*
SF36 (Quality of Life)								
Physical functioning	75.16±22.83	89.50±13.91	<0.001*	80.00±18.24	79.83±18.68	0.801	<0.001 (0.258)*	<0.001 (0.267)*
Role limitations (Physical)	61.66±35.19	79.16±27.91	0.008*	70.66±30.78	72.50±32.92	0.326	0.004 (0.136)*	0.027 (0.081)*
Role limitations (Emotional)	61.03±41.19	78.90±25.56	0.005*	67.77±41.54	71.10±40.81	0.184	0.002 (0.158)*	0.028 (0.081)*
Energy and vitality	53.50±18.24	58.66±18.75	0.045*	54.06±19.26	55.66±20.83	0.118	0.013 (0.101)*	0.184 (0.030)
Mental health	58.66±20.91	65.20±17.23	0.019*	60.53±16.39	63.06±16.96	0.149	0.005 (0.127)*	0.206 (0.027)
Social functioning	61.36±25.63	74.66±18.65	0.002*	71.66±20.48	72.91±21.29	0.326	0.001 (0.179)*	0.005 (0.130)*
Bodily pain	63.30±22.55	78.43±20.36	<0.001*	69.66±23.49	71.16±25.19	0.388	<0.001 (0.249)*	0.001 (0.182)*
General perception of health	53.16±16.32	57.16±13.17	0.045*	61.66±19.57	62.16±20.15	0.415	0.028 (0.080)*	0.086 (0.080)

p^a: paired sample t-test, p^b: mixed-design ANOVA, ES: effect size, *p<0.05.

Scapular KT application was found to be statistically significant in improving upper extremity functionality. In this respect, it can be thought that scapular KT application improves scapular movement and muscle performance and contributes to upper extremity functionality.

Upper extremity movements are performed by the shoulder complex, which consists of the humerus, scapula, and clavicle. The scapula is a center of shoulder activities, and a disorder in the position of the scapula negatively affects the optimal functioning of the rotator cuff muscles. A deterioration in the alignment and stabilization of the scapula on the thorax significantly affects the range of motion and functions of the upper extremity. The scapula contributes to the elevation of the upper extremity by causing upward rotation in the glenoid fossa. Scapula movement is created by the balance of force between the serratus anterior and trapezius muscles. These muscles work synergistically for the upward rotation of the scapula[18]. Naviwala et al. studied the immediate effect of KT on upper extremity movements in post-stroke hemiplegic patients. They applied KT to the upper trapezius, middle trapezius, rhomboids, and serratus anterior muscles of 30 patients and evaluated their upper extremity movements with the Fugl Meyer Assessment Scale. According to the results of the study, they concluded that KT application significantly improves upper extremity movements (i.e., coordination, hand, wrist, etc.)[19]. In our study findings, we concluded that scapular KT may cause a significant improvement in upper extremity functionality and hand motor skills, in line with the literature. We can explain the reason for this by the fact that scapular KT application can increase the intramuscular blood flow by elevating the epidermis and thus may cause improvement in joint functions.

There is limited evidence to support that scapular control can be altered by taping[20,21]. Selkowitz et al.[20] reported that scapular taping decreased the activity of the upper trapezius muscle and increased the activity of the lower trapezius muscle in individuals with suspected shoulder impingement syndrome during a functional overhead reaching activity. On the contrary, in a study involving healthy individuals, Alexander et al.[21] concluded that scapular taping reduces the amplitude

of the H-reflex in the lower trapezius muscle. In another study, Cools et al.[22] concluded that scapular taping on the three parts of the trapezius muscle and the serratus anterior muscle had no effect on muscle activity. In our study, KT application was performed on the upper, middle, and lower trapezius, increasing the upper extremity functionality. We think that KT applied on the muscle can change muscle activation and thus upper extremity functionality by stimulating the muscle spindle, which allows the movement of the muscles in that region to be perceived.

The effect of KT on quality of life is controversial in the literature. Vergili et al. concluded that glenohumeral and scapular KT application significantly improved the quality of life in patients with shoulder impingement[23]. Kul et al. compared the effectiveness of KT and traditional physiotherapy application in patients with shoulder impingement syndrome, and stated that KT application did not provide a significant improvement in the quality of life[24]. In our study, we concluded that the SF-36 quality of life questionnaire improved significantly, except for two subgroups (i.e., emotional role limitations and energy vitality). It is expected to increase the quality of life with the increase in upper extremity functionality.

CONCLUSION

This is the first study to examine the upper extremity functionality of scapular KT in healthy active subjects. Scapular KT application is effective in improving upper extremity functionality and quality of life in healthy active subjects. Also, KT application can be preferred by clinicians and researchers because it is a fast, non-invasive, and inexpensive method. Scapular KT application can be preferred as an effective treatment method in improving upper extremity functionality and movements. Long-term results investigating the effect of scapular KT application on upper extremity functionality in healthy and various pathological conditions are needed in future studies.

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AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Programmed cell death protein 1 is a marker for neoadjuvant chemotherapy response in triple-negative breast cancer

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SUMMARY

OBJECTIVE: Tumor-infiltrating lymphocytes are detectable in up to 75% of triple-negative breast cancer. The composition of these infiltrates may influence prognosis and is not known regarding regulatory or effector lymphocytes. The objectives of this study were to describe and quantify the composition of the tumor-infiltrating lymphocytes before and after chemotherapy (neoadjuvant chemotherapy) and to evaluate their association with complete pathological response and overall survival.

METHODS: This was a retrospective observational study. Clinical and pathological data from 38 triple-negative breast cancer patients treated with neoadjuvant chemotherapy at the University Hospital (HUCFF/UFRJ), between November 2004 and November 2018, were analyzed. The Stromal tumor-infiltrating lymphocytes (Stromal tumor-infiltrating lymphocytes) have been identified on hematoxylin and eosin-stained sections according to the guidelines of the "International tumor-infiltrating lymphocytes Working Group." Immunohistochemistry studies were performed to identify T-cell subsets (i.e., CD3, CD4, CD8, and FOXP3) and T-cell exhaustion (i.e., programmed cell death protein 1).

RESULTS: Statistically significant changes in stromal tumor-infiltrating lymphocyte categories were observed before and post-neoadjuvant chemotherapy, with 32% of intermediate cases becoming high. The correlation between pre-neoadjuvant chemotherapy stromal tumor-infiltrating lymphocytes and pathological response, pre-neoadjuvant chemotherapy and post-neoadjuvant chemotherapy, and stromal tumor-infiltrating lymphocytes and overall survival was not statistically significant. However, we noticed an increase of cells that favor the antitumor activity (i.e., CD3, CD8, and CD8/FOXP3 ratio) and decreased levels of cells inhibiting tumor activities (i.e., FOXP3 and programmed cell death protein 1) post-neoadjuvant chemotherapy. Importantly, programmed cell death protein 1 expression pre-neoadjuvant chemotherapy showed an association with pathological response.

CONCLUSION: In this study, we observed that chemotherapy significantly increases stromal tumor-infiltrating lymphocytes, CD8 T cells, as well as CD8/FoxP3 ratio. Most importantly, programmed cell death protein 1 expression before neoadjuvant chemotherapy positively correlates with pathological response suggesting the use of programmed cell death protein 1 as a prognostic marker before neoadjuvant chemotherapy.

KEYWORDS: Triple-negative breast cancer. Tumor-infiltrating lymphocytes. PD 1 protein.

INTRODUCTION

Breast cancer is the most frequent neoplasm and the leading cause of mortality among women worldwide[1]. Recent studies have demonstrated the importance of the tumor microenvironment (TME) and its prognostic implication regarding the behavior of various tumors, including breast cancer. Although breast cancer is not typically an immunogenic disease, tumor-infiltrating lymphocytes (TILs) are detectable in up to 75% of tumors, and approximately 20% of these tumors present particularly dense infiltrate[2].

There is growing evidence regarding the prognostic values of TILs correlating with survival, especially in triple-negative breast cancer (TNBC) cases and amplified HER2 cases[3]. Randomized studies comparing neoadjuvant treatment protocols in HER2+ and TN tumor cases have demonstrated that there is a significant correlation between TIL intensity in biopsies and better response to chemotherapy, as measured by the number of cases with complete pathological response (pCR)[4,5]. Thus, TILs have been shown to be biomarkers for the response to chemotherapy treatment and, consequently, survival[6].

A recently published meta-analysis that included individual patients from nine large studies confirmed the prognostic role of TILs in TN cases. Thus, it was suggested that TILs could be considered biomarkers for clinical use[7]. This recommendation

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was endorsed by the 16th International Breast Cancer Conference in St. Gallen, and it was proposed that TIL analysis in TN cases should be incorporated into the 8th edition of the American Joint Committee on Cancer staging system[8].

The most abundant cell population in TILs is T lymphocytes (75%). However, depending on the composition of these lymphocytes, i.e., whether they are effectors or regulators, the prognosis of breast cancer may vary. Studies have suggested that the best characterization of the immune infiltrate is obtained through immunohistochemistry (IHC), in terms of the levels of CD3 (total T lymphocytes), CD8 cytotoxic cells, and FOXP3 expressing Treg. The CD8/FOXP3 ratio in TILs correlates with a better response to neoadjuvant chemotherapy (NEOCT) and a greater chance of achieving a pCR[9]. Programmed cell death protein 1 (PD-1) can be overexpressed on the TILs[10]. The PD-1/PD-L1 pathway is crucial for the development of immune tolerance. In fact, blockage of the PD-1/PD-L1 interaction releases T-cell activity, and this is clear in many cancers where anti-PD-1 treatment with monoclonal antibodies allows a good clinical response mediated by T cells[11].

The predictive and prognostic function of immune biomarkers in TNBC remains unclear. This study aimed to quantify and identify the TILS components, along with their relationship with NEOCT.

METHODS

Patients and study design

This was an observational retrospective cohort study. We evaluated 133 patients who were treated at HUCFF/UFRJ with a diagnosis of initial or locally advanced breast cancer. These individuals underwent NEOCT followed by surgery between November 2004 and November 2018. From these, 40 patients with TN breast cancer defined through IHC, who were hormone receptor-negative and HER2-negative (0, 1+, or 2+ and FISH-negative) in accordance with the ASCO/CAP criteria, were selected.

The NEOCT regimen used was based on anthracycline and docetaxel, usually consisting of the FEC 3 docetaxel regimen (PACS protocol 01)[12]. At the end of chemotherapy, the patients were referred for breast surgery (conservative or radical) and axillary surgery (sentinel lymph node biopsy or axillary lymphadenectomy), at the surgeon's discretion. Out of the 40 TN patients, 38 were eligible for this study because sufficient histopathological material from before and after chemotherapy was available. This study was approved by the ethics committee of the UFRJ (CAAE:2800.3420.1.0000.5257).

Quantification and identification of tumor-infiltrating lymphocytes

TILs were identified in the biopsy material and surgical specimens by pathologists, using sections stained with hematoxylin and eosin at magnifications of 200-400×(10×ocular lens with 20-40×objective lens). Stromal TILs (sTILs) within the edge of the tumor scar were analyzed, after the exclusion of areas of ductal carcinoma in situ and tumor zones with necrosis and artifacts. The mean percentage of the stromal area occupied by mononuclear cells was scored, using the guidelines of the "International TILs Working Group," for the evaluation of TILs within the pre-treatment and post-chemotherapy scenarios. The quantity of sTILs was analyzed as a continuous measurement, using three predefined categories: low sTILs (0–10%), intermediate sTILs (10–40%), and high sTILs (40–90%)[13]. The sTILs were quantified blindly by two experienced pathologists at UFRJ.

The composition of the sTILs was identified by means of IHC. Counting of immunostained cells was performed in 3 fields of the stromal area (200–400× magnification). To evaluate CD3, CD4, and CD8 expressions, the following antibodies were used: Dako CD3 antibody (A0452) at a dilution of 1:800, CD8 SP clone (M3162) at a dilution of 1:100, and Bioscience FOXP3 (14-4777-82) at a dilution of 1:100.

Statistical analysis

The statistical assessment of the data was performed using R version 4.1.3 (R Development Core Team: http://www.R-project.com). For comparisons between strata (categories of variables), Student's t-test was used. p-value<0.05 were considered statistically significant.

The Kaplan-Meier statistical method was used for survival analysis. The start date for counting the length of survival was the time when the diagnosis was recorded. The observations began on the date when the first case included was diagnosed.

RESULTS

Cohort description

Out of the 40 TN breast cancer patients, 38 were eligible for inclusion because histopathological material from before and after chemotherapy was available.

The clinical and pathological features of the patients are described in Table 1.

Regarding the overall survival (OS) of the TN patients studied, 50% of the patients were still alive at 60 months. A pCR

Table 1. Clinical and pathological features of 38 patients.

All patients n=38		
Age	Mean (range)	54 (33-81)
Staging	IIA	1 (2.6%)
	IIB	10 (26%)
	IIIA	19 (50%)
	IIIB	3 (7.8%)
	NI	5 (13%)
Ki67	<20%	2 (5%)
	>20%	24 (63%)
	NI	12 (32%)
Histological type	Medullary	2 (5%)
	Metaplastic	1 (2.6%)
	Micropapillary	1 (2.6%)
	Infiltrating ductal carcinoma SOE	34 (89%)
Breast surgery	Mastectomy patey's	31 (82%)
	Conservative surgery	7 (18%)
Axillary surgery	Lymphadenectomy	30 (79%)
	Sentinel lymph node biopsy	8 (21%)
PCR		5 (13%)

Clinical staging based on the TNM of the International Union Against Cancer (UICC) 7th edition. Ki 67 considered low ≤20% and high >20%. N/I: without information. IDC NOS: infiltrating ductal carcinoma not otherwise specified.

was obtained in five patients (13%). In accordance with the literature, patients who achieved a pCR after NEOCT had better survival (p=0.030).

Quantification of tumor-infiltrating lymphocytes pre- and post-neoadjuvant chemotherapy and association with outcome

A total of 100% of the initial biopsy samples studied presented sTILs. Statistically significant changes in sTIL categories from before to after chemotherapy were observed in initially intermediate TILs patients only (p=0.016). At biopsy, sTILs were low in 10 (26%) cases, moderate in 22 cases (58%), and severe in 6 cases (16%). In the post-chemotherapy surgical specimen, we observed that 70% of low TIL cases remained low, 66% of high cases remained high, while 32% of intermediate cases became high and low in 12 cases (32%).

There was no statistically significant association between the intensity of sTILs in pre-chemotherapy biopsies and pCR (p=0.673). In addition, there was no statistically significant association between the intensity of sTILs in pre-chemotherapy biopsies and OS (p=0.98) or between post-chemotherapy sTILs and OS (p=0.24).

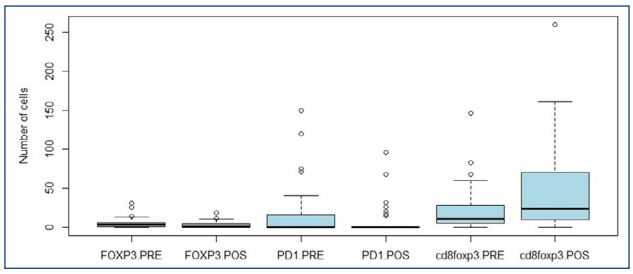

Figure 1. A box plot comparing immunophenotypes in pre- and post-chemotherapy biopsy samples: FOXP3, programmed cell death protein 1, and CD8/FOXP3, which shows significant increase in the pre- and post-CD8/FOXP3 ratio (p=0.001) and significant decrease in regulatory markers: FOXP3 (p=0.027) and programmed cell death protein 1 (p=0.01).

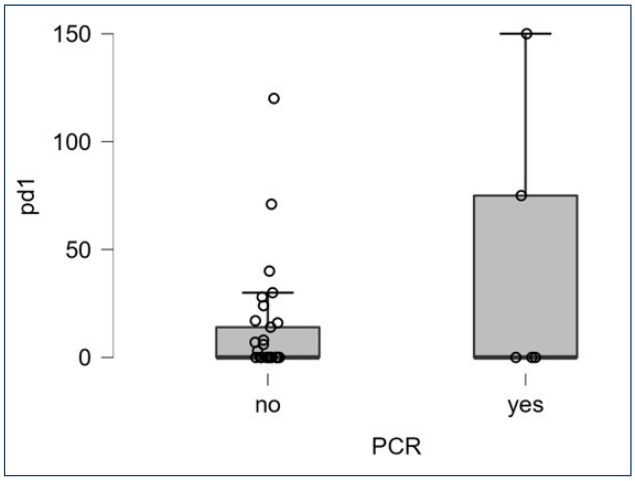

Figure 2. A box plot comparing programmed cell death protein 1 quantification (programmed cell death protein 1+cells) and pathological response. Programmed cell death protein 1 showed a significant correlation with complete pathological response (p=0.039).

Immunophenotypic analysis on stromal tumor-infiltrating lymphocytes

Immunophenotype analyses comparing pre-chemotherapy biopsies with post-chemotherapy surgical samples were performed. We observed non-significant increases in total T cells (CD3: 75.4-88) and in CD8+ T cells (CD8: 58.3-71.4). We also observed a significant decrease in FOXP3+cell levels (p=0.027) and PD-1+cells: 16-7.2 (p=0.011) leading to a significant increase in CD8/FOXP3 (p=0.001) (Figure 1).

However, when patients were separated according to their pathological response, and PD-1 expression quantified, high PD-1 expression was clearly correlated with complete response (p=0.039), while low pre-NEOCT expression was present in non-responding patients (Figure 2).

DISCUSSION

In this retrospective study, we evaluated 38 patients who had been diagnosed with TN breast cancer and were treated with NEOCT, in a single institution. Approximately 84% of them had tumors larger than 5 cm or positive lymph nodes. These findings were probably due to delayed diagnosis and late start of treatment. Only five patients achieved a pCR (13%), a result much lower than that has been reported internationally[14] and in Brazil[15]. The large tumor volumes may explain the poorer response to chemotherapy. However, even with the small number of pCRs, we were able to demonstrate, in accordance with the literature, that patients with a pCR had higher survival rates.

The association between pre-chemotherapy sTILs and pCR was not statistically significant (0.673). In addition, the correlations between pre-chemotherapy sTILs and OS (p=0.98) and between post-chemotherapy sTILs and OS (p=0.24) were not statistically significant, which were different from the literature that shows a correlation between TILs and OS[7].

There were statistically significant changes in the categories of sTILs from before to after chemotherapy. About 70% of low TILs remained low, 66% of high cases remained high, and 32% of intermediate cases became high, making us believe that this group of tumors is the one that best benefits from the immunogenic activation of NEOCT and subsequent immunotherapy. Only the intermediate group turned "cold" neoplasms into "warm" ones with chemotherapy induction. We believe that preexisting antitumor immunity is activated or enhanced during the initial cycle of chemotherapy, but only if infiltrating T cells were initially present at a certain level.

When we immunophenotyped the TILS, an increase in the profile of cells favoring immunity and antitumor activity and a significant decrease in the numbers of cells inhibiting tumor activities (FOXP3 and PD-1) were observed, and consequently, an increase in the CD8/FOXP3 ratio (Figure 1) was observed after NEOCT. This finding is compatible with the literature, in which chemotherapy is described as stimulating the immune response[16].

From all the markers used for immunophenotyping, only PD-1 in pre-chemotherapy samples showed a significant correlation with pCR. PD-1 receptor can be expressed in T cells, whereas PD-L1 is expressed in activated T and B cells, tumor-infiltrating macrophages or fibroblasts, and tumor cells. In the literature, the correlation of PD-1/PD-L1 with immunotherapy (ICB) has been widely explored, especially regarding the treatment of metastatic breast cancer, where PD-1/PD-L1 was used as a predictive biomarker to ICB therapy. The combination of an anti-PD-1 monoclonal antibody with NEOCT significantly increased the pCR rate and event-free survival[17,18] independently of the PDL1 level. These conflicting findings can be justified by the complex interaction between PD-1/PDL1, TILs, TME, and other immune checkpoints such as cytotoxic T-lymphocyte-associated antigen-4 and PD-L2, which are less studied targets in breast cancer[11]. More robust studies are needed to validate this finding. Based on this result, prospective randomized studies could test the addition of adjuvant immunotherapy only in PD-1+ patients without pCR or residual tumor[19]. A better understanding of this complex network can help in the use of new therapeutic targets.

The retrospective design, the small number of patients included, and the small number of pCRs obtained were the limitations of this study and may have influenced the finding of a correlation between the variables and outcomes. The strengths of this study were the use of a homogeneous population, and patients with locally advanced TNBC who underwent NEOCT in a single institution with quantification and immunophenotypic identification of TILs, along with their relationship with the treatment. Over the period covered by this study, NEOCT protocols for TN breast cancer did not undergo major changes.

CONCLUSION

Our study suggests that PD-1 levels in sTILs could be a candidate as a prognostic marker in response to NEOCT, independently of checkpoint inhibitor immunotherapy.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Colonoscopy following the positron emission tomography/computed tomography scan in patients with incidental colorectal uptake: what is the most effective management?

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SUMMARY

OBJECTIVE: Colorectal cancer is one of the most common malignancies. Survival rates are directly related to the stage of cancer at the time of diagnosis, emphasizing the value of early diagnosis. Positron emission tomography with 18F-fluorodeoxyglucose is the gold standard imaging technique in staging, monitoring after treatment, and follow-up. We aimed to assess the importance of incidental ^{18}F-fluorodeoxyglucose uptake by colon and rectum in positron emission tomography–computed tomography imaging to determine a significant cutoff value for further investigation using colonoscopy and histopathological assessment.

METHODS: We performed a retrospective analysis of patients with both ^{18}F-fluorodeoxyglucose-positron emission tomography/computed tomography scan and colonoscopy during 1 year and included the cases who had undergone a colonoscopy within 3 months following the positron emission tomography/computed tomography scan due to an incidental positive finding. Patients with a diagnosed colorectal malignancy or with a history of previous colorectal operations were excluded.

RESULTS: A total of 81 patients were included in this study. Among 81 colonoscopic evaluations, histopathology revealed malignancy in 8 patients, and the prevalence of incidental colorectal cancer ^{18}F-fluorodeoxyglucose uptake was found to be 9.87%. SUVmax was found to be significantly related to malignancy and other colonoscopic findings ($p<0.001$). SUVmax cutoff value to suggest colorectal cancer was found to be median [7.9 (4.1–12.7)] ($p<0.001$).

CONCLUSION: Regarding the studies determining a significant cutoff value, incidental colonic ^{18}F-fluorodeoxyglucose uptake on positron emission tomography/computed tomography should lead the clinician to further investigation with colonoscopic biopsy, although the cutoff values for SUVmax are not certain and different in almost every published study, and negative positron emission tomography.computed tomography findings should not completely rule out malignancy, especially in high-risk patients.

KEYWORDS: Colonoscopy. Colorectal cancer. Positron emission tomography. Screening.

INTRODUCTION

Colorectal cancer (malignancies of the colon and rectum, CRC) is the third most common malignancy in the world[1,2]. Adenomatous colorectal polyps are also considered malignant precursors of CRC[3]. Survival rates are directly related to the cancer stage at the time of diagnosis[4].

Colonoscopy is the gold standard screening method in CRC with high sensitivity and specificity, because it provides not only early detection of precursor lesions but also the ability to remove them[1]. For CRC, the main precursor lesion is adenomas[5]. Colorectal screening with colonoscopy reduces both CRC incidence and mortality. The decrease in these rates depends on the removal of the precursor lesion. In a recent systematic review and meta-analysis, Jodal et al. showed reduced CRC mortality in a 15-year follow-up with colonoscopy[6].

Positron emission tomography (PET-CT) with ^{18}F-fluorodeoxyglucose (FDG) is generally the gold standard imaging technique in staging, monitoring after treatment, and follow-up in cancer patients[7]. A whole body scan with PET-CT is performed for a cancer suspicion or the staging of a diagnosed cancer, and sometimes it may show an incidental FDG uptake in any part of the body. PET-CT is considered a useful technique in CRC and has been shown to have extra value in the detection of disease recurrence[8]. Following the first report by Yasuda et al. in 1998 showing an increased ^{18}F-FDG uptake in a colonic adenoma[9], several studies have evaluated its ability

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to detect CRC. Although a premalignant colorectal lesion can be incidentally detected by standard PET-CT performed for other indications, the technique is not recommended for routine CRC screening or initial diagnosis in patients with high suspicion of CRC[10]. PET-CT is not appropriate for the evaluation of the colonic wall for staging because of its limited resolution in bowel wall layers, and it may also be false positive in cases of colitis, diverticulitis, and even because of physiological colon metabolism and anal canal uptake[11]. Studies have shown that up to 45% of the patients with FDG uptake did not have any lesions in colonoscopic control[12].

Therefore, a possible optimal cutoff SUVmax value may be helpful. This study aimed to assess the importance of incidental FDG uptake by the colon and rectum in PET-CT imaging in patients with a non-CRC diagnosis and to determine a significant cutoff value for further investigation using colonoscopy and histopathological assessment as the gold standard.

METHODS

Following the approval of the local ethics committee, we performed a retrospective analysis of patients with both ^{18}F-FDG-PET/CT scan and colonoscopy during 1-year period (May 2021 to June 2022). The patients who had undergone colonoscopy within 3 months following the PET/CT scan due to an incidental positive finding were included in the study. Patients with a diagnosed colorectal malignancy or with a history of colorectal operation were excluded. In our study group, the PET/CT scans had been performed for staging or the evaluation of response to treatment or screening.

Protocols and criteria for 18F-fluorodeoxyglucose-positron emission tomography/computed tomography imaging

The 18F-FDG-PET/CT scanning was performed as a standard procedure, similar to the recent studies[13,14]. PET-CT scanning was performed using Philips Ingenuity TFÒ (Koninklijke Philips N.V., The Netherlands). The patients were included due to a follow-up because of a primary malignancy rather than a CRC or a suspicion of malignancy following laboratory tests or other imaging techniques. All the reports were reviewed by the same nuclear medicine specialist. Positive FDG findings were classified into patient demographics, the reason for undergoing the PET/CT scan, the localization of the suspected finding, and later to be matched with colonoscopic findings. A false positive PET/CT finding was defined if there were no colonoscopic findings on the colonic segment with abnormal FDG uptake, while a true positive PET/CT finding referred to a relevant colonoscopic finding.

Colonoscopic procedures

Colonoscopic procedures were carried out after adequate bowel preparation, with the addition of dietary recommendations, and performed by experienced endoscopists. A total colonoscopic procedure was defined as evaluating all colon parts adequately. Abnormal mucosal findings were excised or biopsied and sent for histopathological examination. Each specimen was studied by experienced pathologists. We included the procedures with a total colonoscopy with sufficient bowel cleaning and performed them within 3 months following the PET/CT scan with incidental positive findings. Colonoscopic findings were classified as normal, polyp, inflammation (diverticulitis), and malignancy. Patients with a history of colorectal operation were excluded.

The study was conducted according to the Declaration of Helsinki.

Statistical analysis

Data were shown in means of numbers (percentage), mean±standard deviation, and median (minimum-maximum). Statistical analysis was performed with the Kruskal-Wallis test. Conover's test of multiple comparisons was used to define the difference between the two groups. A ROC (receiver operating characteristic) curve was used to define a cutoff point for Suvmax value in patients with and without malignancy. A p-value less than 0.05 was accepted as statistically significant. The IBM SPSS Statistics 26.0 software was used for statistical analysis (SPSS, Inc., version 26.0, Chicago, IL, United States).

RESULTS

Our retrospective study included a total of 81 patients (43 men and 38 women) with a mean age of 60,741 (±14,207) years.

In 1-year period, a total of 7,097 PET/CT scans and 17,144 colonoscopic procedures had been performed in our tertiary center. The most common indication for performing the PET/CT scan was the follow-up or staging of a primary malignancy rather than CRC (65.43%). The most common malignancy diagnosed was lung cancer, followed by breast cancer and prostate cancer. Although it is not recommended in international guidelines, other indications for PET/CT include the need for further investigation when a suspicious finding was positive in other imaging techniques or high values of tumor markers. The left colon was found to be the most common colonic segment with pathological FDG uptake (40.74%). The most common colonoscopic finding was a normal colonoscopic evaluation. The PET/CT scan indications, localization of FDG uptake, and colonoscopic findings are

shown in Table 1. Significant colonic lesions were observed only in 27 patients. Among 81 colonoscopic evaluations, histopathology revealed malignancy in 8 patients, and the prevalence of incidental CRC FDG uptake was found to be 9.87%. In those patients, tumor localizations were found to be 4 in the rectum and 4 in the left colon (Table 2).

While evaluating the values of SUVmax, statistical analysis demonstrated the results with a sensitivity of 100% and a specificity of 90.3% as shown on the ROC curve (Figures 1A, B). Per-lesion analysis of SUVmax values in colonoscopic findings is shown in Table 2. Suvmax was found to be significantly related to malignancy and other colonoscopic findings ($p<0.001$). SUVmax cutoff value to suggest CRC was found to be median [7.9 (4.1–12.7)] ($p<0.001$).

Table 1. Positron emission tomography/computed tomography scan indications, localization of ^{18}F-fluorodeoxyglucose uptake, and colonoscopic findings.

Variables	Classification	Number (%)
PET/CT indication	Screening	28 (34.57%)
	Primary malignancy*	53 (65.43%)
Localization	Rectum	26 (32.10%)
	Left colon	33 (40.74%)
	Transverse colon	10 (12.35%)
	Right colon	12 (14.81%)
Colonoscopic finding	Normal	54 (66.67%)
	Polyp	16 (19.75%)
	Malignancy	8 (9.88%)
	Diverticulitis	3 (3.70%)

*Primary malignancy other than colorectal carcinom.

DISCUSSION

Individuals over 50 years of age are accepted to be at average risk for CRC even if they have no complaints, and therefore screening should be performed for possible early diagnosis[15]. PET/CT is more sensitive than laboratory tests, including tumor markers, which makes the technique more reliable for surveillance in CRC patients when compared to colonoscopy plus computerized tomography. However, there are still debates about the accuracy of PET/CT, and consensus recommendations in various guidelines are still in progress[16].

Table 2. Analysis of SUVmax using colonoscopic findings.

Variable*	Colonoscopic findings				p-value
	Normal	Polyp	Malignancy	Diverticulitis	
SUVmax	6 (1.9–13.4)	7.1 (4.1–14.4)	14.2 (5.1–35)	7.9 (4.1–12.7)	<0.001

*Variables are shown as median (min–max).

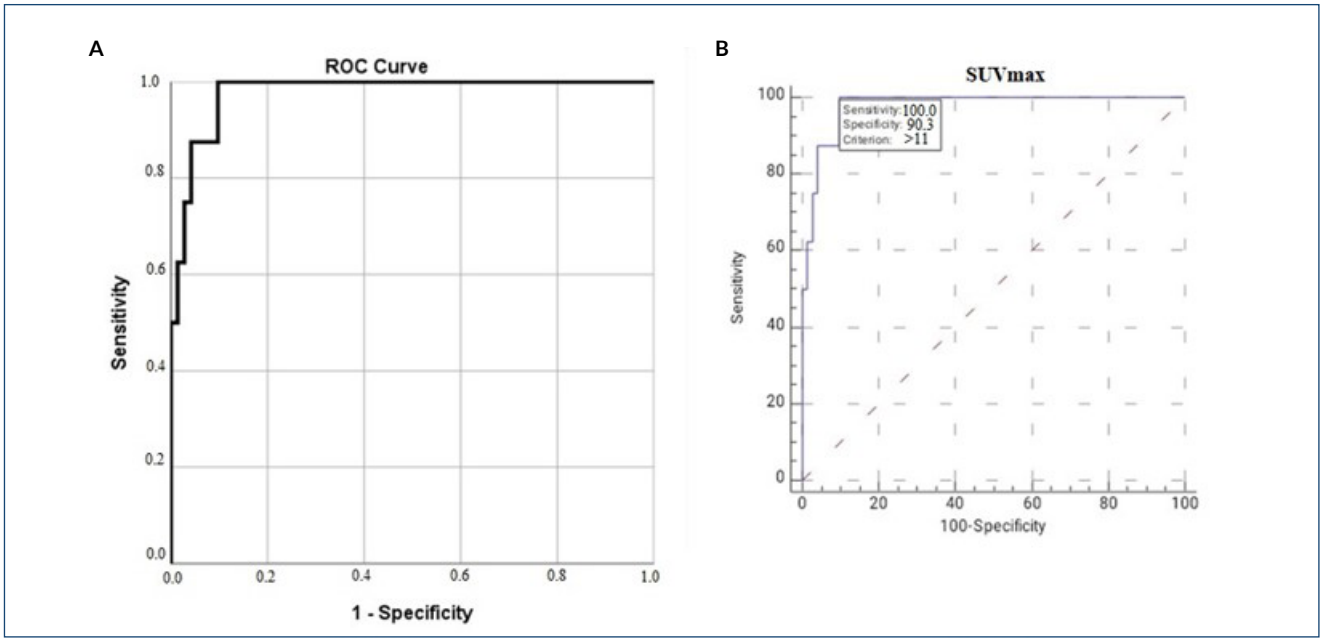

Figure 1. Statistical analysis results with a sensitivity and specificity on the receiver operating characteristic curve **(A)** and SUVmax **(B)**.

Colonoscopy is accepted as the gold standard diagnosing technique for CRC, which led the researchers to be able to control and decrease the rate of false positive results of PET/CT and to avoid the potential bias in studies addressing the efficacy of the technique in detecting CRC, similar to our study[13]. In previous studies, incidental focal colonic ^{18}F-FDG uptake and false positive results on PET/CT have been reported to be 1.3–2.7 and 16–33%, respectively[12]. These results may be indicative of the weak accuracy of PET/CT in specific colonic lesions when compared to endoscopic evaluation. In our study, we excluded patients with a diagnosed CRC, and we found 81 incidental colonic uptakes among 7,097 PET/CT studies in 1 year. In 81 PET/CT studies, significant colonic lesions had been detected in 27 patients (33.3%), and malignancy had been certified in 8 patients (29.6% in detected lesions and 9.87% overall), which shows better results when focusing on a specific patient group in the design of the study. In their study about the capacity of PET/CT in colonic pathologies, Weston et al. included the PET/CT scans with incidental colonic activity followed by colonoscopy; their rate was 0.6%, lower than previous studies and our results[13]. In their systematic review, Kousgaard et al. evaluated the correlation between FDG uptake and colonoscopic findings in four studies and found a rate of 82% when the lesion was located in the same colonic segment in both techniques[17]. In our study, the malignant lesion was found to be at the same segment in both PET/CT and colonoscopy, suggesting the high specificity of PET/CT in CRC. The most frequent use of PET/CT in our group was for staging or surveillance of patients with primary cancer rather than CRC (65.43%), similar to previous studies.

The mean SUVmax values between various types of lesions revealed some significant differences between benign and malignant lesions, similar to our study[18], while others found no significant differences[19]. In our data, the lowest SUVmax value to detect malignancy was 4.1 (mean cutoff value: 7.9). Na et al. found the same value as 2.5[13], while Luboldt et al. determined it as ≥5 in their retrospective study concerning the accuracy of PET/CT in CRC[20]. Our study design and aim do not suggest that CRC can always be diagnosed in PET/CT, but our mean SUVmax value for incidental malignancy may contribute to the existing literature, although it is different from previous studies.

Study limitations

The study is a retrospective analysis of a single-center experience. Our detected cutoff value may be strongly associated with the scanner type. The study is not designed to assess the accuracy of PET/CT in CRC as a screening method because of the selection bias in the study group, as our main aim was to guide incidental colonic findings when detected. However, the statistical analysis can be more accurate with a control group. Additionally, colonoscopies were planned to detect suspected lesions reported to be in specific segments. Prospective studies with large study and control groups may be more effective in determining a cutoff value for incidental FDG uptakes to lead patients to colonoscopy for a more cost-effective screening.

CONCLUSION

Although PET/CT has no significant role in detecting primary cancers, incidental colonic uptake is commonly encountered and leads clinicians to further investigations. We, therefore, assessed the significance of incidental focal FDG colonic activity in the PET/CT scans in diagnosing CRC by comparing it with colonoscopy and histopathology. In conclusion, incidental colonic FDG uptake on PET/CT should lead the clinician to further investigation with colonoscopic biopsy, although cutoff values for SUVmax are not certain and differentiate in almost every published study, and negative PET/CT findings should not completely rule out malignancy, especially in high-risk patients. Similar to our study, a cutoff value for an institute or a department may be accepted as the most cost-effective management to lead the clinician to further investigation with colonoscopy.

AUTHORS' CONTRIBUTIONS

YD: Conceptualization, Data curation, Writing – original draft, Writing –review & editing. **HK:** Writing – original draft, Writing – review & editing. **MKD:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **SA:** Data curation, Formal Analysis. **SS:** Data curation, Formal Analysis.

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ORIGINAL ARTICLE

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En bloc enucleation of the prostate with early apical release using a high-power (200 W) thulium device: studying a learning curve

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SUMMARY

OBJECTIVE: The aim of this study was to reveal the learning curve of early apical release en bloc laser prostatectomy using a high-power thulium (200 W) laser device.

METHODS: We obtained data on the initial 60 patients who had thulium laser enucleation of the prostate by a single surgeon between October 2021 and August 2022 to treat the signs and symptoms of benign prostatic hyperplasia at our clinic. The cases were split into three groups, each consisting of 20 patients. Prostate volumes, prostate-specific antigen and hemoglobin levels, the International Prostate Symptom Score, Quality of Life scores, the International Index of Erectile Function-5 scores, and uroflowmetry parameters were documented preoperatively. The enucleation weight, the enucleation and morcellation times, as well as the efficiency, hospitalization, and catheterization durations were calculated. The patients were re-evaluated at 6 months postoperatively, examined for functional results, and compared to baseline conditions.

RESULTS: Enucleation times, morcellation times, enucleation weight, and enucleation efficiency were significantly different among the groups. However, there was no statistically significant difference in total operative time and morcellation efficiency. In terms of postoperative statistics, the reduction in hemoglobin was significantly greater in Group 1 compared to Group 2. Six months after surgery, all groups had comparable validated ratings (International Prostate Symptom Score, Quality of Life, and the International Index of Erectile Function-5) on postoperative examinations. There were no long-term complications in either group throughout the perioperative period.

CONCLUSION: Completing 40 first cases would be sufficient for managing the learning curve for early apical release en bloc thulium laser enucleation of the prostate.

KEYWORDS: Laser. Prostatectomy. Thulium. Hyperplasia. Learning.

INTRODUCTION

A century after its anatomical description in 1,550, Herr theorized that an enlarged prostate could lead to urinary retention by impeding urine flow[1]. Since then, there has been an enormous increase in our understanding of the pathophysiology of benign prostatic hyperplasia (BPH) and its treatment options. More than 210 million men worldwide are currently diagnosed with BPH[2]. Transurethral resection of the prostate (TUR-P) remains the gold standard for the interventional treatment of symptomatic BPH, despite the remarkable advances in technology and surgical equipment that have led to the emergence of numerous new choices[3]. Studies suggest that laser enucleation of the prostate (LEP) is safer than monopolar transurethral resection of the prostate (TURP) for small to medium-sized prostate glands due to reduced catheter time and decreased risk of bleeding, even in patients receiving anticoagulation or antiplatelet therapy[4]. In conclusion, LEP has been incorporated into recommendations for prostates of more than 80 mL[5].

Thulium:yttrium aluminum garnet (Tm:YAG) lasers are one of the tools in the LEP field to this day[6]. Theoretically, depending on the manufacturer, Tm:YAG continuous wave lasers can produce beams between 2010 and 2013 nanometer wavelengths. At these wavelengths, with an optical penetration depth of about 0.2 mm, electromagnetic energy is converted into heat, causing evaporation of the prostate tissue[7,8]. It has been reported that the relatively short depth of penetration of the Thulium laser compared with the Holmium laser (0.45 mm) makes it more reliable and easier to learn[9,10].

As crucial as the type of laser energy utilized in LEP is the enucleation method employed. The three-lobed procedure, which was initially created and popularized in this operation, consists of three longitudinal incisions through which the median lobe is excised, and subsequently both lateral lobes are enucleated. Later, many adaptations of this procedure and en bloc enucleation techniques were documented and presented to the urological society. None of the recent procedures have been adopted as the standard for LEP as of yet[11].

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After acquiring a 200 W Tm:YAG laser and training under an experienced mentor, our urology clinic shifted its enucleation method preference toward the previously reported 'en bloc with early apical release'[12]. Based on our observations that this procedure might have a steep learning curve, we report the outcomes of the initial 60 consecutive patients of a single surgeon in groups for a comparative analysis in this study.

METHODS

Between October 2021 and August 2022, we collected information regarding the initial 60 patients who underwent Thulium LEP (ThuLEP) by a single surgeon to treat symptoms of benign prostatic hyperplasia in our clinic. The research project we conducted was sanctioned by the university's board of ethics (80576354-050-99/177). The Helsinki Declaration's ethical guidelines were strictly followed. The study did not include patients with a history of urethral stricture or bladder outlet obstruction surgery, neurogenic bladder, or prostate cancer. The specialist undertaking surgical operations has a background in endourology spanning over a decade. The operative surgeon worked as an assistant for approximately 40 cases with an endourologist who had performed over 200 en bloc LEPs before beginning the operations.

Patients had both a thorough physical examination and a set of laboratory tests, including measurement of prostate-specific antigen (PSA), and data were recorded alongside demographic information. If the patient had a high PSA level or a suspicious digital rectal examination, a 12-core transrectal ultrasonography-guided prostate biopsy was performed. In addition, preoperative uroflowmetry and postvoid residual volume (PVR) evaluations were carried out as part of the standard preoperative procedures (if the patient did not have a catheter). Also, all patients had to go through a detailed ultrasonic evaluation, and prostate volumes were calculated. Additionally, patients were asked to complete three validated questionnaires preoperatively and at postoperative follow-up. These were the International Index of Erectile Function (IIEF)-5, the International Prostate Symptom Score (IPSS), and the IPSS-Quality of Life Index (QoL).

Technique

All operations were performed under general anesthesia. A Cyber TM 200 W device (Quanta System, Solbiate Olona, Varese, Italy) was used for every surgery, and a 26 French resectoscope (Karl Storz™) was used to send a 550 m laser fiber through it. Enucleation was done using the earlier-described en bloc technique with an early apical release technique[12]. Beginning at 1'o clock and extending clockwise and counterclockwise, the first incision in the en bloc with the early apical release method separates the prostate adenoma's apex from the urinary sphincter. The whole prostatic adenoma is enucleated in a retrograde direction, circumferentially going toward the bladder neck, after the proper plane has been identified. Later, the bladder neck is entered anteriorly. Finally, the fibers of the bladder neck from the prostate are divided clockwise and counterclockwise, sending the whole adenoma into the bladder in one piece. A Hawk morcellator (Hawk Medical Instrument Co. Ltd.) was used for all morcellation processes. Each patient had a 22 Fr three-way urethral catheter inserted, and their bladder was irrigated continuously until the urine turned a clear color. Enucleation time, morcellation time, and specimen weight were recorded for every instance.

Follow-up

Patients were assessed with PSA levels, uroflowmetry, and PVR as a part of the periodic examination. Additionally, the two valid questionnaires (IIEF and IPSS) that patients completed before the procedure were repeated, taking into account their altered condition, in the sixth postoperative month.

The cases were split into three groups, each consisting of 20 patients, consecutively (Group 1, Group 2, and Group 3). We used the modified Clavien-Dindo Scoring System to evaluate and classify the complications. All demographic data, laboratory findings, and valid questionnaire scores were given in a comprehensive manner.

SPSS version 22.0 was used for the statistical analysis (SPSS Inc., Chicago, IL, United States). The continuous variables were reported as median (25–75 IQR) and were then compared using the Kruskal-Wallis test. *Post hoc* comparisons were made using the Tukey's and Dunnett's tests. The p-value of 0.05 was defined as the threshold for statistical significance.

RESULTS

There was no significant difference between the groups' mean ages. A significant difference in preoperative PSA levels was seen between Group 1 and Group 3. Regarding the preoperative data such as hemoglobin levels, prostate volumes, PVR, and Q-max ratios, there were no statistically significant differences between the groups. Similarly, the groups' preoperative IPSS, QoL, and IIEF ratings were similar.

Enucleation times, morcellation times, enucleation weight, and enucleation efficiency were significantly different among the groups. However, there was no statistically significant difference in total operative time and morcellation efficiency.

Some specific parameters that differed significantly between groups are demonstrated in Figure 1.

In terms of postoperative statistics, the reduction in hemoglobin was significantly greater in Group 1 compared to Group 2. Six months after surgery, all groups had comparable validated ratings (IPSS, QoL, and IIEF-5) on postoperative examinations. Likewise, during the 6-month review, the groups exhibited comparable performance on metrics such as PVR Q-max. Group 3 had shorter hospitalization and catheterization times on an hourly basis. In addition, the percentage of PSA drop relative to the value at baseline was substantially greater in Group 3 compared to Groups 1 and 2. Patient characteristics, and perioperative and postoperative follow-up data are presented in Table 1.

According to Clavien-Dindo Scoring, there is a significant difference between groups when complications are classified as present or absent (chi-square test, p=0.025). Complications such as capsular perforation, injury to the bladder mucosa during morcellation, re-catheterization, and the need for resectoscope-assisted cauterization were numerically higher in Group 1. The categorization of complications according to the Clavien-Dindo classification and the distribution of some specific complications among the groups are presented in Table 2.

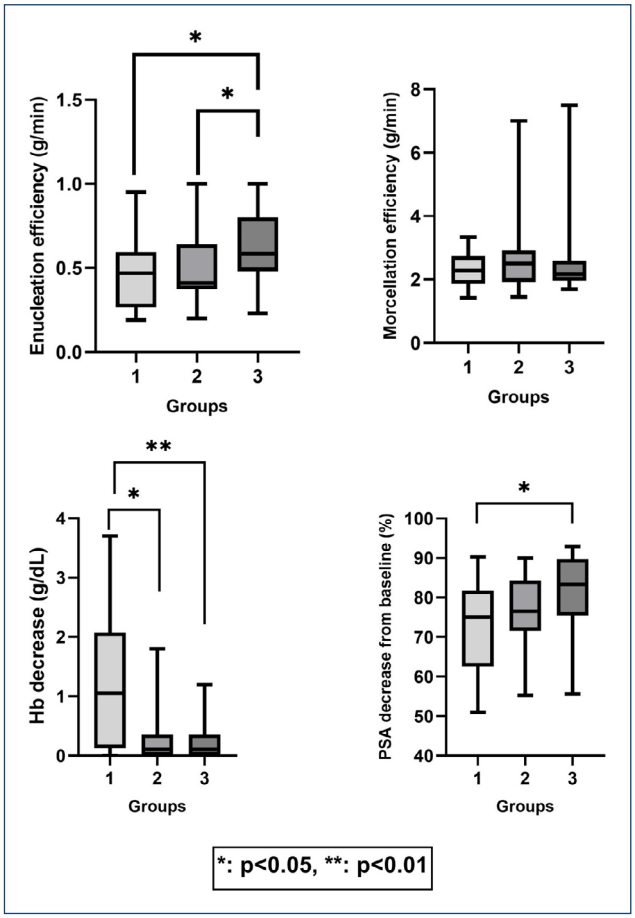

Figure 1. Some specific parameters that differed significantly between the groups.

DISCUSSION

The en bloc LEP learning curve following early apical release utilizing a high-power (200 W) Tm:YAG laser has not yet been the subject of any studies that have been reported in the literature. We must admit that before we begin the procedures, we believe this procedure may have a challenging learning curve. However, as the key conclusion of our research, we can state that after 40 instances, certain significant problems, such as capsular perforation, mucosal damage, and transitory stress incontinence, significantly decreased. After 20 cases, there was a considerable decline in Hb levels. In our final 20 patient group, a significant increase in enucleation efficiency was seen. One measure of enucleation effectiveness is the decline in PSA percentage levels from the baseline level, and this parameter significantly increased after the first 20 instances[13]. After the first 20 instances, we began to spend more attention on post-enucleation hemorrhage management. Thus, we obtained a clear image during morcellation. Therefore, while our enucleation times gradually decreased, no significant difference was observed between the groups in terms of total operation times. On the contrary, our mucosal injury rates decreased after the first 20 cases.

The learning curve for ThuLEP might be managed after more than 20 first instances, according to Aydogan et al. In their study, a high-power (200 W) T:YAG laser was employed, the same as in our investigation, but the three-lobe method of resection was selected. According to this study, complication rates started to drop after 20 cases. After 40 instances in our study, it can be claimed that we are in a safer region when parameters like capsular perforation, temporary incontinence (which in our experiment lasted for a maximum of 4 months), and resectoscope-assisted cauterization requirements are considered[14]. Tuccio et al.'s holmium LEP (HoLEP) series were evaluated comparatively according to the enucleation technique used. They showed that en bloc with an early apical release strategy can significantly have a shorter enucleation time and lower energy delivered. The stress incontinence rate at the 1-month follow-up was found to be significantly reduced in the en bloc group compared with the three-lobe technique. As a result, they stated that both methods can be used safely and effectively in the treatment of BPH[15]. According to Rapaport et al., due to the quick

identification of the surgical capsule and the appropriate layer, en bloc holmium enucleation of the prostate resulted in a shorter enucleation and overall surgical time compared to typical HoLEP. By employing this method, surgical trainees can learn holmium laser enucleation of the prostate more effectively[16]. Our study similarly showed that an experienced endourologist made significant progress for en bloc ThuLEP after 40 cases of experience. No complications were found on Clavien 3 in this process.

Kampantais et al. analyzed the results of 24 studies evaluating the learning curve of HoLEP in their published systematic review. With the caveat of careful case selection, such as avoiding patients with prostates larger than 80 cm^3, cancer or postradiotherapy cases, anticoagulant users, or catheterized patients, the caseload for a surgeon to safely perform the procedure with satisfactory efficiency and outcomes may be estimated at 50 cases. This can drop to less than 25 cases in the presence of a structured mentoring program and simulation training[17]. Inclusion in this study is not contingent on a particular method of enucleation. Neither the trilobed bilobed nor the en bloc approaches were differentiated from one another. Using high-energy thulium, this study aims to highlight the steepness of the learning curve associated with the en bloc approach. Our results revealed that after 40 cases

Table 1. Patient characteristics and perioperative outcomes between the groups.

	Group 1		Group 2		Group 3		p	p (Group 1–2)	p (Group 1–3)	p (Group 2–3)
Age (years)	66	(62–75)	70	(64–73)	69	(62–80)	0.614			
PSA (ng/mL)	4.20	(2.35–7.40)	2.70	(1.30–4.40)	1.70	(1.20–2.50)	**0.015**	0.317	**0.014**	0.604
Prostate volume (mL)	99	(68–120)	78	(67–95)	73	(55–119)	0.339			
PVR preop. (mL)	250	(135–316)	168	(95–333)	109	(66–200)	0.180			
Q-max preop. (mL/s)	9.7	(6.9–12.1)	5.7	(1.8–10)	8.6	(8.1–12.8)	0.316			
Enucleation time (min)	120	(97–128)	70	(58–79)	48	(42–69)	**<0.001**	**0.001**	**<0.001**	0.076
Morcellation time (min)	20	(10–28)	14	(10–15)	10	(10–20)	0.101			
Total operative time (min)	80	(51–116)	68	(53–81)	59	(44–85)	0.503			
Enucleation weight (g)	50	(34–60)	30	(26–35)	24	(20–40)	**0.002**	**0.012**	**0.001**	0.362
Preop. Hb (g/dL)	14.3	(12.6–14.8)	12.9	(11.5–14.7)	13.1	(12.4–14.1)	0.315			
Postop. Hb (g/dL)	12.3	(11.4–13.9)	13.1	(11.1–14.3)	13.3	(12.1–13.9)	0.622			
Enucleation efficiency (g/min)	0.47	(0.28–0.59)	0.41	(0.38–0.64)	0.59	(0.48–0.80)	**0.019**	0.986	**0.015**	**0.014**
Morcellation efficiency (g/min)	2.28	(1.87–2.66)	2.50	(1.95–2.91)	2.18	(1.98–2.58)	0.766			
Hb decrease (g/dL)	1.05	(0.15–1.95)	0.10	(0–0.30)	0.10	(0–0.30)	**0.003**	**0.008**	**0.002**	0.627
IPSS preop.	20	(18.5–25)	20.5	(17–27)	22.5	(19.5–24)	0.614			
IPSS postop. 6 months	6	(3.5–9)	4.5	(3–7)	5	(2–6)	0.093			
QoL score preop.	4	(3–4)	4	(3–4)	4	(3.5–4)	0.635			
QoL score postop.	1	(1–2)	1	(1–2)	1	(1–1.5)	0.426			
Catheter stay time (h)	42.5	(36.5–48)	40.5	(31.5–44)	24.5	(17–36.5)	**<0.001**	0.674	**<0.001**	**0.024**
Hospitalization (h)	50	(44.5–55.5)	46	(35.5–52)	28	(21–41)	**<0.001**	0.402	**0.005**	0.282
PSA postop. 6 months (ng/mL)	1.13	(0.55–1.74)	0.55	(0.22–1.52)	0.29	(0.10–0.46)	**0.006**	0.317	**0.011**	0.604
PSA decrease from the baseline (%)	75.05	(63.1–81.1)	76.55	(71.6–83.8)	83.3	(75.8–89.4)	**0.026**	0.139	**0.030**	1.000
Q-max postop. 6 months (mL/s)	23.5	(17.4–31.4)	22	(19–31.6)	24	(21–32)	0.420			
PVR postop. 6 months (mL)	23	(0–50)	0	(0–50)	0	(0–25)	0.252			
IIEF-5 score preop.	14	(12–16)	14	(11.5–17.5)	15	(13–17)	0.533			
IIEF-5 score postop. 6 months	14	(12–16)	15	(13.5–17.5)	15	(13.5–17.5)	0.291			

Data are given as median-(IQR); the Kruskal-Wallis test was used. PSA: prostate-specific antigen; PVR: post-void residual urine; Preop.: preoperative; Postop.: postoperative; Hb: hemoglobin; QoL: quality of life; IPSS: International Prostate Symptom Score; IIEF-5: International Index of Erectile Function-5; Q-max: maximal flow rate. Bold indicates statistically significant p-value.

Table 2. Number and classification of perioperative and postoperative complications.

	Group 1	Group 2	Group 3
Clavien-Dindo classification			
Absent*	9 (45%)	14 (70%)	17 (85%)
1	8 (40%)	4 (20%)	2 (10%)
2	2 (10%)	2 (10%)	0
3a	1 (5%)	0	1 (5%)
Column sclerosis	1 (5%)	0	0
Urethral stricture	0	0	1 (5%)
Capsular perforation	3 (15%)	1 (5%)	0
Re-catheterization	2 (10%)	0	0
Mucosal injury during morcellation	2 (10%)	0	0
Cauterization with resectoscope	3 (15%)	1 (5%)	1 (5%)
Stress incontinence	1 (5%)	1 (5%)	0

Data are presented as numbers and percentages. *According to Clavien-Dindo Scoring, there is a significant difference between groups when complications are classified as present or absent (χ^2 test, p=0.025).

of en bloc ThuLEP, an experienced endourologist made significant improvements.

Saredi et al. used a simulator program to assess the learning curve for ThuLEP without the assistance of a mentor. Visits to several centers with HoLEP and ThuLEP expertise served as the starting point for the learning process. The ThuLEP method was then practiced using the brand-new simulator Cybersim. The majority of surgical complications were from morcellation rather than the actual enucleation and were quickly resolved. The study's findings are consistent with other case studies for laser treatments of BPH in the literature. Their findings indicate that fewer instances than those for HoLEP are required for an endoscopically skilled urological surgeon to learn ThuLEP, and mentoring is not required for this method. They discovered that a single operator may pass the learning curve after practicing the process in 30 cases[18].

The functional outcomes of our investigation demonstrated that erectile functions were comparably retained 6 months following surgery as compared to the preoperative period. On the contrary, the patient's symptoms in the lower urinary tract dramatically improved. Our functional results and those from prior research on this topic in the literature show remarkable overlap[19].

The purpose of this study was to measure the steepness of the learning curve by tracking how many cases were handled. However, it is important to keep in mind that the learning curve may also be influenced by other factors. According to the research, the threshold for expert performance varies across different outcome metrics. Prostate size before surgery, total number of procedures, and case density are the primary factors affecting the shape of the curve. Increased success rates would result from intensive one-on-one training for surgeons and a high number of cases[20].

CONCLUSION

Our research confirmed that an experienced endourologist can perform ThuLEP surgery with the en bloc early apical release technique without a steep learning curve. Due to training from an experienced mentor, findings at the start of the learning curve are similar to those of other methods. In the early postoperative period, complications are manageable, but beyond 40 cases, results can be in a safer area regarding complications and efficacy.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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A comparison of the rates of and indications for cesarean delivery between Syrian refugee women and Turkish women

Kemal Hansu[1*]

SUMMARY

OBJECTIVE: The aim of this study was to compare the rates of and indications for cesarean delivery among Syrian refugee women and local Turkish women.

METHODS: The study included 74,864 pregnant women, of whom 52,145 were Turkish and 22,719 were Syrian refugee women and who gave birth at our hospital between January 2013 and December 2021. In this study, the pregnant women were divided into two groups: Syrian refugee women and Turkish women, and primary cesarean delivery rates were calculated separately for each group. Cesarean delivery rates for Syrian refugee women and Turkish women were compared separately for each year. Indications for cesarean delivery were determined separately for each group and compared between the groups.

RESULTS: The overall cesarean delivery rate was 56% among Turkish women and 32% among Syrian women ($p<0.05$). The primary cesarean delivery rate was 18.4% for local Turkish women versus 10.7% among Syrian refugee women ($p<0.05$). The most common indication for cesarean delivery among both Syrian refugee women and local Turkish women was previous cesarean delivery, followed by acute fetal distress and cephalopelvic disproportion.

CONCLUSION: Indications for cesarean delivery were similar for Syrian refugee women and local Turkish women, but both overall and primary cesarean delivery rates were higher among local Turkish women compared with Syrian refugee women.

KEYWORDS: Cesarean section. Refugees. Pregnancy. Pregnancy rate. Labor presentation.

INTRODUCTION

Cesarean delivery is defined as the delivery of the fetus through an abdominal incision and is used when vaginal delivery is not safe and when there is an increased risk of morbidity and mortality for the mother or child[1,2].

Cesarean section is one of the most common surgical procedures across the world. Although the ideal cesarean delivery rate has been declared to be 10–15% by the World Health Organization (WHO), the incidence of cesarean delivery is rapidly increasing worldwide, particularly in middle- and high-income countries[3,4]. While cesarean delivery rates are increasing in middle- and high-income countries, this increase remains low in less-developed countries, particularly in African countries. Differences in cesarean delivery rates between countries may be attributed to pregnant women's access to health care or the policies implemented by governments.

One of the most common indications for cesarean delivery is previous uterine surgery and previous cesarean delivery[5,6]. Studies from both Turkey and other countries have reported that the most common causes of primary cesarean delivery were malpresentation followed by fetal distress and cephalopelvic disproportion[7-9]. Although there are no significant differences in indications for cesarean delivery globally, there can be significant differences in cesarean delivery rates between countries or even between different regions within the same country. Approximately 5 million Syrians were forced to flee their country due to the civil war that started in Syria in 2011[10]. As of 2022, Turkey has hosted more than 3.5 million Syrians, of whom around 100,000 lived in Kahramanmaras.

This study aimed to compare the rates of and indications for cesarean delivery among local Turkish women and Syrian refugee women who are from different countries and have different cultural patterns.

METHODS

Our study included 74,864 pregnant women who gave birth at the Kahramanmaraş Necip Fazıl City Hospital between January

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The study was approved by Kahramanmaras Sutcu ImamUniversity Faculty of Medicine Clinical Researches Ethics Committee with the ethical committee decision dated November 14, 2022, and numbered 2022/25-09. The study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of the country of origin; Turkey.

1, 2013, and December 31, 2021. Data were extracted from the hospital information management system using retrospective screening. The study received approval on December 14, 2022 (decision number 2022/25-09) from the clinical research ethics committee of the Kahramanmaraş Sütçü İmam University School of Medicine. The pregnant women included in the study were divided into two groups: Syrian refugee women and Turkish women, after which overall and primary cesarean delivery rates were calculated separately for each group. The overall cesarean delivery rate was calculated using the formula: total number of cesarean deliveries/total number of deliveries, and the primary cesarean delivery rate was calculated using the formula: primary cesarean deliveries/total number of deliveries. The primary cesarean delivery rates for Syrian refugee women and Turkish women were compared separately for each year. Indications for cesarean delivery for local Turkish women and Syrian refugee women were determined and compared between the two groups.

The collected data were analyzed using the IBM SPSS Statistics 22 software. Quantitative variables were reported in mean values and percentage, whereas categorical variables were reported in frequency and percentage. Cesarean delivery rates by year were analyzed, and indications for cesarean delivery were compared between the groups using the chi-square test. Statistical significance was set at $p<0.05$ for all statistical evaluations.

RESULTS

This study included 74,864 pregnant women (of whom 52,145 were Turkish and 22,719 were Syrian refugee women) who gave birth at our hospital between January 2013 and December 2021. Of all the 74,864 pregnant women, 51% (38,156) had vaginal delivery and 49% (36,707) had cesarean delivery. Of the Turkish women, 44% (21,166) had vaginal delivery and 56% (29,374) had cesarean delivery, while 68% (14,991) of the Syrian women had vaginal delivery and 32% (7,332) of them had cesarean delivery. The overall cesarean delivery rate was statistically higher for Turkish women compared with Syrian refugee women (56 vs. 32%; $p<0.05$).

A comparison of the primary cesarean delivery rate between Turkish women and Syrian women revealed a statistically significantly higher rate of primary cesarean delivery among Turkish women than that Syrian women (18.4 vs. 10.7%; $p<0.05$). Additionally, an analysis of primary cesarean delivery rates by year revealed that Turkish women had statistically significantly higher rates of primary cesarean delivery compared with Syrian women during all years except in 2013 ($p=0.093$) (Table 1 and Figure 1).

The analysis of the indications for cesarean delivery for Turkish and Syrian women showed that the most common indication for cesarean delivery was repeated cesarean delivery in both groups and the second most common indication was acute fetal distress. A comparison of indications for cesarean delivery between Turkish and Syrian women showed that Turkish women had significantly higher rates of cesarean delivery due to repeated cesarean delivery, prolonged labor, cephalopelvic disproportion, acute fetal distress, malpresentation, and placental abnormality ($p<0.05$) (Table 2 and Figure 2).

An analysis of the number of births by year showed that, in 2013, 7,920 Turkish women gave birth at our hospital, and this number decreased thereafter; however, only 4,491 Turkish women gave birth in 2021. On the contrary, 916 Syrian women gave birth at our hospital in 2013 versus 2,921 in 2021 (Figure 3).

DISCUSSION

Cesarean section is a surgical procedure used to preserve maternal and fetal health when vaginal delivery cannot be performed safely. While cesarean delivery is a life-saving procedure when performed for medical indications, it is also associated with increased maternal and fetal morbidity and mortality, including anesthesia complications, peripartum hemorrhage, bowel and bladder injuries, venous thromboembolism, placental adhesion anomalies, surgical site infection, and fetal respiratory problems when performed without appropriate indications[11].

Cesarean delivery rates are increasing all over the world, and this increase is influenced by changes in lifestyles and health policies in each country[12]. A 2016 study of 150 countries found that the global average cesarean delivery rate was 18.4%. The lowest cesarean delivery rate was 3% in West Africa, and the highest was 56.4% in the Dominican Republic. Turkey was found to have a cesarean delivery rate of 47.5%, the second

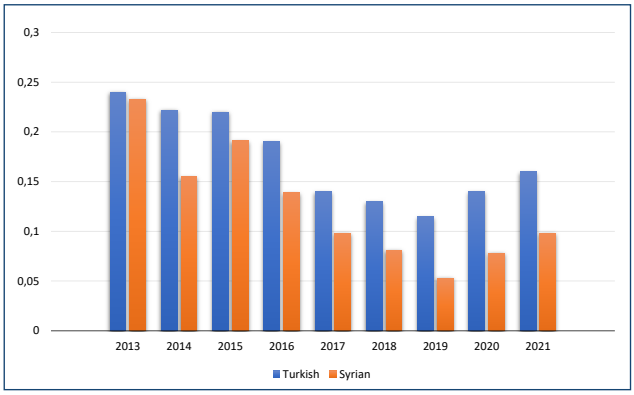

Figure 1. Primary cesarean section rates of Turkish and Syrian pregnant women (Kemal Hansu).

Table 1. Comparison of primary cesarean section rates of Syrian refugee pregnant women and Turkish pregnant women (Kemal Hansu).

Year	Turkish pregnant women	Syrian pregnant women	p-value
	Primary cesarean sections/total birth	Primary cesarean sections/total birth	
2013	24% (1,905/7,920)	14.5% (133/570)	0.093
2014	22% (2,745/7,838)	15.5% (259/1,669)	**<0.05**
2015	22% (1,509/6,709)	19.1% (411/2,142)	**<0.05**
2016	19% (1,220/6,354)	13.9% (368/2,644)	**<0.05**
2017	14% (771/5,336)	9.8% (314/3,181)	**<0.05**
2018	13% (592/4,578)	8.1% (268/3,291)	**<0.05**
2019	11.5% (521/4,506)	5.3% (165/740)	**<0.05**
2020	14% (639/4,443)	7.8% (226/2,881)	**<0.05**
2021	16% (729/4,491)	9.8% (288/2,921)	**<0.05**
Total	18.4% (9,631/52,145)	10.7% (2,432/22,728)	**<0.05**

Statistically significant values are indicated in bold.

Table 2. Comparison of cesarean section indications of Syrian refugee pregnant women and Turkish pregnant women (Kemal Hansu).

Indications	Percentage of Turkish pregnant women (n/N)	Percentage of Syrian pregnant women (n/N)	p-value
Repetitive	70.2% (20,643/29,374)	67% (4,901/7,332)	**<0.05**
Abnormal labor progress	1% (313/29,374)	0.3% (22/7,332)	**<0.05**
Cephalopelvic disproportion	2% (611/29,374)	1.5% (113/7,332)	**<0.05**
Acute fetal distress	24% (7,095/29,374)	26.7% (1,959/7,332)	**<0.05**
Abnormal fetal presentation/position	1.5% (439/29,374)	1% (71/7,332)	**<0.05**
Umbilical cord prolapse	0.02% (7/29,374)	0.014% (1/7,332)	0.59
Preeclampsia	0.18% (54/29,374)	0.12% (9/7,332)	0.25
Multiple pregnancy	0.85% (249/29,374)	1% (78/7,332)	0.8
Placental anomaly	0.63% (185/29,374)	0.12% (15/7,332)	**<0.05**
Patient preference	1% (316/29,374)	0.8% (61/7,332)	0.066
Elective	1.2% (353/29,374)	1.3% (101/7,332)	0.229

n: number of cesarean sections; N: total number of cesarean sections. Statistically significant values are indicated in bold.

highest rate in Asia after Iran[3]. Statistics from Turkey show gradually increasing cesarean delivery rates; it was around 7% in 1993 and reached 58.4% in 2021, while the primary cesarean delivery rate was 29.1%[13,14]. In 2015, Turkey had the highest cesarean delivery rate among all the Organisation for Economic Co-operation and Development countries.

As a result of the civil war that broke out in Syria in 2011, approximately 5 million Syrians were forced to flee their country. Turkey hosts approximately 3.5 million Syrian refugees, of whom approximately 100,000 live in Kahramanmaras. Cesarean delivery rates are known to be influenced by lifestyles, beliefs, cultural patterns, level of access to health services, and health policies implemented by governments. In this study, the cesarean delivery rate was found to be 56% among local Turkish women versus 32% among Syrian refugee women. Although the ideal cesarean delivery rate was declared to be 15% in a 1985 recommendation by the WHO, this rate seems to have changed today as a result of changes in lifestyles, increase in sedentary lifestyle, advanced maternal age, increase in pregnancies achieved with assisted reproductive techniques, development of antenatal tests, and an increase in medical malpractice lawsuits[4]. These developments prompted the WHO to recommend in 2015 that cesarean delivery should be used only for women who need it instead of trying to reach a certain cesarean delivery rate. In Turkey, both overall and primary cesarean delivery rates are higher in Western Anatolia and the Aegean

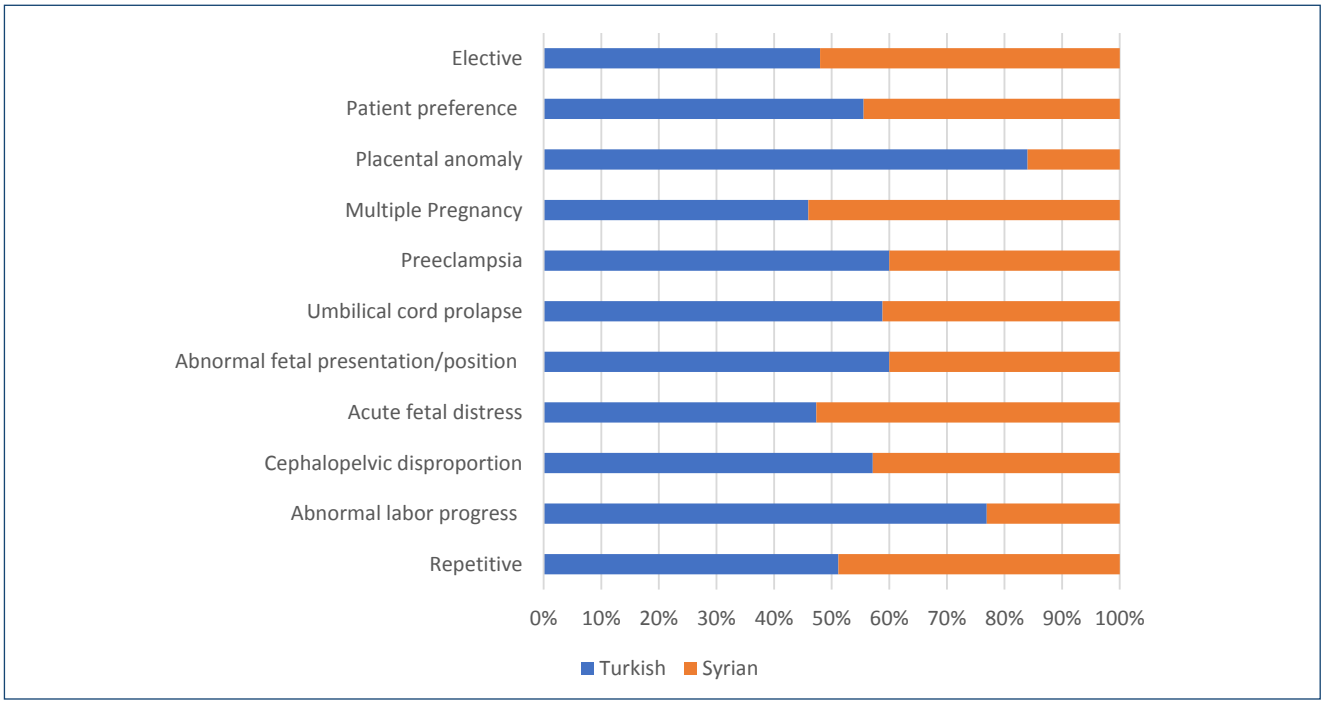

Figure 2. Comparison of cesarean section indications in Turkish pregnant women and Syrian pregnant women (Kemal Hansu).

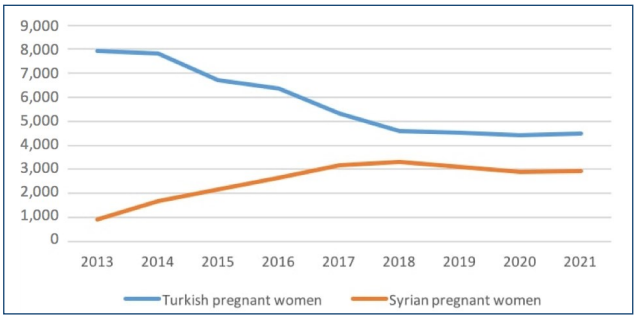

Figure 3. Change in the total number of births of Turkish and Syrian pregnant women in our hospital according to the years (Kemal Hansu).

region than those in Central and Eastern Anatolia. This points out the importance of factors affecting differences in cesarean delivery even within the same country. In this study, the overall cesarean delivery rate was 49% and the primary cesarean delivery rate was 16.1% at our hospital. Although these rates are below the national average, they are well above the targets set by the WHO. It is also noteworthy that both overall and primary cesarean delivery rates were significantly higher among local Turkish women compared with Syrian refugee women. This result may be attributed to the limited access to health services and antenatal follow-up for Syrian refugee women compared with local Turkish women[15]. Lower cesarean delivery rates among Syrian refugee women may also be attributed to their tendency to have a greater number of children due to their cultural and social patterns, which may result in them preferring or insisting on vaginal delivery[16]. In line with our study, previous studies from Turkey comparing cesarean delivery rates among Syrian refugee women and local Turkish women have found lower cesarean delivery rates among Syrian refugee women compared with local Turkish women[17-20]. Akin et al. reported a higher primary cesarean delivery rate among Syrian women compared with Turkish women, but their study included only 328 Syrian women and 9,086 Turkish women[10]. Their report may not reflect the reality because of the significant difference between the number of Turkish and Syrian women.

The analysis of primary cesarean delivery rates by year found that the primary cesarean delivery rate was statistically significantly higher among Turkish women compared with Syrian refugee women in all the years analyzed except for 2013. Primary cesarean delivery rates decreased among both local Turkish women and Syrian refugee women after 2016 but started to increase again after 2020 (Table 1). However, it should also be noted that the number of local Turkish women giving birth at our hospital has been decreasing since 2013 (Figure 3). In 2013, 7,920 local Turkish women gave birth at our hospital, and this number decreased to 4,578 in 2018 and remained stable during the following years. The change in cesarean delivery rates in this study may be due to more local women turning to private clinics and university hospitals and to the decrease in the number of pregnant women who presented to hospitals after

2019 as a result of the COVID-19 pandemic, which naturally led to less antenatal follow-up. This may show the impact of access to health services and antenatal follow-up on cesarean delivery rates. This result may also be attributed to low rates of pregnant Syrian women presenting to a hospital or receiving antenatal care due to their cultural characteristics. In addition, the physicians' preference for cesarean delivery to normal birth due to increasing medicolegal problems may have caused an increase in the rate of cesarean section in both Turkish and Syrian refugee pregnant women after 2020.

In this study, the most common indication for cesarean delivery among both Turkish and Syrian women was previous cesarean delivery, followed by acute fetal distress, cephalopelvic disproportion, malpresentation, and obstructed labor. Although some studies[21,22] have reported similar results in terms of the incidence of indications for cesarean delivery, others have reported that the second most common indication was cephalopelvic disproportion or obstructed labor[9,23]. The difference between these studies may be due to the clinical approach of the physicians or to differences in the equipment available in obstetric clinics. Cesarean delivery rates were shown to be significantly higher among women who received continuous fetal monitoring compared with women who received intermittent auscultation[24]. In our clinic, all pregnant women in labor received continuous fetal monitoring, which may have caused an increase in false-positive diagnoses of acute fetal distress. It has also been shown that cesarean section rates may increase due to physicians not waiting long enough before establishing a diagnosis of cephalopelvic disproportion[25]. This study found similar rates of indications for cesarean delivery among both Syrian refugee women and local Turkish women.

The strength of this study is that it included a large number of Turkish and Syrian women and covered a 9-year period. Our study has also some limitations: its retrospective design prevented us from finding out in which cases and for which indications cesarean delivery was decided and precluded the tracking of the perinatal outcomes of the patients.

CONCLUSION

In conclusion, the indications for cesarean delivery were similar for both Syrian refugee women and local Turkish women, but the rates of both overall and primary cesarean delivery were higher among local Turkish women compared with Syrian refugee women.

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ORIGINAL ARTICLE

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Association between microRNA expression and risk of male idiopathic infertility in Iraq

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SUMMARY

OBJECTIVE: The World Health Organization defines infertility as the inability to get pregnant after 12 months of unprotected sexual activity. This study was conducted to estimate the levels of gene expression for two mature miRNAs (i.e., miR-122 and miR-34c-5p) to evaluate susceptibility to male infertility.

METHODS: This study included 50 male patients with idiopathic infertility who were admitted to hospital from the period November 2021 to May 2022 and another group consisting of 50 apparently healthy individuals used as controls.

RESULTS: miR-122 level was significantly highest in azoospermia and followed by oligospermia, 39.22 (31.88) versus 37.34 (20.45), respectively. In addition, there was a very significant difference in miR-34c-5p levels between the study groups ($p<0.05$).

CONCLUSION: Two miRNAs, namely, miR-34c-5p and miR-122, can be used as predictive and diagnostic biomarkers for infertility.

KEYWORDS: Male infertility. MicroRNAs. Gene expression. Azoospermia. Oligospermia.

INTRODUCTION

Failure to become pregnant following a year of unprotected sexual intercourse is known as infertility. On average, it affects 15% of married couples. About 30% of instances involve men and 35% include women[1]. Male infertility is not a single pathological condition but rather reflects several different pathological conditions, and the majority of men who are investigated for infertility cannot identify the cause because the causation of male infertility is obviously diverse and could be immunological, accidental, pathological, physiological, or even nutritional[2]. Most cases appear as a lack of sperm in the ejaculate or a decline in sperm count without evident cause so considered idiopathic male infertility[3].

Male infertility may be due to a defect in sperm morphology and their motility and concentration, as these causes can contribute to 40-50% of male infertility. On this basis, male infertility can be classified into azoospermia, oligozoospermia, asthenozoospermia, and teratozoospermia[4].

MicroRNAs are short non-coding RNAs (i.e., less than 22 nucleotides) that control the amount of proteins by preventing the translation of mRNA and/or accelerating its degradation. The seed sequence of the microRNA, which is found from the second to the eighth nucleotide from the 5'-end of microRNAs, pairs with a complementary sequence in the target mRNA transcript, which is typically found in the 3' untranslated region, to recognize the target mRNA[5].

Spermatogenesis requires a highly organized gene expression. Organization of gene expression during spermatogenesis occurs at several levels such as transcriptional, post-transcriptional, and even epigenetic modification. Studies have identified around thousands of genes that are coding proteins responsible for the regulation of spermatogenesis[6]. Regulation of gene expression by miRNA is one of the most important levels of post-transcriptional regulation levels. miRNA expression varies at each stage of spermatogenesis, so there are specific types of miRNAs at each stage of sperm production and maturation and the aberration of miRNA is considered a molecular etiology of male infertility and dysregulated expression of miRNA can be inherited by progeny[7].

Previous studies have discovered changed miRNAs in the seminal plasma, spermatozoa, and total semen of asthenozoospermia males[8]; therefore, miRNA emerged as a promising biomarker that can ravel out many molecular etiologies underlying cases of idiopathic infertility[9]. Defects in miRNA expression can explain many problems associated with sperm production, motility, and fertilization ability. Sertoli cells and Leydig cells, which provide the niche for spermatogonial stem cells (SSCs) and subsequently give nutritional and structural support for germ cells, are regulated in their functionality and development by miRNAs. As a result, miRNAs in somatic

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cells play crucial roles in spermatogenesis through the control of genes, which in turn encode proteins with significant functions in spermatogenesis[7].

In recent years, several studies have focused on the role of miRNA in sperm maturation within the epididymis. These studies indicated that miRNA is a communication language between cells of epididymis epithelium and sperm, as transcription of sperm is repressed due to condensation of chromatin, and during spermatogenesis, miRNA plays an important role in the regulation of sperm activities after repressing of its genome and also a key role in early embryogenesis[10].

CBL, a tumor suppressor gene called Casitas B-lineage lymphoma, is the miR-122-5p target gene. SSCs' proliferation and DNA synthesis are enhanced and apoptosis is prevented when CBL is inhibited by miR-122-5p (SSCs)[11].

Deleted in azoospermia-like (dazl) gene is one of miR-34b and miR-34c's likely targets[12]. Members of the MiR-34/449 family perform important functions in spermatogenesis by controlling testicular functionality and spermatozoa maturation. Numerous features of idiopathic male infertility extending from oligozoospermia to non-obstructive azoospermia (NOA) are related to dysregulation of miR-34/449[13].

METHODS

Subject

This study included 50 male infertile patients with ages ranging from 20 to 43 years, who attended to the infertility and IVF center in Al-Sader City Hospital for the period from November 2021 to May 2022. Other (50) healthy subjects without any history of systemic disease were included as a control group. Any infertile man with reproductive system diseases, surgery history, diagnosed varicocele, patients having cryptorchidism and testicular tumors, or undergoing chemotherapy and radiotherapy were excluded from this study. Blood samples were taken; 3 mL of blood per case was drawn through vein puncture. The miR-34c-5p and miR-122 expression levels were then measured using real-time PCR after RNA had been extracted. After explaining the purpose and potential outcomes of the study to each study participant, their informed agreement was acquired.

Stem-loop RT-qPCR

The miRNA-122 and miRNA-34c-5p expressions in the serum of patient and control groups were measured by using the stem-loop RT-qPCR, and the housekeeping gene (GAPDH) was used as a reference. The "TRIzol® reagent kit" was used to extract the total RNA from serum samples following the manufacturer's guidelines. A Nanodrop spectrophotometer (THERMO, USA) was used to measure the purity and concentration of the extracted RNA by reading the absorbance at 260/280 nm. DNase I enzyme was used to treat the extracted RNA to eliminate any remaining genomic DNA in the eluted total RNA and done according to the instructions provided by Promega Company (USA). The Sanger Center miRNA database registry was used to choose the miRNA sequence, and the miRNA primer design tool was used to design the primers for miRNA-34c-5p and miRNA-122. The M-MLV Reverse Transcriptase kit was used to synthesize cDNA from the DNase-I treated RNA samples according to manufacturer's instructions. The housekeeping gene (GAPDH) used in the normalization of the expression analysis of miRNA-34c-5p and miRNA-122 was quantified using a quantitative real-time PCR.

Statistical analysis

Data were either normally distributed or not normally distributed and recorded in a Microsoft Excel spreadsheet. Statistical analysis was carried out with the SPSS v 0.26 software (chi-square, independent sample t-test, Spearman, and Pearson correlation coefficient) after translating data into codes. The results were analyzed and assessed using appropriate statistical methods. When a p-value is less than 0.05, the significance level is considered.

RESULTS AND DISCUSSION

Demographic and clinical parameters

The infertile patients and controls had a similar age range. This study revealed that the majority of patients (50%) belonged to the age group of less than 30 years and 34% of the patients had the age group of 30-39 years (Table 1). The data showed that semen volume was not significant ($p>0.05$) when compared between oligospermia and azoospermia (Table 2).

Previous research showed a rise in infertile males between the ages of 26 and 35 years (63.5%) because the youngest age groups are seeking more medical advice, are more focused

Table 1. Distribution of control and patients according to their age.

Age groups(years)	Controls N (%)	Patients N (%)
<30	24 (48%)	25 (50%)
30-39	18 (36%)	17 (34%)
≥40	8 (16%)	8 (16%)
Total	50 (100%)	50 (100%)

on fathering children, and are also under more psychological stress due to family obligations. Sperm functions, ejaculation frequency, and semen quality were found to gradually decline with older age and begin to decline after 35 years[14]. This active age group may be highly susceptible to infertility due to economic difficulties, stress, and pollution[15].

Semen volume in patients did not significantly alter, which is in agreement with the research by Majed et al.[16], which found no significant increases in semen volume. The mean seminal fluid volume between the investigated groups of controls and infertile is larger than the World Health Organization (WHO)'s more stringent recommendations for how to determine a suitable seminal fluid volume, which is estimated to be at about 1.5 mL. The prostate gland and seminal vesicles and a small amount from the bulbourethral glands are mainly contributed to the volume of the ejaculate (WHO)[1].

Comparison of hsa-miR-122 and hsa-miR-34c-5p among patients and control groups

The levels of hsa-miR-122 and hsa-miR-34c-5p are compared between study groups (Table 3). miR-122 was significantly highest in azoospermia and followed by oligospermia, 39.22 (31.88) versus 37.34 (20.45), respectively, and the statistical significance level was high ($p<0.05$). In addition, there was a very significant difference in miR-34c-5p levels between study groups ($p<0.05$); the highest level was found in the oligospermia group followed by the azoospermia group, 36.64 (22.24) versus 22.31 (17.73), respectively (Table 3).

Table 2. Seminal fluid volume in controls and infertile individuals (oligozoospermia and azoospermia).

Groups	No.	Volume (mL)		
		Mean±SE	Minimum	Maximum
Oligospermia	25	2.31±0.11	1.3	3.2
Azoospermia	25	2.42±0.22	1.2	3.8
Controls	50	2.50±0.15	1.5	3.9

According to a recent study by Fang et al.[17], patients with NOA who had unsuccessful sperm retrievals had lower expression of miR-34c-5p in their testicular tissue than those who had successful retrievals. Also, the expression profile of miR-34c-5p was similar between patient-specific testicular tissue and seminal plasma samples.

In NOA patients' seminal fluid compared with controls, the expression of all miRNAs examined, namely, miR-34c-5p, miR-34b-3p, miR-122-5p, and miR-509-5p, was significantly decreased ($p<0.001$)[18]. Seminal plasma exosomes showed differential expression of miR-34c-5p and miR-122-5p in normozoospermic and azoospermic individuals[19]. Patients with NOA had significantly lower levels of the miRNAs (hsa-miR-202-3p and hsa-miR-34c-5p) in their seminal plasma compared with healthy fertile individuals ($p<0.05$)[20]. Recent research by Liu et al.[21] revealed 173 miRNAs that differed in their expression between spermatids and spermatocytes in human azoospermic patients. Among those, miR-34b-5p and miR-34c-5p significantly decreased in round spermatids as compared with spermatocytes, and it has been suggested that these miRNAs are essential for spermatocyte meiosis.

Mokánszki et al.[22] demonstrated that infertile males (asthenozoospermic and oligozoospermia groups) had significantly lower expression levels of three microRNAs (i.e., miR-122, miR-34b, and miR-15b) in their seminal plasma and spermatozoa than normal men. Significant differences between asthenozoospermic and oligozoospermia ejaculates were found in the relative expression level of these miRNAs in spermatozoa ($p<0.05$). In the comparison of microarray and qRT-PCR analyses, we found that miR-122 expression was downregulated, whereas miR-141 expression was upregulated in the oligoasthenozoospermia group. Moreover, miR-141 expression was upregulated and miR-122 expression was downregulated in the asthenozoospermic comparison of microarray and qRT-PCR analyses[23]. Abu-Halima et al.[24] showed that hsa-miR-429 was significantly upregulated, while three miRNAs (i.e., hsa-miR-122, hsa-miR-34c-5p, and hsa-miR-34b) were significantly downregulated. When compared with control males,

Table 3. Comparison of the protein expression for miRNAs in the study groups.

miRNA	Control *n*=50	Azoospermia *n* = 25	oligospermia *n* = 25	P1	P2	P3
miR-122						
Median (IQR)	1 (0.11)	39.22 (31.88)	37.34 (20.45)	p<0.05 HS	p<0.05 HS	p<0.05 HS
Range	0.28—1.77	4.69—99.55	2.32—95.46			
miR-34c-5p						
Median (IQR)	1 (0.08)	22.31 (17.73)	36.64 (22.24)	p<0.05 HS	p<0.05 HS	p<0.05 HS
Range	0.16—1.12	3.66—97.80	1.03—99.14			

the expression levels of the downregulated miRNAs in the spermatozoa of subfertile men ranged from 3.84-fold for hsa-miR-122, 2.67-fold for hsa-miR-34b, and 2.56 for hsa-miR-34c-5p, up to 7.73-fold for hsa-miR-34b.

As miRNAs are found in the seminal plasma, extracellular vesicles, sperm cells, testis, and epididymis, they may affect spermatogenesis and embryogenesis by altering the target gene's expression. Clearly, such disturbances have the potential to lead to a variety of infertility conditions[25].

CONCLUSIONS

There was a very significant difference in miR-34c-5p and miR-122 levels between study groups. The prevalence of infertility has significantly increased worldwide. Therefore, it is advised that the health institution undertake a prescreen for the genes of these miRNAs, which may serve as a prognostic and diagnosed marker of patients with infertility.

ETHICAL APPROVAL

This study obtained ethical approval from the University of Al-Qadisiyah, and written consent was taken from all participants in the research (patient group and control group).

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AUTHORS' CONTRIBUTIONS

MMK: Conceptualization, Data curation, Formal Analysis, Funding, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **AAM**: Conceptualization, Data curation, Formal Analysis, Funding, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of thyroid functions and obesity in obstructive sleep apnea syndrome

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SUMMARY

OBJECTIVE: Obstructive sleep apnea syndrome is associated with many chronic diseases.

METHODS: Obesity and thyroid function tests were evaluated retrospectively and cross-sectionally for 782 obstructive sleep apnea syndrome patients.

RESULTS: The mean patient age was 49.3±11.5 years, and the majority were obese (67.9%) or overweight (26.6%). The mean age of the patients in Group 2 (moderate/severe obstructive sleep apnea syndrome) was higher than that of Group 1 (simple snoring/mild obstructive sleep apnea syndrome). The rate of severe obstructive sleep apnea syndrome among obese patients (35.2%) was significantly higher than that of normal-weight (11.6%) and overweight (18.3%) patients (p=0.001). The oxygen desaturation index/apnea-hypopnea index and levels of leukocytes and C-reactive protein were significantly higher, while mean/minimum saturation values and hemoglobin, hematocrit, and free triiodothyronine levels were significantly lower among obese patients compared with overweight and normal-weight patients (p=0.001). Leukocytes, C-reactive protein, and apnea-hypopnea index/oxygen desaturation index values were higher, and mean/minimum saturation values were lower in Group 2 than in Group 1.

CONCLUSION: There were relationships between obstructive sleep apnea syndrome severity and body mass index. Obesity could be a critical predisposing factor for sleep disturbances. The prevention and control of obesity is important while being treated for obstructive sleep apnea syndrome.

KEYWORDS: Body fat distribution. Body mass index. Obesity. Sleep apnea, obstructive.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a frequent sleep disorder in the middle-aged population[1]. Obesity, with increased body fat ratio (BFR), has been associated with numerous chronic diseases[1]. OSAS is related to not only primary obesity but also many chronic diseases[1].

Many endocrine and inflammatory parameters are suspected to be associated with obesity and ultimately OSAS[2]. Hypothyroidism and OSAS are both common in the general population and show some clinical overlap[3,4]. This study investigates the relationships among obesity, thyroid function tests (TFTs), and OSAS using polysomnography (PSG).

METHODS

Study design and ethical approval

In this retrospective cross-sectional study, medical documents of inpatients who underwent PSG in the Polysomnography Unit of Sivas Numune State Hospital, Turkey, in 2012-2018 were evaluated. The study was planned in accordance with the recommendations of the Declaration of Helsinki and good clinical practice guidelines, and the Institutional Ethics Committee for Clinical Research approved the study (Date: 05.05.2021, No.: E-70632468-050.01.04-70430/2021/229).

Study population

The medical records of 960 inpatients who underwent PSG were reviewed. Patients who had active systemic infections or endocrinological and hematological disorders were excluded. These cases were analyzed according to body mass index (BMI) values (i.e., normal weight, overweight, and obese) and OSAS severity (i.e., Group 1: simple snoring/mild OSAS and Group 2: moderate/severe OSAS).

BMI values were calculated as weight (kg)/height^2 (m^2) and grouped as follows: BMI <25, normal weight; BMI 25-30, overweight; and BMI >30, obese. BFR was estimated using the Deurenberg equation [(1.2×BMI)+(0.23×years of age)-(10.8×G)-5.4].

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Polysomnography studies

Under the surveillance of a technician, PSG was performed during an overnight stay. An Embla S4500 PSG device was used for visual and auditory recording. Electromyogram (EMG/submental), electromyogram (EMG/right/left tibialis), two-channel electrooculogram (right/left EOG), and four-channel electroencephalogram were used. Pulse oximetry and nasal airflow were monitored overnight for blood oxygen saturation levels. The mean and minimum values of oxygen saturation were calculated from overnight records.

Mean oxygen saturation of at least 4% below the basal value per hour reflects the oxygen desaturation index (ODI). Data were manually scored, and PSG results were classified according to the American Academy of Sleep Medicine guidelines[5].

The number of total episodes of apnea and hypopnea per hour of sleep constitutes the apnea-hypopnea index (AHI), which was evaluated by PSG and oxygen saturation records. Apnea is defined as a lack of nasal airflow for at least 10 s. The presence of any of the following criteria defines hypopnea: 50% decrease in airflow for at least 10 s, oxygen desaturation of at least 3%, development of arousal, more than 30% reduction in airflow for at least 10 s, or 4% reduction in oxygen saturation. AHI values were classified as follows: AHI <5, simple snoring; 5≤ AHI <15, mild OSAS; 15≤ AHI <30, moderate OSAS; and AHI ≥30, severe OSAS.

Laboratory studies

Laboratory values of free thyroxine (FT4), free triiodothyronine (FT3), thyroid-stimulating hormone (TSH), C-reactive protein (CRP), and complete blood count (CBC) were obtained from the medical records of patients.

Statistical analysis

Kolmogorov-Smirnov and Shapiro-Wilk tests were used to determine normality. Descriptive data were evaluated as percentage (%), number (n), and mean±standard deviation (minimum-maximum). For mean values of data with and without normal distribution, independent-samples t-tests and Mann-Whitney U tests were used, respectively. The comparison of more than two normally distributed groups was performed using one-way ANOVA. Differences between more than two groups without normal distribution were analyzed using the Kruskal-Wallis test. Mann-Whitney U tests and Bonferroni correction were performed for post-hoc analysis. Numerical data were analyzed using Spearman correlation tests. Correlations (r) were accepted as weak at r=0.05-0.30, weak to moderate at 0.30-0.40, moderate at 0.40-0.60, strong at 0.60-0.70, very strong at 0.70-0.75, and perfect at 0.75-1.00. The results were evaluated using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Values of $p<0.05$ were accepted as statistically significant.

RESULTS

The mean age of the 782 patients in this study was 49.3±11.5 years, and most of them were males (55.2%). The number of patients with OSAS (83.1%) was greater than the number of patients with simple snoring (16.9%). The majority of patients were either overweight (26.6%) or obese (67.9%). Flowchart of OSAS patients with their BMI is shown in Figure 1. Laboratory results of all patients are shown in Table 1.

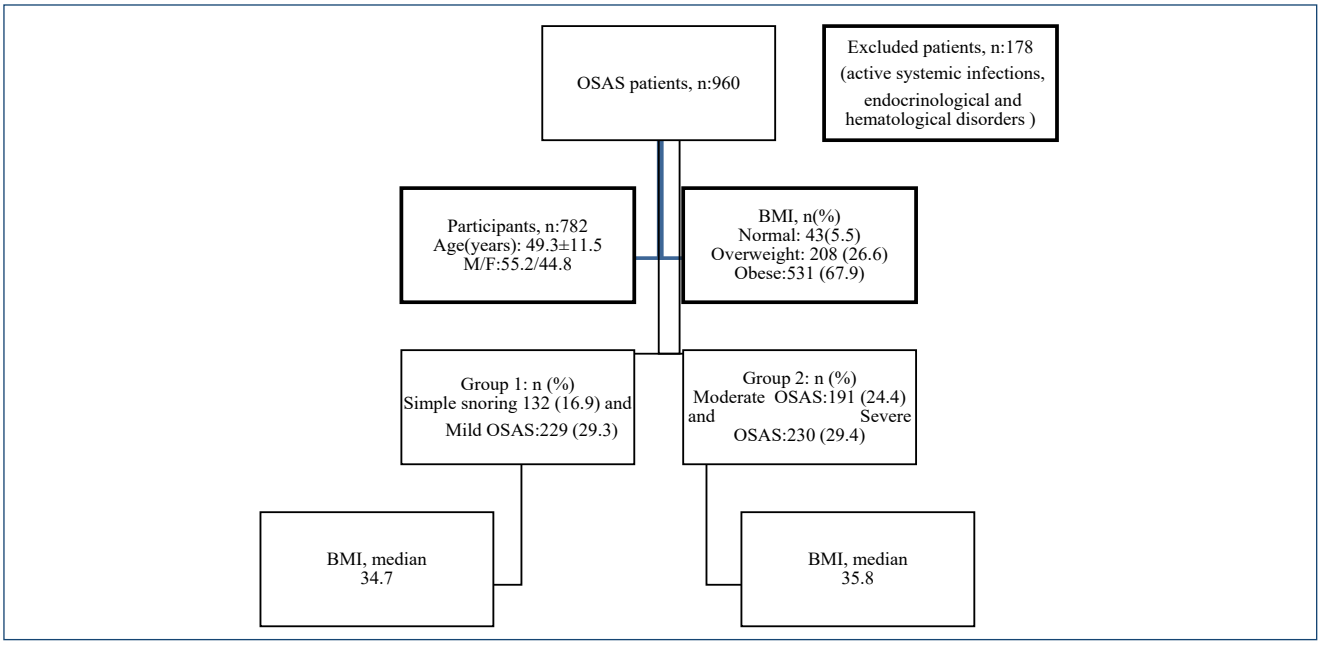

Figure 1. Flowchart of obstructive sleep apnea syndrome patients with their body mass index.

Table 1. Demographics and clinical characteristics of patients grouped according to the severity of obstructive sleep apnea syndrome.

Parameters	All patients	Group 1	Group 2	p
Age (years)	49.3±11.5	46.4±11.4	51.8±11	0.001*
Hemoglobin (g/dL)	14.7 (8.2-19.7)	14.3 (8.2-18.5)	14.3 (10.6-19.7)	0.810
Hematocrit (%)	43.4±5.2	42.4±5.7	43±5.3	0.260
Leukocyte (K/UL)	7.9 (0.5-20.5)	7.8 (3.9-16.6)	8.5 (0.5-20.5)	**0.002**[α]
CRP (mg/L)	0.5 (0.0-23.1)	0.5 (0.0-11.8)	0.6 (0.0-23.1)	**0.001**[β]
TSH (mU/L)	1.7 (0.0-11.3)	1.7 (0.1-10.8)	1.7 (0.0-10.3)	0.772
FT4 (ng/dL)	1.2 (0.1-5.5)	1.2 (0.8-2.7)	1.2 (0.1-3.7)	0.143
FT3 (ng/dL)	3.1±0.5	3.1±0.5	3.0±0.5	0.159
Body mass index	32.6 (20.1-57.8)	34.7 (30.0-51.1)	35.8 (30.0-57.8)	**0.002**[γ]
Body fat ratio	40.0±11.8	43.5±10.3	46.2±10.1	**0.003**[¶]
AHI	16.5 (0.0-109.8)	6.4 (0.0-15)	32.0 (15.0-109.8)	**0.001**[§]
Mean saturation	91.6 (61.4-97.6)	92.5 (66.3-97.6)	90.3 (61.4-96.5)	**0.001**μ
Minimum saturation	80.0 (50.0-95.0)	85.0 (50.0-95.0)	75.0 (50.0-89.0)	**0.001**[ε]
ODI	18.9 (0.1-113.9)	7.8 (0.1-58.6)	36.3 (5.7-113.9)	**0.001**[¥]

Data are shown as Mean±SD or median (minimum–maximum). *p<0.05: significant; BMI: body mass index; CRP: C-reactive protein; TSH: thyroid-stimulating hormone; FT3: free triiodothyronine, FT4: free thyroxine AHI: apnea-hypopnea index; ODI: oxygen desaturation index; OSAS: obstructive sleep apnea syndrome; Group 1: simple snoring and mild OSAS; Group 2: moderate and severe OSAS. *Age was significantly higher in group 2 than group 1; [α]leukocyte count was significantly higher in group 2 than group 1; [β]CRP was significantly higher in group 2 than group 1; [γ]BMI was significantly higher in group 2 than group 1; [¶]BFR was significantly higher in group 2 than group 1; [§]AHI was significantly higher in group 2 than group 1; μmean saturation was significantly lower in group 2 than group 1; [ε]minimum saturation was significantly lower in group 2 than group 1; [¥]ODI value was significantly higher in group 2 than group 1.

Age, BMI, BFR, leukocyte, CRP, AHI, and ODI values were higher, and mean/minimum saturations were lower in Group 2 compared with Group 1 (Table 1).

Estimated BFR values were significantly higher in obese patients (p=0.001), as was the incidence of severe OSAS (35.2%), in comparison with normal-weight and overweight patients (p=0.001). ODI and AHI values (18.4 and 22.6, respectively) were significantly higher, while mean and minimum saturation values (90.6 and 77, respectively) were significantly lower in obese patients (p=0.001) (Table 2).

Obese patients had significantly lower hemoglobin and hematocrit than the overweight and normal-weight patients (p=0.001). In addition, mean leukocyte and CRP levels were significantly higher, and mean FT3 was lower in obese patients than in overweight and normal-weight patients (p=0.001) (Table 2).

No significant correlations between laboratory tests and AHI, ODI, or mean and minimum saturations were found (r<0.05). AHI was weakly positively correlated with age (r=0.262), BMI (r=0.267), and BFR (r=0.233), and ODI and age were also weakly positively correlated (r=0.285). Weak to moderate positive correlations existed between ODI and BMI (r=0.363) and BFR (r=0.324). Mean saturation was moderately negatively correlated with age (r=-0.451), BMI (r=-0.457), and BFR (r=-0.447). Minimum saturation was weakly-moderately negatively correlated with age (r=-0.365) and moderately negatively correlated with BMI (r=-0.449) and BFR (r=-0.451) (p=0.001 for all correlations).

DISCUSSION

In this study, relationships between obesity, TFTs, and OSAS were investigated. Severe OSAS was more common, and mean/minimum oxygen saturation levels were lower in obese patients.

Obesity is known to increase the risk of systemic disease, and there are various studies of its effects on surgical outcomes[2,6]. BMI is a risk factor for complications in the perioperative and postoperative periods of abdominal surgery[6]. However, the relationships between obesity and thoracic surgery outcomes remain unclear. Lung cancer is the most common cause of cancer-related deaths worldwide, and surgical resection is important in its treatment[6,7]. Obese patients who underwent lung lobectomy due to lung cancer were found to have longer operative times compared with non-obese patients and higher rates of postoperative morbidities[6]. However, the rates of perioperative and postoperative complications and postoperative mortality were similar between the groups and higher BMI did not affect the chosen surgical approach[6]. In another study comparing patients undergoing thoracoscopic anatomic lung cancer surgery, the differences between

Table 2. Clinical characteristics of patients grouped according to body mass index.

	Normal weight (n=43)	Overweight (n=208)	Obese (n=531)	p
Hemoglobin (g/dL)	15.1 (11.3-17.4)	15.3 (10.5-18.5)	14.3 (8.2-19.7)	**0.001***
Hematocrit (%)	44.7 (34.1-53.8)	45.0 (33.7-55.0)	42.8 (0.0-60.6)	**0.001**[α]
Leukocyte (K/UL)	7.7 (1.0-12.4)	7.5 (0.0-19.0)	8.2 (0.5-20.5)	**0.001**[α]
CRP (mg/L)	0.2 (0.0-6.7)	0.3 (0.0-14.7)	0.5 (0.0-23.1)	**0.001**[γ]
TSH (mU/L)	1.8 (0.6-4.8)	1.8 (0.0-11.3)	1.7 (0.0-10.8)	0.338
FT4 (ng/dL)	1.2 (1.0-1.5)	1.2 (0.3-5.5)	1.2 (0.1-3.6)	0.065
FT3 (ng/dL)	3.1±0.4	3.3±0.6	3.1±0.5	**0.001**[¶]
AHI	3.0 (0.0-45.4)	14.5 (0.3-87.5)	18.4(0.0-109.8)	**0.001**[§]
Mean saturation	94.2 (79.7-96.8)	92.5 (63.9-96.4)	90.6 (61.4-97.6)	**0.001**μ
Minimum saturation	89.0 (51.0-94.0)	84.0 (50.0-92.0)	77.0 (0.0-95.0)	**0.001**[ε]
ODI	3.8 (0.1-60.4)	14.3 (0.3-97.8)	22.6 (0.2-114)	**0.001**[¥]
Simple snoring, n (%)	25 (58.1)	43 (20.7)	64 (12.1)	**0.001**[†]
Mild OSAS, n (%)	10 (23.3)	68 (32.7)	151 (28.4)	
Moderate OSAS n (%)	3 (7.0)	59 (28.4)	129 (24.3)	
Severe OSAS, n (%)	5 (11.6)	38 (18.3)	187 (35.2)	
Body fat ratio	22.3 (14.4-38)	29 (20.0-46.0)	44.9 (25.1-74.8)	**0.001**[•]

Data are shown as Mean±SD or median (minimum–maximum). BMI: body mass index; CRP: C-reactive protein; TSH: thyroid-stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; AHI: apnea-hypopnea index; ODI: oxygen desaturation index; OSAS: obstructive sleep apnea syndrome; SD: standard deviation. p<0.05: significant. *Obese patients had significantly lower hemoglobin than the overweight and normal-weight patients; [α]obese patients had significantly lower hematocrit than the overweight and normal-weight patients; [§]mean leukocyte was significantly higher in obese patients than overweight and normal-weight patients; [γ]CRP levels were significantly higher in obese patients than overweight and normal-weight patients; [¶]mean FT3 was lower in obese patients than overweight and normal-weight patients; [§]AHI values were significantly higher in obese patients; μmean saturation values were significantly lower in obese patients; [ε]minimum saturation values were significantly lower in obese patients; [¥]ODI values were significantly higher in obese patients; [†]severe OSAS was higher in obese patients than overweight and normal-weight patients; [•]BFR values were significantly higher in obese patients than overweight and normal-weight patients.

intraoperative transfusions, conversion rates, and postoperative outcomes were not significant, although higher rates of intraoperative hypoxemia and new-onset arrhythmias were reported among obese patients compared with non-obese patients[8].

Obesity is also related to sleep disturbances, particularly OSAS. OSAS can lead to obesity due to increased appetite by affecting the levels of obesity-related hormones[4]. Male gender and advanced age are associated with OSAS[9], and abdominal obesity was shown to be associated with increased AHI[10,11]. However, no studies of the relationships between OSAS and BFR as a measure of obesity were found in the literature. In this study, severe OSAS was more common, and mean/minimum oxygen saturation levels were lower in obese patients. Furthermore, increased AHI and decreased mean/minimum saturation levels were found in relation to increased BFR.

Leukocytes are the main elements of inflammatory processes. Adipose tissue is a source of inflammatory proteins, and a positive correlation was shown between adipose tissue and inflammatory markers[12,13]. In this study, levels of CRP and leukocytes were found to be higher in obese patients than in normal-weight and overweight patients. The increased frequency of OSAS with obesity, which eventually leads to hypoxia, triggers systemic inflammation. Vascular diseases, especially coronary artery and cerebrovascular diseases, are commonly seen in obese patients with OSAS, and this situation was shown to be associated with sympathetic activation, systemic inflammation, and endothelial dysfunction. These findings suggest that OSAS is not a simple obesity-related phenomenon[1,14,15]. The finding of increased CRP and leukocytes among obese patients with severe OSAS in this study supports the previous results.

Thyroid dysfunction is closely related to many systemic diseases, particularly obesity. It was reported that the TFT values of patients with OSAS were not different from those of the general population[16]. Moreover, no difference in TSH levels was detected in healthy populations with and without obesity. Resta et al.[17] reported that OSAS prevalence and severity were not affected by thyroid hormone treatment or subclinical hypothyroidism. However, an increase in sleepiness was shown in the absence of treatment of subclinical hypothyroidism[17]. On the other hand, some researchers have shown differences in T3 and T4 levels, mainly due to low T4, and decreased serum FT4 or increased serum TSH levels may increase the risk of obesity in women[18].

Thyroid functions may also be affected by estrogen. In a study conducted on rats, it was reported that conjugated equine estrogens and tamoxifen increased T4 and T3 levels and had a proliferative effect on thyroid follicular cells[19]. Another study found lower FT4 and higher FT3 among euthyroid postmenopausal women with more visceral adipose tissue[20]. The effect of adipose tissue on the 5'-deiodinase enzyme was suggested as the source of that difference[21]. However, in this study, there were no associations of obesity with the levels of TSH, FT4, or BMI, but FT3 levels were lower in obese patients. These variations among studies might be due to different BMI values being accepted for obesity criteria. Differences in T3 and T4 levels can also be explained by the effect of adipose tissue on the 5'-deiodinase enzyme.

The association between obesity and anemia was suggested to be due to increases in hepcidin, shown to impair iron absorption[22,23]. Our results, which demonstrate a relationship between increased obesity and decreased hemoglobin and hematocrit levels, support the previous studies in this regard[24,25].

Limitations of the study

Participants were divided into three groups in terms of BMI values, but obese patients were not classified into further subgroups. While investigating the relationship of OSAS with obesity, relationships with underlying diseases should also be examined at the same time.

CONCLUSION

This study has shown associations between OSAS severity, BMI, and BFR. Therefore, obesity could be a critical predisposing factor for sleep disturbances. FT3 levels were significantly lower in obese patients in this study, but no significant relationships were found between FT4 or TSH and obesity. Based on these results, the relationship between obesity and OSAS seems to be more complicated than previously believed. Patients should be informed about the prevention and control of obesity while being treated for OSAS. These results may offer a valuable contribution to the literature due to the large number of patients included in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

AUTHORS' CONTRIBUTIONS

DF: Conceptualization, Data curation, Investigation Methodology, Formal Analysis, Writing– original draft, Writing – review & editing. **EF:** Data curation, Formal Analysis, Writing – review & editing.

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ORIGINAL ARTICLE

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Blood group as a novel predictor of postoperative atrial fibrillation after off-pump coronary artery bypass grafting

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SUMMARY

OBJECTIVE: The objective of this study was to reveal whether there was a possible relationship between the blood group and postoperative atrial fibrillation after off-pump coronary artery bypass grafting.

METHODS: Between January 2020 and January 2022, 452 patients undergoing off-pump coronary artery bypass grafting surgery consisted of the research population. Patients were divided into two groups based on the occurrence of new-onset atrial fibrillation from the time of operation until discharge. Group 1 (atrial fibrillation group) had 122 patients, whereas group 2 (non-atrial fibrillation group) contained 350 patients. Patients' baseline clinical characteristics and operative and postoperative data were recorded and then compared between the groups. Moreover, a multivariate logistic regression analysis was also conducted to identify the predictors of postoperative atrial fibrillation.

RESULTS: Non-O blood groups were substantially more common in the atrial fibrillation group than in the non-atrial fibrillation group. Patient age differences between the atrial fibrillation and non-atrial fibrillation groups were statistically significant, and patients in the atrial fibrillation group were detected to be older. Mean left atrial diameter, rates of obesity and prior percutaneous coronary intervention history, and perioperative intraaortic balloon pump requirement were significantly greater in the atrial fibrillation group than in the non-atrial fibrillation group. According to logistic regression analysis, blood group, age, left atrial diameter, obesity, and prior percutaneous coronary intervention were identified as predictors of postoperative atrial fibrillation.

CONCLUSION: We demonstrated for the first time in the literature that ABO blood type was a novel and significant predictor of new-onset atrial fibrillation after off-pump coronary artery bypass grafting.

KEYWORDS: Atrial fibrillation. Blood group. Coronary artery bypass grafting.

INTRODUCTION

Atrial fibrillation (AF) is a supraventricular tachyarrhythmia caused by irregular atrioventricular activity, which is characterized by atrial mechanical functional loss[1]. Depending on the atrioventricular node's conduction function, the ventricular rate is variable and irregular. Instead of P waves, an electrocardiogram shows rapid, irregular fibrillation waves of various shapes and sizes[2]. AF is the most common arrhythmia after coronary artery bypass grafting (CABG). It occurs most frequently on the second and third days after the operation[3]. Previously, cardiac surgery-related postoperative atrial fibrillation (POAF) was considered a minor complication. However, in recent research, POAF has been shown to increase early and late mortality and morbidity. POAF is associated with lower left ventricular hemodynamic performance and an increased risk of heart failure and cerebrovascular events, resulting in more extended hospital stays, higher costs, and higher mortality[3]. As a result, it causes problems for both patients and medical professionals and increases the expense of healthcare[4]. Therefore, identifying individuals at risk of POAF and taking appropriate precautions during the perioperative phase is critical.

The presence of A and B antigens on the surface membranes of red blood cells determines the ABO blood group. In addition to red blood cells, the corresponding antigens are expressed on platelets, vascular endothelium, and epithelium. Furthermore, they are found in saliva and body fluids[5]. According to the current literature, there appears to be a link between the ABO blood groups and several autoimmune diseases including Crohn's disease (CD), psoriasis, multiple sclerosis (MS), lupus erythematosus, and type 1 diabetes mellitus (DM)[6-10]. In addition, according to a recent review, many studies have demonstrated the links between ABO blood groups and thromboembolic diseases. Myocardial infarction, atherosclerotic vascular disease, venous thromboembolism, and cardiovascular ischemic events have all been connected, and those with non-O blood groups were shown to be at a higher

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risk than those with O blood groups in all cases[11]. Moreover, a recent study also showed a significant relationship between blood groups and idiopathic high-degree atrioventricular block associated with myocardial fibrosis and sclerosis[12].

To the best of our knowledge, there is no study in the literature examining a possible relationship between the blood group and POAF, which is associated with inflammatory, thrombogenic, and fibrotic processes. Therefore, we designed this study to reveal whether there was a relationship between the blood group and new-onset POAF after off-pump CABG.

METHODS

Ethical considerations

The study obtained approval from the local ethics committee (Decision No: 2022/97, Date: 12/04/2022). All patients were informed about the operation and perioperative process, and their verbal and written consent was taken before the operation. The study was conducted in accordance to the ethical principles of the Declaration of Helsinki.

Study design and population

This cross-sectional study population included 452 patients who underwent off-pump beating-heart CABG surgery in our institution between January 2020 and January 2022. The patients' medical data were gathered from the hospital records and then evaluated retrospectively. The patients were divided into two groups according to the development of new-onset AF during the postoperative period until discharge. There were 122 patients in group 1 (AF group) and 350 individuals in group 2 (non-AF group). Both groups were compared in terms of the baseline clinical parameters, intraoperative data, and postoperative results.

For this study, POAF was defined as new-onset AF of any duration that required pharmacological treatment (i.e., beta-blockers, calcium channel blockers, amiodarone, anticoagulants, etc.).

Exclusion criteria of the study were as follows: a history of paroxysmal, persistent or permanent AF prior to surgery, conventional on-pump CABG surgery, emergency surgery, re-operative surgery, concomitant valve surgery, and surgical techniques other than isolated off-pump CABG.

Surgical procedure

All patients were operated under general anesthesia. Internal thoracic artery and vena saphena magna were the most common harvested bypass grafts. Following the standard median sternotomy and pericardiotomy, coronary arteries and ascending aorta were examined. Coronary anastomoses were initiated following an intravenous heparin administration of 200 IU/kg and obtaining a target-activated clotting time of above 300 s. Distal anastomoses were performed first, followed by proximal anastomoses. Octopus tissue stabilizer was used for providing the proper position of the beating heart. Bulldog clamp and air blowing were used providing a blood-free space during distal anastomosis. The effect of unfractionated heparin was neutralized by protamine infusion after the anastomoses were completed, and the procedure was terminated in the standard fashion.

Arrhythmia monitoring

After the operation, all patients were transferred to the intensive care unit (ICU) and were continuously monitored with a cardiac rhythm monitor during ICU follow-up. All patients had standard 12-lead ECG recordings taken before the operation, immediately after the operation, and then twice a day during their postoperative hospital stay in both the ICU and the acute inpatient ward. Additional ECG recordings were obtained when there was a clinical suspicion of arrhythmia.

Laboratory analysis

The AB0-Rh blood groups were identified using the lam agglutination technique with a blood grouping reagent (Dia-Gast, Loos, France), the microplate agglutination procedure with the Galileo System (Stratec, Frankfurt, Germany), or the gel centrifugation assay with the IH-1000 Fully Automated System (DiaMed, Cressier, Switzerland).

Statistical analysis

Data were collected, tabulated, and statistically analyzed. Before statistical analysis, the distributional properties of the data were evaluated using the Shapiro-Wilk test. For normally distributed data, continuous variables were expressed as mean±standard deviation, and group comparison was performed using an independent two samples t-test. Non-normally distributed data were expressed as median (min.–max.), and group comparison was performed using the Mann-Whitney U test. Categorical variables were presented as the frequency and percentage, and the ^{2} test was used for bivariate comparison. The single and multiple explanatory variable(s) logistic regression (LR) analysis methods were employed. In the single explanatory variable LR analysis, we estimated the odds ratios with 95% confidence intervals of SSI for each study variable, and the significance level of each factor/covariate was determined. In the multiple explanatory variable LR analysis, the initial model was fit including all significant independent variables. Then, a backward-elimination

approach in a multiple explanatory variable LR model was conducted to evaluate the model for potential confounding effects. In this model, the factors/covariates were removed one at a time, starting with the factor/covariate that had the largest p-value, until all remaining factors had a two-sided p-value<0.05. The goodness of fit was tested using the Hosmer-Lemeshow test. The result was considered significant when the P-value was less than 0.05. The statistical software package 21.0 SPSS was used.

RESULTS

The study comprised 122 subjects with POAF and 330 subjects without POAF, and the frequency of POAF was 27% (122/452). When the blood types of the patients were analyzed, the O blood group (23.9%) and the non-O blood group (i.e., A, B, and AB blood group) were detected (73.9%). The majority of patients (86.2%) were Rh (D) positive, whereas 13.7% were Rh (D) negative. The AF and non-AF groups' preoperative demographic and clinical features, as well as intraoperative and postoperative data, were compared (Tables 1 and 2).

Patients in the AF group were significantly older than those in the non-AF group (67.9±7.7 vs. 63.5±10.5 years, p<0.001). A non-O blood group (p<0.004) was significantly more prevalent in the AF group than in the non-AF group. Additionally, mean left atrial (LA) diameter, incidences of obesity, previous percutaneous coronary intervention (PCI) history, and intraaortic balloon pump requirement (IABP) requirement were found to be significantly higher in the AF group than in the non-AF group. Other preoperative demographics and clinical characteristics, as well as all intraoperative

Table 1. Preoperative baseline characteristics of the study groups.

Variables	Non-AF group (n=330)	AF group (n=122)	p-value
Demographic			
Age (years)	63.53 (±10.55)	67.93 (±7.76)	<0.001
Gender, female/male	80/250	32/90	0.664
Blood group type			
0/Non-0 group	98/232	20/102	0.004
Rh positive	284 (86.0%)	102 (83.6%)	0.512
Medical history			
Hypertension	164 (49.0%)	62 (50.8%)	0.888
Diabetes mellitus	136 (41.2%)	42 (34.4%)	0.122
Hyperlipidemia	82 (24.8%)	38 (31.1%)	0.178
Obesity	80 (24.2%)	50 (41.0%)	0.002
Smoking	164 (49.7%)	38 (31.1%)	<0.001
Heart failure	98 (29.6%)	30 (24.6%)	0.225
LVEF level (%)	51.5 (±9.32)	51.26 (±8.82)	0.996
LA diameter (cm)	3.79 (±0.51)	4.39 (±0.85)	<0.001
Peripheral arterial disease	30 (9.1%)	16 (13.1%)	0.209
Myocardial infarction	150 (45.4%)	56 (45.9%)	0.932
Chronic renal dysfunction	38 (11.5%)	18 (14.7%)	0.321
Chronic liver disease	2 (0.06%)	0 (0.0%)	0.389
Chronic pulmonary disease	20 (6.1%)	8 (6.6%)	0.846
Previous CVE	54 (16.4%)	20 (16.4%)	0.994
Previous PCI	52 (15.8%)	34 (27.9%)	0.004
Beta-blocker usage	132 (40.0%)	50 (41.0%)	0.850
LMCA disease	46 (13.9%)	14 (11.5%)	0.193

AF: atrial fibrillation; LVEF: left ventricular ejection fraction; LA: left atrium; CVE: cerebrovascular event; PCI: percutaneous coronary intervention; LMCA: left main coronary artery. p-value<0.05 is considered as statistically significant.

and postoperative variables, revealed no significant differences between the groups.

The independent effects of probable demographic and perioperative parameters associated with POAF were evaluated by univariate and multivariate LR analysis. After applying the univariate analysis, six parameters were found as statistically significant for POAF (i.e., age, non-O blood groups, obesity, LA diameter, previous PCI, and IABP requirement), and then these variables were included in the multivariate analysis. The multivariate analysis revealed that age, non-O blood groups, obesity, LA diameter, and previous PCI were independently linked with POAF (Table 3).

DISCUSSION

This study was designed to examine whether there was a potential predictive relationship between blood groups and the development of new-onset POAF after off-pump CABG. Our findings suggested that patients with non-O blood group types had a higher risk for developing POAF than O blood group patients, as expected. According to multivariate regression analysis, age, LA diameter, obesity, and previous PCI history also caused a higher risk of developing POAF, which is consistent with the literature. The most intriguing and significant finding of our study was that blood group types independently predicted the development of POAF, for the first time in the literature.

The pathophysiology of POAF after CABG is still being researched. According to current evidence, POAF is thought to be caused by a combination of factors. Increased inflammation, Ischemia, oxidative stress, atrial fibrosis, excessive catecholamine delivery to the systemic circulation, autonomic tonus imbalance, and changes in connexin expression all contribute to forming a predisposing anatomic substrate[3].

Previous research has found a link between blood type and diseases caused by inflammation, thromboembolism, and fibrosis. Rumley et al. described that patients with non-O blood groups have lower plasma levels of factor VIIIc and von Willebrand factors than those with blood group O, so their

Table 2. Intraoperative and postoperative variables.

Variables	Non-AF group (n=330)	AF group (n=122)	p-value
LIMA usage	288 (87.3%)	106 (86.9%)	0.193
Complete revascularization	308 (93.3%)	110 (90.2%)	0.257
Inotrope requirement	72 (21.8%)	38 (31.1%)	0.056
In-hospital mortality	8 (2.4%)	2 (1.6%)	0.615
Number of distal bypasses	3.64 (±1.19)	3.72 (±1.17)	0.528
IABP requirement	16 (4.8%)	12 (9.8%)	0.045

AF: atrial fibrillation; LIMA: left internal mammary artery; IABP: intraaortic balloon pump. p-value<0.05 is considered as statistically significant.

Table 3. Results of multivariate logistic regression analysis for the prediction of atrial fibrillation.

Variables	Univariate	p-value	Multivariate	p-value
	OR (95%CI)		OR (95%CI)	
Non-O blood group	0.464 (0.272–0.792)	0.005	0.421 (0.216–0.820)	0.011
Rh positive	1.211 (0.683–2.144)	0.512		
Age	1.047 (1.024–1.071)	0.001	1.039 (0.008–1.071)	0.015
Hypertension	1.031 (0.679–1.565)	0.888		
Obesity	0.468 (0.308–0.773)	0.002	0.535 (0.311–0.922)	0.024
Smoking	2.294 (1.476–3.567)	0.001	1.904 (1.051–3.448)	0.034
Heart Failure	0.745 (0.463–1.199)	0.226		
LA diameter	3.927 (2.712–5.688)	0.001	4.049 (2.630–6.236)	0.001
Previous PCI	0.484 (0.295–0.794)	0.004	0.289 (0.151–0.554)	0.001
IABP requirement	0.459 (0.210–1.000)	0.049		

CI: confidence interval; AF: atrial fibrillation; LA: left atrium; PCI: percutaneous coronary intervention; IABP: intraaortic balloon pump. p-value<0.05 is considered as statistically significant.

risk of thrombosis differs[13]. In a population-based meta-analysis, Kole et al. reported an association between ABO blood groups and cardiovascular disease risk profiles[14]. Astarcıoğlu et al. found that non-O blood type was an independent risk factor for prosthetic valve thrombosis in research that included patients with mechanical prosthetic valve thrombosis[15]. According to Clark et al., the ABO blood group altered the pathogenesis and prognosis in individuals with cerebral Ischemia of arterial origin[16]. In the Framingham study population, peripheral vascular disease was also more prevalent in people with non-O blood types[17].

According to a previous study, there appears to be a link between the ABO blood types and a number of autoimmune illnesses. Studies have shown that ABO blood type significantly impacts circulating glycoprotein levels, which are essential for endothelial function and inflammation[18]. Type A blood was linked to high-sensitivity C-reactive protein levels and the number of antibiotic purchases yearly in prospective cohort research by Parente et al., which looked at ischemic heart disease occurrences in 4531 type 1 DM patients[10]. Furthermore, according to Oner et al., type A blood was the most prevalent genotype in all diabetes groups in both genders. However, type AB was substantially more common among type 1 DM patients[19]. This points to an inflammatory/infectious mediator in diabetes individuals that connects the non-O blood association to specific outcomes. The AB0 antigens have been related to the fructosyltransferase 2 (FUT2) gene, which is also a recognized CD locus. A study on CD patients found that individuals who lacked the FUT2 gene and were non-O blood-type carriers were less protected than type O blood group carriers[6].

Similarly, another study showed that non-O blood type was linked to an increased risk of complications such as invasive illness and structures[20]. Several studies have also shown a link between MS and the ABO blood group. In Basque research, blood group 0 was protective against MS compared with blood groups A, B, or Rh(+)[8]. As a result, evidence indicates that ABO blood types can alter the prognosis of various diseases by influencing the inflammatory status. Most research indicates type 0 as a protective factor and a risk factor for people who do not have type 0.

Furthermore, some research suggests that the AB0 gene may have a role in the fibrotic/sclerotic process. According to Hakyemez et al., blood types may be a hereditary risk factor for progression to severe hepatic fibrosis and cirrhosis.[21] Again, some links between blood types and cancer fibrosis have been discovered[22]. A significant association was found between blood types and the cardiac conduction system related to myocardial fibrosis and sclerosis in a recent study by Acar et al., with the non-O blood group being associated with increased risk[12].

The findings of our research are relevant and useful in clinical practice. Due to the rise in hospitalization and complications, POAF is now highly significant. Before the procedure, it is critical to identify high-risk individuals. According to this research, POAF is linked to fibrosis and inflammation. Fibrosis and inflammation are more likely in people with blood group 0 than those with other blood groups. Consequently, by looking at blood group values that are regularly evaluated during pre-operative evaluation, we may anticipate individuals at risk for POAF.

Limitations

Our study has the following limitations. First, the major limitations of the study were retrospective design and a limited number of cases which included reflection of the experience of a single institution. The second limitation was that due to the need for mid- and long-term data from the research population, it was unable to establish a link between POAF and mid- and long-term survival. Finally, postoperative cardiac rhythm monitoring was not performed constantly after the ICU stay. It is possible to miss the asymptomatic short and silent attacks of POAF.

CONCLUSION

This research demonstrated for the first time in the literature that the ABO blood group was a novel and independent predictor of new-onset AF after CABG surgery. The blood group determination is an inexpensive and easily applicable test routinely performed in the preoperative period, and it may help us predict the new-onset AF after CABG surgery based on the results of our study. Nevertheless, further prospective, large-scale, well-designed studies are required to support our findings and obtain stronger scientific evidence.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Validation of a Turkish version of the fathers' breastfeeding attitude and participation scale*

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SUMMARY

OBJECTIVE: This study aimed to determine the validity, structure, and reliability of a Turkish version of the Fathers' Breastfeeding Attitude and Participation Scale. The Fathers' Breastfeeding Attitude and Participation Scale consists of two parts, namely, Fathers' Breastfeeding Attitude and Fathers' Participation in Breastfeeding Process. Totally, the scale consists of 28 items, of which 14 items belong to Fathers' Breastfeeding Attitude and 14 items belong to Fathers' Participation in Breastfeeding Process. There is no report of a validity and reliability study in the original scale. Currently, there is no validated and reliable scale to assess Fathers' Breastfeeding Attitudes and Involvement in Turkish literature and other languages. In this context, the psychometric properties of the Fathers' Breastfeeding Attitudes and Involvement Scale were examined.

METHODS: The instruments were translated and adapted according to the WHO guidelines.

RESULTS: The Turkish version of the Fathers' Breastfeeding Attitude and Participation Scale demonstrated acceptable validity and reliability.

CONCLUSION: The use of the validated instrument to examine fathers' breastfeeding attitudes and participation in the breastfeeding process will provide data to guide as it is a determinant of breastfeeding behavior.

KEYWORDS: Breastfeeding. Fathers. Attitude. Scales.

INTRODUCTION

Breastfeeding plays a key role in the Sustainable Development Goals that countries expect to reach by 2030 and is critical for the realization of many of the goals[1,2]. Although 96% of children in our country have been breastfed for a while, breastfeeding still continues at a rate of 66% at the age of 1 year, while only 34% of children are breastfed until their second birthday[3-5]. Since families do not know enough how to cope with the problems they experience during the breastfeeding process, they have wrong beliefs and attitudes about breastfeeding, and mothers cannot receive adequate support from their environment, especially from their spouses[6]. There has been increasing evidence that fathers will affect the breastfeeding process[7-12]. It is stated that fathers have a significant effect on the choice of feeding method of the child, the decision of mothers to start breastfeeding, and the duration of breastfeeding[7-9]. In addition, it is stated that mothers' breastfeeding attitudes are related to the attitudes of their spouses[10-12]. While it is stated in the literature that fathers' effects on mothers' decision to breastfeed and breastfeeding attitudes[10-13] and fathers' participation in the breastfeeding process are important for breastfeeding success[14], there is no scale that evaluates fathers' attitudes toward breastfeeding and father participation in the breastfeeding process together. Filling this gap in the literature will also make an important contribution to midwives, nurses, and other health professionals who take an active role in breastfeeding counseling. Therefore, the aim of this study was to make the Turkish Version of the Fathers' Breastfeeding Attitude and Participation Scale (F-BAPS).

METHODS

In this methodological study, a cross-sectional design was used to perform the psychometric test of the Turkish translation of F-BAPS.

Fathers who were at least primary school graduates, had at least one breastfeeding experience, and whose spouses thought to stop breastfeeding before the age of 2 years were included in the study. Fathers who experienced the breastfeeding process with their twin children, had any discomfort in the child or the mother in the postpartum period, had a condition that prevented breastfeeding (such as galactosemia,

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cleft palate, cleft lip in the child, syphilis, tuberculosis in the mother), and whose spouses became pregnant during the breastfeeding period were excluded from the study. The purpose of the study was explained to the fathers, and an informed consent form was signed. First, the "introductory information form"[6,15-18] was filled in 5-6 min using the face-to-face interview technique. Second, information was given about "F-BAPS," and it took 20-25 min for the fathers to answer the items in the scale with the self-report method.

F-BAPS was developed by Abu-Abbas et al.[19]. Totally, the scale consists of 28 items, of which 14 items belong to F-BA and 14 items belong to F-PB. Responses on this scale are evaluated on a five-point Likert scale. The total score to be taken under the F-BA and F-PB varies between 14 and 70, and the cut-off point of the scale is 58. Those with a total score of ≥58 are called positive, and those with a total score of <58 are called negative.

The instruments were translated and adapted according to the WHO guidelines[20]. F-BAPS's original language is Arabic. It was translated into Turkish by three independent linguist translators who knew Turkish and Arabic well, and it was back-translated from Turkish into Arabic by a different translator.

Ethical aspect of research

The study, which was conducted in accordance with the Declaration of Helsinki and the country's ethical standards, was approved by Aydın Adnan Menderes University Ethics Committee (Date: 31.10.2018, No: 6).

RESULTS

The population of this research, between 15 December 2019 and 30 July 2020, consisted of fathers who applied to the same hospital with their spouses, and the sample consisted of 28×10=280 fathers. The age range of the 280 fathers participating in the study was 20-48 years [mean 34.47, standard deviation 4.70]. The rate of fathers who have experienced the breastfeeding process once is 70.0%, the rate of male children is 53.2%, and the rate of cesarean section is 63.9% in the last breastfeeding experience.

As a result of the analysis made to determine the adequacy of the sample size used in the research for factor analysis—F-BA, KMO coefficient=0.807, p=0.000 and Bartlett's sphericity test result χ^2=1362.758, df=91, p=0.000 were found; F-PB, KMO coefficient=0.814, p=0.000 and Bartlett's sphericity test result χ^2=1366.579, df=91, p=0.000 were found.

Factor analysis was conducted for the F-BA scale. As a result of the analysis, a four-factor structure with an eigenvalue above 1 was determined for the 14 items that were taken as the basis of the analysis. The contribution of the factors to the total variance was found to be 66.731% (Table 1). After this stage, the 14-item scale (Appendix 1) included in each factor was examined and the sub-dimensions were as follows: Factor 1 as "Cognitive" (5 items), Factor 2 as "Experience" (3 items), Factor 3 as "Emotion" (3 items), and Factor 4 as "Culture" (3 items).

Then, factor analysis was conducted for the F-PB scale.

As a result of the analysis, a four-factor structure with an eigenvalue above 1 was determined for the 14 items that were taken as the basis of the analysis. The contribution of the factors to the total variance was found to be 66.470% (Table 1). After this stage, the 14-item scale (Appendix 1) included in each factor was examined and the sub-dimensions were Factor 1 as "Understanding Breastfeeding" (5 items), Factor 2 as "Help" (3 items), Factor 3 as "Motivation" (3 items), and Factor 4 as "Sensitivity" (3 items).

First-order confirmatory factor analysis (CFA) was then performed for the scales whose factor structure was determined.

First, CFA was conducted for the F-BA. Chi-square (χ^2=116,788, n=280, SD=71, p=0.001) value was obtained as a result of the first-level CFA analysis. Fit indices were found as χ^2/SD=1.645, NFI=0.916, TLI/NNFI=0.955, IFI=0.965, CFI=0.965, RMSEA=0.048, GFI=0.945, AGFI=0.918, and RMR=0.039 (Table 2).

Subsequently, CFA was conducted for F-PB. Chi-square (χ^2=140,644, n=280, SD=71, p=0.000) value was obtained as a result of the first-level CFA analysis. Fit indices were found as χ^2/SD=1,981, NFI=0.899, TLI/NNFI=0.931, IFI=0.947, CFI=0.947, RMSEA=0.059, GFI=0.932, AGFI=0.899, and RMR=0.042 (Table 2).

After CFA, CR values were used as indicators to determine the concurrent validity: CR values of the factors of the F-BA subscale were;0.90 for "Cognitive," 0.85 for "Experience," 0.73 for "Emotion," and 0.66 for "Culture." CR values of the factors of the F-PB subscale were;0.75 for "Understanding Breastfeeding," 0.82 for "Help," 0.73 for "Motivation," and 0.86 for "Sensitivity."

AVE values were calculated for the discriminant validity of the F-BA and F-PB subscales. When the social structure-experience factor pairs with the largest square of the correlation coefficient between the factors according to F-BA were compared with the AVE values, AVE-social structure=0.51>0.19 and AVE-experience=0.55>0.19 were found. When the sensitivity-assistance factor pair, which has the largest square of the correlation coefficient between the factors according to F-PB, and the AVE values are compared, AVE-help=0.55>0.19 and AVE-sensitivity=0.50>0.19 are found (Table 3).

Table 1. Factor pattern of expressions for Fathers' Breastfeeding Attitude and Fathers' Participation in Breastfeeding Process parts (vertical rotation-varimax) (n=280).

F-BA items	F-BA factor load values			
	Factor 1	Factor 2	Factor 3	Factor 4
4	**0.797**		0.123	
8	**0.763**		0.145	0.148
13	**0.760**	0.104		0.108
3	**0.745**		0.186	
5	**0.731**	0.109	0.102	0.164
1	0.102	**0.835**		0.119
10	0.110	**0.830**		
11		**0.774**		0.186
7	0.121		**0.857**	
6			**0.811**	
14	0.217	0.247	**0.764**	
12				**0.831**
2	0.157	0.270		**0.780**
9	0.142		0.131	**0.778**
Explained variance	21.660	15.553	14.998	14.540
Total variance explained	66.731			
F-PB Items	**F-PB factor load values**			
	Factor 1	Factor 2	Factor 3	Factor 4
1	**0.795**		0.127	0.129
3	**0.788**	0.174		
9	**0.779**	0.128		0.170
14	**0.740**	0.195	0.102	0.107
10	**0.724**		0.158	0.145
4		**0.817**		0.192
11	0.161	**0.808**	0.183	
5		**0.784**	0.262	0.135
6			**0.846**	
13	0.122		**0.799**	0.111
2	0.109	0.234	**0.725**	
12				**0.828**
7	0.156	0.213		**0.787**
8	0.171	0.124	0.188	**0.737**
Explained variance	21.883	15.409	14.829	14.349
Total variance explained	66.470			

The factor loads obtained as a result of the rotation process are "0.32–0.44=bad", "0.45–0.54=normal", "0.55–0.62=good", "0.63–0.70=very good," and "0.70 and above=excellent." Bold values indicate "excellent" value. Subscales consist of four factors, and each color represents each factor.

The homogeneity of F-BAPS was assessed using Cronbach's α and item-to-total correlations. The Cronbach's α reliability coefficients were 0.807 for F-BA and 0.824 for F-PB.

Item-total correlations of the F-BA scale ranged between 0.345 and 0.541. The item-total correlations of the subscales range between 0.607 and 0.676 for "cognitive," 0.584 and

0.651 for "experience," 0.572 and 0.684 for "emotion," and 0.568 and 0.618 for "culture." Item-total correlations of the F-PB scale ranged between 0.364 and 0.556. The item-total correlations of the sub-dimensions ranged between 0.589 and 0.638 for "understanding breastfeeding," 0.607 and 0.653 for "help," 0.546 and 0.624 for "motivation," and 0.545 and 0.593 for "sensitivity."

DISCUSSION

This study determines the validity, structure, and reliability of a Turkish version of the F-BAPS.

The construct validity of the scale was also evaluated by first examining the factor structure. With the exploratory factor analysis, it was determined that the F-BA and F-PB subscales of F-BAPS had a four-factor structure. It is considered sufficient that the variance explained in multifactorial scales is between 40 and 60%[21-23]. In this framework, it is observed that the contribution of the factors defined for the F-BA and F-PB subscales of the scale to the total variance is sufficient. Factor load values above 0.45 are considered an appropriate criterion, and 0.70 and above are classified as "excellent"[21,23] and are defined as loads that can explain the structure well[22]. The smallest factor load value obtained as a result of the rotation process of this study is above the value accepted as the lower limit in the literature. All these EFA findings show that the four-factor F-BA and four-factor F-PB subscales of F-BAPS meet the construct validity criteria.

The four-factor structure of the scale was also supported by CFA. According to the fit indices made to determine whether the model structure of the F-BA and F-PB subscales after the CFA was consistent with the data, as a result of the first- and second-level CFA of the expressions for the F-BA and F-PB part, it was concluded that the model fit indices were in the range of acceptable and good fit values, and the factor loadings of the items in the F-BA subscales consisting of four factors and F-PB subscales consisting of four factors were statistically significant[20].

In the literature, it is stated that concordance and discriminant validity must also be provided in order to say that a scale structure that has been revealed by EFA and confirmed by CFA has construct validity. It has been reported that if the calculated CR coefficient is greater than 0.70, high structure reliability is achieved, and if it is between 0.60 and 0.70, an acceptable reliability level and concordance validity are provided[21,23].

Table 2. Fit indices before and after modification.

Fit indices	Before modification	After modification
Chi-square (χ^2)	140.644	117.982
χ^2/SD (CMIN/DF)	1.981	1.685
NFI	**0.899**	**0.915**
NNFI	0.931	0.952
IFI	0.947	0.964
CFI	**0.947**	**0.963**
RMSEA	0.059	0.050
GFI	0.932	0.943
AGFI	0.899	0.915

χ^2: Chi square; SD: degrees of freedom; NFI: normed fit index; NNFI: non-normed fit index; TLI: Turker-Lewis index; IFI: incremental fit index; CFI: comparative fit index; RMSEA: root-mean-square error of approximation; GFI: goodness-of-fit index; AGFI: adjusted goodness-of-fit index. Bold values: After the modification process, the NFI and CFI values of the model showed an acceptable fit.

Table 3. The squares of the correlation coefficients and average variance extracted values of the expressions for the Fathers' Breastfeeding Attitude and Fathers' Participation in Breastfeeding Process parts.

BET factors	Knowledge	Experience	Emotion	Culture
Knowledge (AVE=0.50)	1.000			
Experience (AVE=0.55)	**0.09**	1.000		
Emotion (AVE=0.55)	**0.16**	**0.10**	1.000	
Culture (AVE=0.51)	**0.13**	**0.19**	**0.08**	1.000
BEK factors	**Understanding breastfeeding**	**Help**	**Motivation**	**Sensitivity**
Understanding breastfeeding (AVE=0.50)	1.000			
Help (AVE=0.55)	**0.11**	1.000		
Motivation (AVE=0.50)	**0.08**	**0.19**	1.000	
Sensitivity (AVE=0.50)	**0.18**	**0.19**	**0.09**	1.000

AVE: average variance extracted. Bold values: According to their AVE values, the factors measure independent and separate features and have discriminant validity.

Considering the CR values of the factors in the F-BA subscale of the F-BAPS, it can be said that the cognitive, experience, and emotional factors provide high structural reliability and the social structure factor provides an acceptable level of structural reliability and congruent validity. Considering the CR values of the factors in the F-PB subscale of F-BAPS, it can be said that it provides high construct reliability and concordance validity in four factors.

In order to ensure discriminant validity, the AVE value of both factors should be greater than the square of the correlation coefficient between these two factors[21,23]. AVE values of both F-BA and F-PB parts of F-BAPS are greater than the square of the correlation coefficient between all factor pairs. Therefore, it is observed that the discriminant validity condition is met. As a result, the fact that the F-BA and F-PB subscales of the F-BAPS have both concurrent validity and discriminant validity is a strong proof of construct validity.

The Cronbach's α reliability coefficients of the F-BA and F-PB subscales were 0.807 and 0.824, respectively, with a high level of reliability between 0.80 and 1.00[21,23]. This shows that F-BAPS is a reliable scale and the scale provides internal consistency.

According to a criterion accepted in the literature, it is stated that the item-total correlation coefficient of an item should not be negative way and items with an item-total correlation coefficient higher than 0.30 should remain in the scale[21,23]. The values obtained do not carry a negative charge and are above the desired item-total correlation value of all the items in the F-BA and F-PB subscales. Therefore, it is concluded that all items move in the same direction as the scale, the additiveness of the scale is not impaired, and the internal consistency of the scale is ensured.

This study also has some limitations. First, the test-retest reliability analysis of the scale could not be performed due to the COVID-19 pandemic conditions. In the original scale, it is stated that the interviews were conducted with fathers who had been breastfeeding for a maximum of 5 years. However, the fact that fathers whose breastfeeding attitude and experience have passed for more than 5 years due to the COVID-19 pandemic conditions have to be included in the study is another limitation of the study.

CONCLUSION

The Turkish version of the F-BAPS (Appendix 1) demonstrated acceptable validity and reliability and thus provides a means of better understanding the breastfeeding attitude and participation in the breastfeeding process of Turkish fathers.

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AUTHORS' CONTRIBUTIONS

HUH: Conceptualization, Methodology, Project Administration, Supervision, Formal Analysis, Investigation, Resources, Software, Validation, Writing – original draft, Writing – review & editing. **SG:** Conceptualization, Methodology, Data curation, Formal Analysis, Investigation, Resources, Software, Validation, Writing – original draft.

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APPENDIX 1

BABALARIN EMZİRME TUTUMU VE KATILIMI ÖLÇEĞİ

Lütfen aşağıdaki ifadeleri okuyunuz ve sizin görüşlerinize en uygun olanını "X" işareti koyarak cevaplayınız. Bu soruların cevaplandırılmasında doğru veya yanlış cevapların olmadığını bilmeniz (hatırlamanız) önemlidir. Biz emzirmeye ilişkin tutumunuzu ve emzirmeye ne kadar katıldığınızla ilgileniyoruz.

Bölüm (1): Babaların emzirmeye ilişkin tutumları	Kesinlikle Katılmıyorum	Katılmıyorum	Kararsızım	Katılıyorum	Kesinlikle Katılıyorum
1.1. Emzirme, hazır mama ile beslemeden daha rahattır.					
1.2. Ev dışında çalışan bir anne bebeğini emziremeye güç yetiremez.					
1.3. Çoğu annenin emzirme için yeterli sütü vardır.					
1.4. Hazır mama bir bebek için anne sütü kadar sağlıklıdır.					
1.5. Anne sütü, hazır mamadan daha kolay sindirilir.					
1.6. Emzirme, evlilik ilişkisini olumsuz etkileyebilir.					
1.7. Kadın emzirmeden dolayı çekiciliğini kaybeder.					
1.8. Emzirme, annenin sağlığı için yararlıdır.					
1.9. Emzirme annenin sorumluluğundadır ve babanın bu konuda bir rolü yoktur.					
1.10. Emzirme anneye, hazır mama ile beslemeye göre daha çok zaman kazandırır.					
1.11. Emzirme anneyi kısıtlar ve sosyal yaşamını engeller.					
1.12. Tanımadığım bir kadın önümde bebeğini emzirdiğinde utanırım.					
1.13. Emzirme, bebeği hastalıklardan korur.					
1.14. Anne emzirirken, baba kendini dışlanmış hisseder.					
Bölüm (2): Babaların emzirme sürecine katılımı	**Kesinlikle Katılmıyorum**	**Katılmıyorum**	**Kararsızım**	**Katılıyorum**	**Kesinlikle Katılıyorum**
2.1. Eşimle emzirmeye ne kadar devam edeceği hakkında konuştum.					
2.2. Başkalarına ziyaretimiz sırasında eşimin bebeğimizi emzirmesini kolaylaştırdım.					
2.3. Emzirme problemlerini çözmeye çalışırken eşimle fikir alışverişinde bulundum.					
2.4. Eşim emzirirken, diğer çocuklarımız veya evdeki diğer sorumluluklarımızla ilgilendim.					
2.5. Eşime ev işlerinde yardım ettim ve bebeğimizin ağlamasına cevap verme, yıkanmasına yardım etme gibi bakım işleriyle ilgilendim.					
2.6. Eşim emzirmeye başladığında, konforu için yastık verme, bir bardak su getirme gibi işlemlerde bulundum.					
2.7. Eşimin uykusunun bölünmesi veya cinsel aktivite sırasında memelerindeki süt dolgunluğu ile ilgili huzursuzluğunu anlayışla karşıladım.					
2.8. Bebeğimizle aynı odada uyumayı, karşı çıkmadan, kabul ettim.					
2.9. Bebek emmeye devam ederken eşimin emzirmeyi kesme isteğini onayladım.					
2.10. Bebeğimizin hazır mama ile beslenmesini kabul ettim.					
2.11. Bebeğimizin bakımını üstlenerek eşimin bir süre uyuyabilmesi için bir zaman dilimi verdim.					
2.12. Emzirme sürecinde diğer ev işleri yapılmadığında mutsuz oldum.					
2.13. Eşim emzirirken sevinç ve memnuniyet gösterdim. (gülümseme, izleme, eşimi tutma gibi .....)					
2.14. Eşime emzirmenin, kendisi veya bebeğimiz için olan, faydalarını belirttim.					

ORIGINAL ARTICLE

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Is post-traumatic stress disorder related to the severity of physical trauma?

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SUMMARY

OBJECTIVE: Trauma can cause physical morbidity and even result in death. Besides, it can lead to serious mental problems as well. The most well-known mental health problem is post-traumatic stress disorder. Through this study, it was primarily aimed to find out whether the severity of physical trauma is effective on post-traumatic stress disorder and other risk factors if any.

METHODS: The reports of the patients who were transferred to the Turkish Council of Forensic Medicine Third Speciality Board between January 01, 2019, and December 31, 2020, for post-traumatic invalidity or disability evaluation and whose psychiatric examinations were performed were retrospectively analyzed in the electronic environment.

RESULTS: It was found that 34 (26.4%) of the patients had a diagnosis of post-traumatic stress disorder (under treatment for at least 6 months), while 76 (58.9%) of them did not have a psychiatric disease and 19 (14.7%) of them had mental disorders not associated with trauma (i.e., affective disorder, anxiety disorder, etc.). No significant correlation was found between trauma scores and post-traumatic stress disorder ($p>0.05$).

CONCLUSION: Based on the results of our study, post-traumatic stress disorder and the severity of physical trauma are not significantly correlated. Being of female gender, sustaining a non-accidental injury, and witnessing a fatal event stand out as significant risk factors.

KEYWORDS: Injury severity score. Trauma severity indices. Post-traumatic stress disorder.

INTRODUCTION

Traumatic events may lead to physical morbidities and even death. They may also result in serious psychological problems, such as post-traumatic stress disorder (PTSD), which is one of the most common mental health conditions.

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), PTSD is a psychiatric disorder that can develop following exposure to a traumatic event involving actual or threatened death, serious injury, or sexual violence. The disorder is characterized by intrusive re-experiencing of the traumatic event through distressing memories, flashbacks, or nightmares, avoidance of trauma-related stimuli, negative alterations in mood and cognition, such as persistent negative beliefs or feelings, distorted blame, or diminished interest in activities, and alterations in arousal and reactivity, including hypervigilance, exaggerated startle response, and sleep disturbances[1].

Some of the factors that facilitate PTSD development include adverse life experiences before a traumatic event, traumatic events during childhood, contact with a familiar and trusted individual experiencing a traumatic event, insufficient social support, and pre-existing mental disorders[2,3]. There is also ongoing research into the impact of the severity of physical trauma[4].

Several scoring systems have been developed to objectively determine the severity of physical trauma. Anatomic scoring systems have been developed based on the injured body region and injury types. The Abbreviated Injury Scale (AIS) is a scoring system that classifies each injury by body region on a 6-point scale. Scoring systems such as the injury severity score (ISS) and new injury severity score (NISS), which have been developed based on AIS, are commonly used in retrospective trauma studies.

ISS is considered the "gold standard" anatomic scoring system, which helps to determine the severity of injury[5,6]. The ISS is calculated as the sum of the squares of the highest AIS code in each of the three most severely injured areas in each of the six body regions. In 1997, NISS was introduced to improve its accuracy[7]. The NISS is calculated as the sum of the squares of the three highest AIS scores for each patient, regardless of body region[8].

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The aim of this study was to determine whether the severity of physical trauma has an impact on PTSD and other risk factors. The findings of this study may facilitate the early identification of patients who might require psychiatric follow-up/treatment in the post-traumatic period.

METHODS

A retrospective examination was made of the electronic records of patients referred to the (…) for disability assessment after trauma (i.e., occupational accidents, physical violence, and traffic accidents, among others) and who underwent psychiatric examinations between January 01, 2019 and December 31, 2020. The physical examinations of the patients regarding their medical conditions were conducted by various specialists (such as orthopedists, neurologists, and ophthalmologists) at our institution. Psychiatric evaluations were also performed by psychiatrists. Diagnoses were established according to the criteria of DSM-5. Psychiatrists did not possess detailed knowledge of trauma scores beyond the patients' medical history. The trauma scores for all patients were retrospectively calculated by a forensic medicine specialist following the examinations.

In line with the AIS, the ISS and NISS values were calculated based on a minimum score of 0 and a maximum of 75. Patients with psychiatric disorders who exhibited simulated behaviors (patients who simulate illness, express complaints incongruent with their clinical condition, and exhibit exaggerated symptoms during psychiatric or physical examinations, while lacking a trustworthy attitude during the interview) during the examination and those who had been diagnosed with trauma-related mental health conditions other than PTSD were excluded from the study.

Data analyses were performed using the SPSS version 24 software. The conformity of the data to normal distribution was tested using the Shapiro-Wilk test. The Mann-Whitney U test was used to compare non-normally distributed variables between the two groups. Relationships among categorical variables were tested using the chi-square test. A value of $p<0.05$ indicated statistical significance.

Ethical declaration

This study was reviewed and approved by the Ministry of Justice Council of Forensic Medicine Scientific Research Committee (21589509/2021/307).

RESULTS

A total of 129 patients were included in the study, comprising 101 (78.3%) males and 28 (21.7%) females, in an age range of 9–72 years (median age, 32 years; mean age, 32.39±10.83 years). Of the total patients, 86 (68.6%) were married and 43 (33.3%) were single.

Of the patients who had provided information on their educational level, 44 (34.1%) were primary-school graduates or lower, 20 (15.5%) were secondary-school graduates, 36 (27.9%) were high-school graduates, and 21 (16.3%) were university graduates.

The traumatic events were determined as 67 (51.9%) occupational accidents, 36 (27.9%) traffic accidents, and 26 (20.2%) were in the "other" category, which included injury caused by a sharp object, firearms injury, fall from height, and bomb explosion.

Physical trauma exposure resulted in 0–210 days of hospitalization (mean, 19.02±27 days; median, 9 days). A total of 37 (28.7%) patients did not undergo surgery, whereas the remaining patients underwent 1–13 surgical procedures (mean, 1.47±1.80; median, 1).

A total of 30 (23.3%) patients suffered trauma-induced head and neck injuries, 37 (28.7%) suffered face injuries, 28 (21.7%) suffered injuries to the thoracic region, 17 (13.2%) suffered abdominal injuries, 68 (52.7%) suffered injuries to the pelvis and extremities, and 39 (30.2%) suffered burns.

The severity of physical trauma exposure was scored using anatomic trauma scoring systems. The mean and median ISS and NISS were 10.99±8.63 and 9 and 11.64±8.88 and 9, respectively. In total, 27 (20.9%) patients lost consciousness at the time of the event, and 9 (7%) witnessed a fatal event.

The medical history, medications used, and the psychiatric re-examination by our board revealed that 34 (26.4%) patients were diagnosed with PTSD and it was determined that they had been under treatment for at least 6 months, 76 (58.9%) had no psychiatric disease, and 19 (14.7%) demonstrated mental health conditions (i.e., affective and anxiety disorders) that were not related to the trauma.

The comparisons of the sociodemographic and injury data of the groups with and without a diagnosis of PTSD are shown in Tables 1-3.

In our examination, the functionality of PTSD patients under treatment was evaluated for the determination of disability. Mild functional impairment was observed in 7 (20.6%) of the 34 patients diagnosed with PTSD, moderate functional impairment was observed in 7 (20.6%) patients, and severe functional impairment was observed in 1 (2.9%) patient. It was determined that the functionality of 19 (55.9%) patients was good with treatment.

Table 1. Comparisons of the sociodemographic data of the groups with and without a diagnosis of post-traumatic stress disorder.

	PTSD (n=34)	Other (n=95)	p
Age (years)	30.47±12.17	33.08±10.3	0.197
Duration of hospitalization (days)	20.52±39.06	18.48±21.22	0.650
Number of surgeries	1.42±1.71	1.49±1.85	0.877
Injury severity score	9.74±6.77	11.44±9.2	0.607
New injury severity score	10.65±6.86	12±9.52	0.784

Mann-Whitney U test.

Table 2. Comparisons of cases with and without a diagnosis of post-traumatic stress disorder in terms of epidemiological data.

		PTSD		Other		p
		n	%	n	%	
Sex	Male	21	61.8	80	84.2	0.006*
	Female	13	38.2	15	15.8	
Marital status	Married	22	62.8	64	68	0.458
	Single	13	37.2	30	32	
Educational status	Primary-school graduates and lower	9	30.0	35	38.5	0.737
	Secondary-school graduates	5	16.7	15	16.5	
	High-school graduates	9	30.0	27	29.7	
	University graduates	7	23.3	14	15.4	
Witnessed a fatal event	Yes	6	17.6	3	3.2	0.008*
	No	28	82.4	92	96.8	

*p<0.05; χ^2 test.

Table 3. Comparisons of cases with and without a diagnosis of post-traumatic stress disorder in terms of injury sites, type, and treatment.

		PTSD		Other		p
		n	%	n	%	
Head and neck injury	Yes	4	11.8	26	27.4	0.065
	No	30	88.2	69	72.6	
Facial injury	Yes	11	32.4	26	27.4	0.581
	No	23	67.6	69	72.6	
Thoracic injury	Yes	10	29.4	18	18.9	0.204
	No	24	70.6	77	81.1	
Abdominal injury	Yes	4	11.8	13	13.7	0.774
	No	30	88.2	82	86.3	
Pelvic and extremity injury	Yes	18	52.9	50	52.6	0.975
	No	16	47.1	45	47.4	
Other injuries**	Yes	10	29.4	29	30.5	0.903
	No	24	70.6	66	69.5	
History of at least one surgery	Yes	9	26.5	28	29.5	0.740
	No	25	73.5	67	70.5	
Event type	Accident	23	67.6	80	84.2	0.039*
	Non-accidental trauma***	11	32.4	15	15.8	

*p<0.05; χ^2 test. **Injuries involving the skin, such as burn trauma, as defined by the injury severity score. ***Physical violence, stab wounds, explosion-blast injury, and firearm injury.

DISCUSSION

Physical and mental traumas may have numerous short- and long-term consequences. The prevalence of psychiatric morbidities following traumatic injury has been reported to vary between 17.5 and 42% in the first 6 months and between 2 and 36% in the first 12 months[4,9]. The most well-known trauma-induced psychiatric morbidities are PTSD and depression. In addition to the clinical diagnosis, follow-up, and treatment of these diseases, the medico-legal implications are also crucial. These mental health morbidities can also be the subject of criminal cases or disability assessments. The detection of PTSD risk factors may aid in the early follow-up of high-risk patients and disability assessments.

In this study, the relationship between various variables and the diagnosis of PTSD was examined. The median and mean ages of patients were 32 and 32.39±10.83 years, respectively, and the study group consisted of 101 (78.3%) males and 28 (21.7%) females. Although the mean age of the patients with PTSD was relatively lower than that of patients without a diagnosis of PTSD, there was no statistically significant difference (Table 1). There was a significant predominance of the female sex in the PTSD group (Table 2). PTSD typically affects females and young adults[10]. It has been stated that females (11–20%) are at higher risk of developing PTSD following trauma than males (4–8%)[11]. The results of this study are in line with the literature.

Several studies have reported that a low education level and socio-economic status are risk factors for PTSD. Furthermore, married individuals have been reported to exhibit a relatively low risk for PTSD owing to the presence of better social support[12]. However, no significant relationship between marital status and PTSD diagnosis was found in this study (Table 2). Similar results were obtained regarding educational status. A high or low education level was not found to be a significant risk factor for PTSD.

Owing to the fact that PTSD is a highly heterogeneous psychiatric disorder, investigating the relationships between trauma type and clinical findings is imperative to better understand disease etiology and improve treatment approaches[13]. The type of event responsible for causing the physical trauma and how it occurred are important factors for the development of PTSD. For example, sexual trauma is known to be a serious risk factor for PTSD[13]. Risk levels of PTSD have been specified for attack incidents and disasters[14]. A previous study reported that victims of physical violence exhibit subclinical presentations of PTSD and require treatment[15]. In this study, a significant difference was observed in terms of the development of PTSD between accidental incidents and non-accidental injuries, favoring non-accidental injuries (Table 3).

The relationship between lesion localization and PTSD was examined (Table 3). More than half of the patients suffered injuries to the pelvis and extremities. Approximately a quarter (26.5%) of patients with fractures have been reported to experience PTSD[16]. In this study, 30.2% of the cases had a history of burns and scars. The prevalence of psychiatric complaints among burn patients has been shown to range between 28 and 75%[17]. The most common psychological disorders observed in the early period following burn trauma include acute stress reactions, anxiety disorder, depression, behavioral disorders, and delirium. Psychiatric conditions depend on both the primary effect of the burn trauma and the events that occur during treatment[18]. In addition, burns and scars after the treatment are considered possible causes of psychiatric conditions[19]. In some studies, burn lesions involving the hands and face have been shown to increase the risk of PTSD because they remind the patient of the trauma experienced[20]. However, in this study, no significant relationship between the injury region and the PTSD diagnosis was identified.

In this study, PTSD was significantly more frequently observed in patients who witnessed a fatal event (Table 2). This was considered a stand-alone risk factor for PTSD development, regardless of the physical trauma experienced.

The relationship between the severity of physical trauma exposure and PTSD has been examined in many studies using trauma scoring systems, such as AIS and ISS. In a study conducted on traffic accident victims, no significant relationship between PTSD and mean AIS values was reported[21]. Similarly, no correlation has been determined between ISS and psychiatric consequences[4,22]. In this study, the mean ISS and NISS were not significantly higher in the PTSD group (Table 1), thereby indicating that, contrary to expectations, severe trauma did not result in an increased probability of psychological conditions. In other words, trauma with low physical severity may also have an impact on the development of PTSD. Therefore, the effect of trauma on the victim and the psychological outcome should not be correlated with just the severity of the trauma.

CONCLUSION

The prediction of post-traumatic conditions has the advantages of both close and remote follow-up and early rehabilitation of patients at risk. This study was conducted on a group of patients whose disability was evaluated as part of the judicial process and who had experienced trauma at least six months prior. The suitable identification of risk factors in this patient group might also be beneficial in establishing the relationship between post-traumatic mental health complaints and the traumatic event. The findings of this study suggest that PTSD is not significantly associated with the severity of physical trauma.

ETHICAL DECLARATION

This study was reviewed and approved by the Ministry of Justice Council of Forensic Medicine Scientific Research Committee (21589509/2021/307). The study was conducted in accordance with the criteria of the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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miR604A>G gene polymorphism is associated with recurrent pregnancy loss in Turkish women

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SUMMARY

OBJECTIVE: Recurrent pregnancy loss is considerably a reproductive health problem for couples. Genetic, epigenetic, and environmental factors play an important role in the development of recurrent pregnancy loss. While there are many causes, genetic and epigenetic factors are common. In this study, we aimed to examine the association between miR604 (rs2368393) A>G gene polymorphism and the risk of recurrent miscarriage in the Turkish population.

METHODS: The study included 250 participants (i.e., 150 patients and 100 controls). DNA samples were isolated from peripheral blood, and polymerase chain reactions and restriction fragment length polymorphism methodologies were applied.

RESULTS: The genotype distribution and allele frequencies of miR604A>G gene showed statistically significant differences between patients and control groups (p=0.002 and p<0.002, respectively).

CONCLUSION: As a result of the study, we found that the AA genotype and A allele of the miR604A>G gene were statistically significant for the risk of recurrent pregnancy loss in Turkish women.

KEYWORDS: Gene polymorphism. microRNA. Recurrent miscarriages.

INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as two or more spontaneous pregnancy loss until the 20th gestational week. Generally, pregnancy losses occur in the first trimester. The risk of recurrence of pregnancy loss is higher in individuals whose first pregnancy resulted in spontaneous abortion. There are many causes of RPL, including genetic factors, anatomical disorders, hormonal disorders, placental structure, infection, and other reasons[1,2]. Genetic factors are of great importance among them. In recent years, epigenetic factors have become more popular. Especially microRNAs (miRNAs) have recently been studied intensively. miRNAs are short, 17-25-nucleotide-long, non-coding small RNA molecules that regulate gene expression. miRNAs have been reported to have critical regulatory roles in controlling genes associated with cellular and molecular activity and maintenance of pregnancy[3]. It has been revealed that most of the pregnancy-associated miRNAs are expressed in reproductive tissues[4,5]. miR604A>G showed a relation that it binds targets to related placenta retention. Based on these data, we aimed to investigate the relationship between miR604 (rs2368393) A>G gene polymorphism and the risk of RPL in the Turkish population.

METHODS

Study sample

The study was carried out with women who applied to the obstetrics and gynecology department between 2019 and 2021 with a history of idiopathic recurrent miscarriage and women in the control group who had a healthy pregnancy and did not have any miscarriages. A questionnaire was applied to the participants in which age, body mass index, number of miscarriages, number of pregnancies, smoking and alcohol use, occupation, and hormone values were questioned. The individuals participating in the study signed an informed consent form, and the study included 250 female volunteers (i.e., 150 patients and 100 controls). Women with a history of two or more pregnancy losses were defined as having a recurrent miscarriage. The control group women have a healthy pregnancy and no history of pregnancy loss. The mean age of the patients was 33.21±4.52 years, and the mean age of the control group was 33.61±5.38 years. The study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University Faculty of Medicine on May 31, 2023 (OMU KAEK 2023/178).

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DNA isolation and polymerase chain reaction-restriction fragment length polymorphism methods

A total of 2 mL of peripheral blood was collected from participants in EDTA tubes. DNA was isolated from the peripheral blood sample by kit methodology (Invitrogen). The obtained DNAs were stored in the freezer. Following DNA isolation, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methodology was performed. The miR604 (rs2368393) A>G polymorphism was detected using the forward and reverse primers, namely, 5′-CTT GGC TCA GTG GTC TGT TT-3′ and 5′-GTA CAG GGA CTG AAA GGT GAA G-3′, respectively. The 243 bp PCR product was digested with BssSI enzyme (New England BioLabs, Ipswich, MA, USA) to AA type (169 and 74 bp), AG type (243, 169, and 74 bp), and GG type (243 bp), under conditions of initial denaturation at 95°C for 15 min, 40 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 40 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Genotypes were visualized on 2% agarose gel with an image analysis system.

Statistical analysis

The SPSS.20 (Chicago, IL, USA) program and OpenEpi Info software package program were used for statistical analysis. Genotype distribution and allele frequencies are calculated and compared by chi-square (χ^2) analysis. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Statistical results reached the significant value of $p<0.05$ (two-tailed).

RESULTS

The clinical and demographic findings of the patient and control groups are shown in Table 1. Genotype distribution and allele frequencies of miR604A>G gene are shown in Table 2.

Table 1. Clinical and laboratory findings of the patient and control groups.

Characteristics	Patients (n=150) (mean±SD)	Median (min–max)	Controls (n=100)	Median (min–max)
BMI (kg/m²)	25.52±3.572	28.00 (12-45)	28.38±4.186	28 (26-42)
Age at menopause (years)	45.22±5.824	47.90 (35-60)	46.56±4.674	45 (33-58)
Age at menarche (years)	13.52±1.371	13.0 (9-18)	13.46±1.51	12 (10-17)
Number of birth (n)	3.56±1.828	3.0 (0-10)	3.0±1.468	3 (0-6)
Serum calcium (mg/dL)	9.63±0.540	9.75 (8.35-11.6)	9.57±0.481	9.55 (8.5-10)
Serum phosphorus (mg/dL)	3.73±2.55	3.52 (2.1-25.40)	3.78±0.876	4 (2.00-5.32)
Serum ALP (U/L)	187.03±61.83	187.355 (2.42-430)	177.65±45.146	186.6 (96-310)
Serum PTH (pg/mL)	77.03±53.95	77.23 (11.70-563)	75.32±27.563	72.7 (30-167)
Homocysteine (µmol/L)	7.28±1.95	8.05 (6.30-8.2)	NA	NA
Folate (mg/mL)	14.25±11.85	15.25(12.42-15.4)	NA	NA
PAI-1 (ng/mL)	10.43±5.81	11.15(9.95-15.0)	NA	NA

NA: not applicable.

Table 2. Genotype and allele frequencies of miR604A>G gene polymorphism.

Genotype/Allele	Patients (n=150)		Controls (n=100)		χ^2 (95%CI)	p-value	OR
	n	%	n	%			
Genotype							
AA	48	32.0	22	22.0	χ^2=12.325	p=0.002*	
AG	78	52.0	43	43.0			
GG	24	16.0	35	35.0			
Total	150		100				
Allele							
A	171	57	87	44	χ^2=9.486 (1.226-2.53)	p<0.002*	1.761
G	126	42	113	56			

*p<0.05 is statistically significant.

Patients' body mass index and mean standard deviation (SD) were 25.52±3.572 and 28.38±4.186 in control. The mean age±SD was 33.21±4.52 years in patients and 33.61±5.38 years in the control group, respectively. The mean homocysteine±SD was 7.28±1.95. The mean folate±SD was 14.25±11.85 and PAI-1±SD was 10.43±5.81 in patients. Table 2 represents the distribution of miR604A>G gene polymorphism genotypes in patients and control groups. miR604A>G gene AA, AG, and GG genotype frequencies of patients were 32, 52, and 16%, respectively. In the control group, AA, AG, and GG genotype frequencies were 22, 43, and 35%, respectively. AA genotype frequency was high in the patient group (p=0.002), and A allele frequency was also higher in the patient group (p<0.002), which reached a statistically significant value.

DISCUSSION

RPL is affecting 1-3% of pregnancies. Its exact etiology is poorly understood due to different definitions and limitations in access to abortion material. Few factors such as parental chromosomal abnormalities, untreated hypothyroidism, uncontrolled diabetes mellitus, anatomical anomalies of the uterus, and antiphospholipid antibody syndrome are important for pregnancy losses. Other possible etiological factors include endocrine disorders, hereditary and/or acquired thrombophilia, immunological abnormalities, infections, and environmental factors. However, the cause of almost half of RPLs is still unexplained. Many couples in recent days suffer from recurrent miscarriages that also considerably affect their quality of life. Diagnostic biomarkers, which are increasing day by day, are beneficial for patients in terms of determining the predisposition and taking the necessary precautions. SNPs in miRNA genes, genes encoding miRNA machinery proteins, or miRNAs that target genes involved in miRNA synthesis or function will affect processes regulated by miRNAs. They can adversely affect downstream gene expression[6,7]. Various studies showed that miRNAs play an important role in RPL. In Cho et al.'s study, a construct containing the 3'UTR MTHFR gene was performed using luciferase to measure the binding affinity of variant and major alleles of miR604 G>A to MTHFR[8]. These analyses showed that the binding affinity of miR604 was found to be stronger in cell lines transfected with the major A allele compared with those transfected with the minor G allele. The data therefore suggest that miR604A>G expression may affect the binding affinity of this miRNA to the 3'-UTR of MTHFR. It is also suggested that the liberation of MTHFR expression by the A to G change in miR604 (rs2368393) may have an effect on one-carbon metabolism. One-carbon metabolism is also associated with vascular and defects in blood coagulation factors, low, abnormal plasma urate, folate, and homocysteine levels, which are risk factors for RPL. The results of Cho et al. suggest that the miR604A>G polymorphism and its effect on MTHFR may contribute to RPL and therefore should be considered when evaluating RPL patients[8]. In our study, we aimed to examine whether the miR604A>G gene polymorphic region could be a potential biomarker for RPL (CHOO). Our results revealed that AA genotype and A allele of the miR604A>G gene were statistically significant for the risk of RPL. Several studies have provided evidence supporting a critical role for miRNAs in RPL[9,10]. According to the findings of Cho et al., the miR604A>G polymorphism is linked to RPL[8]. They reported that the miR604A>G polymorphism is associated with recurrent miscarriage, which is consistent with our findings. Few associated studies have been conducted in this field, and the results of this investigation do not definitively prove the significance of these polymorphisms in recurrent miscarriages[11,12]. Further research into this and other pre-miRNA polymorphisms in other ethnic populations, in combination with functional studies, will aid in understanding the role of miRNA polymorphisms in RPL and its susceptibility. In a recent study, Kim et al. investigated miR604A>G polymorphism and found that miR604A>G polymorphism was statistically significant for recurrent implantation failure[13]. To confirm our results, further studies are needed by novel and detailed analysis[14,15].

CONCLUSION

Only limited studies exist which investigated miR604A>G gene polymorphism and its relation with RPL, and as we know that this is the first study examining the miR604A>G gene polymorphism in relation with RPL in the Turkish population, our results would contribute to the literature.

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AUTHORS' CONTRIBUTIONS

ET: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Writing - original draft. **UC:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision. **MO:** Funding acquisition, Resources, Software, Supervision, Visualization. **ST:** Data curation, Formal Analysis, Funding acquisition, Resources, Software, Validation.

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ORIGINAL ARTICLE

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Does the presence of radiculopathy affect sleep quality and lower extremity functionality in neuropathic low back pain?

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SUMMARY

OBJECTIVE: Sleep disturbance in chronic neuropathic low back pain is a well-known condition. In this study, we aimed to investigate the effect of lumbar radiculopathy on sleep quality and lower extremity functionality in the presence of neuropathic low back pain.

METHODS: A total of 79 patients diagnosed with disk herniation, needle electromyography, and neuropathic pain were included in the study. Visual Analog Scale, Pittsburg Sleep Quality Index, and Lower Extremity Functionality Scale were applied to the patients.

RESULTS: Of the 79 patients who participated in the study, 34 (43%) were females and 45 (57%) were males. No significant difference was found between the group with and without radiculopathy in terms of sleep quality and lower extremity functionality (p=0.245 and p=0.092, respectively). In our study, a negative correlation was found between night pain and the presence of radiculopathy (p=0.006). The number of lumbar herniated disk levels was higher in the group without radiculopathy and was statistically significant (p=0.023).

CONCLUSION: We found that the presence of radiculopathy did not affect sleep quality and lower extremity functionality in disk herniation patients with neuropathic pain. Although it was not statistically significant in our study, we think that the degree of herniation may affect sleep and lower extremity functionality rather than the number of disk herniation levels with the available data. The fact that neuropathic pain is not limited to disk herniation and radiculopathy, and that neuropathic pain is intertwined with clinical conditions such as anxiety, sleep disorders, and depression are among the conditions that make the studies difficult.

KEYWORDS: Radiculopathy. Sleep quality. Back pain with radiation.

INTRODUCTION

Neuropathic pain (NP) is defined as pain that occurs as a result of injury, dysfunction, or change in excitability of a part of the peripheral or central nervous system. The definition of pain arising from somatosensory system lesions or diseases has also been used for NP[1]. The ability of NP to cause pain away from the affected area, the absence of continuous nociceptive stimulation, and its neuroanatomical distribution are the features that distinguish NP from nociceptive pain. Stroke, spinal cord injury, syringomyelia, multiple sclerosis, and cortical atrophy are among the causes of central NP. Diabetes, hypothyroidism, HIV, rheumatoid arthritis, collagen tissue diseases, vasculitides, radiculopathies, and entrapment neuropathies may be the causes of peripheral NP. Symptoms of NP include loss of tendon reflexes, muscle atrophy, weakness, allodynia, hyperalgesia, burning, tingling, and sudomotor changes. Conditions such as wind, temperature changes, friction of clothing, or emotional stress can trigger NP[2].

In chronic low back pain (CLBP), NP may occur due to inflammatory mediators released from the disk, or untreated nociceptive pain as well as NP may occur with direct compression of the nerve by the radiculopathy[3].

Lumbar radiculopathy (LRP) is a clinical condition that can lead to dermatomal pain, muscle weakness, and a decrease in deep tendon reflexes due to compression of spinal nerve roots. The most common cause is herniated disk, and infection, malignancy, and inflammations can cause radiculopathy. The most frequently affected levels are L5 and S1 roots. The diagnosis is made by visualization of the pressure with magnetic resonance imaging (MRI) and electrophysiological examinations[4].

In the literature, studies on chronic pain, CLBP, LRP, and NP show that patients' sleep can be disturbed, and sleep problems are common, especially in patients with chronic neuropathic low back pain[5-7].

In this study, we aimed to examine the effect of radiculopathy on sleep quality and lower extremity functionality in patients with lumbar disk herniation (LDH) with NP. This is the first study in the literature to examine the effect of radiculopathy on sleep quality and lower extremity functionality in patients with LDH and NP.

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METHODS

Study design

This prospective case-control study was conducted in Şanlıurfa Training and Research Hospital Physical Medicine and Rehabilitation outpatient clinic.

Participants

Patients aged between 18 and 75 years were admitted to an outpatient clinic with complaints of CLBP and radicular pain, and 148 patients who had lumbar spinal MRI in the last 3 months and were diagnosed with LDH, who underwent needle electromyography (EMG) with a preliminary diagnosis of radiculopathy in the last 3 months, and who had no motor deficit were analyzed.

Patients with LDH who have conditions such as fibromyalgia, stroke, multiple sclerosis, spinal cord injury, vasculitis, diabetes, hypothyroidism, HIV, herpes simplex, cirrhosis, and malignancy that can cause central or peripheral NP, who have been operated for LDH, who have known sleep disorders, and who are under treatment were not included in the study. A total of 79 patients with NP were included in the study after verbal and written consent.

Data collection

Socio-demographic and anthropometric parameters of the patients were analyzed. Visual Analog Scale (VAS) was used to measure pain. VAS is a well-known, easy-to-implement method. On a 100-mm line, no pain is written on one end and very severe pain on the other end, and the patient marks his current condition on this line. The length of the distance from the point where there is no pain to the point marked by the patient indicates the patient's pain in centimeters. The NP Diagnostic Questionnaire (DN4) was applied to detect NP. DN4 is an open-access, validated, and reliable inventory in Turkish. A score of 4 or higher indicates NP[8].

Pittsburgh Sleep Quality Index (PSQI) and Lower Extremity Functionality Scale (LEFS) were applied to the patients included in the study. PSQI is an open-access index consisting of 18 questions and 7 components, and Turkish validity and reliability studies have been conducted using this index which evaluate sleep disorders and sleep quality in the last 1 month. As a result of the questionnaire, if the total score is above 5, it indicates poor sleep quality[9]. LEFS is an open-access questionnaire consisting of 20 questions, and Turkish validity and reliability studies have been conducted using this questionnaire which evaluate functional impairment in the lower extremities. The total score is between 0 and 80. A high score indicates good lower extremity functions, and a low score indicates poor lower extremity functions[10].

Statistical analysis

Analyses were evaluated in 22 package programs of SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL). Descriptive data are shown as n and percentage values in categorical data, and mean±standard deviation (mean±SD) values in continuous data. Chi-square analysis (Pearson chi-square) was used to compare categorical variables between groups. The conformity of continuous variables to normal distribution was evaluated by the Kolmogorov-Smirnov test. Student's t-test was used to compare paired groups, and one-way ANOVA was used to compare more than two variables. The statistical significance level in the analyses was accepted as $p<0.05$.

Ethical considerations

Written permission was obtained from the Harran University Faculty of Medicine Clinical Research Ethics Committee (HRU.23.02.23) and the institution where the study was conducted prior to data collection. In addition, all study participants were informed about the nature of the study and that participation was on a voluntary basis. Informed consent was obtained from all participants.

RESULTS

A total of 79 patients with a mean age of 44.1±12.7 years were included in the study. Of the patients, 43% (n=34) were females and 57% (n=45) were males. The mean body mass index (BMI) of the patients was 28.7±5.3 kg/m^2, and the mean duration of pain was 17.8±23.6 months. There was a history of low back pain at night in 78.5% (n=62) of the patients and a family history of low back pain in 22.8% (n=18). There was radiculopathy in needle EMG in 53.2% (n=42) of the patients. In needle EMG, 2.5% (n=2) of the patients with radiculopathy were L3-L4, 8.9% (n=7) were L4-L5, 48.1% (n=38) were L5-S1, and 2.5% (n=2) had multiple root involvement. Root involvement was on the right in 27.8% (n=22) of patients with radiculopathy, on the left in 20.3% (n=16), and bilateral in 5.1% (n=4). A total of 60.8% (n=48) of the patients had good sleep and 39.2% (n=31) had poor sleep quality. The mean LEFS of the patients was 35.0±15.3. A total of 51.9% of the patients had 1 level, 35.4% had 2 levels, and 12.7% had 3 levels of LDH (Table 1).

LRP was found to be significantly higher in women (76.5%) than in men (35.6%) ($p<0.001$). The incidence of night pain in patients with LRP (66.7%) was significantly lower than the

Table 1. General characteristics and parameters of the patients.

			Number	%
Age, Mean±SD			44.1±12.7	
Sex		Female	34	43.0
		Male	45	57.0
BMI, Mean±SD			28.7±5.3	
Pain duration (months), Mean±SD			17.8±23.6	
Night pain		Yes	62	78.5
		No	17	21.5
Family low back pain history		Yes	18	22.8
		No	61	77.2
Needle EMG radiculopathy		Yes	42	53.2
		No	37	46.8
VAS, Mean±SD			6.3±1.1	
Needle EMG affected root levels	L3-4	Yes	2	2.5
		No	77	97.5
	L4-5	Yes	7	8.9
		No	72	91.1
	L5-S1	Yes	38	48.1
		No	41	51.9
	Multiple	Yes	2	2.5
		No	77	97.5
Pittsburg Sleep Quality Index		Poor sleep	48	60.8
		Good sleep	31	39.2
LEFS, Mean±SD			35.0±15.3	
Affected root side		No LRP	37	46.8
		Right	22	27.8
		Left	16	20.3
		Bilateral	4	5.1
Affected disk herniations levels number		1 level	41	51.9
		2 levels	28	35.4
		3 levels	10	12.7

SD: standard deviation; BMI: body mass index; EMG: electromyography; VAS: Visual Analog Scale; LEFS: Low Extremity Functional Scale; LRP: lumbar radiculopathy.

rate of night pain in patients without radiculopathy (91.9%) (p=0.006). The mean VAS score of those with radiculopathy was found to be significantly higher than the VAS score of those without radiculopathy (p=0.002). There was no significant difference between the groups in terms of pain duration, BMI, and family history of LBP. While 64.3% of those with radiculopathy had 1 level, 31% had 2 levels, and 4.8% had 3 levels of LDH, 37.8% of those without radiculopathy had 1 level, 40.5% had 2 levels, and 21.6% had 3 levels of LDH. The number of affected disk levels was significantly higher in patients without radiculopathy compared with those who had (p=0.023) (Table 2).

There was no significant relationship between the sleep quality of the participants in terms of age, gender, BMI, duration of pain, night pain, VAS, affected root level, and direction. The LEFS score of those with good sleep quality was found to be significantly lower than those with poor sleep quality (p=0.008). Poor sleep quality was observed in 63.4% of those with 1 level of LDH, 14.3% of those with 2 levels of LDH, and 10% of those with 3 levels of LDH, and there was a significant difference between the number of LDH levels in terms of sleep quality (p<0.001) (Table 3).

DISCUSSION

The incidence of NP in CLBP varies according to countries in the literature, and 53.3% (n=79) of 148 patients with CLBP examined in our study had NP. In a study conducted in a different city in Türkiye using DN4 in 2021, the frequency of NP in CLBP was found to be 43.9%[11]. The reason for this difference may be that NP is associated with conditions such as socio-cultural level and lifestyle.

A total of 53.2% of the patients included in our study had radiculopathy. In our study, the incidence of LRP in females (76.5%) was found to be significantly higher. The most common level of radiculopathy was L5-S1 roots. Our study is compatible with the literature with these results[4,12,13].

In our study, there was no statistical difference between the group with and without radiculopathy in terms of PSQI and LEFS. An increase in the affected disk levels increases the pressure and inflammation in the nervous system and may cause an increase in nerve damage[3,6]. In our study, it was observed that the disk levels in the group with radiculopathy were significantly lower than the group without radiculopathy, and similarly, the affected levels in the patients without radiculopathy were found to be higher. The reason for this situation may be the low number of volunteers and the bulging stages in MRI are considered as herniated disks. It is known in the literature that herniation causing radiculopathy is mostly caused by disk disorder in the protrusion stage[12]. In this respect, our study seems to be compatible with the literature. In terms of sleep quality, we found that patients with a high number of herniated disks had less radiculopathy and better sleep quality. Although it is not statistically significant, we can say that disk herniations that do not reach the stage of radiculopathy have better sleep with our data.

In our study, the rate of night pain in patients with radiculopathy (66.7%) was found to be significantly lower than the rate of night pain in patients without radiculopathy (91.9%). Root irritation symptoms are known to decrease with rest or in positions that reduce the load on the root. Although disk herniation is considered to be the cause of NP in the patient group without radiculopathy, in many studies with CLBP and

Table 2. Comparison of various parameters according to the presence of radiculopathy.

		With radiculopathy		Without radiculopathy		p-value
		Number	%	Number	%	
Age, Mean±SD		46.6±12.1		41.2±12.8		0.056*
Sex[a]	Female	26	76.5	8	23.5	**<0.001****
	Male	16	35.6	29	64.4	
BMI, Mean±SD		28.7±4.5		28.6±6.2		0.950*
Pain duration (months), Mean±SD		16.3±23.3		19.5±24.1		0.544*
Night pain	Yes	28	66.7	34	91.9	**0.006****
	No	14	33.3	3	8.1	
Family history of low back pain	Yes	10	23.8	8	21.6	0.817**
	No	32	76.2	29	78.4	
VAS, Mean±SD		6.7±0.9		5.9±1.2		**0.002***
PSQI[b]	Good sleep	23	54.8	25	67,6	0.245**
	Poor sleep	19	45.2	12	32,4	
LEFS, Mean±SD		32.2±11.8		38.2±18.2		0.092*
Affected disk herniations levels number[b]	1 level	27	64.3	14	37.8	**0.023****
	2 levels	13	31.0	15	40.5	
	3 levels	2	4.8	8	21.6	

*Student's t-test, **Chi-square tests were applied. [a]Row percentage; [b]Column percentage. SD: Standard deviation; BMI: body mass ındex; VAS: Visual Analog Scale; PSQI: Pittsburg Sleep Quality Index; LEFS: Low Extremity Functional Scale. Statistically significant p-values are indicated in bold.

Table 3. Comparison of sleep quality of various parameters[a].

		Good sleep		Poor sleep		p-value
		Number	%	Number	%	
Age, Mean±SD		43.2±11.9		45.4±13.9		0.457*
Sex	Female	19	55.9	15	44.1	0.440**
	Male	29	64.4	16	35.6	
BMI, Mean±SD		29.3±5.6		27.7±4.8		0.201*
Pain duration (months)		16.8±18.6		19.4±30.0		0.629*
Night pain	Yes	41	66.1	21	33.9	0.062**
	No	7	41.2	10	58.8	
Family history of low back pain	Yes	12	66.7	6	33.3	0.559**
	No	36	59.0	25	41.0	
VAS, Mean±SD		6.5±1.2		6.2±1.0		0.242*
LDH level L3-4	Yes	1	50.0	1	50.0	1.000**
	No	47	61.0	30	39.0	
LDH level L4-5	Yes	2	28.6	5	71.4	0.105**
	Yok	46	63.9	26	36.1	
LDH level L5-S1	Yes	22	57.9	16	42.1	0.616**
	No	26	63.4	15	36.6	
Multiple LDH levels	Yes	1	50.0	1	50.0	1.000**
	No	47	61.0	30	39.0	
LEFS, Mean±SD		31.4±14.5		40.6±15.0		**0.008***
In LRP affected root side	No	25	67.6	12	32.4	0.159**
	Right	15	68.2	7	31.8	
	Left	6	37.5	10	62.5	
	Bilateral	2	50.0	2	50.0	
Affected disk herniations levels	1 level	15	36.6	26	63.4	**<0.001****
	2 levels	24	85.7	4	14.3	
	3 levels	9	90.0	1	10.0	

*Student's t-test, **Chi-square test was applied. [a]Row percentage. SD: standard deviation; VAS: Visual Analog Scale; BMI: body mass ındex; LDH: lumbar disk herniation; LEFS: Low Extremity Functional Scale; LRP: lumbar radiculopathy. Statistically significant p-values are indicated in bold.

NP, these patients had disturbed night sleep, frequent painful awakenings, frequent accompanying psychiatric disorders such as depression and anxiety, and central sensitization, with or without radiculopathy. Syndromes have been shown to be more common than the general population[5-7,14].

In our study, 39.2% of the patients had poor sleep quality. When our study is viewed from the perspective of CLBP and NP, it is compatible with the literature[6,11,13].

The LEFS score of those with good sleep quality was found to be significantly lower than those with poor sleep quality. This may be due to the fact that patients with NP who do not have radiculopathy due to LDH, which may be accompanied by central sensitization, were grouped in the poor sleep quality group.

In a study comparing patients with NP due to LRP and fibromyalgia patients with healthy volunteers, it was found that pain threshold values in both fibromyalgia and LRP were lower than the healthy control group, pain modulations were weaker than the healthy control group, and the results of LRP and fibromyalgia were similar[15]. From our study and literature review, we observed that the symptoms in LRP without motor loss are caused by NP and its accompanying clinical problems.

CONCLUSION

We found that the presence of radiculopathy did not affect sleep quality and lower extremity functionality in patients with LDH with CLBP with NP component. The major limitation of our study is the inability to clearly define the etiology of NP in patients. In the presence of disk herniation, NP due to non-disk origin and untreated nociceptive pain may also develop. In the presence of radiculopathy, there were also patients whose DN4 result was not found to be NP and who were excluded from the study. In which of the two patients with radiculopathy at the same root level, the same disk herniation classification, no motor deficit, and similar socio-demographic and anthropometric parameters, does NP occur? The fact that we did the NP research with a single inventory in our study may have caused these questions. Studies with a large number of patients and using more than one inventory will help to find answers to these questions. Although we did not include patients with known psychiatric sleep disorders and fibromyalgia-like chronic pain disorders in our study, it is an important problem affecting the results of our study that low back pain, which has the character of NP, creates similar situations independent of etiology.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Maternal temperament and anxiety sensitivity in children with foreign body aspiration

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SUMMARY

OBJECTIVE: Foreign body aspiration is one of the childhood emergencies that thoracic surgeons are interested in, and it can cause morbidity and mortality. Although the relationship between various behavioral problems related to children and foreign body aspiration has been investigated so far, there is no study investigating the relationship between maternal temperament and anxiety sensitivity. This study aimed to investigate the relationship between maternal emotional temperament, anxiety sensitivity, and foreign body aspiration.

METHODS: Mothers of 18 children with foreign body aspiration have been evaluated by a thoracic surgeon, and 18 healthy controls have also been included in the study. Maternal emotional temperament has been measured with the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego – Auto questionnaire scale, and anxiety sensitivity has been measured with the Anxiety Sensitivity Index-3.

RESULTS: There has been no statistically significant difference between groups in terms of maternal emotional temperament and anxiety sensitivity. In the logistic regression analysis conducted to determine the predictors of foreign body aspiration, it is determined that the mother's anxious temperament has predicted foreign body aspiration significantly.

CONCLUSION: As a result of the study, it can be concluded that mothers' anxious temperament can be considered a risk factor for foreign body aspiration because it affects parenting skills and children's ability to manage behavioral problems. Consistent results could be able to be obtained with studies including larger samples on the subject.

KEYWORDS: Foreign bodies. Child. Mothers. Temperament. Anxiety.

INTRODUCTION

Foreign body aspiration (FBA) is a common cause of emergency admissions and is most common during the early childhood period. It is the leading cause of distress, morbidity, and accidental infantile deaths in children, as well as being the fourth leading cause of death in primary school children[1]. In the United States reports, children who have foreign bodies in their airways exhibit similar risk factors to those previously mentioned, such as being male, being under 5 years old, and not having private insurance. The mortality rate was reported as 2.75%, and older age, urban hospital settings, and teaching hospital status increased mortality risks[2].

It is a known fact that maternal psychopathology is a serious factor that affects both the mother's and the child's health. There are data suggesting that maternal psychiatric disorders might be associated with injuries in children. In a recent population-based study, it has been mentioned that maternal depression was linked to a higher risk of injury in offspring throughout childhood when compared with offspring of mothers who had no history of depression. In the mentioned study, the strongest correlation was observed during the first year of life[3]. Taking these data into consideration, it can be thought that injuries in children might also be related to psychopathology in mothers. In addition to anxiety and depression, anxiety sensitivity could be an important maternal characteristic among children with injury and FBA. Anxiety sensitivity describes how much people find anxiety-related feelings upsetting and how seriously they take these feelings as posing a social, psychological, or physical threat. Anxiety sensitivity differs from state and trait anxiety in that it expresses a relatively stable structural feature and reveals the occurrence of an anxiety disorder in the future[4]. To the best of our knowledge, there is no study in the

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literature that investigates the association between maternal anxiety sensitivity and childhood injury or FBA.

Several psychiatric disorders and behavioral challenges have been found to be related to FBA. Turgut et al. have reported a potentially high prevalence of ADHD symptoms among children presenting to emergency services due to unintentional ingestion of foreign bodies. This could be interpreted as children with ADHD are at an increased risk for FBA[5]. In addition, psychological problems are found more common in children with foreign body ingestion than in the controls[6], and when compared with healthy children, children who had ingested caustic had impulsive behavior. In addition to impulsivity, hyperactivity is a risk factor for caustic ingestion in children under the age of 5 years[7].

In addition to psychopathology, the association between children's temperament profiles and injuries has also been investigated. In a former study, it was suggested that aggressive behavior was linked to all accidental injuries after adjusting for psychosocial factors such as social class, crowding, mother's psychological distress, age, and marital status, as well as child's sex. After accounting for the covariates, overactivity was only linked to injuries that did not necessitate hospitalization. Compared with children with low scores on both behavioral scales, children with high activity and high aggression scores had a 1.9 relative risk of injuries that required hospitalization[8]. However, in a recent study, none of the three temperament types identified by the Childhood Behaviour Questionnaire have been linked to a higher risk of fracture[9]. Similarly, Zhang et al. have also suggested that parenting style could play an important role in mediating children's temperament and recurrent unintentional injury[10]. When these data are evaluated together, it can be thought that not only the temperament of the child but also the factors related to parents might play a role in accidents and injuries.

It appears that psychological factors associated with both parents and children might also be associated with FBA. Although there have been several studies that examine the association between mothers' psychiatric disorders and children with FBA, there is no study conducted on the temperament characteristics of mothers. In addition, there is no study on anxiety sensitivity, which is considered a temperament trait, either. This study aimed to compare the temperament characteristics and anxiety sensitivity of mothers of children treated for FBA with mothers of healthy children.

METHODS

A total of 36 mothers have been included in this study (18 mothers of children with FBA were considered the patient group and 18 mothers of healthy children were considered the control group). The patient group was chosen from the mothers of children with FBA that admitted to thoracic surgery clinics of Niğde Training and Research Hospital and Uşak Training and Research Hospital. The control group was chosen from the mothers of children with mild symptoms (i.e., consultancy, upper respiratory tract infection, and so on) that were admitted to Niğde Training and Research Hospital Pediatric Outpatient Clinic. The sample consisted of only the mothers of the children with FBA and the healthy ones. As FBA is a condition that is usually managed under emergency department circumstances, the participants were asked to fill out the scales during the follow-up appointments. At the follow-up appointment, after the child was evaluated, the mother was asked to fill in the scales in a suitable room. Moreover, maternal mental health status was evaluated via an interview. Due to the possibility of a confounding effect, participants with any current psychiatric disorders, those receiving medical treatment, and those with long-term medical conditions have been excluded from our study. The history of any psychiatric disorder was determined by self-report. Our findings would have been tainted if we had included participants who had any pre-existing psychiatric disorders because a diagnostic interview had not been conducted with them. The questionnaires have been completed by the researchers. The protocol of this study has received approval from the Nide Training and Research Hospital Ethics Committee (approval number: 2022/46 and approval date: 28.04.2022), and each participant has given their informed consent before participating in the study.

Tools

Sociodemographic Data Form: The data form that includes questions about age, family characteristics, and clinic characteristics has been used in this study. Using this form, the child's age, type of family, father's and mother's age, occupation, educational level, and family income have been evaluated, and the form has been completed by researchers.

Anxiety Sensitivity Index (ASI-3): The ASI-3, developed by Taylor et al.[11], has been used to evaluate AS. This 18-item scale is divided into three six-item subscales (physical, cognitive, and social); total ASI-3 scores range from 0 to 54, with higher scores indicating higher levels of AS. This scale is a self-report scale. The ASI-3 has undergone validation and reliability testing in Turkey[12]. The total score is obtained by summing the scores obtained from all items.

Temperament Evaluation of Memphis, Pisa, Paris, and San Diego – Auto questionnaire (TEMPS-A): Akiskal et al.[13] have developed the TEMPS-A in order to assess affective temperament

dimensions. It addresses five temperament dimensions: depressive, cyclothymic, hyperthymic, irritable, and anxious. TEMPS-A is a dichotomous scale. Questions can be answered as yes or no. The answer "yes" is scored as 1 and "no" as 0. Subscale scores and total scores are obtained by summing the scores obtained from various questions. Vahip et al. have established the validity and reliability of the TEMPS-A for Turkish speakers[14].

SPSS has been used to perform statistical analysis on the collected data. All the analyses have been conducted with scales, total scores, and TEMPS-A subscale scores. Demographic variables have been analyzed by using descriptive statistics. The Kolmogorov-Smirnov and Shapiro-Wilk tests are used to determine the normality of the data. In order to examine the differences between the groups, the independent-sample T test and Mann-Whitney U test are used, and to examine the differences between categorical variables, the chi-square test is used. The potential predictors of FBA have been identified by using logistic regression analysis. A statistically significant two-tailed p-value of 0.05 has been considered.

RESULTS

The study sample consisted of 36 children and their mothers, of which 18 were patients (16 boys and 2 girls) and 18 were healthy controls (18 boys). There has been no significant difference between the groups in terms of gender (p=0.146, χ^2=2.118). There has been no significant difference between the groups in terms of mothers' age (28.83±5.41 years for the patient group and 29.11±4.76 years for the control group, p<0.871). The median age of patients is 24 months (IQR=72), and the median age of controls is 36 months (IQR=30). There has been no significant difference between groups in terms of age (p=0.141, U=116.000). The demographic data are demonstrated in Table 1. According to the test of normality results, age and mothers' irritable, anxious temperament scores have not been normally distributed. Moreover, depressive, cyclotomic, hyperthymic temperament and anxiety sensitivity index scores are normally distributed. When the two groups are compared in terms of mothers' temperament dimension and anxiety sensitivity, it can be seen that there is no significance between the two groups. More detailed statistics are given in Table 2.

The predictors of FBA have been assessed via the logistic regression analysis. FBA diagnosis is defined as the dependent variable (0=no diagnosis and 1=FBA), and all temperament dimensions and anxiety sensitivity are defined as independent variables. According to this analysis, mothers' anxious temperament has been a significant predictor of having epilepsy (p=0.05, odds ratio=1.299, 95% confidence interval=1–1.688). The results of the logistic regression analysis are shown in Table 3.

DISCUSSION

In this study, we have investigated the association between FBA and mothers' temperament characteristics and anxiety sensitivity.

Table 1. Demographic and clinical characteristics.

	Patient	Control	Patient	Control	U	p
	Median	IQR	Median	IQR		
Age (months)	24	72	36	30	116.000	0.141
	n	%	n	%	χ^2	p
Sex (female/male)	2/16	11.1/88.9	0/28	0/100	2.118	0.146

Table 2. The comparison of mothers' temperament dimensions and anxiety sensitivity.

	Patient		Control		Statistics	p
	Mean	SD	Mean	SD	t	
Depressive temperament	6.28	4.74	6.22	3.50	-0.40	0.968
Cyclothymic temperament	6.83	4.23	8.78	3.73	1.462	0.153
Hyperthymic temperament	11.44	7.30	10.72	5.41	-0.337	0.738
Anxiety sensitivity index	15	10.31	21.5	12.07	1.737	0.091
	Mean rank	Sum of rank	Mean rank	Sum of rank	U	p
Irritable temperament	20.5	369	16.50	297	126.000	0.249
Anxious temperament	20	360	17	306	135.000	0.391

Table 3. Logistic regression analysis about the predictor of foreign body aspiration.

	B	SE β	Wald's χ2	p	OR	95%CI OR
Depressive temperament	-0.010	0.149	0.004	0.947	0.990	0.739–1.327
Cyclothymic temperament	-0.260	0.145	3.206	0.073	0.771	0.580–1.025
Hyperthymic temperament	0.097	0.080	1.464	0.226	1.102	0.941–1.290
Irritable temperament	0.113	0.129	0.771	0.380	1.120	0.870–1.441
Anxious temperament	0.262	0.134	3.845	0.050	1.299	1–1.688
Anxiety sensitivity index	-0.066	0.047	2.035	0.154	.936	0.854–1.025
Constant	-0.117	1.686	0.005	0.945	.890	

The dependent variable in this analysis is foreign body aspiration diagnosis (0=no diagnosis 1=foreign body aspiration). OR: odds ratio; CI: confidence interval.

As a result, it is determined that there is no significant difference between patients and controls in terms of temperament subdimensions and anxiety sensitivity. In logistic regression analysis, anxious temperament is found as a significant predictor of FBA. To the best of our knowledge, this is the first study in the literature that examines the association between FBA and mothers' temperament characteristics and anxiety sensitivity.

There are several studies in the literature about FBA and child psychopathology. Turgut et al. proposed a potentially elevated occurrence of ADHD symptoms among children who were referred to emergency services subsequent to the inadvertent ingestion of foreign bodies[5]. Similarly, Çelenk et al. also mentioned that ADHD might be associated with the self-insertion of nasal and aural foreign bodies and suggested that clinicians should be aware of the possibility of ADHD in children, particularly those between the ages of 5 and 9 years who present with self-inserted nasal and aural foreign bodies[15]. In a more detailed study, which investigated the psychological status of children with foreign body ingestion, it has been found that there is a significant increase in SDQ scores between the two groups in total score, emotional symptoms, hyperactivity disorders, conduct problems, and prosocial behaviors[6]. Therefore, by taking these studies into consideration, it can be suggested that children with FBA would be separated from their peers who have no history of FBA in the psychological dimension. Increased emotional difficulties and increased ADHD symptoms could be risk factors for FBA. Moreover, this study has investigated maternal risk factors such as temperament and anxiety sensitivity. In another recent study that investigated the relationship between maternal affective temperament and ADHD and comorbidities, the results have shown a positive association between maternal anxious and irritable temperament and child inattention, hyperactivity-impulsivity, and oppositional defiant disorder (ODD) scores[16]. There are other studies in the literature which support the association between maternal anxious temperament and ODD behaviors[17]. In our study, we have also found that maternal anxious affective temperament significantly predicts FBA. Considering the close relationship between FBA and ADHD, our finding is consistent with the literature. Despite the lack of clarity regarding the mechanisms underlying the link between maternal anxiety or an anxious temperament and oppositional-defiant behaviors, it has been suggested that maternal anxiety might have increasing effects on these behaviors by adversely affecting parenting techniques[18]. It is determined that maternal anxious affective temperament characteristics of the mother might affect her parenting skills and thus create a risk for FBA. Also, it is known that the parenting skills are crucial in preventing FBA, so it can be said that improving parenting skills through various sources increases awareness about FBA[19]. However, we could not determine any association in terms of anxiety sensitivity. Our relatively small sample size might have interfered with founding a significant association.

This study has certain limitations. First, our sample size is relatively small. As FBA is a relatively rare condition and most cases are being treated with various different methods (e.g., Heimlich maneuver) without thoracic surgery consultation, we have had difficulty in collecting a larger sample group. This limitation could have interfered with our comparisons between two study groups. It is a cross-sectional study, so the results might lack the ability to infer causality and/or risk. Further prospective studies are required to clarify the association between maternal affective temperament and FBA. Second, a structured diagnostic evaluation has not been conducted with children, but instead self-report measuring tools have been used. This could be considered a methodological limitation that might interfere with the results.

As a result, no study has been conducted specifically on children with FBA and possible maternal temperament and anxiety sensitivity association, which would be an important factor considering the issue. We think that our study is important in terms of determining the temperament profile of children with FBA. We think that, in such a situation in which preventive measures can be life-saving, it is important to identify the risk

factors and intervene in those in the risk group. It is thought that there is a need for studies investigating maternal affective temperament and FBA association with larger sample groups in which diagnostic evaluations are also conducted.

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AUTHORS' CONTRIBUTIONS

MÇ: Data curation, Project administration, Supervision, Writing – review & editing. **SBA:** Conceptualization, Investigation Methodology, Project administration, Supervision, Writing – original draft. **AE:** Data curation, Validation, Visualization. **ZC:** Data curation, Validation, Visualization. **NS:** Formal Analysis, Resources, Software. **İT:** Funding acquisition, Project administration, Resources, Software, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Impact of catheter ablation procedure on optical coherence tomography angiography findings in patients with ventricular arrhythmia

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SUMMARY

OBJECTIVE: Catheter ablation procedure may cause retinal complications associated with the risk of thromboembolism. We aimed to evaluate retina and optic disc microvascularity with optical coherence tomography angiography before and after the catheter ablation process in patients with ventricular arrhythmia.

METHODS: A total of 40 eyes of 21 ventricular arrhythmia patients were included in this cross-sectional study. Demographic characteristics and ophthalmic examination findings of patients were recorded. optical coherence tomography angiography measurements were evaluated before (group 1) and after (group 2) catheter ablation. Optical coherence tomography angiography was applied to all eyes with 6×6 mm sections for the macula and 4.5×4.5 mm sections for the optic nerve head. Foveal retinal thickness, peripapillary retinal nerve fiber layer thickness, vessel density in different parts of the retina, and optic disc were analyzed.

RESULTS: The mean age of ventricular arrhythmia patients was 53.48±13.02 years. In all, 13 (61.9%) of the patients were males and 8 (38.1%) were females. There was no significant difference between the groups in terms of average, inferior, superior, and temporal retinal nerve fiber layer thicknesses, foveal avascular area, flow areas, superficial and deep vessel densities, and optic disc capillary densities of the optic disc. However, when compared with group 1, significantly lower values in foveal retinal thickness and higher values in nasal retinal nerve fiber layer thickness were observed in group 2 (248.42±20.50 vs. 247.20±20.44, p<0.001 and 94.22±18.43 vs. 96.12±20.18, p=0.044, respectively).

CONCLUSION: Although foveal retinal thickness and nasal retinal nerve fiber layer thickness are affected in patients undergoing catheter ablation for ventricular arrhythmia, the stable retinal and optic disc vessel densities can be explained by the administration of effective anticoagulants during the procedure.

KEYWORDS: Arrhythmia. Catheter ablation. Angiography. Retina. Optic disc. Microvasculature.

INTRODUCTION

Catheter ablation (CA) by radiofrequency is the most effective treatment modality for ventricular arrhythmias (VAs) as premature ventricular contractions or ventricular tachycardia[1-4]. However, endocardial CA procedures may cause cardiac complications[5,6] as well as cerebral[7,8] and ocular[9] complications. Ablation in the left ventricle is associated with a risk of thromboembolism[10].

Optical coherence tomography angiography (OCTA) is an innovative, rapid, and high-resolution method that shows the retinal and choroidal perfusion in minimal acquisition time[11]. This noninvasive tool can be valuable to assess cardiovascular status having a promising biomarker as retinal vascular density[12].

There is a report about OCTA findings before and after CA in patients with atrial fibrillation[13]. However, to the best of our knowledge, there is no data regarding the effect of CA on optic nerve head and retinal microvasculature in patients with VA. Given the fact that OCTA is a noninvasive relatively simple and cost-effective examination, it may provide a useful tool for the evaluation of the risk of CA procedure. Therefore, in this study, we aimed to compare the OCTA parameters of VA patients before and after CA and to have an opinion about the safety of radiofrequency CA procedure for optic disc and retinal perfusion.

METHODS

This cross-sectional study was approved by the Ethics Committee of our institution (approval number: 2022/25) and conducted in accordance with all the relevant tenets of the Declaration of Helsinki. Patients with VA (including ventricular tachycardia or repetitive premature ventricular contractions), before and 24 h

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after the CA procedure, referred to the ophthalmology outpatient clinic from the department of cardiology were included in the study. The mapping and CA strategy and periprocedural anticoagulation management were applied as described in the literature[7].

Eyes with a refractive error of ≥±2.00 diopters, amblyopia, previous ocular surgery, ocular trauma, and other ocular diseases such as uveitis, cataract, or glaucoma, any systemic disease such as diabetes except regulated hypertension and dyslipidemia, and history of anticoagulant drug use were excluded.

All subjects underwent full ophthalmologic examination including best-corrected visual acuity, biomicroscopic anterior segment, and fundus examination. Intraocular pressure (IOP) and central corneal thickness measurements were assessed by a noncontact tonometer.

The OCTA images were obtained by a single technician using a spectral-domain OCT system with the AngioVue OCTA software (Avanti RTVue-XR 100, OptovueInc, Fremont, CA). The OCTA image protocol involved two scans covering a 6×6 mm area centered on the macula and a 4.5×4.5 mm area centered on the optic nerve head. Foveal retinal thickness (FRT), vessel density in the fovea of superficial and deep capillary plexus, and 300 µm width around the foveal avascular zone (FAZ) were measured. Flow areas of the outer retina and choriocapillaris were noted, too. Radial peripapillary capillary (RPC) densities (including whole image, inside disc, and peripapillary capillary plexus densities) and retinal nerve fiber layer (RNFL) thickness were also obtained. The exclusion criteria were OCTA scans with a quality level of less than 8, artifacts, or decentered. Baseline and 24 h after the CA, OCTA measurements of all patients were taken.

Statistical analysis

All analyses were performed using SPSS 21.0 for the Windows software. Continuous variables were described using mean value±standard deviation. Categorical data were expressed as number and percentages. As for inferential statistics, in order to compare the OCTA measurements at baseline and 24 h after the CA procedure, paired-samples T test was used. A p-value<0.05 was considered significant.

RESULTS

A total of 40 eyes of 21 VA patients were enrolled. The mean age of VA patients was 53.48±13.02 years (range 27–75). In all, 13 (61.9%) of the patients were males and 8 (38.1%) were females. The baseline characteristics of VA subjects are shown in Table 1. There was no significant difference between the groups in terms of average, inferior, superior, and temporal RNFL thicknesses, foveal avascular area flow areas, superficial and deep vessel densities, and optic disc capillary densities of the optic disc. However, when compared with group 1, significantly lower values in FRT and higher values in nasal RNFL thickness were observed in group 2 (248.42±20.50 vs. 247.20±20.44, p<0.001 and 94.22±18.43 vs. 96.12±20.18, p=0.044, respectively). The outcomes of retina and optic disc parameters by OCTA are shown in Table 2 and Table 3, respectively.

DISCUSSION

This OCTA study demonstrated no significant difference between the groups in terms of retinal and optic disc vessel densities. However, when compared with group 1, significantly lower values in FRT and higher values in nasal RNFL thickness were observed in group 2.

OCTA has increasing use in clinical practice and also enables quantitative analysis of blood flow in the retina and optic nerve head. The different studies in the literature have evaluated changes in OCTA parameters in different cardiovascular problems[12,14] and cardiac interventions[11,13].

CA is an effective treatment option for VA. One of the most devastating complications of this procedure is cerebral thromboembolism[7]. Borišincová et al.[7] reported significant asymptomatic brain injury in one-fifth of subjects after ablation of ventricular tachycardia. Embolism can also occur in the retinal vasculature as in the cerebral vascular bed. In a study, the authors evaluated whether retinal microemboli were visible on OCTA following transcatheter aortic valve implantation[11]. They mentioned 28.6% of new capillary dropout lesions in OCTA scans after transcatheter aortic valve implantation. However, quantitative measurements of macular and peripapillary flow remained stable after transcatheter aortic valve

Table 1. Baseline characteristics of ventricular arrhythmia subjects.

	Mean±SD
Number of subjects, n	21
Number of eyes, n	40
Age, years (Mean±SD) Range	53.48±13.02 (27–75)
Female/male (n, %)	8/13 (38.1/61.9)
Intraocular pressure (mmHg) (Mean±SD)	18.78±4.8
Central corneal thickness (µm) (Mean±SD)	543.53±22.4
Body mass index (kg/m^2) (Mean±SD)	23.42±4.8
Hypertension, n	5
Smoking, n	7

VA: ventricular arrhythmia; SD: standard deviation.

Table 2. The outcomes of retina parameters by optical coherence tomography angiography.

	Before CA (Group 1)	After CA (Group 2)	p
FRT (μm) (Mean±SD)	248.42±20.50	247.20±20.44	**<0.001**
Superficial vessel density (%) (Mean±SD)			
Whole image	48.13±5.64	48.98±5.16	0.111
Fovea	20.77±6.44	20.04±6.85	0.068
Parafovea	50.65±6.17	50.79±7.95	0.904
Perifovea	48.61±5.72	49.35±5.43	0.177
Deep vessel density (%) (Mean±SD)			
Whole image	50.94±7.53	51.45±7.55	0.631
Fovea	37.67±7.44	36.96±8.49	0.191
Parafovea	55.19±4.78	55.84±5.06	0.424
Perifovea	52.13±8.34	52.77±8.33	0.598
FAZ area (mm^2) (Mean±SD)	0.26±0.08	0.28±0.16	0.344
Flow area for outer retina (mm^2) (Mean±SD)	0.70±0.43	0.72±0.51	0.789
Flow area for choriocapillaris (mm^2) (Mean±SD)	2.10±0.78	2.03±0.32	0.225

OCTA: optical coherence tomography angiography; CA: catheter ablation; FRT: foveal retinal thickness; FAZ: area of 300 μm width around the foveal avascular zone; SD: standard deviation. Bold indicates statistically significant p-values.

Table 3. The outcomes of optic disc parameters by optical coherence tomography angiography.

	Before CA (Group 1)	After CA (Group 2)	p
RNFL average thickness (μm) (Mean±SD)	106.51±17.60	109.12±21.48	0.134
Inferior quadrant (μm)	138.87±24.95	141.92±30.35	0.266
Superior quadrant (μm)	127.95±23.22	129.30±24.23	0.131
Temporal quadrant (μm)	68.05±11.97	69.22±14.67	0.438
Nasal quadrant (μm)	94.22±18.43	96.12±20.18	**0.044**
RPC density (%) (Mean ± SD)			
Whole image	47.80±4.56	48.15±5.22	0.515
Inside disc	48.45±6.43	48.78±5.89	0.730
Peripapillary	49.71±5.32	50.17±6.32	0.501

OCTA: optical coherence tomography angiography; CA: catheter ablation; RNFL: retinal nerve fiber layer; RPC: radial peripapillary capillary; SD: standard deviation. Bold indicates statistically significant p-values.

implantation, possibly indicating effective ocular blood flow regulation within the range of left ventricular ejection fraction. In concordance with this study, stable results of retinal and optic disc vessel densities were also obtained in our study. Moreover, we did not observe any capillary dropout lesions in OCTA scans. We thought that it can be explained by the administration of effective anticoagulants during the procedure. A loading dose of unfractionated heparin (10,000 IU) was given.

Then, heparin was administered by intermittent boluses to maintain the activated clotting time in the range of 300–350 s[7].

On the contrary, IOP alterations of patients during the CA process can be responsible for lower values in FRT. However, we did not have a chance to follow up IOP of patients during the procedure. Additionally, higher values in nasal RNFL thickness may be due to transient capillary rarefaction and limitation of blood flow through sparse capillary networks.

Our study has some limitations. First, the number of patients was relatively small. Second, it would be better if there was a control group. Third, the results can be affected by smoking status[14] and presence of hypertension[15] and dyslipidemia[16]. In this study, seven of the patients had a smoking history, and five had hypertension and dyslipidemia. As each compared measurement result is evaluated on the same patient basis and the hypertension and dyslipidemia were controlled, we thought that it would not affect the results. Finally, we do not have long-term results after the CA procedure. This can be the subject of another study.

CONCLUSIONS

Although the FRT and nasal RNFL thickness are affected in patients undergoing the CA for VA, the CA procedure appears to be safe due to the stable results of retinal and optic disc vessel densities. This stability can be explained by the administration of effective anticoagulants during the procedure. Larger prospective longitudinal OCTA studies are needed to determine the safety of radiofrequency CA procedure for optic disc and retinal perfusion.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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The prognostic significance of the heterologous component in uterine carcinosarcomas

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SUMMARY

OBJECTIVE: Uterine carcinosarcomas are aggressive, rare biphasic tumors with malignant epithelial and malignant sarcomatous components. The prognostic significance of the presence of extrauterine sarcoma (heterologous component) is controversial. Therefore, the aim of this study was to investigate the effect of heterologous components in uterine carcinosarcomas on disease-free survival, overall survival, and other prognostic factors.

METHODS: Clinical and histopathological data from patients treated for uterine carcinosarcoma in a tertiary cancer center in Turkey between July 2000 and January 2020 were collected. Independent risk factors affecting overall survival and disease-free survival were analyzed by univariate and multivariate Cox regression analyses.

RESULTS: A total of 98 patients were identified. The median follow-up was 21.8 (1.2–233.1) months. In the multivariate analysis, the median overall survival and disease-free survival were 23.8 and 20.7 months in those with homologous mesenchymal components and 17.6 and 9.7 months in those with heterologous mesenchymal components, respectively. It was found that the presence of heterologous mesenchymal components significantly reduced both overall survival and disease-free survival (odds ratio [OR], 2.861; 95% confidence interval [CI] 1.196–6.841; p=0.018 and OR, 3.697; 95%CI 1.572–8.695; p=0.003, respectively). In addition, both lymphadenectomy and adjuvant radiotherapy were found to significantly increase overall survival and disease-free survival. Age was found to increase only disease-free survival.

CONCLUSION: The results obtained in this study showed that the presence of heterologous components in uterine carcinosarcoma is a prognostic factor that adversely affects both overall survival and disease-free survival. Lymphadenectomy and adjuvant radiotherapy have beneficial effects on both overall survival and disease-free survival.

KEYWORDS: Uterine neoplasm. Cancer of uterus. Carcinosarcoma. Prognosis. Survival.

INTRODUCTION

Uterine carcinosarcomas (UCs), which are also known as malignant mixed mesenchymal tumors, are extremely aggressive and rare tumors[1]. Although carcinosarcomas account for only 5% of all uterine tumors, they are responsible for 15% of deaths due to uterine corpus malignancies[2]. UCs are biphasic tumors with malignant epithelial and sarcomatous components[3]. The sarcomatous component can be homologous (uterine-type mesenchymal tissue) or heterologous (non-gynecological mesenchymal tissue)[3].

UCs are classified as endometrial cancer, and surgical staging is performed according to the recommendations of the International Federation of Gynecology and Obstetrics (FIGO)[4]. The main treatments for UCs are total hysterectomy, bilateral salpingo-oophorectomy (BSO), systematic pelvic and paraaortic lymph node dissection (PPLND), omentectomy or omental biopsy, and resection of the entire gross mass[5]. Adjuvant therapy is indicated for patients in stages IB–IV and is closely associated with overall survival (OS)[6].

Various clinical features and prognostic factors that affect treatment response and determine prognosis have been evaluated in previous studies. Cancer stage, epithelial component grade, performance status, cancer antigen (CA) 125 level, lymphovascular site invasion (LVSI), depth of myometrial invasion, lymphadenectomy, lymph node metastasis (LNM), presence of residual tumor, and adjuvant therapy have all been associated

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with survival[6-10]. However, the prognostic effect of the heterologous component is controversial[2,3,5,11,12].

Therefore, the aim of this study was to investigate the effect of the heterologous component on disease-free survival (DFS) and OS in UCs and examine other clinical and histopathological features that affect prognosis.

METHODS

The records of patients treated for UC in the gynecological oncology clinic of a tertiary cancer center between July 2000 and January 2020 were reviewed. The files of 107 patients diagnosed with carcinosarcoma were reviewed. A total of 98 patients who underwent surgical staging in our clinic, whose paraffin blocks were evaluated by pathologists specializing in gynecological oncology, and who received adjuvant treatment in our hospital were included in the study. Four patients who underwent surgery at another center but received adjuvant treatment at our clinic were excluded from the study.

Patients' demographic characteristics, surgical and histopathological characteristics, and clinical results were retrieved from hospital records. Age at diagnosis, date of diagnosis, body mass index (BMI), CA 125 level, stage, tumor diameter, tumor grade, myometrial invasion, LVSI, cervical invasion, whether lymphadenectomy was performed, LNM, omental metastasis presence, histopathological features, presence of residual disease, adjuvant treatments, recurrence status, date of last follow-up, date of recurrence, and time of death were recorded.

Staging was carried out according to the FIGO 2009 staging system. Surgical staging included TAH, BSO, PPLND, total omentectomy, or omental biopsy. Optimal cytoreduction surgery was indicated for residual tumors of <1 cm, while suboptimal surgery was indicated for residual tumors of >1 cm. Among the histopathological features, epithelial and mesenchymal components were examined separately. The epithelial component was initially divided into two groups: endometroid and non-endometroid. Endometrioid types were classified histologically into grades 1, 2, and 3. In the non-endometroid group, cases were categorized as serous carcinoma, clear cell carcinoma, squamous cell carcinoma, undifferentiated carcinoma, and serous+clear cell carcinoma. The mesenchymal component was classified as either homologous or heterologous. Homologous-type sarcomas include leiomyosarcoma, endometrial stromal sarcoma, and high-grade/undifferentiated sarcoma. Heterologous types included rhabdomyosarcoma, chondrosarcoma, osteosarcomas, and double or triple comorbidities.

The time from surgery to recurrence was used to calculate DFS. The time from surgery to the date of the last follow-up or death was used to calculate OS. The time from the date of first diagnosis to the date of the last follow-up or death was used to calculate the mean follow-up period. The study was performed in accordance with the World Medical Association Declaration of Helsinki, and ethical approval was obtained from the ethics committee of Tepecik Training and Research Hospital with decision number 2021/02-25.

Data were analyzed using IBM SPSS V23. Independent risk factors affecting OS and DFS were analyzed by univariate Cox regression analysis. The Kaplan-Meier test was used to compare the prognostic factors with OS duration. Categorical data were expressed as frequency and percentage, while quantitative data were presented as mean, standard deviation, median, minimum, and maximum. $p<0.05$ was considered statistically significant in all analyses.

RESULTS

A total of 98 patients were evaluated. The median age of the patients was 63 (45–84) years. The median follow-up was 21.8 (1.2–233.1) months. The median CA 125 level was 21.07 (2.98–3290). According to the histopathological features of the patients, 75 (76.5%) had LVSI. Deep myometrial invasion was detected in 69 (70.4%) patients, whereas cervical invasion was detected in 39 (39.8%) patients. Lymph node dissection was performed in 87 (88.8%) patients, and LNM was detected in 22 (22.4%) patients. Optimal cytoreductive surgery (residual tumor>1 cm) could not be performed in 18 patients (18.4%) because of their poor general condition or diffuse nonresectable tumor (Table 1).

Independent risk factors affecting OS and DFS were analyzed by univariate Cox regression analysis. Stage 4, presence of LVSI, cervical invasion, increased tumor diameter, increased CA 125 level, presence of LNM, lack of optimal cytoreduction (residual disease>1 cm), presence of non-endometrioid epithelial component, and lack of radiotherapy significantly reduced OS and DFS. While lymphadenectomy was associated with OS, it did not significantly affect DFS. The heterologous mesenchymal component was found to be associated only with DFS. Age, BMI, and adjuvant chemotherapy did not have a statistically significant effect on either OS or DFS (Table 2).

When the independent risk factors affecting OS and DFS were analyzed by multivariate Cox regression analysis (Table 2), median OS and DFS were found to be 23.8 (3.02–31.7) and 20.7 (3.0–231.7) months in those with a homologous mesenchymal component and 17.6 (1.2–233.1) and 9.7 (1.2–233.1) months in those with a heterologous mesenchymal component, respectively. The heterologous mesenchymal component had a

Table 1. Demographic and clinical characteristics of patients.

	%/Median (Range)
Age (years)	63 (45–4)
BMI	31.6 (20.7–51.4)
Average follow-up time (months)	21.8 (1.2–233.1)
CA 125	21.07 (2.98–3290)
Tumor diameter (cm)	6 (1–30)
Stage	
Stage 1	43.9
Stage 2	14.3
Stage 3	19.4
Stage 4	12.4
Lymphadenectomy	
Yes	88.8
No	11.2
Lymph node metastasis	
Yes	22.4
No	66.3
Residual disease (cm)	
>1	18.4
<1	81.6
Epithelial component	
Endometrioid type	45.9
Grade 1	2.0
Grade 2	23.5
Grade 3	20.4
Non-endometrioid	54.1
Serous carcinoma	44.9
Squamous cell carcinoma	4.1
Undifferentiated carcinoma	3.1
Clear cell carcinoma	1.0
Serous + clear cell carcinoma	1.0
Mesenchymal component	
Homologous	74.5
Endometrial stromal sarcoma	4.1
Leiomyosarcoma	14.3
High-grade/differentiated sarcoma	56.1
Heterologous	25.5
Rhabdomyosarcoma	13.3
Chondrosarcoma	8.2
Osteosarcoma	1.0
Rhabdomyosarcoma/ chondrosarcoma /osteosarcoma	2.1
Rhabdomyosarcoma/ chondrosarcoma	1.0

BMI (kg/m^2): body mass index; CA 125 (IU/mL): cancer antigen 125.

significant effect on both OS and DFS (Figure 1) (odds ratio [OR], 2.861; 95% confidence interval [CI] 1.196–6.841; p=0.018 and OR, 3.697; 95%CI 1.572–8.695; p=0.003, respectively).

DISCUSSION

Most UC cases have a single epithelial component, a poorly differentiated serous carcinoma. However, endometrioid, clear cell, mucinous, squamous, and undifferentiated histological types can all be observed. Similar to the epithelial component, the mesenchymal component often has a single sarcomatous component, with the most common homologous component being high-grade stromal sarcoma. Rhabdomyosarcoma is the most common heterologous component, followed by chondrosarcoma, osteosarcoma, and liposarcoma[1]. A consensus has emerged in UC regarding some prognostic factors such as stage, lymphadenectomy, residual disease, and adjuvant therapy that affect survival[6,10,12,13]. In this study, which was conducted in a tertiary reference center with more than 20 years of experience in the field of gynecological oncology, we found that the presence of a heterologous component in UC shortened both OS and DFS compared with the presence of a homologous component. Multivariate analysis showed that the heterologous mesenchymal component had a significant effect on both OS and DFS.

To the best of our knowledge, this is the first study to report that the heterologous component negatively affects both OS and DFS in all carcinosarcoma stages. Fergusson et al. evaluated only stage 1 patients and found that patients with heterologous components had worse DFS and 3-year OS[11].

In a multicenter retrospective study that accumulated the largest data in the United Kingdom, Marsoo et al. evaluated 1,192 patients[14]. The patients were divided into four groups. The 5-year progression-free survival (PFS) rate was 50.6% for low-grade/homologous, 50.6% for low-grade/heterologous, 45.8% for high-grade/homologous, and 34.0% for high-grade/heterologous cases. For low-grade carcinoma cases, the presence of heterologous components showed worse PFS but did not make a statistically significant difference. In high-grade carcinoma cases, the presence of heterologous components resulted in significantly worse outcomes.

Revirosa et al. investigated the pathological prognostic factors of 81 stage 1–3 patients, and the multivariate analyses revealed no significant results for age, heterologous components, or myometrial invasion, except for the disease stage[12]. Temkin et al. evaluated 47 patients and reported that the heterologous components did not affect either OS or DFS in stage 1–2 patients[10]. This result could be attributed to the inclusion

Table 2. Cox regression analysis results.

	Overall survival		Disease-free survival	
	OR (95%CI)	p	OR (95%CI)	p
Univariate				
Age	1.016 (0.981–1.052)	0.375	1.018 (0.983–1.054)	0.321
BMI	1.022 (0.968–1,090)	0.422	1.016 (0.964–1.071)	0.547
Stage (reference: Stage 1)				
Stage 2	0.776 (0.288–2.093)	0.617	0.755 (0.280–2.034)	0.578
Stage 3	0.977 (0.406–2.352)	0.959	1.054 (0.437–2.538)	0.907
Stage 4	3.242 (1.660–6.332)	**0.001**	3.917 (1.994–7.696)	**<0.001**
Tumor diameter	1.048 (1.003–1.095)	**0.034**	1.051 (1.006–1.098)	**0.025**
CA 125	1.001 (1–1.001)	**0.048**	1.001 (1–1.002)	**0.001**
Lymphadenectomy	0.249 (0.073–0.852)	**0.027**	0.425 (0.129–1.402)	0.160
Lymph node metastasis	2.372 (1.325–4.244)	**0.004**	2.598 (1.45–4.655)	**0.001**
Residual disease	2.029 (1.088–3.784)	**0.026**	2.076 (1.111–3.88)	**0.022**
Mesenchymal component (heterologous)	1.820 (0.996–3.328)	0.052	1.995 (1.093–3.644)	**0.025**
Epithelial component (non-endometrioid)	2.394 (1.32–4.344)	**0.004**	2.608 (1.432–4.749)	**0.002**
Multivariate				
Age	1.037 (0.993–1.082)	0.099	1.053 (1.011–1.097)	**0.014**
Lymphadenectomy	0.081 (0.021–0.314)	**<0.001**	0.094 (0.024–0.361)	**0.001**
Mesenchymal component (heterologous)	2.861 (1.196–6.841)	**0.018**	3.697 (1.572–8.695)	**0.003**

OR: odds ratio; CI: confidence interval; BMI (kg/m^{2}): body mass index; CA 125 (IU/mL): cancer antigen 125. Statistically significant values are indicated in bold

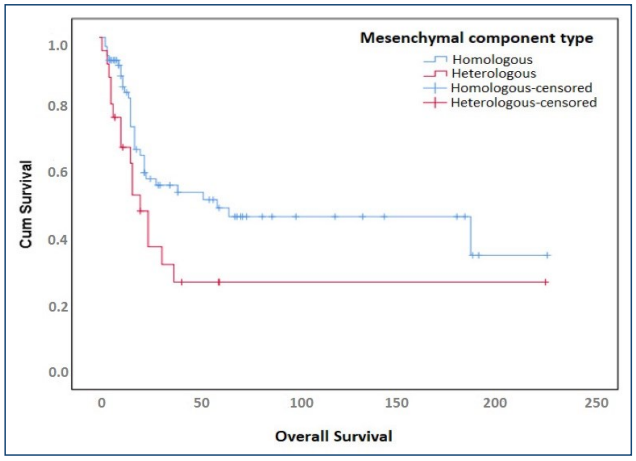

Figure 1. Overall survival graphics according to the type of mesenchymal component.

of early-stage patients in the study and the presence of heterologous components increasing disease progression and stage, resulting in detection at a lower rate in the early stages.

As supported by many studies in the literature, we also found that lymphadenectomy had a positive effect on OS in this study[1,10,13,15]. Although some studies claim that the number of lymph nodes resected is also effective, others claim that lymphadenectomy is strongly associated with OS[13,15]. However, in a similar study conducted by Ross et al., it was reported that pelvic lymph node dissection did not contribute to PFS but only increased OS, while paraaortic lymph node dissection had no effect on OS and PFS[16]. In contrast, the authors reported that the presence of LNM affected survival. In this study, the presence of LNM had a negative effect on both OS and DFS.

In UCs, adjuvant treatment is generally performed with radiotherapy, chemotherapy, or a combination of both[17,18]. In this study, multivariate analysis revealed that radiotherapy significantly improved OS and DFS. However, contrary to the literature, it was found that chemotherapy had no effect on survival[17,18]. This could be attributed to the fact that carcinosarcomas are already aggressive cancers and the vast majority of patients have received chemotherapy. However, 62.2% of our patients had received radiotherapy as adjuvant treatment.

The median age of UC onset is 62–67 years[19]. The median age of onset in the present study was consistent with the literature. In the multivariate analysis, age had no effect on OS and decreased DFS. Chen et al. evaluated 81 patients and reported that advanced age was a significantly worse prognostic factor in terms of both OS and PFS[20]. There are also studies in the

literature stating that age has no prognostic value in carcinosarcomas[11-13]. Worse DFS in advanced age could be attributed to the better response to adjuvant treatment in younger patients.

In uterine carcinomas, as in high-grade endometrial cancers, cancer stage, CA 125 elevation, LVSI positivity, presence of cervical invasion, presence of large tumor, presence of omental metastasis, and presence of residual tumor after surgery are associated with poor prognosis[1,3,5,6,16,19,21]. Consistent with the literature, it was also found in this study that these prognostic factors affected survival.

This study has several limitations. The study was designed retrospectively and conducted in a single center. In addition, although we were able to analyze the tumoral components of the patients effectively, we were unable to obtain clearer results because we could not detect how much of the tumor was covered by sarcoma or epithelial types in all patients. There is a need for further studies with a larger patient group and more detailed histopathological analysis that can specify the percentage of the tumor's heterologous component.

In conclusion, the results obtained in this study show that the coexistence of extrauterine sarcoma types with uterine tumors reduces life expectancy. It is difficult to conduct prospective studies because of the aggressive progression and short survival of carcinosarcomas. However, the results of this study are valuable because of the sufficient number of patients in a single tertiary center and the expert pathologists involved in the study. According to the findings of our study, a more decisive application of radiotherapy and chemotherapy combinations may improve survival and life expectancy in cases with a heterologous component.

ETHICAL APPROVAL

Ethical approval was obtained from the ethics committee of Tepecik Training and Research Hospital with decision number 2021/02-25.

AUTHORS' CONTRIBUTIONS

BC: Investigation, Project administration, Writing – original draft. **VK:** Conceptualization, Resources, Writing – original draft, Writing – review & editing. **İÇ:** Formal Analysis, Funding acquisition, Writing – original draft. **SS:** Data curation, Methodology, Writing – original draft. **KH:** Software, Validation, Writing – original draft. **OK:** Supervision, Visualization, Writing – original draft.

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ORIGINAL ARTICLE

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The use of artificial intelligence to improve the scientific writing of non-native english speakers

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SUMMARY

OBJECTIVE: Scientific writing in English is a daunting task for non-native English speakers. The challenges of writing in a foreign language are evident in the scientific literature where texts by non-native English-speaking scientists tend to be less clear and succinct, contain grammatical errors, and are often rejected by prestigious journals.

METHODS: We conducted a non-systematic review of the most recent literature using the terms "Artificial Intelligence," "Scientific Writing," and "Non-English Speaking" to create a narrative review.

RESULTS: Artificial intelligence can be a solution to improve scientific writing, especially for non-native English-speaking scientists. Artificial intelligence can assist in the search for pertinent scientific papers, generate summaries, and help with the writing of different sections of the manuscript, including the abstract, introduction, methods, results, and discussion. Artificial intelligence-based programs can correct grammatical errors and improve writing style, both of which are particularly helpful for non-native English speakers. Two artificial intelligence programs that can help with the search for pertinent scientific papers on the internet are Elicit and ResearchRabbit. Scispace Copilot can be used to summarize the retrieved reference. The artificial intelligence software programs such as Grammarly and Paperpal can correct grammatical and spelling errors, while ChatGPT can also restructure sentences and paragraphs, reword text, and suggest appropriate words and phrases.

CONCLUSION: Overall, artificial intelligence can be an effective tool to improve the clarity, style, and coherence of scientific writing, helping non-native English-speaking scientists to communicate their research more effectively.

KEYWORDS: Artificial intelligence. Language. Writing, medical. Internet.

INTRODUCTION

Most scientific papers in the world literature are written in English[1] by non-native English speakers[2]. Non-native English-speaking scientists face many difficulties in writing clearly, succinctly, and without grammatical errors[3,4]. Nevertheless, despite the use of word processors and spell checkers, the final text still does not compare favorably with those created by native English speakers, thus contributing to the lower chances of acceptance of these papers in prestigious scientific journals[5,6].

Artificial intelligence (AI) involves the development of algorithms and computer programs that can learn from and make predictions or decisions based on real-world data, mimicking human intelligence. Furthermore, AI systems can be trained to recognize data patterns, make predictions, and learn from experience. AI can thus perform tasks that typically require human-like reasoning and decision-making.

Natural language processing is a type of AI that enables machines to understand, interpret, and generate human language in a manner that is natural to humans[7,8]. For example, ChatGPT (Generative Pre-Trained Transformer) is a language model developed by OpenAI that was designed to generate natural language responses to text prompts. In addition, ChatGPT's underlying AI system is pre-trained on vast amounts of text data from the Internet that enables it to learn the patterns and structures of languages. Therefore, it learns how to generate coherent and contextually appropriate responses to a wide range of text prompts.

With all these capabilities, the AI programs can assist scientists in the search for pertinent scientific papers on the Internet, summarize them, and help with the writing of abstracts, titles, and parts of the introduction, methods, results, and discussion. In addition, the AI programs can correct mistakes and improve the writing style of previously written texts, both of which are particularly helpful to non-native English-speaking scientists[9-12].

In this paper, we will briefly describe how AI-based programs can help with the preparation of research papers, especially considering how they can help improve scientific writing skills.

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METHODS

Due to the very recent widespread access to AI-based programs such as ChatGPT, there was no enough evidence in the medical literature to pursue with a systematic review. We therefore elected to conduct a non-systematic review of the most recent literature using the terms "Artificial Intelligence," "Scientific Writing," and "Non-English Speaking" to create a narrative review using Google Scholar, Google, and AI-powered reference retrieval programs such as Elicit (www.elicit.org) and ResearchRabbit (https://www.researchrabbit.ai/).

RESULTS

Artificial intelligence programs supporting scientific writing

Drafting a research manuscript entails several steps to be taken after the scientist has all the experimental or clinical trial results already tabulated, analyzed, and displayed in tables and/or figures. It is also important to conduct a recent and thorough review of the literature and have all the references that will be used in the paper stored in the citation software for future use. Most of the experts then suggest writing the results, followed by methodology sections[13,14]. These sections are the easiest ones to write as they are readily available to the researcher when he or she becomes ready to start drafting the paper. Therefore, these sections, which represent the essence of the paper, are not going to benefit as much from AI text-generating capabilities, but they still can be much improved by AI-powered grammar and spelling correctors. Then comes the discussion section and finally the introduction, abstract, and title[13,14]. These parts of a paper can be drafted with AI help, once the AI-powered program is fed with the methods and results previously written. Several authors have already outlined which are the parts that every one of the sections should have and what type of content they should include for a paper to convey all the scientific information properly and clearly as it is expected to report[15-18].

Artificial intelligence and bibliographic reference

Before even starting to write a paper, it is necessary to assemble all the pertinent references already published in the literature to learn about the field and at the same time to find still unanswered questions that can be the subject of future research. AI is particularly useful for this purpose, as it can find references related to a specific article from its content, authors, or citations and quickly build a new list of references that would otherwise be much more difficult to collect. Two AI programs can be helpful for this purpose, namely, ResearchRabbit (https://www.researchrabbit.ai/) (Figure 1) and Elicit (www.elicit.org). Both programs will suggest references many times not yet known by the scientist because they can go "beyond the horizon" by a self-learning approach based on several retrieved papers using their contents, keywords, and citations[19] to retrieve other papers.

AI can also help summarizing selected references by using both Elicit and Scispace Copilot (https://typeset.io/). Scispace Copilot is an AI chrome extension that can also explain highlighted parts of a paper and the statistical results obtained and allows the scientist to ask his own questions regarding the text.

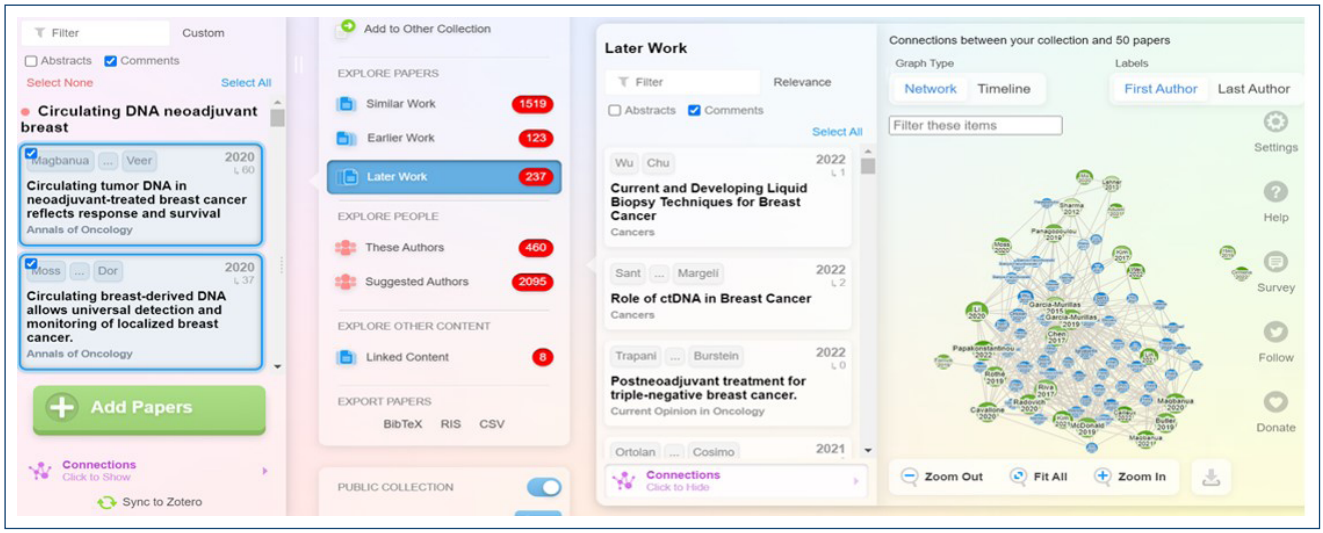

Figure 1. Reference searching with ResearchRabbit.

All the selected papers should then be stored in a reference management system (RMS). Although RMSs do not normally use AI, they are powerful tools to help in scientific writing. Examples of RMSs are Zotero (www.zotero.org) and Mendeley (www.mendeley.com), both of which are free and compatible with the most widely used word processors such as Microsoft Word. Zotero can also be used with Google Docs. Zotero and Mendeley allow the scientist to store his or her notes on each of the stored papers that could be useful later when writing the paper. Both these RMSs can import articles found with Elicit and ResearchRabbit. Many other RMSs such as Endnote and Paperpile, which are widely used by scientists, are not free. With the help of the RMS software, we can insert citations in the text as we are writing it and at the end of the paper produce a bibliography including all references that were cited in the text in a specific format such as Vancouver. Moreover, if editing changes are made later in the paper, these RMSs can automatically change the order of the references in the text and the bibliography.

Artificial intelligence and correction and improvement of english texts

Several software programs also use AI that can correct grammatical and spelling errors as well as improve the text with suggestions such as Grammarly (app.grammarly.com) and Paperpal (www.paperpal.com). Despite suggesting useful changes in the text, these programs, however, do not change the main structure of the article or generate new text. The AI tools such as ChatGPT, however, can correct spelling, punctuation, and grammatical errors; in addition, it can also summarize the text, paraphrase it, and even create a section of a paper based on another, for example, creating an abstract or an introduction based on the results of the paper. Most interesting to us is the possibility of a non-native English speaker loading his text into ChatGPT and asking the AI program to improve it so that it will not be perceived anymore as a paper written by a foreigner. ChatGPT can also write code to analyze data present in a Microsoft Excel spreadsheet or in a statistical program such as R and advise on what statistical tests to use to analyze data and help explain the obtained results.

ChatGPT works with prompts and knowing some of them as related to scientific writing may be especially useful to maximize its output toward a desired goal. For instance, before loading the text of a paper or a section of it to ChatGPT within quotation marks, we can ask the AI program to proceed with several tasks through prompts that we type in the chatbot window. For example, we can ask ChatGPT to summarize the following paper "paste the text of the paper here." Likewise, we can ask it to Create an introduction; Create a title; Create 250 words abstract; Reduce an abstract; Create a title for an abstract (Figure 2); Create a discussion; and so on.

ChatGPT is a very promising resource and will certainly improve with all the technological improvements that can be foreseen in the next few years. Nevertheless, we can already point out some of the advantages and potential disadvantages that it can bring to scientific writing (Table 1).

Ethical considerations

AI can help the scientist in these steps differently. In fact, at present, AI can be an accessory, a facilitator, but should never be expected to write any of the sections of a scientific paper as an author would. As amazing as it can be that AI-generated output can be rapidly produced, grammatically correct, and sometimes even insightful in terms of its content, several errors can occur that limit the usefulness of AI-generated texts without due correction and editing[10].

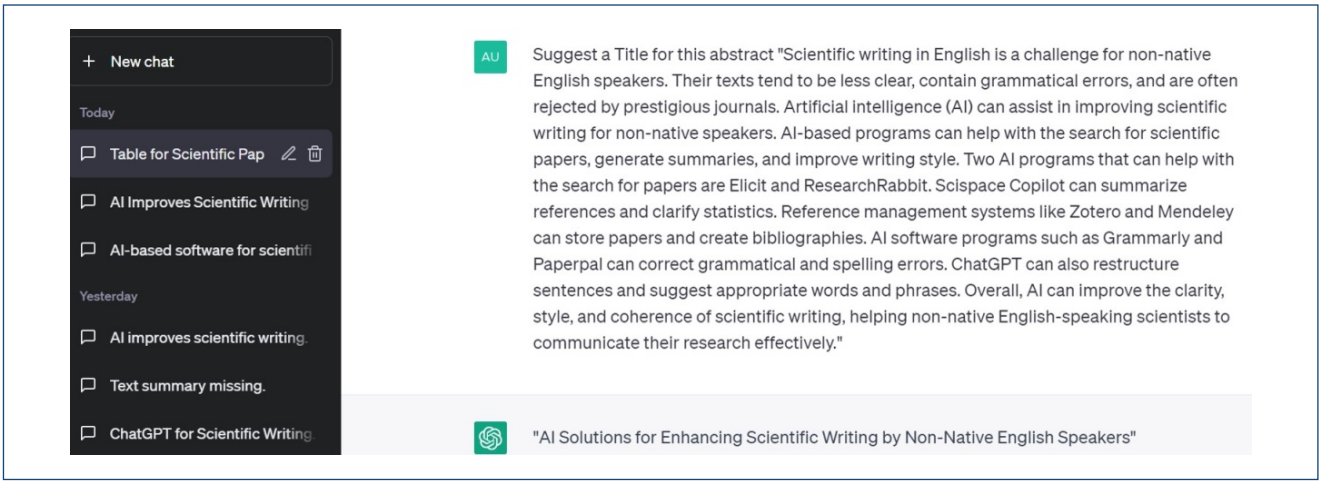

Figure 2. Using ChatGPT for creating a title for an abstract.

Table 1. Benefits and disadvantages of the artificial intelligence software programs in scientific writing.

Benefits	Disadvantages
AI-based programs can help break mental logjams when writing by suggesting a first draft of a text. These programs can also enhance readability by breaking up a difficult topic into smaller pieces.	Incapable of understanding added information, generating novel insights and deep analysis which would limit the discussion within a scientific paper. AI tools are more adequate for regurgitating conventional wisdom than for identifying or generating unique outcomes.
Programs such as Elicit or ResearchRabbit can identify references for a specific topic that might be missed by conventional literature searches.	Superficial, and over-reliance on the output could decrease scientific creativity by reducing the impact of papers in generating real contributions to a specific knowledge field.
Be used effectively to improve your manuscript's title, abstract, and conclusion and tailor it to match the journal parameters and better match its scope or readership.	AI might be worse at assessing whether a unique outcome is spurious or ground-breaking. Therefore, reliance upon AI for this purpose will reduce the frequency of future disruptive scientific breakthrough.
Help a writer be more thorough when covering a topic by reminding them of aspects they had not considered and by providing knowledge in areas not yet familiar to the author in an easy and understandable way.	AI-based programs may harm the development of writing skills by young researchers and facilitate plagiarism.
Facilitating composition by non-native English speakers and decreasing inequalities existing with native-speaking researchers. The language resources of AI will certainly be included soon directly in other interfaces, such as Microsoft Office 365.	
Develop code for Python and other programs such as R and Excel. This code-generating ability can help in the analysis of statistical data and in creating figures to represent experimental results.	

ChatGPT cannot be considered a coauthor in a scientific paper. Authorship requires contributions to the conception, design, analysis, interpretation, and writing and approval of a definitive version of the manuscript. Furthermore, authorship also requires that authors take public responsibility for the content of the paper[20]. As ChatGPT cannot be responsible for what it writes, the responsibility regarding the content of a scientific paper rests completely upon all the human coauthors who must check and edit all the generated content.

Nevertheless, it is the authors' opinion that the editorial help provided by the AI-powered software programs such as ChatGPT can be of use to improve their writing. Authors need, however, to properly disclose at the end of the paper in which of the article's sections the AI-powered software programs were used and for what reasons. In addition, it is important to state that AI-generated output was duly checked by the authors. Acknowledgment regarding the responsibility for all the paper content needs to be explicitly recognized by all the authors as well. We believe that, if all these precautions are taken, AI can be safely and productively used by non-native English-speaking researchers to improve their scientific writing[21,22]. At the end of this paper, we added a paragraph based on a model suggested by Elsevier Company[21] to acknowledge the use of AI in this article that may be used by other researchers as well.

Another ethical concern with AI-generated texts is the potential for plagiarism[22]. Plagiarism detectors exist[23] to help in identifying texts or parts of texts that were copied from other sources. Unfortunately, however, automatic rephrasing of paragraphs can potentially elude plagiarism detection[24]. A potential problem with this technology that can circumvent plagiarism detection is the proliferation of papers with few real contributions to add to the overall knowledge that may further contribute to the relative decrease in scientific breakthroughs we are currently observing[25].

CONCLUSION

Writing scientific articles in English is a challenging task for non-native speakers. However, with the help of the AI tools, non-native English speakers can improve the clarity, style, and coherence of their writing. The AI-based programs can assist with various aspects of the scientific writing process, including searching for relevant papers, generating summaries, correcting grammatical errors, improving writing style, and creating bibliographies. Elicit, ResearchRabbit, Scispace Copilot, Zotero, Mendeley, Grammarly, Paperpal, and ChatGPT are some of the AI tools that can be particularly helpful for non-native English-speaking scientists. Using the AI-powered software programs can not only enhance the quality of scientific writing but also help researchers produce papers with higher chances of publication in major scientific journals and thus help effectively communicate their research to a wider audience.

Artificial intelligence disclosure

During the preparation of this work, the authors used CHATGPT to generate a draft of the abstract and conclusion sections. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

AUTHORS' CONTRIBUTIONS

ADG: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. **MUPC:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Role of increased plasminogen activator inhibitor-1 and vitronectin in gestational diabetes mellitus

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SUMMARY

OBJECTIVE: The aim of this study was to analyze the second-trimester levels of vitronectin and plasminogen activator inhibitor-1 in gestational diabetes mellitus.

METHODS: This study was conducted between September 2020 and December 2020 at the University of Health Sciences, Bursa Yuksek Ihtisas Research and Training Hospital, Department of Obstetrics and Gynecology. A total of 30 pregnant women with gestational diabetes mellitus and 60 healthy controls between 24 and 27/6 weeks of gestation were included. The inclusion criteria were as follows: being between 18 and 45 years old and 24–27/6 gestational weeks, having singleton pregnancy, diagnosed with gestational diabetes mellitus by using a two-step challenge test. The exclusion criteria of this study were as follows: chronic inflammatory or infectious disease, fasting blood glucose>126 mg/dL, intolerance to glucose tolerance testing, abnormal liver or kidney function tests, as well as pregnancy with pre-gestational diabetes history of adverse perinatal outcomes. Serum vitronectin and plasminogen activator inhibitor-1 levels were measured using the enzyme-linked immunosorbent assay method.

RESULTS: Vitronectin and plasminogen activator inhibitor-1 levels were higher in the gestational diabetes mellitus group compared with controls [91.85 (23.08) vs. 80.10 (39.18) ng/mL, for vitronectin and 6.50 (1.05) vs. 4.35(1.0) ng/mL, for plasminogen activator inhibitor-1 (for both p<0.001)]. vitronectin >84.7 ng/mL was found to predict gestational diabetes mellitus with a sensitivity of 70% and specificity of 63.3%. Moreover, vitronectin had a significant positive correlation with fasting blood glucose (r=0.476, p<0.001), postprandial blood glucose (r=0.489, p<0.001), HbA1c (r=0.713, p<0.001), and plasminogen activator inhibitor-1 (r=0.586, p<0.001).

CONCLUSION: This study revealed that second-trimester vitronectin and plasminogen activator inhibitor-1 are increased in gestational diabetes mellitus and vitronectin could be a candidate for the prediction of gestational diabetes mellitus.

KEYWORDS: Biomarkers. Gestational diabetes mellitus. Second trimester.

INTRODUCTION

Gestational diabetes mellitus (GDM), the incidence of which varies from 2 to 10%, can be defined as glucose intolerance with onset or first recognition in pregnancy[1]. Beta-cell dysfunction in pancreatic tissue, insulin resistance, low-grade inflammation, and endothelial dysfunction are the main pathophysiological mechanisms in GDM[2,3]. As GDM is tightly associated with short- and long-term perinatal mortality and morbidity such as cardiovascular diseases, type 2 diabetes mellitus, birth complications, cesarean delivery, and endocrine disorders of neonate, new biomarkers elucidating the etiology of GDM have been suggested in the literature[4,5].

Vitronectin (Vn), which is encoded by the Vn gene, is a 75-kDa cellular adhesion glycoprotein with its N-terminal somatomedin-B domain, central hemopexin-like domain, and C-terminal domain[6-8]. It has been found in many tissues including plasma, extracellular matrix, platelets, liver, blood vessels, embryonic lungs, renal basal membrane, muscles, and human skin[9]. Vn plays crucial roles in many processes such as regulation of coagulation cascade, oncogenic formation, fibrinolysis, inflammation, wound healing, fibrosis, and insulin signaling[10,11]. It interacts with integrins and urokinase plasminogen activators and leads to neutrophil adhesion and migration[12]. Somatomedin B domain of Vn stabilizes plasminogen activator inhibitor-1 (PAI-1) which plays a primary role in the inhibition of plasminogen activators and the in vivo conversion of plasminogen to plasmin[13]. In the literature, high PAI-1 levels have been demonstrated to predict the risk of type 2 diabetes, and deficiency in PAI-1 has a protective role against insulin resistance[14]. Recent studies have suggested that GDM triggers the expression and release of PAI-1, which is associated with GDM severity due to insulin

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The study was carried out Department of Obstetrics and Gynecology University of Health Sciences, Bursa Yuksek Ihtisas Research and Training Hospital, Bursa, Turkey.

resistance development and exaggerated proinflammatory and inflammatory cytokines. High PAI-1 levels in GDM may cause hypofibrinolysis and thrombotic complications[15-17].

As insulin resistance and inflammation are the main etiological mechanisms for GDM, we hypothesized that Vn and PAI-1 are increased in cases with GDM. There is no study evaluating the levels of these markers together for GDM in the second trimester. In this study, we first aimed to analyze second-trimester Vn and PAI-1 levels together in pregnant women.

METHODS

This observational case-control study was conducted between September 2020 and December 2020 at the University of Health Sciences, Bursa Yuksek Ihtisas Research and Training Hospital, Department of Obstetrics and Gynecology. This study was approved by the local ethics committee (2011-KAEK-25 2020/09-13), and written informed consent was obtained from all study participants.

Study population

A power analysis was performed, and the analysis revealed that the minimum patient number was 30 for each group with 80% power to detect a 30% difference in cases with a value of 0.05. In this prospective case-control study, a total of 30 pregnant women with a diagnosis of GDM and 60 pregnant women without GDM were included in the study. GDM was diagnosed if the patient had a glucose level of >200 mg/dL at 50 g oral glucose challenge test or 95 mg/dL for fasting, 180 mg/dL at the first hour, 155 mg/dL at the second hour, and 140 mg/dL at the third hour in 100 g testing for patients who have 50 g challenge test value of 140–200 mg/dL. The control group was composed of pregnant women who had normal 50 g oral glucose testing. The inclusion criteria were as follows: being between 18 and 45 years old and 24–27/6 gestational week, having singleton pregnancy, diagnosed with GDM by using a two-step challenge test. The exclusion criteria of this study were as follows: chronic inflammatory or infectious disease, fasting blood glucose>126 mg/dL, intolerance to glucose tolerance testing, abnormal liver or kidney function tests, as well as pregnancy with pre-gestational diabetes history of adverse perinatal outcomes (Figure 1). The sociodemographic and obstetric features and laboratory characteristics were recorded.

Definition of gestational diabetes mellitus

In our clinic, we routinely screen pregnant women for GDM between 24 and 28 gestational weeks by a two-step protocol that

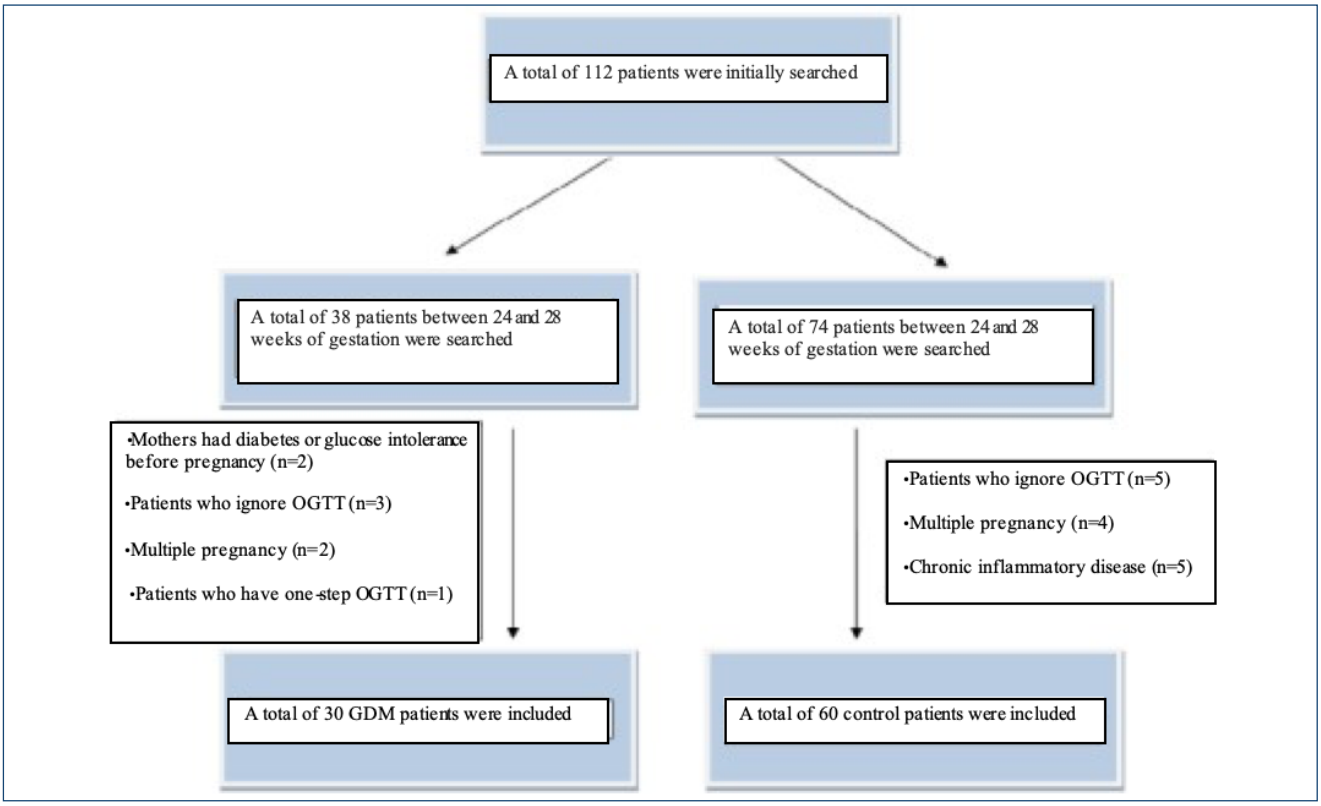

Figure 1. Flowchart showing selection of gestational diabetes mellitus and non-gestational diabetes mellitus cohorts.

was suggested by the 2018 American College of Obstetricians and Gynaecologists Guidelines[18]. In a two-step protocol, 50 g oral glucose tolerance test was used in the first step followed by a 100 g oral glucose tolerance test if blood glucose levels were above 140 mg/dL at 1 h in 50 g testing. GDM was diagnosed if two abnormal glucose levels were detected according to the Carpenter and Coustan criteria in 100 g tolerance testing. The diagnostic glucose levels were 95 mg/dL for fasting,180 mg/dL at the first hour, 155 mg/dL at the second hour, and 140 mg/dL at the third hour in 100 g testing. Consequently, pregnant women who had normal 50 g oral glucose testing were assigned to the control group, whereas pregnant women diagnosed with GDM by a two-step protocol were assigned to the GDM group.

Vitronectin and plasminogen activator inhibitor-1 measurement

Patients serum samples were obtained from the antecubital vein after 12 h of fasting and the sera were stored for Vn and PAI-1 measurement at -80°C after centrifuged at 3,500 rpm for 10 min to be analyzed after the patient was examined for GDM. Serum Vn and PAI-1 levels were measured using a commercially available kit, namely, Human Vn and PAI-1 kit, with the enzyme-linked immunosorbent assay method.

Statistical analysis

Statistical analyses were performed on the SPSS software. Shapiro-Wilk's test was used to determine whether the obtained data were normally distributed or not. Variables were defined as mean±standard deviation for normally distributed quantitative variables and median (IQR) for non-normally distributed quantitative variables. Student's t-test and Mann-Whitney U tests were used for two-group analysis. Categorical variables were compared with chi-square or Fisher's exact test. The correlation between Vn and clinical variables was evaluated by performing Spearman correlation analysis. The predictive value of Vn for GDM patients was determined by receiver operating curve analysis. A p-value<0.05 was considered statistically significant.

RESULTS

The sociodemographic features and the perinatal outcomes of the GDM (n=30) and control group (n=60) are demonstrated in Table 1. Gestational age at delivery was significantly lower in the GDM group compared with the control group [37 (1.47) vs. 38.18 (1.44) weeks, p<0.003]. The laboratory characteristics of the GDM and control groups are shown in Table 2. Fasting glucose postprandial glucose, HbA1c, C-reactive protein

Table 1. Clinical characteristics and perinatal outcomes of the gestational diabetes mellitus and control groups.

Variables	GDM group (n=30)	Control group (n=60)	p
Age (years)	30.46 (5.37)	28.45 (5.62)	0.107[a]
Gravida (n)	2 (1.25)	2 (2)	0.291[b]
Parity (n)	1 (1.25)	1 (2)	0.993[b]
Body mass index (kg/m²)	26.97 (1.87)	26.39 (1.96)	0.107[a]
Cesarean section (n, %)	14 (46.7%)	20 (33.3%)	0.219[c]
Gestational age at delivery (weeks)	37 (1.47)	38.18 (1.44)	**<0.003[a]**
Birth weight (g)	3,200 (2,150–4,560)	3,165 (2,060–4,350)	0.840[b]
Polyhydramnios (n,%)	4 (13.3%)	5 (8.3%)	0.474[c]
Macrosomia (n, %)	4 (13.3%)	5 (8.3%)	0.474[c]
Apgar score first min	7 (1)	8 (2)	0.430[b]
Apgar score fifth min	9 (1)	9 (1)	0.682[b]
Apgar first min <7	4 (13.3%)	6 (10%)	0.726[d]
Apgar fifth min<7	2 (6.7%)	2 (3.3%)	0.598[d]
NICU admission (n, %)	15 (30%)	9 (15%)	0.058[c]
Neonatal sepsis (n, %)	3 (10%)	5 (8.3%)	1.000[d]
RDS (n, %)	8 (16%)	4 (6.7%)	0.183[c]
Adverse perinatal outcome (n, %)	10 (33.3%)	12 (20%)	0.165[c]

NICU: neonatal intensive care unit; RDS: respiratory distress syndrome. aIndependent-samples t-test; bMann-Whitney U test; cChi-square test; dFisher's exact test. Statistically significant value is indicated in bold.

Table 2. Laboratory characteristics of the gestational diabetes mellitus and control groups.

Variables	GDM group (n=30)	Control group (n=60)	p
Fasting glucose (mg/dL)	87 (15)	75 (13)	**<0.001[b]**
Postprandial glucose (mg/dL)	165.5 (45.75)	112 (26)	**<0.001[b]**
HbA1c (%)	6.1 (1.13)	5.5 (0.97)	**<0.001[b]**
C-reactive protein (mg/L)	3.3 (1.43)	2.4 (1.28)	**<0.001[b]**
Urea (mg/dL)	26.14 (7.39)	25.73 (7.29)	0.773[a]
Creatinine (mg/dL)	0.71 (0.2)	0.6 (0.2)	0.077[b]
AST (IU/L)	18 (14.25)	17 (5.75)	0.406[b]
ALT (IU/L)	16 (8.25)	13 (6)	0.151[b]
Hemoglobin (g/dL)	10.99 (1.46)	10.82 (1.17)	0.468[a]
Hematocrit (%)	32.89 (4.47)	32.43 (2.88)	0.521[a]
Platelet count (10^3/L)	238 (51.25)	209 (51.75)	**0.024[b]**
Vn (ng/mL)	91.85 (23.08)	80.10 (39.18)	**<0.001[b]**
PAI-1 (ng/mL)	6.50 (1.05)	4.35 (1.0)	**<0.001[b]**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; Vn: vitronectin; PAI-1: plasminogen activator inhibitor-1. aIndependent samples t-test; bMann-Whitney U test. Statistically significant values are indicated in bold.

levels, and platelet levels were significantly higher in the GDM group (p<0.05). Furthermore, median Vn and PAI-1 levels were higher in the GDM group compared with the control group [91.85 (23.08) vs. 80.10 (39.18) ng/mL, for Vn and 6.50 (1.05) vs. 4.35 (1.0) ng/mL, for PAI-1 (for both of them p<0.001)]. The predictive value of Vn for GDM patients was determined by receiver operating curve analysis. Vn was found to predict GDM with a cutoff value >84.7 ng/mL with a sensitivity of 70% and specificity of 63.3%. The area under the curve was 0.647 and the 95% confidence interval was 0.550 and 0.736 with a p-value of 0.005. The receiver operating curve for the predictive value of Vn to diagnose GDM is presented in Figure 2.

Another finding of the study was the correlation of Vn with clinical parameters. Vn had a significant positive correlation with fasting blood glucose (r=0.476, p<0.001), postprandial blood glucose (r=0.489, p<0.001), HbA1c (r=0.713, p<0.001), CRP (r=0.245, p<0.001), and PAI-1 (r=0.586, p<0.001).

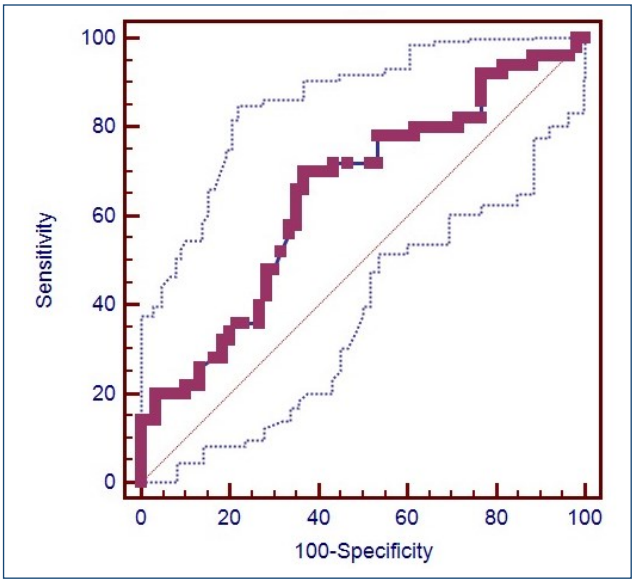

Figure 2. Receiver operating curve for the predictive value of vitronectin to diagnose gestational diabetes mellitus.

DISCUSSION

This study evaluated the role of Vn and PAI-1 in the second trimester of gestation in GDM. The main findings of the study demonstrated that both Vn and PAI-1 levels were increased in GDM. Vn was found to predict GDM with a cutoff value of >84.7 ng/mL with a sensitivity of 70% and specificity of 63.3%. Moreover, Vn levels were correlated with fasting blood glucose, postprandial glucose level, HbA1c, CRP, and PAI-1.

Gestational diabetes is a metabolic disorder that increases women's risk and likelihood of developing type 2 diabetes and cardiovascular disease following life. Inflammatory and metabolic changes that occur in normal pregnancy altered and exaggerated secondary to the excessive systemic inflammatory process in GDM initiated by diffuse endothelial dysfunction[19]. There are several factors regulating PAI-1 expression in GDM,

such as hyperglycemia, hyperinsulinemia, proinflammatory cytokines, and elevated angiotensin II[19]. Vn is an adhesive extracellular glycoprotein with binding sites or PAI-1, the urokinase-type plasminogen activator receptor, and various integrins and is circulated as a high-molecular-weight complex[20]. In studies performed with fibroblast culture, it has been shown that PAI-1 inhibits the interaction of Vn and integrin and modulates the properties of Vn. Studies testified that Vn induces insulin secretion independently of glucose and a significant reciprocal decrease in insulin content in fetal beta cells[2].

There are limited studies in the literature evaluating the role of Vn and PAI-1. Ekmekçi et al. reported high PAI-1, t-PA, and CRP levels and low Vn levels in patients with early- and late-onset preeclampsia (PE). They suggested that increased Vn complex formation led to the increment in PAI-1 level and PAI-1 activity, and decreased Vn levels contributed to the progression of inflammation and hypercoagulability in PE[21]. Contrary to this study, Blumenstein et al. found high Vn and high-molecular-weight quinolone levels in early pregnancy before the development of PE and small gestational age. They stated that Vn provides material endothelin repair by increasing platelet adhesion and aggregation following cell damage in areas with vascular endothelial damage developing in PE[22].

Yaghoubi et al. reported high Vn levels in patients with coronary artery disease, which correlated significantly with the severity of the disease[23]. These results can be attributed to the regulatory role of Vn in the vascular hemostatic response and thrombus formation in vascular injury in atherosclerotic lesions. Evaluating the role of these markers in diabetes, Alessi et al. found higher basal Vn and PAI-1 levels in patients with metabolic syndrome (MetS) and type 2 diabetes mellitus compared with the control group in their 9-year follow-up. They concluded that Vn is a valuable predictive marker for MetS, independent of PAI-1[6]. In another recent study by Ravnsborg et al., it was confirmed that Vn significantly increased in GDM in their study in pregnant women with BMI>27 m^2/kg in the early trimester of pregnancy[17]. In this study, we found higher Vn and PAI-1 levels in GDM, which supports the result of the previous study, and demonstrated a significant correlation with fasting blood glucose, postprandial glucose level, HbA1c, CRP, and PAI-1. Although the results are similar, according to our opinion, this study will contribute to the literature as it does not only consist of patients with high BMI but also includes second-trimester measurements. We suggest that this study could contribute to the literature by evaluating second-trimester Vn levels and assessing the patients regardless of BMI. Results regarding PAI-1 level in GDM are inconsistent. In a study, it was shown that t-PA level increased in GDM, but PAI-1 did not change[23]. Liu et al. verified that t-PA was lower and PAI-1 was significantly higher in GDM patients[24].

In our study, similar to the literature, we found that the PAI-1 level was statistically significantly higher in the GDM group compared with healthy pregnant women.

This study has some limitations. It had a small sample size arising from the same center from a local region. Second, first-trimester measurements of these markers would be more beneficial for the early prediction of GDM. Finally, not knowing whether the cases of GDM were clinically controlled at the time when the Vn was evaluated was another challenging issue.

CONCLUSION

GDM is a progressive and long-term pregnancy complication with a risk of mortality and morbidity for both mother and fetus. The dynamics of the coagulation cascade and fibrinolysis mechanism at the pathophysiological level in GDM are still not fully clarified. By developing diagnostic biomarkers, elucidating the emerging pathogenic mechanisms for the development and consequences of GDM enables to improved early risk prediction. This study revealed that second-trimester Vn and PAI-1 are increased in GDM and vitronectin could be a candidate for the prediction of GDM.

ETHICAL APPROVAL

This study was approved by the local ethics committee (Number: 2011-KAEK-25; Date: 2020/09-13).

AUTHORS' CONTRIBUTIONS

LO: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **GO:** Data curation, Writing – original draft. **BD:** Formal Analysis, Methodology, Supervision. **FB:** Data curation, Formal Analysis.

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ORIGINAL ARTICLE

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CHA2DS2-VASc score, P-wave indexes, and echocardiographic parameters in sinus rhythm patients without valvular heart disease

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the correlation between P-wave indexes, echocardiographic parameters, and CHA2DS2-VASc score in patients without atrial fibrillation and valvular disease.

METHODS: This retrospective cross-sectional study included patients of a tertiary hospital with no history of atrial fibrillation, atrial flutter, or valve disease and collected data from June 2021 to May 2022. The exclusion criteria were as follows: unavailable medical records, pacemaker carriers, absence of echocardiogram report, or uninterpretable ECG. Clinical, electrocardiographic [i.e., P-wave duration, amplitude, dispersion, variability, maximum, minimum, and P-wave voltage in lead I, Morris index, PR interval, P/PR ratio, and P-wave peak time], and echocardiographic data [i.e., left atrium and left ventricle size, left ventricle ejection fraction, left ventricle mass, and left ventricle indexed mass] from 272 patients were analyzed.

RESULTS: PR interval (RHO=0.13, p=0.032), left atrium (RHO=0.301, p<0.001) and left ventricle diameter (RHO=0.197, p=0.001), left ventricle mass (RHO=0.261, p<0.001), and left ventricle indexed mass (RHO=0.340, p<0.001) were positively associated with CHA2DS2-VASc score, whereas P-wave amplitude (RHO=-0.141, p=0.02), P-wave voltage in lead I (RHO=-0.191, p=0.002), and left ventricle ejection fraction (RHO=-0.344, p<0.001) were negatively associated with the same score. The presence of the Morris index was associated with high CHA2DS2-VASc (p=0.022).

CONCLUSION: Prolonged PR interval, Morris index, increased left atrium diameter, left ventricle diameter, left ventricle mass, and left ventricle indexed mass values as well as lower P-wave amplitude, P-wave voltage in lead I, and left ventricle ejection fraction values were correlated with higher CHA_2DS_2-VASc scores.

KEYWORDS: Heart function tests. Electrocardiography. Echocardiography. Risk factors.

INTRODUCTION

The CHA_2DS_2-VASc score is used to assess the risk of stroke in patients with atrial fibrillation (AF)[1,2]. However, recent studies have shown validation of this score as a predictor of cardiovascular outcomes (including the development of AF), thromboembolic events, and death, even in the absence of AF[3-7].

Electrocardiographic parameters such as P-wave duration (PWD), variability and dispersion, interatrial block, maximum P (Pmax), and P-wave voltage in lead I (PVL1) have been studied as risk stratifiers for AF[8-11]. Echocardiographic parameters, including left atrial (LA) and left ventricular (LV) size, LV ejection fraction (LVEF), and LV mass, have also been associated with the risk of developing AF as well as all-cause mortality, myocardial infarction, and stroke or transient ischemic attack (TIA)[12-14].

Despite the data presented, there are no enough data on the correlation between these parameters and the CHA_2DS_2-VASc score in the population without AF.

The aim of this study was to evaluate the correlation between P-wave indexes [i.e., Morris index, mean duration, standard deviation (SD) and variability of P-wave, Pmax, minimum P (Pmin), P-wave dispersion, PVL1, PR interval (PRi), P/PR ratio (PPRi), and P-wave peak time] and echocardiographic findings (i.e., LA and LV size, LVEF, LV mass, and LV indexed mass) and CHA_2DS_2-VASc score in patients without AF and without valvular disease.

METHODS

The research project that resulted in this article was sent to Plataforma Brasil, received the number CAAE

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46451521.2.0000.5462, and was approved on June 01, 2021 by the Research Ethics Committee of Dante Pazzanese Cardiology Institute. All patients included in the study signed an informed consent form.

This was a retrospective cross-sectional study that included patients with no history of AF, atrial flutter, or valve disease, who were followed up at Dante Pazzanese Cardiology Institute and underwent electrocardiogram (ECG) and echocardiogram at the same institution. Patients with unavailable medical records, pacemaker carriers, absence of echocardiogram report, or with uninterpretable ECG were excluded from the study. Overall, 321 patients were included in the study period and data collection was performed in the same period (06/01/2021 to 05/01/2022).

The insufficient data on the correlation of ECG parameters and CHA_2DS_2-VASc score made it impossible to calculate the sample size before carrying out this study, which, in turn, may serve as a basis for sample calculations for other future studies with similar objectives. Therefore, the sample size of this study was defined by convenience.

Calculation of the CHA_2DS_2-VASc score

The CHA_2DS_2-VASc score was calculated based on the data available on medical records. Information about heart failure (HF), hypertension (HTN), diabetes mellitus (DM), vascular disease, history of stroke or TIA, gender, and age at the time of ECG were obtained. A high CHA_2DS_2-VASc score was considered if ≥2 for males and ≥3 for females.

ECG analysis

All ECGs were analyzed by an investigator to determine the P-wave indexes using the CardioCalipers® program. A second investigator performed the same measurements on 20% of the sample in order to assess the interobserver agreement. Both investigators were unaware of the patients' clinical data.

PWD was measured in all 12 leads. The highest value was chosen to determine Pmax and the lowest for Pmin. P-wave dispersion was calculated by the difference between Pmax and Pmin. The mean PWD and SD were also calculated. P-wave variability was obtained by dividing the SD by the mean PWD.

The PRi was measured in lead II. The PPRi ratio was calculated by dividing the PWD by the PRi.

P-wave peak time was measured from the beginning to the peak of the P-wave in lead II. P-wave voltage (PVL1) was measured in lead I, while P-wave amplitude was measured in lead II.

The presence of the Morris index was considered when the product of the amplitude (mm) and time (ms) of a terminal negative P-wave in V1 was>40.

Echocardiographic analysis

The following echocardiographic variables were collected: LA dimension, LV diastolic diameter, LVEF, LV mass, and LV indexed mass.

Statistical analysis

Continuous variables were presented by measures of central tendency (mean and median) and dispersion (variation and SD), and categorical variables were presented by frequency distribution (number of cases and relative percentage).

Categorical variables (high or low CHA_2DS_2-VASc score) were compared in relation to numerical variables (P-wave and echocardiographic measurements) with Student's t-test. Shapiro-Wilk's tests were used to test the normality of the data. If data normality was not verified, the Mann-Whitney U-test was adopted.

The chi-square test was used to verify the association between the categories. To verify the correlation between numerical variables, Spearman's rank correlation coefficient (RHO) was used. The kappa coefficient was applied to measure inter-rater reliability.

RESULTS

A total of 49 patients were excluded due to unavailable medical records (n=40) or the absence of available echocardiogram reports (n=9).

The mean age of the 272 individuals included in the final analysis was 62.4 (12.6) years, 56.6% (n=154) were females, 82% (n=223) of patients had HTN, 72.4% (n=197) had dyslipidemia, 35.3% (n=96) had atherosclerotic disease, 34.2% (n=93) had DM, and 21% (n=57) had HF. The mean CHA_2DS_2-VASc score was 3. The majority of patients [68% (n=185)] were on beta-blockers, and 18% (n=49) were on antiarrhythmics. Beta-blockers were indicated due to coronary disease, HF, and refractory HTN. Antiarrhythmics were indicated for the treatment of ventricular and supraventricular arrhythmias, excluding AF and atrial flutter (Table 1).

The mean LA and LV diameters were 40.1 (5.1) and 51.6 (6.9) mm, respectively. The mean LVEF was 57.9 (11)%. The mean LV mass and LV indexed mass were 209.5 (63.3) g and 117.2 (31.6) g/m^2, respectively. For P-wave indexes, the mean PWD was 110.3 (14.2) ms, PVL1 was 0.79 (0.27) mm, P amplitude was 1.1 (0.39) mm, and the PRi was 174.4 (39.9)

Table 1. Demographic characteristics, echocardiographic parameters, P-wave indexes, and CHA2DS2-VASc score.

Variable	Category/ Measurements	Frequency (%)/ Measurements
Age	Mean (SD)	62.4 (12.6)
Age range	18–64	140 (51.5)
	65–74	89 (32.7)
	≥75	43 (15.8)
Gender	Male	118 (43.4)
	Female	154 (56.6)
BMI (kg/m^2)	Mean (SD)	28.4 (5.5)
HF		57 (21.0)
HTN		223 (82.0)
DM		93 (34.2)
Stroke/TIA		16 (5.9)
Atherosclerotic disease		96 (35.3)
Dyslipidemia		197 (72.4)
Hypothyroidism		43 (15.8)
Smoking	Smoker	15 (5.5)
	Former smoker	69 (25.4)
	Never smoker	188 (69.1)
Beta-blocker		185 (68.0)
Antiarrhythmic		49 (18.0)
LA (mm)	Mean (SD)	40.1 (5.1)
LVEF (%)	Median (variation)	62.0 (22–79)
LV (mm)	Median (variation)	50 (36–88)
LV mass (g)	Median (variation)	198 (36–464)
LV indexed mass (g/m^2)	Median (variation)	113.1 (34–243)
Average PWD (ms)	Mean (SD)	110.3 (14.2)
P SD (ms)	Mean (SD)	14.4 (4.1)
P variability	Median (variation)	0.13 (0.03–0.24)
Maximum P (ms)	Mean (SD)	131.9 (15.2)
Minimum P (ms)	Mean (SD)	85.5 (16.1)
P dispersion (ms)	Median (variation)	44.0 (16–108)
PVL1 (mm)	Median (variation)	0.8 (0.3–2.2)
P amplitude (mm)	Median (variation)	1.1 (0.2–2.6)
PPRi	Median (variation)	0.69 (0.34–1.24)
P-wave peak time (ms)	Median (variation)	60 (28–100)
PRi (ms)	Median (variation)	172 (88–276)
Morris index		42 (15.4)
CHA_2DS_2-VASc	Median (variation)	3.0 (0–7)
CHA_2DS_2-VASc	0	5 (1.8)
	1	31 (11.4)
	2	67 (24.6)
	3	68 (25.0)
	4	59 (21.7)
	5	31 (11.4)
	6	10 (3.7)
	7	1 (0.4)
High CHA_2DS_2–VASc		193 (71.0)

BMI: body mass index; DM: diabetes mellitus; HF: heart failure; LA: left atrium; LV: left ventricle; LVEF: left ventricular ejection fraction; PPRi: P/PR ratio; PRi: PR interval; PVL1: P-wave voltage in lead I; PWD: P-wave duration; HTN: hypertension; SD: standard deviation; TIA: transient ischemic attack.

ms. The presence of the Morris index was observed in 15.4% of patients (Table 1).

The ECG analysis showed a slight correlation between the CHA_2DS_2-VASc score and PRi (RHO=0.13, p=0.032), P-wave amplitude (RHO=-0.141, p=0.02), and PVL1 (RHO=-0.191, p=0.002), when analyzed as continuous variables. The correlation was positive for PRi and negative for P-wave amplitude and PVL1. The other variables showed no correlation with the CHA_2DS_2-VASc score (Table 2).

All echocardiographic parameters analyzed were significantly correlated with the CHA_2DS_2-VASc score. The LA diameter (RHO=0.301, p<0.001), LV diameter (RHO=0.197, p=0.001), LV mass (RHO=0.261, p<0.001), and LV indexed mass (RHO=0.340, p<0.001) were positively correlated with the CHA_2DS_2-VASc score, whereas LVEF (RHO=-0.344, p<0.001) had a negative correlation (Table 2).

The CHA_2DS_2-VASc score was categorized into high (≥2 for males and ≥3 for females) and low (<2 for males and<3 for females). There was a statistically significant comparison between high CHA_2DS_2-VASc score and PRi (median of 176 ms in high CHA_2DS_2-VASc versus 164 ms in low CHA_2DS_2-VASc). A similar finding was observed with a high CHA_2DS_2-VASc score and all studied echocardiographic variables. The presence of the Morris index was also associated with high CHA_2DS_2-VASc. Morris index was observed in 18.6% of the individuals with high CHA_2DS_2-VASc (Table 3).

Interobserver variation analysis revealed CCC of 0.915 for PVL1 and 0.937 for P-wave amplitude and kappa coefficient of 1.0 for the presence of Morris index, 0.7047 for P-wave peak time, 0.9483 for PRi, and 0.9196 for PWD, indicating substantial to almost perfect agreement for all the examined variables.

DISCUSSION

In this study, we found a correlation between P-wave indexes, echocardiographic parameters, and the CHA_2DS_2-VASc score.

P-wave indexes and CHA_2DS_2-VASc score

The analysis of P-wave indexes should be stimulated by the wide availability and reproducibility of ECG in clinical practice, as it is a low-cost test.

The positive and significant correlation between PRi and the CHA_2DS_2-VASc score reflects that patients with cardiovascular comorbidities tend to have a higher occurrence of first-degree atrioventricular block. PRi prolongation alone is associated with an increased risk of AF, pacemaker implantation, and all-cause mortality[15].

Table 2. Correlation between P-wave indexes, echocardiographic parameters, and CHA2DS2-VASc score.

Variable	Variation	Median	Mean (SD)	RHO	p-Value
Mean PWD (ms)	63–153.1	110.3	110.3 (14.2)	0.084	0.167
P dispersion (ms)	16–108	44	46.4 (13.5)	0.026	0.669
P amplitude (mm)	0.2–2.6	1.1	1.1 (0.4)	(-0.141)	**0.020**
PRi (ms)	88–276	172	174.4 (29.9)	0.130	**0.032**
PPRi	0.3–1.2	0.7	0.7 (0.1)	(-0.078)	0.200
P-wave peak time (ms)	28–100	60	59.0 (13.2)	(-0.027)	0.656
P SD (ms)	4.3–29.3	14.2	14.4 (4.1)	0.053	0.379
P Variability	0.03–0.24	0.13	0.13 (0.04)	0.027	0.662
PVL1 (mm)	0.3–2.2	0.8	0.8 (0.3)	(-0.191)	**0.002**
LA (mm)	25–57	40	40.1 (5.1)	0.301	**<0.001**
LVEF (%)	22–79	62	57.9 (11.0)	(-0.344)	**<0.001**
LV (mm)	36–88	50	51.6 (6.9)	0.197	**0.001**
LV mass (g)	36–464	198	209.5 (63.3)	0.261	**<0.001**
LV indexed mass (g/m²)	34–243	113.1	117.2 (31.6)	0.340	**<0.001**

LA: left atrium; LV: left ventricle; LVEF: left ventricular ejection fraction; PPRi: P/PR ratio; PRi: PR interval; PVL1: p-wave voltage in lead I; PWD: P-wave duration; RHO: Spearman's rank correlation coefficient; SD: standard deviation. Statistically significant values are indicated in bold.

Table 3. P-wave indexes, echocardiographic parameters, and high or low CHA2DS2-VASc score.

Variable	Category/Measurements	CHA_2DS_2-VASc		p-Value
		Low	High	
PPRi	Mean (SD)	0.7 (0.1)	0.7 (0.1)	0.153*
Mean PWD (ms)	Mean (SD)	109.3 (13.3)	110.7 (14.6)	0.468*
P SD (ms)	Mean (SD)	13.9 (4.1)	14.6 (4.2)	0.224*
P variability	Median (variation)	0.13 (0.03–0.24)	0.13 (0.05–0.24)	0.292**
P-wave peak time (ms)	Median (variation)	60 (28–88)	60 (28–100)	0.946**
P dispersion (ms)	Median (variation)	44 (16–92)	48 (16–108)	0.339**
PVL1 (mm)	Median (variation)	0.8 (0.4–1.5)	0.7 (0.3–2.2)	0.220**
P amplitude (mm)	Median (variation)	1.1 (0.6–2.3)	1.1 (0.2–2.6)	0.183**
PRi (ms)	Median (variation)	164 (128–256)	176 (88–276)	**0.020****
Morris index		6 (7.6)	36 (18.6)	**0.022*****
LA (mm)	Mean (SD)	36.7 (4.5)	41.5 (4.7)	**<0.001***
LVEF (%)	Median (variation)	64 (32–75)	60 (22–79)	**<0.001****
LV (mm)	Median (variation)	49 (36–61)	51 (38–88)	**<0.001****
LV mass (g)	Median (variation)	172 (53.8–341)	213 (36–464)	**<0.001****
LV indexed mass (g/m²)	Median (variation)	99 (34–179.6)	118.7 (56.6–243)	**<0.001****

*p-value obtained by Student's t-test. **p-value obtained by the Mann-Whitney U test. ***p-value obtained by the chi-square test. LA: left atrium; LV: left ventricle; LVEF: left ventricular ejection fraction; PPRi: P/PR ratio; PRi: PR interval; PVL1: P-wave voltage in lead I; PWD: P-wave duration; SD: standard deviation. Statistically significant values are indicated in bold.

PVL1, when reduced, is associated with recent-onset AF in the population with coronary artery disease[10]. This finding may be related to the propagation of the electrical stimulus of the heart. By means of electrophysiological mapping, it was demonstrated that the electrical impulse of interatrial conduction is more displaced in the area of the Bachmann bundle in

individuals with low PVL1[16]. The negative correlation between PVL1 and CHA_2DS_2-VASc score in individuals without AF reinforces that this P-wave index should be valued in clinical practice.

P-wave amplitude in lead II was also negatively correlated with the CHA_2DS_2-VASc score. The P-wave amplitude, when reduced, is associated with greater rates of early AF recurrence after electrical cardioversion[17].

Echocardiographic parameters and CHA_2DS_2-VASc score

In individuals with AF, echocardiographic abnormalities are commonly found such as changes in LA diameter, LA *strain*, left atrial appendage emptying velocity, presence of spontaneous contrast, and thrombus. Atrial abnormalities are also associated with thromboembolic risk and mortality[18].

$CHADS_2$ and CHA_2DS_2-VASc scores are associated with echocardiographic risk factors for thromboembolism, such as decreased left atrial appendage emptying velocity, presence of spontaneous contrast, and thrombus[19]. Left atrial stasis, the presence of thrombi, and complex aortic plaque were associated with an increased risk of stroke, regardless of $CHADS_2$ and CHA_2DS_2-VASc scores in patients with AF[20]. The addition of echocardiographic risk parameters can complement the clinical assessment to estimate stroke risk in patients with AF[19].

Ventricular abnormalities also predict thromboembolic risk in patients with AF, such as increased LV mass, LV hypertrophy, and left ventricular dysfunction[19,21].

Even in the absence of AF, increased LV mass, abnormal LV geometry, and reduced LVEF are independent risk factors for death and cardiovascular diseases such as myocardial infarction and stroke[13,14,22-24].

In this study, a significant correlation between all echocardiographic parameters and the CHA_2DS_2-VASc score was demonstrated, being positive for LA diameter, LV, LV mass, and LV indexed mass and negative for LVEF.

ECG and echocardiographic parameters in clinical practice

The results of this study highlight the importance of the association of clinical, electrocardiographic, and echocardiographic variables in the stratification of systemic thromboembolism in patients with sinus rhythm.

High-cost and less-available devices, such as implantable monitoring devices, have been gaining ground to identify individuals with silent AF as the identification can prevent stroke with early institution of anticoagulation. However, there is still no well-defined consensus on which patients such devices should be recommended considering health system costs[25].

There is still not enough evidence to establish anticoagulation as a preventive treatment for stroke in the absence of AF; however, the applicability of clinical, ECG, and echocardiographic parameters may be confirmed in the future with the development of randomized clinical trials.

Limitations

The unicentric, observational, and cross-sectional nature is the main limitation of this study. The sample size was not calculated before the start of the study because of insufficient data on the correlation of ECG parameters and CHA_2DS_2-VASc score. Moreover, information about AF and valve disease was based on medical records. Therefore, silent AF patients may be included in the study.

CONCLUSIONS

Prolonged PRi, Morris index, increased LA diameter, LV diameter, LV mass, and LV indexed mass values as well as lower P-wave amplitude, PVL1, and LVEF values were correlated with higher CHA_2DS_2-VASc scores.

AUTHORS' CONTRIBUTIONS

AVD: Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – original draft. **LVA:** Conceptualization, Project administration, Supervision, Writing – review & editing. **DARM:** Conceptualization, Writing – review & editing. **MHS:** Formal Analysis, Investigation, Methodology. **KHV:** Formal Analysis, Investigation, Methodology. **PSG:** Formal Analysis, Investigation, Methodology. RAMB: Formal Analysis, Investigation, Methodology. **MAHV:** Formal Analysis, Investigation, Methodology. **MAD:** Formal Analysis, Investigation, Methodology. **GDC:** Formal Analysis, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Revisiting *ab initio* carcinoembryonic antigen and CA19-9 tumor markers in colorectal carcinoma in association with anatomotopographic location and staging of disease

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SUMMARY

OBJECTIVE: This study purposed to evaluate preoperative two tumor markers, namely, carcinoembryonic antigen and carbohydrate antigen (CA)19-9, in colorectal cancer for anatomotopographic location with disease stage and to assess their utility for diagnostic staging purposes.

METHODS: The study retrospectively incorporated patients who had undergone surgery for colorectal cancer at our department in 2015–2018 and in whom carcinoembryonic antigen and CA19-9 tumor markers had been preoperatively analyzed. The obtained data were then statistically processed using R-project.

RESULTS: A total of 155 patients had been incorporated, of whom 96 (62%) were men and 59 (38%) were women. Rectum was the most common location (74 patients, 48%), and the least represented stage was IV (18, 12%). The marker carcinoembryonic antigen was obtained in all 155 cases, while CA19-9 was in 105. The median carcinoembryonic antigen was 3 (0.34–1104.25), and the median CA19-9 was 12 (0.18–840.00). A significance was recognized between median carcinoembryonic antigen and disease stage (p-value=0.016), with stages I, II, and III (medians 2, 3, and 2) different from stage IV (median 13), while no significance for CA19-9 was recognized (p-value=0.343). No significance between either marker and location (carcinoembryonic antigen: p=0.276; CA19-9: p=0.505) was detected. The testing was performed at a significance level of alpha=0.05.

CONCLUSION: This study revealed a significance between the marker carcinoembryonic antigen, but not CA19-9, and the disease stage, while no relationship of either of these markers with tumor location was found. Herewith, the study confirmed that higher carcinoembryonic antigen values may suggest the finding of more advanced forms of colorectal cancer and thus a worse prognosis of this malignant phenomenon.

KEYWORDS: Colorectal cancer. Tumor markers. Carcinoembryonic antigen. Surgery. Pathology.

INTRODUCTION

Colorectal cancer (CRC) is considered a disease of civilization, representing a serious global health and economic problem. In 2020, more than 1.9 million new cases of colorectal (including anus) cancer were diagnosed worldwide and 935,000 patients died of this diagnosis. It is the most common gastrointestinal malignancy in developed countries, and its global incidence keeps increasing. The current incidence rate, of both sexes, ranks CRC as the third most common malignancy after breast cancer and lung cancer, while it is second only to lung cancer in mortality[1]. In the Czech Republic, approximately 8,000 patients are diagnosed with this disease every year and approximately 4,000 die of it. However, in recent years, there has been a decline in both incidence and mortality. Specifically, in 2018, 7,437 patients were diagnosed with CRC and 3,550 patients died of CRC[2].

Many years of research on the available global data show that the carcinogenesis of CRC is associated with lifestyle, type of diet, smoking, and the influence of the environment in which one lives and works. A sedentary lifestyle and a general

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lack of exercise, an inadequate diet low in fiber and vitamins, as well as stress have a significant impact on the development of the disease[3]. Carcinogenesis is a long, complex, and gradual process. Epithelial cells are subject to abnormal proliferation under genetic influence, leading to the creation of new clones. Of note, if new clones pass unrecognized by suppressor genes (or if these genes are damaged so that they are unable to recognize the changes at the level of DNA), then they proliferate unperturbed and form the basis of a tumor[4].

Before initiating treatment of CRC, it is essential to fully examine the patient using imaging modalities and determine the disease's clinical stage. Staging aims to determine the extent of local tumor spread and the extent of lymph node involvement and to evaluate whether distant metastases are present. Colonoscopy, *per se*, is the gold standard in the diagnosis of colorectal neoplasia due to its high sensitivity and the possibility of performing biopsy as well as therapeutic procedures. As such, colonoscopy is usually followed by other imaging modalities, namely, a CT scan of the lungs, abdomen, and small pelvis, contrast-enhanced ultrasonography in order to determine the presence, number, and size of liver metastases, endoscopic sonography, and magnetic resonance imaging (MRI) of the small pelvis where the rectum is involved. Basic laboratory tests (i.e., blood count and biochemistry) including the tumor markers complement the diagnostic procedures. The therapy is then determined based on carefully performed staging[5].

Although carcinoembryonic antigen (CEA) and carbohydrate antigen (CA)19-9 are the most commonly used tumor markers in CRC staging, the role of these tumor markers in screening, early detection of disease recurrence, or as prognostic or predictive factors for CRC is still debated. *Grammatici certant.* Therefore, this study aimed to determine whether the preoperative levels of the tumor markers CEA and CA19-9 can indicate the location of the tumor within the colon and whether the preoperative levels of these markers can predict the clinical staging of this malignant phenomena and thus the prognosis of the patients.

METHODS

Study design

The study was designed as a retrospective analysis of preoperative CEA and CA19-9 tumor markers in cases who had undergone surgery for CRC at the Department of Surgery, University Hospital Ostrava, Ostrava, Czech Republic, from 2015 to 2018. All the cases had undergone a preoperative colonoscopy and a biopsy, based on which the diagnosis of CRC was established. Colonoscopy and histopathological examination of the preoperative samples had been performed at multiple departments within the region before referring the cases to the Department of Surgery of the University Hospital Ostrava. We had supplemented the previously determined staging with additional imaging methods, especially CT of the lungs, abdomen, and small pelvis, which was supplemented by MRI or endoscopic ultrasonography for rectal tumors.

Using these examinations, the preoperative staging of the tumor was determined and patients were classified according to the current TNM classification. Based on these data, the optimal treatment strategy was determined. All included cases had been indicated for surgical treatment of CRC (i.e., all had undergone resection); in some of them, surgery was performed after neoadjuvant chemoradiotherapy. The CEA tumor marker had been collected preoperatively in all 155 patients, and the CA19-9 tumor marker had been collected in 105. The laboratory samples had been processed and evaluated by the Institute of Laboratory Medicine, University Hospital Ostrava. The surgically obtained tumor (including the entire colon resection) was sent to the Institute of Pathology of the University Hospital Ostrava for processing and final TNM classification.

Statistical analysis

The Shapiro-Wilk test was used to test the normality of the data. Of note, none of the variables had a normal distribution; therefore, nonparametric tests were used for statistical analysis. The Kruskal-Wallis rank test was used as a nonparametric alternative to the one-factor analysis of variance to test significance across multiple groups. In addition, Dunn's multiple comparison test was utilized for *post hoc* analysis in case of multiple comparisons. Testing was performed at the level of significance of alpha=0.05. Statistical analysis was calculated using R-project[6] with the following packages: dplyr, ggplot2, and Dunn's test.

RESULTS

A total of 155 patients who had undergone surgery for CRC in 2015–2018 and in whom CEA (and, in most instances, CA19-9) tumor markers were collected preoperatively were included in the study, of whom 98 patients (62%) were men and 59 (38%) were women. Laparoscopic surgery had been performed in 107 (69%) cases, while the conventional, i.e., open, surgical technique had been employed in 48 (31%) patients. The median CEA for all patients was 3 (range 0.34–1104.25), and the median CA19-9 was 12 (range 0.18–840.00).

The number of cases and CEA and CA19-9 medians concerning disease stage classification are shown in Table 1.

Statistical analysis was performed to evaluate the significance between the medians of CEA and CA19-9 markers and the disease stage and/or disease location. The analysis revealed a statistically significant association between the median CEA tumor markers according to the disease stage (p-value=0.016, Kruskal-Wallis test). Figure 1A demonstrates that stages I–III formed a relatively homogeneous group with similar CEA medians, and CEA median in stage IV patients was found to have no significance (for clarity, a logarithmic scale was used for the marker values), and the conclusion was confirmed by *post hoc* analysis (Dunn's test). No statistical significance was recognized between CA19-9 medians and the disease stage as shown in Figure 1B (p=0.343, Kruskal-Wallis test). In addition, the possible association of tumor markers with tumor location had been investigated. Figure 2 obviates no statistical significance between medians of CEA (Figure 2A; p-value=0.276, Kruskal-Wallis test) or CA19-9 (Figure 2B; p-value=0.505, Kruskal-Wallis test) and location.

DISCUSSION

De facto, the use of tumor markers in screening for CRC, in early detection of the disease recurrence, or as prognostic or predictive factors still remains under debate, and no clear consensus has been established to date. *Ad fontes,* CEA and CA19-9 are well-known tumor markers used in the preoperative staging and postoperative follow-up of CRC, especially in cases undergoing chemotherapy. As such, CEA is an oncofetal tumor marker discovered by Gold and Freedman[7] and remains the only tumor marker with recognized efficacy in the monitoring of the treatment modalities in CRC. Originally, it was considered to be specific for CRC, but its elevated levels were also detected later in other cancers, such as gastric and pancreatic cancer, as well as in inflammatory conditions, such as ulcerative colitis, liver cirrhosis, chronic bronchitis, and smokers. The European Group on Tumor Markers (EGTM), in line with other societies (i.e., European Society of Medical Oncology and American Society of Clinical Oncology), does not recommend utilizing CEA for screening purposes. However, the EGTM recommends the determination of preoperative CEA levels in newly diagnosed CRC cases. Although diagnosis[8-12] remains crucial for this malignant phenomenon, the preoperative CEA level provides some prognostic information and, in addition, serves as a baseline for follow-up[8-10,13-15]. Our study supports

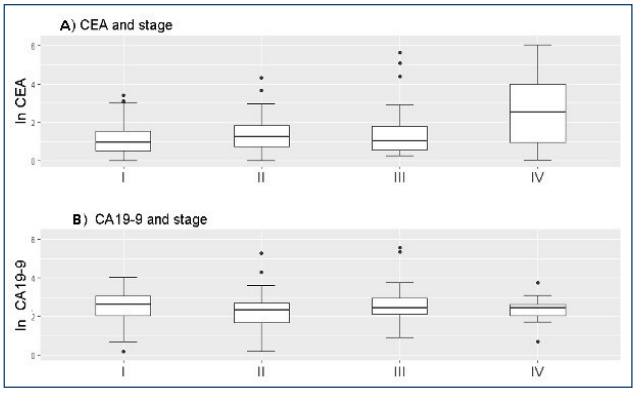

Figure 1. The tumor markers and the stage (logarithm of marker values). (A) Carcinoembryonic antigen – significant association. (B) CA19-9 – no significant association.

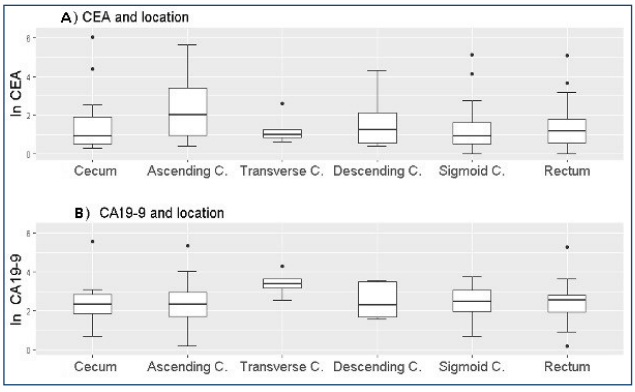

Figure 2. The tumor markers and the location (logarithms of marker values). Neither of the markers has any relationship to the location. (A) Carcinoembryonic antigen; (B) CA19-9.

Table 1. Characteristics of carcinoembryonic antigen and CA19-9 markers according to the disease stage.

Stage	I	II	III	IV	p-value
CEA					
n (%)	47 (30%)	43 (28%)	47 (30%)	18 (12%)	
Median (CI)	2 (2–4)	4 (3–6)	3 (2.5)	31 (8–142)	0.016
CA19-9					
n (%)	30 (29%)	34 (32%)	28 (27%)	13 (12%)	
Median (CI)	14 (11–19)	11 (8–16)	14 (10–20)	22 (9–451)	0.343

these conclusions by demonstrating a statistically significant association between CEA and disease stage (specifically, stage IV). In addition, CA19-9 is a tumor antigen whose elevated serum levels were observed in metastatic CRC, i.e., stage IV[13]. However, this study did not demonstrate an association of CA19-9 with the disease stage. Vukobrat-Bijedic et al.[3], based on their study involving 91 patients, extremely elevated CEA and CA19-9 values in tumors localized in the right colon. On the contrary, Nakatani et al.[16] reported extremely elevated CEA and CA19-9 values in a patient with CRC localized in the sigmoid colon. A CT scan did not reveal metastasis in this case. However, our study did not demonstrate a dependence of either of the two studied markers on tumor location.

The usability of the CEA and CA19-9 tumor markers in the management of CRC is still ambiguous. Several studies focus on these markers but often reach contradictory conclusions. We recorded a higher incidence of tumors in the distal parts of the colon (i.e., rectum and sigmoid colon), which is in accordance with the results mentioned by most authors. Regarding the prediction of tumor location in the colon, however, our study did not confirm any dependence of preoperatively measured values of these markers on tumor location. However, we did demonstrate a statistically significant dependence of preoperative CEA values on the disease stage, i.e., stage IV. Thus, herein, in agreement with most studies, we might postulate that CEA can be utilized as a definite prognostic factor for CRC. However, our study did not state such a relationship for CA19-9.

Limitations

This study has some limitations. CA19-9 had not been collected in all the cases, leading to the sample being limited. As such, it could also be argued that the number of patients included in this study (155 cases) is still relatively limited, especially given the number of cases with tumors in individual locations, but this number of subjects is similar to or higher than those reported in similar studies.

CONCLUSION

This study demonstrated a statistically significant relationship between the tumor marker CEA (but not CA19-9) and disease stage, while neither the CEA nor CA19-9 was associated with the topographic tumor location of CRC. As such, our preliminary outcomes are, therefore, following most of the previously reported studies, i.e., that higher CEA values may suggest the presence of a more advanced form of CRC and, therefore, a worse prognosis which was, however, not valid for marker CA19-9. *Nothing new under the sun.* Nevertheless, although CEA in stage IV disease is significantly elevated compared with others, neither CEA nor CA19-9 appears to be suitable markers for CRC screening. This issue merits further investigation.

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AUTHORS' CONTRIBUTIONS

ML: Conceptualization, Data curation, Formal Analysis, Funding, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. D**S:** Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **IS:** Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **MP:** Methodology, Project administration, Validation, Visualization. **WG:** Methodology, Project administration, Validation, Visualization. **VJ:** Methodology, Project administration, Validation, Visualization. T**M:** Methodology, Project administration, Validation, Visualization. H**T:** Methodology, Project administration, Validation, Visualization. **LP:** Methodology, Project administration, Validation, Visualization. **AP:** Investigation, Methodology, Validation, Visualization.

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ORIGINAL ARTICLE

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Severe acute respiratory syndrome coronavirus 2 seroprevalence among patients with pulmonary tuberculosis

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SUMMARY

OBJECTIVE: The objective of this study was to estimate the seroprevalence of severe acute respiratory syndrome coronavirus 2 antibodies in patients with tuberculosis.

METHODS: This cross-sectional study was conducted at an outpatient tuberculosis clinic in Alvorada, RS, Brazil, with data collection between October and December 2020. Outpatients aged>18 years with active pulmonary tuberculosis, no prior history of coronavirus disease 2019, and no suspected coronavirus disease 2019 were included in the study. Whole blood samples were collected to perform the severe acute respiratory syndrome coronavirus 2 antibodies test.

RESULTS: During the study period, 52 patients met the inclusion and were included in the analysis. Severe acute respiratory syndrome coronavirus 2 antibodies were positive in 16 (30.8%) patients. Male sex was more frequent among patients with negative severe acute respiratory syndrome coronavirus 2 antibodies than in patients with positive severe acute respiratory syndrome coronavirus 2 antibodies (86.1 vs. 56.3%, p=0.031). Contact with coronavirus disease 2019 case was more common in patients with positive severe acute respiratory syndrome coronavirus 2 antibodies compared with patients with negative severe acute respiratory syndrome coronavirus 2 antibodies (87.5 vs. 8.3%, p<0.0001). In a multivariate analysis, in a model including the variables such as male sex and contact with coronavirus disease 2019 case, only contact with coronavirus disease 2019 was independently associated with positive severe acute respiratory syndrome coronavirus 2 antibodies (OR 77.0, 95%CI 11.5–512.4, p<0.0001).

CONCLUSION: This study revealed a seroprevalence of 30.8% severe acute respiratory syndrome coronavirus 2 among patients with tuberculosis.

KEYWORDS: Tuberculosis. SARS-CoV-2. Seroepidemiologic studies. COVID-19. Antibodies.

INTRODUCTION

Tuberculosis (TB) is a worldwide public health concern, with a global incidence of 6.4 million people in 2021[1]. Brazil is among the 30 high TB burden countries, with an incidence of 32 cases/100,000 population in 2021; in the State of Rio Grande do Sul, the incidence is 36.5 cases/100,000 population in 2021[2]. The association between TB and coronavirus disease 2019 (COVID-19) has been described since the beginning of the COVID-19 pandemic[3,4]. Both diseases can present simultaneously and have similar symptoms[3], so diagnosing TB during the COVID-19 pandemic requires a high degree of clinical suspicion. In addition, TB and COVID-19 co-infected individuals may be at a greater risk of morbidity and mortality. The risk of mortality was demonstrated to be 2.17 times higher in patients with concomitant COVID-19 and pulmonary TB[5]. In a large cohort of 767 patients co-infected with TB and COVID-19, from 172 centers in 34 countries, the authors showed that age, male gender, and invasive ventilation were independent contributors to mortality. Among the patients who died, 42 (49.4%) died from COVID-19; 31 (36.5%) from COVID-19 and TB; and 1 (1.2%) died from TB only[6].

Although studies have described the association between TB and COVID-19, emphasizing that the overlap of these diseases can cause more severe clinical conditions, no study has evaluated the prevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies in TB patients. Seroprevalence studies are important because they enable the detection of asymptomatic and subclinical infections, not usually included in the reported cases[7,8]. Identification and isolation

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of asymptomatic carriers and patients with mild COVID-19 are very important in preventing the disease spread, especially in these high-risk populations, helping to prevent COVID-19 morbidity and mortality[9,10]. Therefore, the objective of this study is to estimate the seroprevalence of SARS-CoV-2 antibodies in patients with TB without symptoms suggestive of COVID-19, in an outpatient TB clinic, before the widespread introduction of COVID-19 vaccines.

METHODS

Study design and location

We conducted a cross-sectional study, from October to December 2020, with prospective data collection in an outpatient TB clinic in Alvorada, RS, Brazil. This clinic is the only reference center for TB in the city and treated 252 confirmed cases of TB in 2022. Alvorada is a city with 211,352 inhabitants, located in the metropolitan area of Porto Alegre, which has a TB incidence of 89.9 cases/100,000 inhabitants[2]. Despite the sanitary restrictions in force during the study period, the care service for patients with TB has not changed in Alvorada. The study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre (number 20-0490 – CAAE: 38370330.8.0000.5327). All patients signed informed consent before the start of the study.

Patients and data collection

Outpatients aged>18 years with active pulmonary TB, no history of COVID-19, and no suspected COVID-19 were included in the study. Patients with extrapulmonary TB were excluded from this study. Pulmonary TB was diagnosed according to the Brazilian Guidelines for Tuberculosis[11].

After signing informed written consent, enrolled subjects were interviewed using a standardized questionnaire. The following data were collected: demographic data (i.e., sex and age), medical history (i.e., presence of comorbidities, smoking habits, alcohol abuse, and use of drugs), and history of contact with a suspected or confirmed case of COVID-19.

Whole blood samples were collected to perform the test. The VITROS Immunodiagnostic Products Anti-SARS-CoV-2 Total Test (Ortho Clinical Diagnostics, USA) was performed using the VITROS Anti-SARS-CoV-2 Total Reagent Pack and the VITROS Anti-SARS-CoV-2 Total Calibrator in the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and in the VITROS 5600/XT 7600 Integrated Systems. The test assesses the immune response by qualitatively measuring total antibodies (including IgG, IgM, IgA, and other isotypes) against SARS-CoV-2. The result takes 48 min and is described as <1 (anti-SARS-CoV-2 non-reactive sample) or ≥1 (anti-SARS-CoV-2 reactive sample). According to the manufacturer, the sensitivity of the test ranged from 79.4 to 100% and the specificity from 99.1 to 100%[12].

Statistical analysis

Data analysis was performed using SPSS 18.0 (Statistical Package for the Social Sciences, Chicago, IL). Data were presented as number of cases, mean±standard deviation (SD), or median with interquartile range. Categorical comparisons were performed by chi-square test using Yates's correction if indicated or by Fisher's exact test. Continuous variables were compared using *t*-test or Wilcoxon test. Multivariate logistic regression analysis was performed to assess factors associated with the presence of antibodies against SARS-CoV-2. Hierarchical logistic regression models with predictors added one at a time were examined to assess possible collinearity between the predictors. The predictors selected in the final model were based on numerical and clinical significance. The quality of fit of the multiple logistic regression models was evaluated with the Hosmer-Lemeshow test. Odds ratios (ORs) and 95% confidence intervals (CI) were presented. A two-sided p-value<0.05 was considered significant for all analyses.

To calculate the sample size, we considered a SARS-CoV-2 seroprevalence of approximately 30% based on previous studies[13-16]. Thus, with an alpha error of 0.5 and a power of 80%, it would be necessary to include at least 42 patients in the study.

RESULTS

During the study period, 52 patients met the inclusion criteria and were included in the analysis. Only two patients did not accept to participate in the study. SARS-CoV-2 antibodies were positive in 16 (30.8%) patients. The characteristics of the study population are shown in Table 1, according to the results of SARS-CoV-2 antibodies.

Male sex was more frequent among patients with negative SARS-CoV-2 antibodies than in patients with positive SARS-CoV-2 antibodies (86.1 vs. 56.3%, p=0.031). Contact with COVID-19 case was more common in patients with positive SARS-CoV-2 antibodies compared with patients with negative SARS-CoV-2 antibodies (87.5 vs. 8.3%, p<0.0001). All other characteristics were not statistically different comparing patients with negative SARS-CoV-2 antibodies with patients with positive SARS-CoV-2 antibodies.

In a multivariate analysis, in a model including the variables male sex and contact with COVID-19 case, only the last one was independently associated with positive SARS-CoV-2 antibodies (OR 77.0, 95%CI 11.5–512.4, p<0.0001) (Table 2).

DISCUSSION

We aimed to estimate the prevalence of SARS-CoV-2 antibodies among TB patients with no prior history of COVID-19 and no suspected COVID-19. SARS-CoV-2 seroprevalence was 30.8%. In addition, in a multivariate analysis, a history of contact with COVID-19 case was independently associated with positive SARS-CoV-2 antibodies.

Our result of 30.8% of SARS-CoV-2 seroprevalence is similar to the range of percentage reported in other studies[13-16]. In a serological survey conducted on 1,141 healthcare workers in Brazil, the serum prevalence for the virus was 30%[13]. In another study in Brazil[14], the SARS-CoV-2 seroprevalence among 3,046 asymptomatic and symptomatic individuals, selected from a convenience sample, was estimated at 29.1%. In a national survey in Mexico, the authors found a seroprevalence of 24.9% after the first epidemic wave, from August to November 2020[16]. In a meta-analysis[15] of nine studies from South America (i.e., Argentina, Brazil, Colombia, and Peru), the pooled seroprevalence was estimated at 33.6%.

As the clinical manifestation of SARS-CoV-2 infection is largely variable, ranging from asymptomatic to fatal, the number of reported cases does not reflect the actual number of infections because asymptomatic cases are not regularly tested. In the present study, all included patients were COVID-19 asymptomatic. In a seroprevalence study[16] in Mexico, among seropositive individuals, 67.3% were asymptomatic. Recently published studies confirm that a surveillance strategy that relies only on detected cases by RT-PCR will underestimate the true number of SARS-CoV-2 infections[8,17,18].

History of contact with a COVID-19 case was the most important factor associated with positive SARS-CoV-2 antibodies in our study. According to these results, Halili et al.[19] found that having an infected family member is related to seropositivity to SARS-CoV-2 antibodies. In addition, in univariate analysis, males had more positive SARS-CoV-2 antibodies than females. This is in accordance with other studies that found that males have higher odds of seropositivity[19-21]. In contrast, Airoldi et al.[22] reported higher seropositivity among females.

This study has some limitations. First, it was carried out in a single center; thus, considering the locoregional differences and the different waves of the pandemic, it is possible that these results cannot be generalized. Second, although this was not the focus of the study, we did not collect data on mask use and social distancing, measures that may influence the seroprevalence of SARS-CoV-2. However, this is the first study that evaluated the prevalence of SARS-CoV-2 antibodies in TB patients.

CONCLUSIONS

This study revealed a seroprevalence of SARS-CoV-2 of 30.8% among patients with TB. These data help understand the

Table 1. Characteristics of study patients according to severe acute respiratory syndrome coronavirus 2 antibodies.

Characteristics	SARS-CoV-2 antibodies positive (n=16)	SARS-CoV-2 antibodies negative (n=36)	p-value
Age, years	47.3±23.3	41.3±15.9	0.364
Male sex	9 (56.3)	31 (86.1)	0.031
Active smoking	5 (31.3)	10 (27.8)	0.999
Alcohol abuse	1 (6.3)	3 (8.3)	0.999
Use of drugs	1 (6.3)	4 (11.1)	0.999
Previous TB	3 (18.8)	7 (19.4)	0.999
HIV	2 (12.5)	3 (8.3)	0.637
DM	3 (18.8)	2 (5.6)	0.163
Presence of any comorbidity	8 (50.0)	12 (33.3)	0.406
Contact with COVID-19 case	14 (87.5)	3 (8.3)	<0.0001

SARS-CoV-2: seroprevalence of severe acute respiratory syndrome coronavirus 2; TB: tuberculosis; HIV: human immunodeficiency virus; DM: diabetes mellitus; COVID-19: coronavirus disease 2019.

Table 2. Multivariate analysis of factors associated with positive severe acute respiratory syndrome coronavirus 2 antibodies.

Characteristics	β	SE	Wald	OR (95%CI)	p-value
Male sex	-2.21	1.28	3.01	0.11 (0.01–1.33)	0.083
Contact with COVID-19 case	4.34	0.97	20.18	77.0 (11.5–512.4)	<0.0001

SARS-CoV-2: seroprevalence of severe acute respiratory syndrome coronavirus 2; COVID-19: coronavirus disease 2019; SE: standard error; OR: odds ratio; CI: confidence interval.

epidemiology of COVID-19 in this population and to raise awareness that many patients have asymptomatic disease, with the potential for transmission to other patients in TB clinics and to family members. In addition, due to the high seroprevalence and the possibility of severe COVID-19, vaccination efforts must be intensified in TB patients. Also, other measures to mitigate the transmission of the disease from asymptomatic patients and potentially reduce morbidity and mortality would be the maintenance of the use of masks in TB clinics and low-threshold testing (testing patients with mild symptoms)[23,24].

AUTHORS' CONTRIBUTIONS

NJDD: Conceptualization, Methodology, Investigation, Data curation, Project administration, Writing – review & editing. **MSS:** Conceptualization, Methodology, Investigation, Writing – review & editing. **MSB:** Conceptualization, Methodology, Investigation, Writing – review & editing. **GRP:** Conceptualization, Methodology, Investigation, Writing – review & editing. **DRS:** Conceptualization, Methodology, Investigation, Data curation, Project administration, Supervision, Writing – original draft.

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https://doi.org/10.1590/1806-9282.20230110ERRATUM

In the manuscript "Role of magnetic resonance imaging in the differentiation of mucinous ovarian carcinoma and mucinous borderline ovarian tumors", DOI: 10.1590/1806-9282.20230110, published in the Rev Assoc Med Bras 2023;69(7):e20230110, on pages 4-5:

On Figure 1, where it reads:

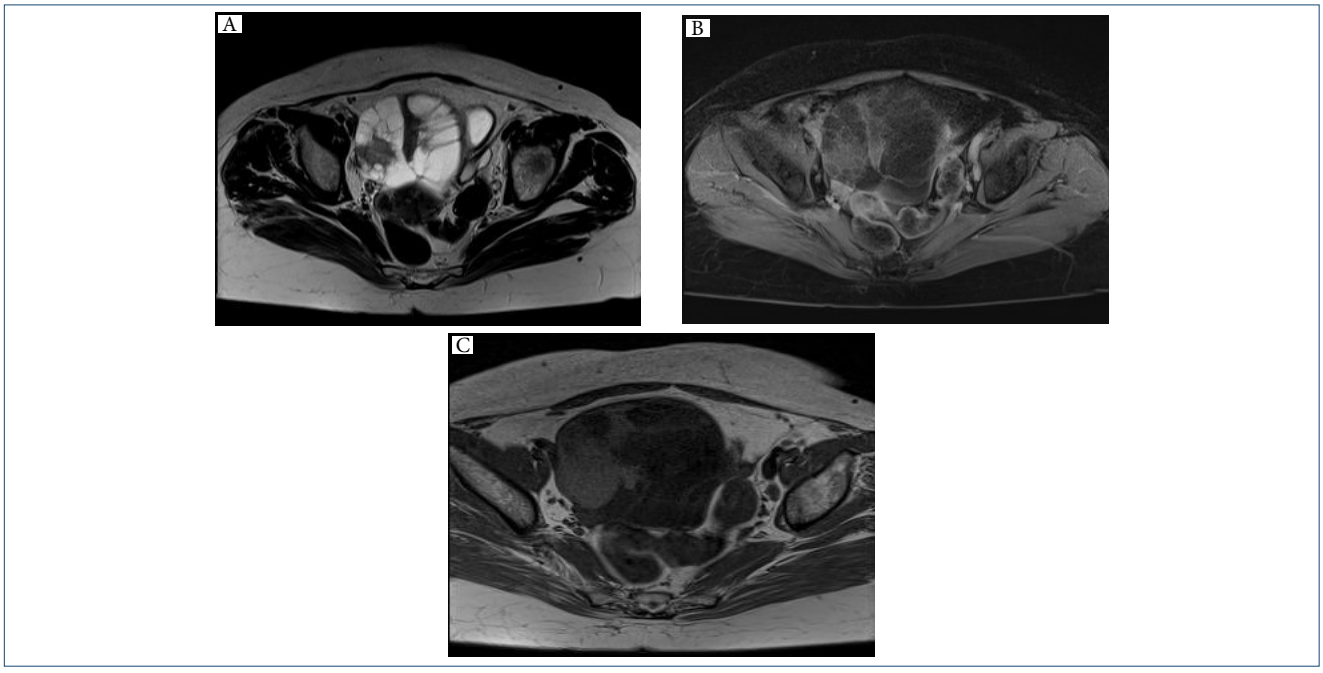
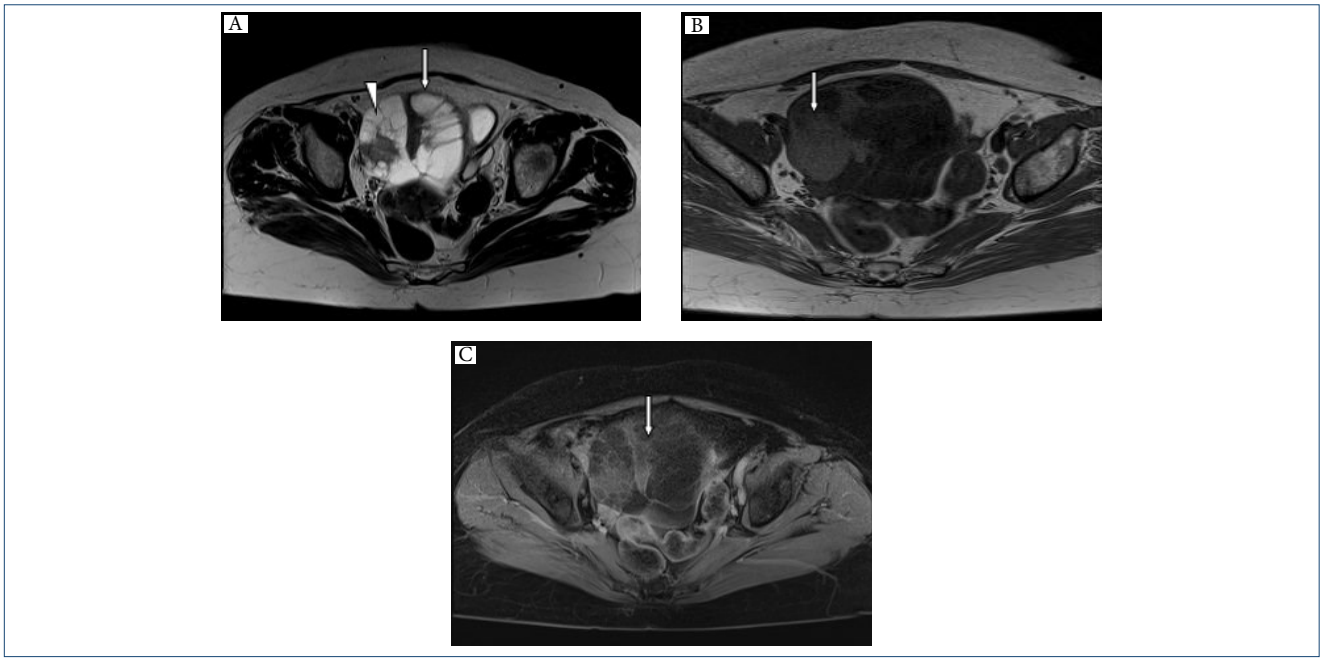


Figure 1. A 42-year-old woman with mucinous borderline tumor. **(A)** Axial T2-weighted image shows multilocular cystic tumor with stained glass appearance (arrow) and honeycomb sign (arrowhead). **(B)** T1-weighted image shows multilocular cystic tumor with stained glass appearance. **(C)** Contrast-enhanced T1-weighted image shows enhanced multiple thin septa.

It should read:



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On Figure 2, where it reads:

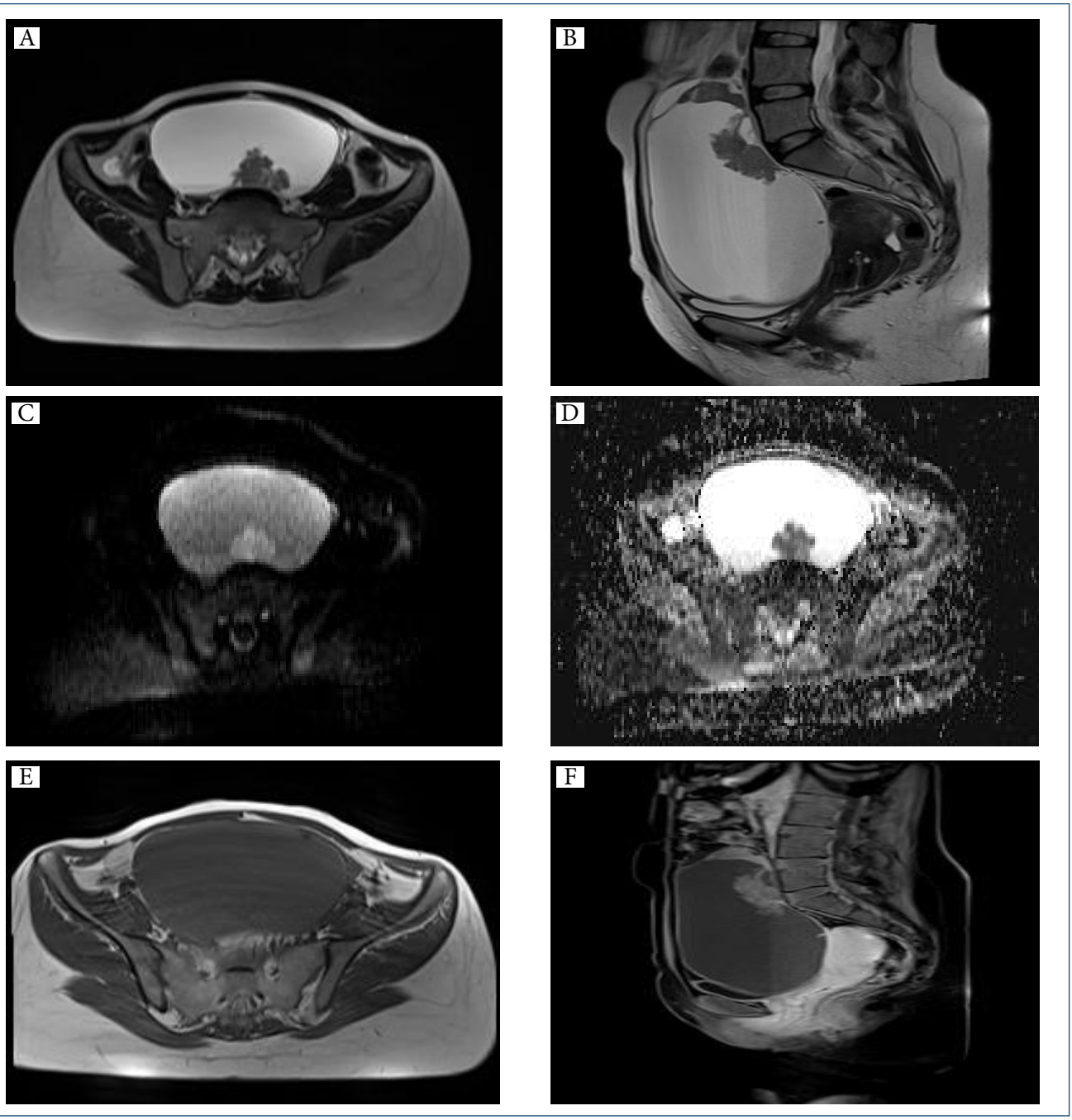
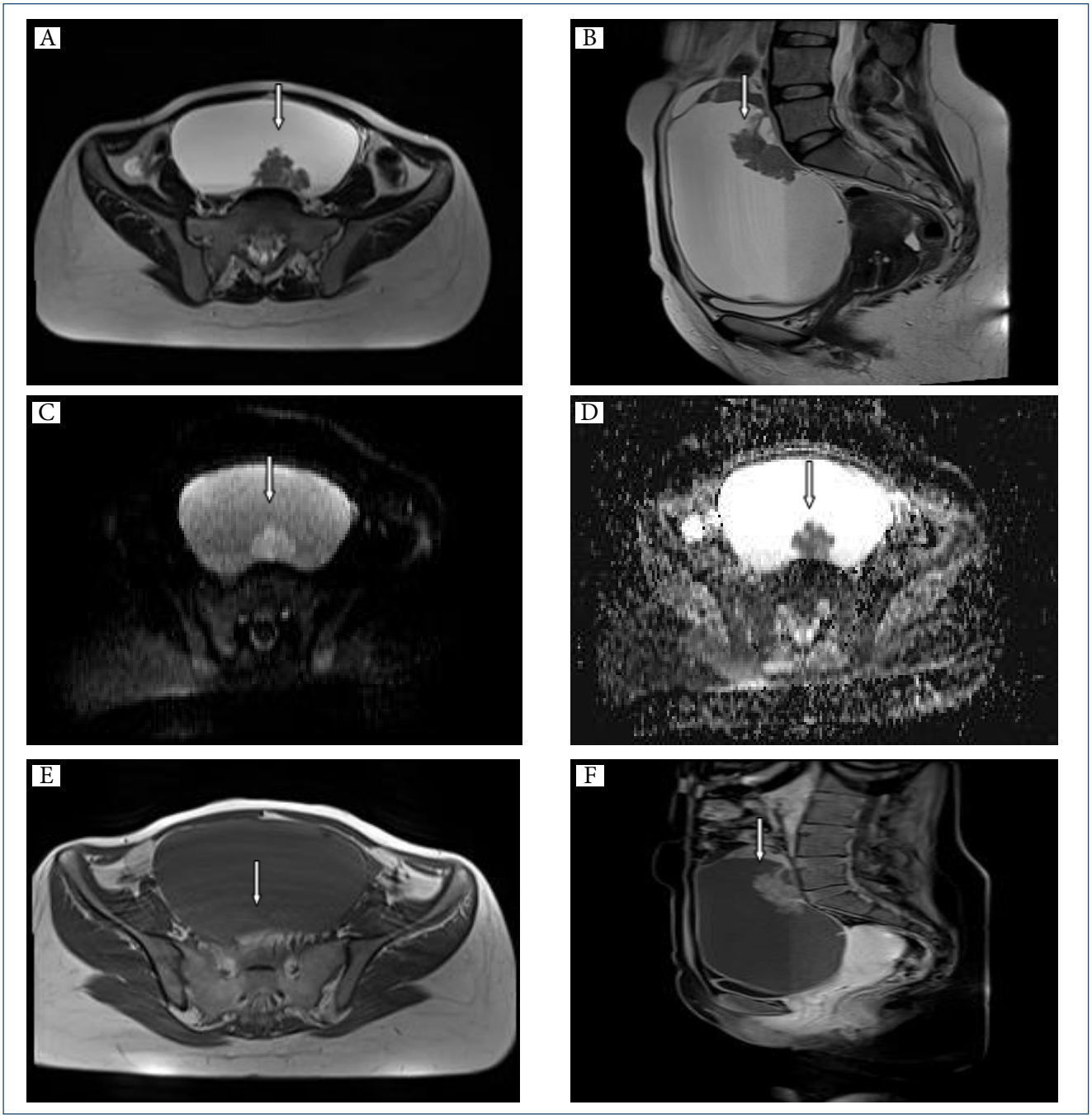


Figure 2. A 53-year-old woman with mucinous carcinoma. **(A and B)** Axial and sagittal T2-weighted image shows a gross multilocular cystic tumor with a mildly hyperintense mural nodule larger than 5 mm (arrow). **(C)** Diffusion-weighted image shows a hyperintense mural nodule (arrow). **(D)** Apparent diffusion coefficient (ADC) map shows a low ADC value. **(E)** T1-weighted image shows a hypointense mural nodule (arrow). **(F)** Contrast-enhanced T1-weighted image shows a moderately enhanced mural nodule (arrow).

It should read:



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