ISSN 0104-4230
ISSN 1806-9282 (On-line)

RAMB

Journal of The Brazilian Medical Association

Volume 70, Number 5
May, 2024

AMB Associação Médica Brasileira

WMA

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ISSN 0104-4230
ISSN 1806-9282 (On-line)

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RAMB - REVISTA DA ASSOCIAÇÃO MÉDICA BRASILEIRA
(JOURNAL OF THE BRAZILIAN MEDICAL ASSOCIATION)

Editors-in-Chief: Renato Deláscio Lopes, José Maria Soares Jr and Roseli Nomura.
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Website: www.ramb.org.br

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The RAMB, Journal of The Brazilian Medical Association, is an official publication of the Associacao Medica Brasileira (AMB – Brazilian Medical Association), indexed in Medline, Science Citation Index Expanded, Journal Citation Reports, Index Copernicus, Lilacs, and Qualis B1 Capes databases, and licensed by Creative CommonsR.
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EDITORIAL

https://doi.org/10.1590/1806-9282.20231558

Application of ChatGPT in reducing vaccine hesitancy and enhancing vaccine acceptance: hope or myth?

Akhilesh Vikram Singh[1*]

Vaccines have always remained the ultimate scope of health recovery from many diseases. In recent years, with the emerging COVID-19 pandemic, several vaccines have been approved by the regulatory agencies of various countries and were administered in different forms[1]. However, before administration of vaccines to the affected ones, several human lives ended up due to lack of vaccines and hesitancy of taking vaccines, which made them step back from taking vaccines[2]. This condition was described as "vaccine hesitancy." World Health Organization (WHO) defined this condition as a state of mind to "the delay in the acceptance or refusal to vaccinate despite the availability of vaccine services[3]." Vaccine hesitancy is reserved not only for a single person but also for several communities due to the spread of misinformation as well as misunderstanding about the vaccines among public. However, it was difficult to control the spread of misinformation. With the help of social media, the benefits of vaccination have been rapidly spread to public by giving examples through case observations that achieved success and gained back their normal health[4,5]. Recently, with the introduction of ChatGPT, which is a text-generative artificial intelligence (AI) tool, awareness has been spread in favor of vaccine acceptance, which has helped several people to assess the benefit–risk of vaccines and encouraged users to get vaccinated and reduce misconceptions[2]. Therefore, it has become essential to study the application of ChatGPT in reducing vaccine hesitancy to enhance vaccine acceptance.

The achievement of this era is the application of AI with the launch of ChatGPT. This supportive online tool was introduced during the COVID-19 pandemic[6]. Evidence-based studies have shown that public's trust on such tools regarding the COVID-19 vaccination has created a range of hesitancy, and only a few people were willing to get vaccinated[7-9]. Recently, a study was performed on COVID-19 vaccination in Cyprus, which reveals that two-thirds of medical practitioners have opposed mandatory COVID-19 vaccination[10], and a similar response was obtained from French hospital workers[11]. This is still a myth in some communities and a challenge for public health professionals due to resistance against vaccination. Recently, common people and health professionals have set a list of questions to be answered by ChatGPT on vaccine hesitancy and found that the responses provided were clear, correct, and concise[12]. Most studies that were correlated and retrieved from ChatGPT reveal that misinformation regarding the COVID-19 vaccines is likely to avoid preventive behaviors, propagating vaccine hesitancy and negative attitudes toward the COVID-19 vaccines[13,14].

Apart from providing accurate information on vaccine hesitancy, surprisingly, ChatGPT also undergoes technical and ethical issues, which are not a substitute for the findings of a scientific, or medical expert. Technically, it can match the information tuned toward information that aligns well with scientific evidence, but practically, one cannot solely rely on it for decisions related to medical practice. In some instances, inaccurate information may be generated depending on variations in the versions of the ChatGPT[15]. So, displaying inappropriate content is possible with AI tools, leading to confusion among the public. There is a possibility that ChatGPT may provide limited information due to a lack of understanding of the globe and events after 2021 and may end up replying, "My knowledge cutoff is 2021." ChatGPT lacks practical patient care options that differ according to ethnicity and medical troubleshooting. Information provided by ChatGPT lacks a descriptive nature, which lacks a match with the quantitative statistical analysis, thereby creating a lag in the technical aspect[16]. Although ChatGPT promisingly provides users with vaccine hesitancy information, it is critical to acknowledge its limits and, when needed, primarily, its availability is neutral when willing to make vaccine acceptance decision. However, for the public with limited medical knowledge, without the consultation of an expert's medical advice, these tools are not accessible from the risk of eliciting misleading responses[12].

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on November 13, 2023. Accepted on November 29, 2023.

Furthermore, in the future, the implementation of ChatGPT in helping the public get vaccinated for COVID-19 becomes a significant medical application in discussing the concerns about immune-based variations globally. Through programming the tool for computer-aided diagnosis, clinicians can provide case studies as reference studies for further investigation. They can check the match line data provided by ChatGPT with clinical variables. Therefore, the healthcare ecosystem is realizing to balance the clinical condition of patients by seeking ChatGPT in the next-generation healthcare technology. It is believed that ChatGPT can bring improvements to any process within healthcare operation and delivery[2].

Overall, fake and irrelevant questions related to vaccines and vaccination protocol can be captured by ChatGPT because the language applied by this AI is not overly technical, and one can understand and make better decision toward vaccine acceptance. Moreover, fearless, supportive opinions are helpful for the public in changing their perceptions about taking vaccines, encouraging users to get vaccinated, and reducing misconceptions.

ACKNOWLEDGMENTS

The author thanks Capsid Medical Communications for language editing support.

AUTHORS' CONTRIBUTIONS

AVS: Conceptualization, Data curation, Formal Analysis, Methodology, Validation, Writing – original draft, Writing – review & editing.

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https://doi.org/10.1590/1806-9282.20231617

Is the neutrophil-to-lymphocyte "ratio"nale from head and neck to thyroidology for thyroidologists?: promise or passé?

Ilker Sengul[1,2], Demet Sengul[3*], Tugrul Kesicioglu[2], Esma Cinar[3], Dzemail Detanac[4], Anton Pelikán[5,6,7], Pavla Kudlova[7]

Dear Editor,

We read with a great deal the research article entitled "Is the neutrophil-to-lymphocyte ratio a marker for differentiating between benign and malignant submandibular gland masses?" by Bora[1]. This research of high quality seems to demand portraying to evaluation of the effect of the neutrophil-to-lymphocyte ratio (N/L) on distinguishing benign from malignant masses in the submandibular triangle of the head and neck which has recently been published in the 69th volume of *Rev Assoc Med Bras*[1]. The author stated that he retrospectively evaluated 48 cases who had undergone surgery for submandibular gland masses with the histopathology of sialolithiasis, sialadenitis, pleomorphic adenoma[2-4], and malignant conditions. He deduced and emphasized that N/L, *per se*, can be used as a biomarker in submandibular gland masses and has prognostic significance in malignant masses. Moreover, he proposed that N/L can be utilized as a biomarker beyond fine needle aspiration cytology. Thereto, he highlighted N/L as being proven in distinguishing between benign and malignant nodules in patients[1] with suspicious thyroid nodules[1,5-17]. Nevertheless, at least a minimal debate is still ongoing on an accurate decision on N/L whether it is a biomarker in discrimination of malignity or not. Exempligratia, we reported that N/L was not a convenient and favorable biomarker in forecasting thyroid malignancy[18] in thyroidology[19-29]. Of note, N/L, particularly in the thyroid with head and neck providers, the growing spectrum of clinical management for challenging conditions remains, especially for thyroidologists and thyroid health as different peas in a pod. *Fide, sed cui vide.* As a matter of fact, this issue merits further investigation. We thank Bora[1] for his valuable study.

ACKNOWLEDGMENTS

We thank all the study participants.

AUTHORS' CONTRIBUTIONS

IS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **DS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **TK:** Investigation, Methodology, Software, Validation, Visualization. **EC:** Investigation, Methodology, Software, Validation, Visualization. **DD:** Investigation, Methodology, Validation, Visualization. **AP:** Investigation, Methodology, Validation, Visualization. **PK:** Investigation, Methodology, Validation, Visualization.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on November 24, 2023. Accepted on November 27, 2023.

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LETTER TO THE EDITOR

https://doi.org/10.1590/1806-9282.20231637

The quality of sleep and digestion in cerebral palsy depends not only on the level of functional independence

Walter Michael Strobl[1], Carla Alexandra Scorza[2], Fulvio Alexandre Scorza[2], Josef Finsterer[3*]

Dear Editor,

We read with interest Gunaydin and Tuncer's article, which is a cross-sectional, single-center, observational study on the association of the levels of functional independence with sleep and constipation in 60 children aged 4–18 years with cerebral palsy (CP) carried out between September 2021 and April 2022[1]. It was found that 46.7% of the cases corresponded to level III on the Gross Motor Function Classification System (GMFCS), 35% to level IV, and 18.3% to level V[1]. There was a negative correlation between the functional independence measure (FIM) for children and pediatric sleep questionnaire (PSQ) and between FIM for children and the constipation severity scale (CSS)[1]. It was concluded that low levels of functional independence correlate with poorer sleep and constipation[1]. The study is impressive, but some points require discussion.

The major limitation of the study is that alternative factors that influence sleep quality and digestion were not adequately included in the analysis. Digestion depends not only on the level of mobility, but also on the type of diet, level of hydration, sympathetic parasympathetic balance, psychiatric status, stress level, current medications, body mass index, and sleep quality. Therefore, we should know the diet of the 60 patients included during the study period, as well as the type and amount of fluids in relation to their body weight. The composition, quality, and quantity of food largely determine the function of the intestines. It is also important to know the level of exogenous and endogenous stress to which the included patients were exposed. Digestion also depends on a person's temperament. Other factors that affect digestion include mood, drive, and threat level. Therefore, it would have been advisable to administer depression and anxiety scales to all enrolled patients.

In addition, there are a number of comorbidities that can affect digestion. Although patients with certain comorbidities or medical conditions (surgery to improve intestinal health, chronic infectious bowel disease, congenital intestinal anomalies, treatment with botulinum toxin in the last 6 months, uncontrolled seizures, constipation in the last 6 months, cardiopulmonary disease) were excluded, multiple comorbidities were not included in the list of exclusion criteria. These include gastritis, diabetes, hypo-/hyperthyroidism, hypo-/hypercorticism, and hypo-/hyperaldosteronism[2].

Sleep quality may depend not only on the level of functional mobility but also on several other factors. These include the level of noise in the bedroom at the beginning and during sleep, the time of last food/liquids intake, the presence/absence of pain during the night, the presence/absence of restless legs, concomitant medications taken at night, degree of sympathetic/parasympathetic activation, presence/absence of nocturia, presence/absence of nocturnal seizures, temperature in the bedroom, lighting conditions in the bedroom, type of daytime experiences and their processing during sleep, air quality in the bedroom (level of pollution, urban or rural area), time of going to bed, and the underlying etiology of CP, i.e., the location of the cerebral lesion[3]. Of particular interest is whether the sleep/wake rhythm center was affected. Therefore, sleep quality should also be correlated with cerebral imaging findings and the EEG.

Additional limitations include that the group size was small, the design was single center, and test-retest reliability was not assessed.

In summary, the interesting study has limitations that put the results and their interpretation into perspective. Clarifying these weaknesses would strengthen the conclusions and could improve the study. Despite all possible objections,

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on November 29, 2023. Accepted on November 30, 2023.

we agree that all necessary measures must be taken to achieve the highest possible level of functional mobility and to enable the affected CP individuals to have the highest possible level of independence.

STATEMENT OF ETHICS

The study was approved by the institutional review board. Written informed consent was obtained from the patients for publication of the details of their medical case and any accompanying images.

DATA AVAILABILITY STATEMENT

Data that support the findings of the study are available from the corresponding author.

AUTHORS' CONTRIBUTIONS

JF: Cenceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **FAS:** Formal Analysis, Methodology, Writing – review & editing. **CAS:** Formal Analysis, Methodology, Writing – review & editing. **WMS:** Formal Analysis, Investigation, Validation, Writing – Review & editing.

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LETTER TO THE EDITOR

https://doi.org/10.1590/1806-9282.20231734

Comment on "The effect of psychological inflexibility on health-related quality of life, depression, and anxiety in patients with chronic tinnitus without hearing loss"

Xiaohong Zhang[1,2], Shenggang Yan[1,2*]

Dear Editor,

We read with great interest the recent study[1] investigating the relationship between psychological inflexibility and health-related quality of life, depression, and anxiety in individuals coping with chronic tinnitus but without accompanying hearing loss. The exploration of such an aspect of the human experience is both timely and essential, given the increasing prevalence of chronic tinnitus and its often-profound impact on individuals' overall well-being. The study[1] analyzes the lesser-explored psychological aspects of chronic tinnitus, highlighting the role of psychological inflexibility in amplifying the difficulties experienced by patients. Through a detailed examination of how psychological inflexibility interacts with health-related quality of life, depression, and anxiety, the researchers offer valuable insights that enhance our understanding of the complex nature of chronic tinnitus. The findings suggest that addressing psychological inflexibility may hold promise as an avenue for intervention and support for individuals grappling with the burdensome effects of chronic tinnitus. This implies that therapeutic approaches aimed at enhancing psychological flexibility could potentially play a crucial role in ameliorating the negative impacts on mental health and overall quality of life for this patient population. Furthermore, the study prompts reflection on the broader implications for healthcare professionals, emphasizing the importance of adopting a holistic and patient-centered approach to the management of chronic conditions such as tinnitus. The integration of mental health considerations alongside traditional medical approaches may prove instrumental in fostering more comprehensive and effective care for these patients. However, we note that the following concerns require further clarification.

First, in reference to Table 1 of the study[1], the mean value for the control group's Beck Depression Inventory (BDI) is reported as 5.29, with a corresponding standard deviation of 6.25. Notably, the standard deviation value (6.25) is significantly higher than its corresponding mean (5.29). In statistical terms, when the standard deviation is markedly higher than the mean, it suggests a skewed distribution of data. In such cases, it is advisable to describe the data using the median and interquartile range and to employ the Wilcoxon rank-sum test for between-group comparisons rather than the t-test. Therefore, it is highly recommended to conduct a normality test for continuous data before initiating data analysis. While acknowledging that the ultimate conclusions may not undergo significant changes, employing appropriate statistical methods contributes to the credibility and replicability of the study. By ensuring the robustness of statistical analyses, we can enhance the overall validity of the research findings. We believe that addressing these statistical considerations will strengthen the methodological rigor of the study and contribute to the robustness of its conclusions.

Second, as outlined in the study, the participants comprised individuals with chronic tinnitus. However, the research does not provide an in-depth exploration of the treatment strategies employed for chronic tinnitus, particularly those related to health-related quality of life, depression, and anxiety. A randomized controlled trial[2] involving 492 tinnitus patients revealed that those receiving specialized treatment exhibited higher health-related quality of life and lower tinnitus severity compared to those receiving usual care. This suggests an association between specialized treatment, improved quality of life, and reduced tinnitus severity. In addition, a study performed by Ibarra-Zarate et al[3]. cautioned against the indiscriminate use of music therapy (MT) as it may exacerbate the condition of tinnitus patients. On the contrary, their results found that for patients with tinnitus who are dealing with stress-related symptoms but not anxiety, it is

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: this work was supported by the Zhejiang Province Traditional Chinese Medicine Science and Technology Plan Project (No. 2024ZF163).
Received on December 15, 2023. Accepted on December 16, 2023.

advisable to consider Binaural Sound Therapy (BST). The evidence presented indicates potential treatment strategies linked to quality of life, stress, and anxiety. However, the current study lacks a detailed account of the treatment strategies employed for individuals with chronic tinnitus.

AUTHORS' CONTRIBUTIONS

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

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https://doi.org/10.1590/1806-9282.20240093

How to demonstrate the impact of ivabradine on suppressing ventricular arrhythmia

Daichi Sannomiya[1], Naoya Kataoka[1], Teruhiko Imamura[1*]

Dear Editor,

Ivabradine, an inhibitor of the I_f current predominantly expressed in the sinoatrial node, improves mortality and morbidity in patients with heart failure and reduced ejection fraction (HFrEF) as well as sinus rhythm by modulating heart rate[1]. However, the impact of ivabradine on reducing the burden of ventricular arrhythmia remains unknown. Pay and colleagues demonstrated that ivabradine therapy was associated with a lower incidence of appropriate implantable cardioverter-defibrillator discharge but was not associated with mortality in the HFrEF cohort[2]. Several concerns have been raised.

The cornerstone randomized controlled study, the SHIFT trial, demonstrated the impact of ivabradine on reducing cardiovascular death or heart failure readmission in the HFrEF cohort[1]. Could the authors explain the reason for the discrepancy between the two studies? In the SHIFT trial, patients with left ventricular ejection fraction <35%, heart rate ≥70 bpm, and sinus rhythm were included and received ivabradine[1]. How many patients in the authors' study satisfy the same inclusion criteria? In the SHIFT trial, the dose of ivabradine was up-titrated to achieve a heart rate between 50 and 60 bpm[1]. Could patients achieve sufficient heart rate reduction during ivabradine therapy? For the achievement of heart rate optimization, our team recently proposed to minimize the overlap between the E-wave and A-wave in the Doppler echocardiographic transmitral flow during ivabradine therapy to maximize cardiac output (echo-guided heart rate modulation therapy)[3].

The mechanism of why ivabradine suppresses ventricular arrhythmia remains uncertain. Another heart failure medication, sacubitril/valsartan, suppresses the incidence of ventricular arrhythmia by facilitating cardiac reverse remodeling[4]. Did the authors have successive echocardiographic data during ivabradine therapy? Ivabradine has the potential to stabilize hemodynamics and give us a chance to up-titrate the dose of beta-blockers, which may assist in suppressing ventricular arrhythmia. Were heart failure medications up-titrated during ivabradine therapy?

The reason for implantable cardioverter-defibrillator implantation is unclear[2]. The incidence of ventricular arrhythmia should be higher in patients who received the devices for secondary prevention versus primary prevention.

AUTHORS' CONTRIBUTIONS

DS: Writing – original draft. **NK:** Writing – original draft. **TI:** Conceptualization, Supervision, Writing – review & editing.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 19, 2024. Accepted on February 04, 2024.

https://doi.org/10.1590/1806-9282.20240103

The reperfusion rates after recombinant human tissue plasminogen activator thrombolysis depend not on a few but on a plethora of influencing factors

Josef Finsterer[1*], Fulvio Alexandre Scorza[2], Carla Alexandra Scorza[2]

Dear Editor,

We read with interest Oliveira et al.'s article, which is a retrospective, cross-sectional, observational study on possible determinants of reperfusion after venous thrombolysis with recombinant human tissue plasminogen activator (rt-PA) based on a review of hospital records of inpatients diagnosed with ischemic stroke[1]. Analysis of 316 patient records revealed that reperfusion following rt-PA treatment occurred more frequently in women than men[1]. The mean admission severity score was higher in patients without reperfusion than in those with reperfusion. Mean ejection fraction (EF) was normal in reperfused and non-reperfused patients, but it was higher in non-reperfused compared to reperfused patients[1]. Reperfusion was associated with reduced mortality after ischemic stroke[1]. The study is impressive, but several points require discussion.

The main drawback of the study is its retrospective design. Retrospective design has several disadvantages. It does not allow control of the accuracy of the data stored, does not systematically apply the same examinations to all included patients, produces missing data, does not allow for the addition of missing data, and is not suitable for generating desirable new data.

The second limitation is that several factors that may additionally determine reperfusion rates were not included in the analysis[1]. Factors determining the reperfusion rates not included in the analysis were the degree of atherosclerosis, the number of risk factors for atherosclerosis, sympathetic activity, door-to-needle time, the extent of mismatch between stroke core and penumbra, which was confirmed by diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI), stroke volume, blood pressure on admission, and rt-PA doses used.

The third limitation is that the modified thrombolysis in cerebral infarction (TICI) scoring system has not been used to assess the degree of reperfusion[2]. According to the modified TICI system, TICI3 means complete reperfusion, TICI2c means almost complete reperfusion except for slow flow in distal branches, TICI2b means partial filling covering >50% of the territory, TICI2a means partial filling of <50% of the territory, and TICI1 means no or minimal reperfusion[2]. The clinical outcome after thrombolysis can strongly depend on the TICI score achieved[3].

A significant risk factor not included in the analysis was atrial fibrillation. Permanent atrial fibrillation can be a strong risk factor for re-occlusion and hence the outcome of thrombolysis. Therefore, it is mandatory to report the number of patients who had paroxysmal, persisting (>7 days response to treatment), or had permanent atrial fibrillation (not amenable to treatment).

Another factor affecting the reperfusion rate after thrombolysis is the presence or absence of pre-stroke antithrombotic or anticoagulant treatment. Reperfusion rates may depend largely on the coagulation status at the time of thrombolysis. Therefore, current medication must be included in the analysis as well as the number of the included patients who had hereditary or acquired coagulopathy.

There is a discrepancy in the number of patients included. In the first paragraph of the results, 316 patients were included (540 included and 224 excluded). In the next sentence, 192 were reperfused, 124 were not reperfused, and 12 did not show data consistent with reperfusion, yielding 328 patients. This inconsistency should be resolved. Why were the 12 patients with data inconsistent with reperfusion not combined with the 124 patients without reperfusion?

To sum up, the excellent study has limitations that should be addressed before final conclusions are drawn. Clarifying the weaknesses will strengthen the conclusions and improve the

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 20, 2024. Accepted on February 04, 2024.

study. Reperfusion after systemic thrombolysis may depend not only on gender, age, and classic cardiovascular risk factors but also on a number of other influencing factors that need to be included in the analysis before drawing final conclusions.

AUTHORS' CONTRIBUTIONS

JF: Conceptualization, Data curation, Writing – original draft.
FAS: Formal Analysis, Validation, Writing – review & editing.
CAS: Formal Analysis, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231006

Factors influencing the positivity of diagnostic tests for congenital syphilis

Rodrigo Soares Ribeiro[1], Natália Sperli Geraldes Marin dos Santos Sasaki[1*], Alessandra Marinela de Abreu Queiroz[1], Ana Cecília Mota Ferreira[2], Gabriela de Souza Segura[1], Maria de Lourdes Sperli Geraldes Santos[1], Lara Helk de Souza[1], Luciano Garcia Lourenção[3]

SUMMARY

OBJECTIVE: The objective of this study was to analyze the factors that influence the positivity of treponemal and non-treponemal tests in cases of congenital syphilis.

METHODS: This cross-sectional and correlational study was carried out from the analysis of the database of Disease and Notification Information System (SINAN, in Portuguese) using the data obtained through the Epidemiological Surveillance Group 29, with 639 notifications of congenital syphilis between 2007 and 2018. The data were analyzed by a descriptive and inferential analysis from logistic regression with a significance level of 5% ($p \leq 0.05$).

RESULTS: The positivity of the treponemal test was higher by 4.5 times in infants living in rural areas and 19.6 times among those whose mothers obtained the diagnosis of syphilis after birth. The treponemal test showed positivity 3.2 times higher for the variable "having been diagnosed between 2007 and 2015" and 5.5 times higher for the variable "having been diagnosed with maternal syphilis in the postpartum period."

CONCLUSION: This study shows that testing during prenatal care is essential for early diagnosis and prevention of syphilis complications.

KEYWORDS: Syphilis. Congenital. Early diagnosis. Prenatal care.

INTRODUCTION

Vertical transmission of syphilis can lead to serious fetal problems such as miscarriage, prematurity, and death, in addition to congenital infections[1,2]. Prevention involves early detection and treatment during pregnancy. If the pregnant woman receives proper treatment, the fetus is easily cured and its adverse effects are minimized, especially before the third gestational trimester. Evidence shows a worldwide decline in mother-to-child transmission of syphilis, due to advances in screening and early detection of infection[1,3].

Prenatal syphilis screening by rapid testing has been a strategy of the World Health Organization (WHO) to improve timely detection and adequate treatment to interrupt vertical transmission. The rapid test does not require laboratory infrastructure and can be easily taken to people in rural areas or those who do not have easily accessible health services. They are simple and affordable tests, making them useful in all types of care[1,3,4]. Despite this, there are barriers such as logistics, infrastructure, professional training, and lack of knowledge among pregnant women about the applicability of rapid tests[5].

In Brazil, women's access to primary health care units is not equal, due to inadequate infrastructure (difficult access, lack of rooms, use of temporary spaces), disproportionate coverage between regions, and restricted opening hours (limited to business hours), as many women are unable to leave work to go to the health service[6].

In view of the above, this study aimed to analyze the factors that influence the positivity of treponemal and non-treponemal tests in cases of congenital syphilis.

METHODS

Study design, period, and location

This cross-sectional, descriptive, and correlational study was carried out between January and March 2019, using the data from the Disease and Notification Information System (SINAN, in Portuguese), obtained through Epidemiological Surveillance Group 29, a regional member of the structure of the

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on February 01, 2024. Accepted on February 04, 2024.

"Prof Alexandre Vranjac" Epidemiological Surveillance Center (CVE/SP), which regulates the Epidemiological Surveillance System in the State of São Paulo. This research was guided by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) tool.

The Epidemiological Surveillance Group 29 covers 67 municipalities belonging to the Regional Management Collegiate of Catanduva, José Bonifácio, Votuporanga, and São José do Rio Preto, where it is headquartered.

Population and sample

The study included the congenital syphilis cases notified by SINAN in the municipalities integrating the Epidemiological Surveillance Group 29, in the period from January 2007 to December 2018. This period was defined in agreement with the GVE coordination, taking into account the quality and completeness of the data available in the system and notification. The rapid test for diagnosis was introduced in 2016.

Study protocol

The variables explored included information from the child, pregnant woman, and sexual partner which were considered as independent variables. Regarding children, the variables comprised general data and individual notification as the period of notification according to the change in diagnostic guidelines (2007–2015 and 2016–2018)[7], area of residence, sex, color, and death. Regarding pregnant women, socioeconomic variables were analyzed (maternal age group according to the classification considered with gestational risk, color, and education in years of schooling), clinical and laboratory variables (prenatal care, diagnosis of maternal syphilis, and title of the non-treponemal test), and concomitant treatment of the partner. For the non-treponemal test titer, the reference values used were greater than 1:8 or less than or equal to 1:8, because false-positive results generally have titers less than 1:84. The dependent variables were the reactivity of the non-treponemal test and the treponemal test.

Analysis of results and statistics

Data analysis was performed using the software Statistical Package for the Social Sciences (SPSS), version 20.0. Initially, a univariate analysis was performed with the calculation of Pearson's or Fischer's chi-square tests when necessary, considering a significance level of 5% ($p \leq 0.05$). Ignored or blank cases were excluded as they appeared in the data cross-analyses. Subsequently, a multivariate analysis was performed using stepwise binary logistic regression in which the variables that obtained $p \leq 0.20$ in the univariate analysis and that did not present multicollinearity were included. The data were presented in contingency tables.

Ethical aspects

The research complies with the ethical precepts and was approved by the Research Ethics Committee under opinion 2.556.704 and CAAE: 85159518.0.0000.5489.

RESULTS

During the study period, there were 639 notifications of congenital syphilis, of which 92.8% had a reagent non-treponemal test and 78.2% had a reagent treponemal test. There was statistical significance between the non-treponemal test only with a zone of residence ($p=0.015$) and the treponemal test with a period of notification ($p=0.001$) and stillbirth, neonatal, and infant death ($p=0.050$). Among maternal variables, there was statistical significance between treponemal and non-treponemal tests related only to maternal syphilis diagnosis ($p<0.001$) (Table 1).

The results showed that 92.5% of non-treponemal test-positive cases followed an inadequate treatment regimen. This practice was observed in 78.4% of the treponemal test-positive cases (Table 1). The concomitant treatment of the partner was not carried out in 92.6% of the non-treponemal test-positive cases and 79.1% of the treponemal test-positive cases (Table 1).

For logistic regression, only variables with $p \leq 0.05$ were included in the step-wise analysis (Table 2). The variables "area of residence" and "maternal diagnosis of syphilis" were included in the model for the positivity of the non-treponemal test. The period of diagnosis, concomitant partner testing, and maternal syphilis diagnosis were included in the model for the positivity of the treponemal test. Living in a rural area and being diagnosed in the postpartum period represented, respectively, 4.5 and 19.6 times greater chances of being positive for the non-treponemal test. On the contrary, the chances of being positive for the treponemal test were 3.2 times higher for the variable "having been diagnosed between 2007 and 2015" and 5.5 times higher for the variable "having been diagnosed with maternal syphilis in the postpartum period." Newborn deaths were five times higher among those who tested positive. Diagnosis at delivery was a protective factor for positivity in both tests (Table 2).

DISCUSSION

This study showed that the positivity of the non-treponemal test was influenced by the area of residence and maternal diagnosis after delivery, while the cases with syphilis maternal diagnosis at delivery had a protective factor. As for the treponemal test, besides the three previous factors, death was also a predictor of greater chances of reactivity. The protective factor related to syphilis diagnosis at the time of delivery is related to

Table 1. Results of treponemal and non-treponemal tests according to sociodemographic, clinical, and treatment variables of the mother and child and treatment of the partner (São Paulo, Brazil).

Variables (n=639)	Non-treponemal test		Treponemal tests	
	Non-reactive	Reactive	Non-reactive	Reactive
	n (%)	n (%)	n (%)	n (%)
	46 (7.2)	593 (92.8)	139 (21.8)	500 (78.2)
Reporting period	p=0.075		p=0.001	
2007–2015	34 (8.4)	369 (91.6)	104 (25.8)	299 (74.2)
2016–2018	12 (5.1)	224 (94.9)	35 (14.8)	201 (85.2)
Living area*	p=0.015		p=0.102	
Urban	37 (6.1)	566 (93.9)	128 (21.2)	475(78.8)
Rural	18 (78.3)	5 (21.7)	8 (34.8)	15 (65.2)
Gender of the child*	p=0.358		p=0.413	
Female	21 (6.8)	286 (93.2)	69 (22.5)	238 (77.5)
Male	22 (8.0)	254 (92.0)	59 (21.4)	217(78.6)
Child's skin color*	p=0.529		p=0.164	
White	34 (7.0)	451 (93.0)	100 (20.6)	385 (79.4)
Other	6 (6.5)	87(93.5)	24(25.8)	69 (74.2)
Stillbirth, neonatal, and infant death	p=0.501		p=0.050	
Yes	6 (7.7)	72 (92.3)	11 (14.1)	67 (85.9)
No	40 (7.1)	521 (92.9)	128 (22.8)	433 (77.2)
Maternal age group*	p=0.280		p=0.664	
<18 years	2 (3.8)	50 (96.2)	12 (23.1)	40 (74.9)
18–34 years	42 (7.9)	493 (92.1)	115 (21.5)	420 (78.5)
35 years or older	1 (2.5)	39 (97.5)	11 (27.5)	29 (72.5)
Mother's skin color*	p=0.248		p=0.409	
White	25 (6.0)	395 (94.0)	90 (21.4)	330 (78.6)
Other	15 (7.8)	178 (92.2)	39 (20.2)	154 (79.8)
Mother's education (in years)*	p=0.553		p=0.108	
Up to 8 years	15 (6.8)	204 (93.2)	53 (24.2)	166 (75.8)
9 years or more	19 (6.8)	262 (93.2)	54 (19.2)	227(80.8)
Prenatal*	p=0.541		p=0.527	
Yes	39 (7.0)	519 (93.0)	120 (21.5)	438 (78.5)
No	5 (6.4)	73 (93.6)	17 (21.8)	61 (78.2)
Maternal syphilis diagnosis*	p<0.001		p<0.001	
Postpartum	12 (54.5)	10 (45.5)	14 (63.6)	8 (36.4)
During delivery	3 (1.7)	177 (98.3)	36 (20.0)	144 (80.0)
Prenatal	26 (6.1)	402 (93.9)	83 (19.4)	345 (80.6)
Non-treponemal test title*	p=0.405		p=0.544	
≤1:8	2 (0.5)	377 (99.5)	72 (19.0)	307 (81.0)
>1:8	0 (0.0)	216 (100.0)	41 (19.0)	175 (81.0)
Treatment scheme	p=0.127		p=0.368	
Adequate	0 (0.0)	27 (100.0)	7 (25.9)	20 (74.1)
Inadequate	46 (7.5)	566 (92.5)	132 (21.6)	480 (78.4)
Concomitant partner treatment	p=0.405		p=0.190	
Yes	8 (6.2)	120 (93.8)	32 (25.0)	96 (75.0)
No	38 (7.4)	473 (92.6)	107 (20.9)	404 (79.1)

*Exclusion of cases with ignored information.

Table 2. Logistic regression analysis between the reacting results of non-treponemal and treponemal tests (São Paulo, Brazil).

Variables			OR	95%CI	p-value
Non-treponemal test	Living area	Rural	4.585	1.388–15.147	0.013
		Urban	1		
	Maternal syphilis diagnosis	Prenatal	1		
		during the birth	0.290	0.086–0.979	0.046
		postpartum	19.619	7.579–50.783	<0.001
Treponemal tests	Reporting period	2007–2015	3.176	1.780–5.667	<0.001
		2016–2018	1		
	Concomitant partner treatment	Yes	1		
		No	0.426	0.245–0.742	0.003
	Maternal syphilis diagnosis	Postpartum	5.573	1.737–17.881	0.004
		During delivery	1.003	0.557–1.774	0.991
		Prenatal	1		

OR: odds ratio; CI: confidence interval.

the identification of treponema before the disease is activated in the newborn and without complications for the mother[8]. This is why it is essential to carry out the rapid test at the time of delivery, even if the pregnant woman has already been tested in the last trimester of pregnancy.

In this regard, it is important to note that the strengthening of primary health care services, by expanding the coverage of the family health strategy and building attention networks, brought greater qualification of care, expanding the monitoring through the assignment of the territory and linkage with the team and making the diagnosis of diseases more accessible to the population[6,9]. However, some challenges are still present, such as the high percentage of cases with inadequate treatment regimens and the low rate of concomitant treatment of the partner, observed in this study and reported in the literature[8].

Syphilis diagnosis is based on direct and immunological tests. Immunological tests are most commonly used in clinical practice and are divided into non-treponemal and treponemal tests. The non-treponemal tests detect the non-specific anticardiolipin of the *Treponema pallidum* antigen that allows quali-quantitative analysis and the result is expressed in progressive fraction, allowing monitoring of the therapeutic response or evolution of the infection. However, late or latent infection has low titers[9]. The treponemal tests detect specific antibodies produced against the *T. pallidum* antigens, which are the first post-infection tests to present reagent results and, in approximately 85% of infected persons, remain reagent for life, thus requiring non-treponemal tests to evaluate the therapeutic response[4].

Rapid treponemal tests are quick to perform, read, and interpret. Performed with a small amount of blood in digital or venipuncture, serum, or plasma, they do not require laboratory structure, ensuring an improvement in screening and early diagnosis[3,4]. The Ministry of Health recommends its use in pregnant women with previous contact to the disease, who may develop a high risk of untreated syphilis. False-negative results may occur in the initial phase of the disease, requiring the association of a treponemal test with a non-treponemal test[10].

Testing for syphilis is recommended during the first prenatal visit, from the 28th week of gestation, at the time of birth, or in cases of abortion, regardless of having been previously tested[4]. The expansion of rapid testing during prenatal care has contributed to the identification of asymptomatic pregnant women and caused a significant increase in cases of acquired, gestational, and congenital syphilis[11].

Early initiation of prenatal care is essential for preventing the development of congenital syphilis. To this end, the access of pregnant women to health services should be expanded, to ensure the prevention of complications during pregnancy and to the conceptus[2,6,12-16].

Access to health services is addressed by the concept of accessibility, which involves aspects of how people enter the health care network and how professional and technological resources are organized to serve them[6]. Accessibility encompasses the organizational, sociocultural, economic, and geographic dimensions. Despite the investments provided by the National Program for Improving Access and Quality of Primary Care (PMAQ-AB), regional disparities in access and accessibility to primary care services in Brazil are still observed, mainly related to the infrastructure of services, which require investment to improve access. These failures cause major difficulties in achieving resoluteness for the population's health problems[6,12,16].

Neglected diseases, such as syphilis, are associated with socioeconomic conditions and people living in poverty, which generate an important condition of vulnerability for the population exposed to the risk of contamination. To overcome this condition, it is important to implement health actions and policies that improve people's knowledge about the problem, arousing interest and creating possibilities to transform concerns into protective practices, including the search for early diagnosis[17,18].

Another crucial factor for the prevention of congenital syphilis is the treatment with benzathine penicillin G, which is considered the first option (gold standard) for the treatment of syphilis in pregnancy. In 2014, there was a shortage due to a worldwide shortage of the drug, and it was re-established the following year[19-21]. Although the drug shortage occurred in almost all the studied municipalities, in one of them, this did not occur due to the organization of the health system, with a provision in the previous year and the structuring of clinical protocols for the detection and control of syphilis. Therefore, health planning is important for the success of policies for diagnosis and control of communicable diseases such as syphilis, which contributes to organize the entire structure of services and standardization of treatments[11].

In this aspect, one can suggest weaknesses in prenatal care with respect to screening for *T. pallidum* infection in the three moments of prenatal care, highlighting the importance of screening at delivery by rapid test[4,22].

At delivery, the maternal diagnosis of syphilis allows the mother and her partner to be treated, avoiding complications. However, at this time, it will not be able to prevent transmission of the disease to the baby; it is no longer timely. Even so, the diagnosis of gestational syphilis at the time of birth offers the possibility of treating the baby, avoiding the severe consequences of late Congenital Syphilis, such as neurosyphilis[4,15].

For years, syphilis has been diagnosed by means of the VDRL test, which is considered simple and of low-cost, but it requires a laboratory structure for its performance. The late initiation of prenatal care associated with delayed results when returning to the clinic may contribute to late access to VDRL results during prenatal care[4].

When titers decrease around two dilutions in 3 months, non-treponemal tests indicate treatment success, such as a result that was 1:64 and dropped to 1:16. Persistent low titers after 1 year of treatment, if there is no possibility of a new infection in this period, is also considered a successful treatment. Persistent low titers indicate serological scarring and may last for a lifetime. However, if the titer is elevated by two dilutions or more, the possibility of reinfection or reactivation of the infection should be considered, requiring drug treatment[4,14].

The reinfection of pregnant women by syphilis is also associated with the non-treatment of their sexual partners and the consequent increase in vertical transmission. The unfavorable outcomes for newborns with congenital syphilis are independent of the treatment of the pregnant woman's sexual partner, considering that the syphilis infection was late in pregnancy[1,15]. The Ministry of Health recommends that, regardless of the syphilis stage diagnosed in the pregnant woman, all sexual partners exposed in the last 90 days before the diagnosis of gestational syphilis should be treated. This extends to sexual partners of contact greater than 90 days and those who had intercourse in the latent phase should be clinically evaluated[4].

For the treatment of sexual partners of pregnant women, it should be assumed that they are infected, even with non-reactive immunological tests. Therefore, they should presumably be treated with only one dose of intramuscular benzathine penicillin. In case of a reactive test for syphilis, one should follow the recommendations for the treatment of adult-acquired syphilis, according to the clinical stage of infection, preferably using benzathine penicillin[4,15].

The study has some limitations, such as the quality of the SINAN database, which generated incomplete data[23], and geographical delimitation, which includes cultural factors that restrict the generalization of the results. However, the results show that congenital syphilis remains a major challenge to public health, stressing that the characteristics linked to the binomial regarding health care point to low effectiveness in prenatal care regarding the appropriate treatment, interruption of vertical transmission, and treatment of sexual partners of mothers infected with *T. pallidum*. These results highlight the need to strengthen public policies aimed at diagnosing congenital syphilis and call for new studies covering other regions of the country.

CONCLUSION

This study showed that living in rural areas, maternal diagnosis of syphilis after birth contributes to a greater chance of having a positive non-treponemal and treponemal test, and the death of the newborn increases the positivity of the treponemal test. Rapid testing in maternity hospitals proved to be effective in detecting the disease.

RESEARCH ETHICS COMMITTEE APPROVAL

Opinion number 2.556.704 – CAAE: 85159518.0.0000.5489.

AUTHORS' CONTRIBUTIONS

RSR: Conceptualization, Formal Analysis, Writing – original draft. **NSGMSS:** Conceptualization, Supervision, Writing – review & editing. **AMAQ:** Conceptualization, Formal Analysis, Writing – review & editing. **ACMF:** Conceptualization, Formal Analysis, Writing – review & editing. **GSS:** Conceptualization, Formal Analysis, Writing – review & editing. **MLSGS:** Conceptualization, Supervision, Writing – review & editing. **LHS:** Conceptualization, Methodology, Writing – review & editing. **LGL:** Conceptualization, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231085

What is the applicability of tubal ligation with the vaginal natural orifice transluminal endoscopic surgery technique and its value in patient comfort?

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SUMMARY

OBJECTIVE: The aim of this study was to observe the feasibility of the tubal/adnexal approach using vaginal natural orifice transluminal endoscopic surgery and compare its contribution with surgeon ergonomics and postoperative patient comfort with that of conventional laparoscopy.

METHODS: We completed this study retrospectively with 47 patients. Patients were followed at their postoperative first month. We analyzed the usability of the vaginal natural orifice transluminal endoscopic surgery method over conventional laparoscopy by comparing the demographics, surgical data, and postoperative findings collected between the two groups.

RESULTS: Patients in the conventional laparoscopy group were older (39.1±3.3 years) than those in the vaginal natural orifice transluminal endoscopic surgery patient group (p=0.005). Pain intensity 24 h after surgery was lower in the vaginal natural orifice transluminal endoscopic surgery group (p=0.003), while sexual function and dyspareunia did not differ between the two groups in the first month. Patients in the vaginal natural orifice transluminal endoscopic surgery group were more relieved about painlessness and the comfort it brought than the conventional laparoscopy group (p=0.027, χ^2=12.56).

CONCLUSION: Patients subjected to the vaginal natural orifice transluminal endoscopic surgery procedure showed higher levels of satisfaction, less postoperative pain, and greater comfort than those subjected to conventional laparoscopy.

KEYWORDS: Natural orifice transluminal endoscopic surgery. Tubal ligation. Laparoscopy. Operative surgical procedures.

INTRODUCTION

Cost-effective methods that emphasize patient comfort and minimize complications in gynecological surgeries are recurrent research areas. Minimally invasive techniques have become common in gynecologic surgery over the past 20–30 years.

Natural orifice transluminal endoscopic surgery (NOTES) is one of the most significant innovations in surgery and gynecology since the advent of laparoscopy[1]. In 2013, Yang et al.[2] reported on the first transvaginal NOTES adnexectomy. Of the seven cases described in these studies, all were conducted successfully without conversion to the traditional laparoscopic approach.

After this study, vaginal NOTES (v-NOTES) has been increasingly embraced as a minimally invasive modality for various gynecological surgeries, including hysterectomies, myomectomies, and uterosacral ligament suspensions. This procedure may be particularly effective and safe for selected populations, such as obese women or those with large uteri[3,4].

Despite the positive results, data on v-NOTES need to be expanded. Unknown factors regarding this technique include the ease of application, its effect on the comfort of the surgeon, the impact on the comfort and pain level of the patient after surgery, and its effects on the comfort of sexual activity after the procedure.

Our aims in this study were to observe the feasibility of the tubal/adnexal approach using v-NOTES, compare it with conventional laparoscopy, and examine its contribution and benefits in terms of surgeon ergonomics, operation time, and postoperative patient well-being.

METHODS

This retrospective study analyzes specific surgeries performed in a third-level hospital during 6 months between August 2022 and January 2023. The Bursa Yüksek İhtisas Training and Research Hospital Ethics Committee approved the applied methods with ethics committee number 2011-KAEK-25 2022/11-18. Informed written consent was obtained from all patients.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 19, 2024. Accepted on February 04, 2024.

Patient selection

We planned to recruit 47 volunteers in total. The study population included women older than 18 who requested permanent surgical contraception and did not have a Grade 2 or greater prolapse according to the POP-Q system. Patients were excluded from the study if they had pelvic inflammatory disease, suspected malignancy, a history of rectovaginal endometriosis, pregnancy, or a pelvic abscess. In addition, a bilateral salpingectomy was offered instead of tubal ligation to reduce the risk of ovarian cancer.

The study was designed to compare conventional laparoscopy with that of v-NOTES based on patient selection. We recorded the age, parity, body mass index, previous surgery history, whole blood parameters, and modified POP-Q findings, including C, Ba, and Bp points of each patient preoperatively on the study forms.

Implementation

All procedures were performed by surgeons. The co-authors of this study followed previously published surgical techniques regarding the CL and v-NOTES approaches[5,6]. All patients were administered a prophylactic antibiotic treatment consisting of 2 g of intravenous cefazolin 30 min before surgery[6,7]. After general anesthesia and endotracheal intubation, the patients were placed in the Trendelenburg position by lithotomy. A single 2.5-cm incision was made in the posterior vaginal fornix, and the pouch of Douglas was opened to insert the NOTES port. This self-constructed device consisted of a 75-mm silicone vaginal ring (pessary) attached to a size eight powder-free surgical glove. We prevented the glove from opening during the procedure by rolling the ring inside the glove inward twice. One finger of the surgical glove was removed to insert a 10-mm reusable trocar for CO_2 insufflation and laparoscopic entry. Three 5-mm reusable trocars were inserted through the remaining fingers of the glove to position the reusable laparoscopic instruments (Figure 1). We used standard 30° 10-mm laparoscopy (Karl Storz SE & Co. KG, Tuttlingen, Germany). The reusable conventional laparoscopic instruments utilized were bipolar forceps, atraumatic forceps, and an aspiration-irrigation cannula (ENDOPATHTM Grasper 5 mm, Ethicon, Inc., Somerville, NJ, USA).

With the aid of a Breisky posterior vaginal retractor, we pushed the pessary ring of the NOTES port into the peritoneal cavity and perpendicular to the base of the pouch of Douglas.

Surgical procedure

An operator controlled the optics during the surgery and manipulated the uterus with the aid of a grasper when required. A second operator conducted the surgery using a bipolar grasper in one hand and a grasper in the other hand. After the ureters were identified, salpingectomy was performed from the fimbria to the uterine horn or ligation with bipolar assistance from two separate areas of the tube. After the procedure, we completed the process by re-visualizing and providing hemostasis with bipolar coagulation if necessary. Then we closed the colpotomy incision with an absorbable polyglactin 1.0 suture in a locked, continuous technique.

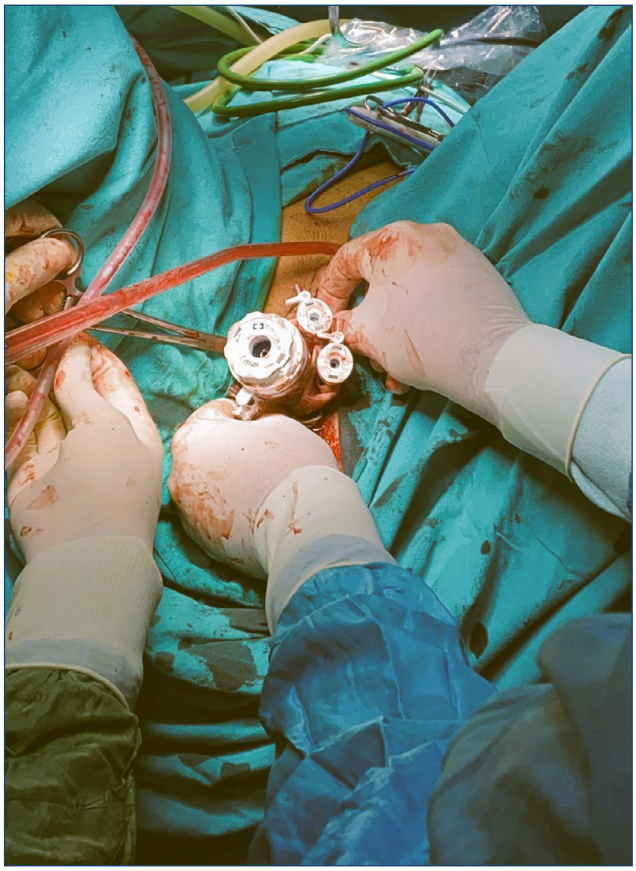

Figure 1. Image of port creation and its position in the patient.

Data analyses

Postoperative patient comfort, postoperative pain, pelvic pain, and sexual function variables were measured. We used the 35-question "multidimensional quality of life scale" questionnaire before surgery and during the first month after surgery to assess patient comfort and satisfaction[8]. The impact of pelvic pain of the patient on the quality of life was assessed using the pelvic pain impact questionnaire (PPIQ)[9]. The assessed domains reflected the energy level, mood, sleep, and gastrointestinal function of the patients, as well as their ability to sit, engage in functional activities and exercise, and wear specific clothing. Sexual discomfort or pain was measured using the female sexual function index-6 (FSFI-6) scale, and we noted a

difference between pre-surgery and the first month after surgery. Total scores range from 2 to 30, with lower scores indicating lower sexual function[10].

Postoperative pain was measured using a Likert-type visual analog score (VAS) after the surgical procedure and 24 h after surgery. VAS scores ranged from 0 (no pain) to 10 (most severe pain)[11]. Additional analgesics were not routinely given to the patients, and we recorded if they were used. In addition, we recorded the level of sexual discomfort or pain during vaginal penetration, the total duration of the surgery (from the first incision to the last suturation, in minutes), the change in hemoglobin levels, and the length of hospital stay over the first month.

RESULTS

We included 47 patients in our study, with 28 from the CL group and 19 from the v-NOTES group, with mean ages of 36.1±3.2 and 39.1±3.3 years, respectively. The body mass indexes and birth numbers were significantly different for both groups. However, the number of previous abdominal surgeries was significantly higher in the v-NOTES group (p=0.035). The pain measurement VAS score at 24 h after surgery was significantly higher in the CL group than in the v-NOTES group (p=0.003). We did not find a significant difference in postoperative sexual function when comparing the score values in the first month with those of the preoperative and postoperative score reduction values of the FSFI-6 scoring scale (p=0.12 and p=0.08, respectively). We evaluated postoperative comfort and general satisfaction of the patients using the MILQ scale and found that the values in the first month after surgery were lower in the CL group than in the v-NOTES group (p<0.01 and p=0.02, respectively). A detailed analysis of numerical data between the groups is summarized in Table 1.

Patients in the v-NOTES group were significantly more satisfied regarding the lack of pain and related comfort level than those of the CL group (p=0.027, χ^2=12.56). Five patients in the v-NOTES group expressed themselves as "very satisfied," whereas two patients in the CL group stated that they were not satisfied. The number of patients expressing the "I am happy" and "very happy" categories was 13 (46%) in the CL group and 13 (68.4%) in the v-NOTES group. The complete analysis is summarized in Table 2.

Four patients from the CL group and five from the v-NOTES group did not have sexual intercourse within 1 month. There was no significant difference between the two groups in pain or discomfort during penetration according to the sixth sub-item of the FSFI-6 scale (p=0.735, χ^2=0.616). Eleven patients (45.8%) from the Cl group and seven patients (50%) from the v-NOTES group stated that they did not feel any pain during this activity (Table 2).

DISCUSSION

In this study, we documented the feasibility of the v-NOTES procedure in patients requesting permanent contraception

Table 1. Comparison of clinical and demographic characteristics of the groups in terms of abdominal cleaning method.

Parameters	Conventional laparoscopy group (n=28)	v-NOTES group (n=19)	p
	Median (min–max)/mean±SD	Median (min–max)/mean±SD	
Age (years)*	36.1±3.2	39.1±3.3	0.005
Body mass index (kg/m^2)*	26.2±4.8	23.7±3.7	0.05
Number of births#	3 (2–6)	4 (3–6)	0.07
Previous abdominal surgery#	0 (0–3)	1 (0–3)	0.035
24th hour VAS score#	4 (1–8)	3 (1–4)	0.003
Duration of surgery (min)#	27 (15–45)	22 (15–40)	0.06
Hemoglobin decrease (mg/dL)*	1.4±0.6	1.2±0.4	0.12
Hospitalization (days)#	1 (1–2)	1 (1–1)	0.95
Postoperative FSFI-6 score*	13.4±7	16.4±5.8	0.12
Postoperative MILQ score*	196.3±26.2	217.6±10	<0.01
FSFI-6 decrease#	4 (0–13)	3 (0–10)	0.08
MILQ decrease#	11 (3–26)	6 (2–18)	0.02
PPIQ score#	5 (1–15)	4 (2–10)	0.11

Student's t-test *p<0.05 and Mann-Whitney U test #p<0.05 significant. min: minimum; max: maximum; SD: standard deviation.

Table 2. Cross-square analysis table of the groups according to the results of the seventh subtitle parameter of the multidimensional quality of life scale.

Among the groups	Are you satisfied with your pain-free state?				
	Conventional laparoscopy group (n=28)	v-NOTES group (n=19)	n	χ^2	p
Not satisfied at all	2 (7.1%)	0 (0%)	2 (4.3%)	12.569	0.027
Partially dissatisfied	1 (3.6%)	2 (10.5%)	3 (6.4%)		
Neither satisfied nor not satisfied	4 (14.3%)	0 (0%)	4 (8.5%)		
Partially satisfied	8 (28.6%)	4 (21.1%)	12 (25.5%)		
Satisfied	13 (46.4%)	8 (42.1%)	21 (44.7%)		
Very satisfied	0 (0%)	5 (26.3%)	5 (10.6%)		
Among the groups	**How often did you experience discomfort or pain during vaginal penetration?**				
	Conventional laparoscopy group (n=24)	v-NOTES group (n=14)	n	χ^2	p
Sometimes pain	1 (4.2%)	0 (0%)	1 (2.6%)	0.616	0.735
Rarely pain	12 (50%)	7 (50%)	19 (50%)		
Never pain	11 (45.8%)	7 (50%)	18 (47.4%)		

Pearson chi-square, $p<0.05$ was considered significant.

through salpingectomy or tubal ligation. Patients who underwent the v-NOTES procedure showed greater satisfaction, less postoperative pain, and a higher level of comfort than those who underwent CL.

There is considerable interest in new methods of minimally invasive surgery, which has led to the emergence of a new field of gynecological surgery, NOTES. Potential benefits of natural orifice surgery in gynecology include less operative pain, shorter hospital stay, lack of abdominal incisions, improved visibility, and extensive lysis of adhesion circumvention to reach the pelvic cavity. Transvaginal access for NOTES is the safest and most feasible of all of the procedures for clinical application. However, the opening of the posterior vaginal space (culdotomy) should be carefully performed to avoid potential complications, especially in the pouch of Douglas obliteration cases[12,13].

Vaginal surgery may have benefits over other techniques for benign gynecological diseases. However, inadequate visualization of the surgical field and difficulty in accessing adnexal structures in a non-prolapsed uterus restrict the use of transvaginal opportunistic bilateral salpingectomy[7]. Data support the usefulness of v-NOTES in the surgical treatment of adnexal pathologies[14]. v-NOTES can reduce the challenges and potential limitations of vaginal surgery and increase the indications for conventional vaginal surgery[12,13,15].

Li et al.[5] published a systematic review on the role of v-NOTES in gynecological surgery in 2019. The current review compared conventional laparoscopy with v-NOTES in two studies and demonstrated that v-NOTES can deliver superior outcomes in terms of blood loss, operative time, and length of stay[16,17]. This review stated that younger patients were more concerned than older patients regarding the v-NOTES procedure, which may be primarily related to anxieties relating to possible sexual repercussions. In this review, postoperative pain was reduced in patients who received the v-NOTES procedure compared with those undergoing the CL treatment. This review concluded that the studied gynecological surgical procedures could be performed safely and consistently by experienced surgeons.

Laganà et al.[12] compared patients who had undergone salpingectomies with those subjected to v-NOTES and CL in 2021. Patients in the v-NOTES group reflected less postoperative pain and more comfortable surgical outcomes; therefore, that study indicated that the v-NOTES procedure could be a suitable alternative to CL.

In our study, we showed that patients felt less pain after v-NOTES. Theoretically, a v-NOTES incision in the vagina would be expected to result in less pain than that of a skin incision. In addition, the pain and comfort levels in the first month after surgery were more positive in the v-NOTES patient group than the CL group, although this may partly relate to preconceptions of patients regarding surgery. The absence of a fascia incision can explain this situation, although it may be a psychological factor related to the lack of a surgical scar. Nevertheless, more data are required in the literature comparing the two surgical procedures to provide additional explanatory reasons for this situation in the coming years.

In this study, the results regarding postoperative sexual life and dyspareunia were similar between the patient groups. The group that chose CL from the two procedures was younger than the group that chose transvaginal surgery. We think that this may be a reflection of concerns concerning sexuality. Although one of the most severe concerns about transvaginal surgery is sexual dysfunction, we found no signs of withdrawal from coitus or pain in our patient group.

The main limitation of this study was the number of patients. We used 30-degree optics as a standard for visualization; however, using an image with angleless optics and determining its effects on the duration of the surgery could be a future option.

Safe and optimal peritoneal access is critical to performing a v-NOTES procedure. Consideration of the importance of patient comfort and recovery speed will increase as technologies are developed. v-NOTES is a candidate for a primary minimally invasive surgical procedure in the coming years.

ETHICAL APPROVAL

This study was approved by the Ethical Committee of the Bursa Yüksek İhtisas Training and Research Hospital (number 2011-KAEK-25 2022/11-18).

PATIENT CONSENT STATEMENT

Patients were informed in detail about the surgical procedure. Their written consent was also obtained regarding the surgical process and its aftermath.

AUTHORS' CONTRIBUTIONS

SSK: Data curation, Formal Analysis, Methodology, Resources, Supervision, Visualization, Writing – original draft. **ÖK:** Conceptualization, Formal Analysis, Project administration, Supervision, Writing – original draft.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231107

Assessment of serum granulysin and cathepsin-L levels in vitiligo patients

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SUMMARY

OBJECTIVE: Cellular and humoral immunity plays a role in the pathogenesis of vitiligo. T lymphocytes and natural killer cells involved in cellular immunity carry out their cytotoxic activities through perforin/granzyme-dependent granule exocytosis, in which granulysin and cathepsin-L are also involved. The aim of this study was to investigate the possible role of serum granulysin and cathepsin-L in the etiopathogenesis of vitiligo and their association with disease activity and severity.

METHODS: This randomized, prospective case-control study was conducted with 46 vitiligo patients admitted to the hospital for vitiligo between January and November 2021 and 46 healthy volunteers of similar age and gender. Serum levels of granulysin and cathepsin-L were measured by the enzyme-linked immunosorbent assay method.

RESULTS: The mean serum levels of granulysin and cathepsin-L were statistically significantly higher in vitiligo patients compared with the control group (p=0.048 and p=0.024, respectively). There was no statistically significant correlation between serum granulysin and serum cathepsin-L levels and disease severity in the patient group (r=0.30, p=0.062 and r=0.268, p=0.071, respectively). Disease activity also showed no significant association with serum granulysin and cathepsin-L levels (p=0.986 and p=0.962, respectively).

CONCLUSION: Although granulysin and cathepsin-L are molecules involved in the pathogenesis of vitiligo, the use of these molecules may not be helpful in assessing disease activity and severity. It may be helpful to conduct comprehensive and prospective studies to find new molecules to fill the gap in this area.

KEYWORDS: Vitiligo. Granulysin. Cathepsin L. Autoimmunity. Cellular immunity.

INTRODUCTION

Vitiligo is an acquired systemic disease characterized by selective destruction of melanocytes[1,2]. Although etiopathogenesis is not clearly known, many theories (genetics, autoimmunity, oxidative stress, production of inflammatory mediators, and melanocyte separation mechanisms) have been proposed to explain the pathogenesis[3]. Among these theories, the autoimmunity theory is the most widely accepted[4].

Both innate immunity and acquired immunity play a role in the pathogenesis of vitiligo[5]. Specifically, natural killer (NK) cells play the main role in innate immunity and melanocyte-specific CD8+ cytotoxic T lymphocytes (CTLs) in acquired immunity[5,6]. Both cells exert their cytotoxic activities through the cytolytic molecules they contain in large amounts[7].

Granulysin is a cytolytic protein found in the granules of CTLs and NK cells, acting synergistically with perforin and inducing apoptosis[7,8]. Serum granulysin level is considered a useful indicator that can be used to assess cytotoxic immunity[7].

Cathepsin-L, which is a member of the lysosomal cysteine protease family, plays an important role in regulating the immune response by contributing to antigen presentation, adhesion, and migration, as well as cytokine and growth factor degradation[9,10]. It is suggested that this molecule may be a useful indicator for assessing cellular immunity[11].

This study investigates whether granulysin and cathepsin-l could be indicators of cell-mediated cytotoxicity in the pathogenesis of vitiligo and whether their levels measured in the acute phase could be markers that can help predict disease activity and prognosis of vitiligo.

METHODS

This randomized prospective case-control study was conducted in a single center between January and November 2021. A total of 46 vitiligo patients and 46 healthy volunteers of similar age and gender were included in the study. Before the study, ethical approval

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: this study was supported by the Gaziosmanpasa University (Project No. 2021/03).
Received on December 28, 2023. Accepted on January 19, 2024.

(20-CREC-297) was obtained from the clinical research ethics committee. All patients and healthy volunteers included in the study gave their verbal and written informed consent. The rules of the Declaration of Helsinki of the World Medical Association were followed during the study. Subjects between the ages of 18 and 65 years diagnosed with vitiligo were included in the study. Subjects with findings/diseases that could affect serum levels of granulysin and cathepsin-L (allergic, autoimmune, and/or chronic inflammatory systemic and/or skin diseases, phototherapy in the past 3 months, infections in the past 1 month, immunosuppression, or malignancy) and pregnant and lactating subjects were excluded from the study.

A dermatologic examination of all study participants was performed by the same physician. In addition to demographic characteristics such as age, gender, and body mass index (BMI) of the patient and control groups, the presence of autoimmune diseases (including vitiligo) and atopy in the family was asked, and the data were recorded. In addition, in the patient group, vitiligo onset age, localization, disease duration, vitiligo subtype (diffuse, acrofacial, or focal), koebnerization, mucosal involvement, leukotrichia, and presence of halo nevus were determined by interview.

The vitiligo area severity index (VASI) was used to evaluate the disease severity of the patient group, and the vitiligo disease activity score (VIDA) was used to evaluate disease activity. If vitiligo started before 10 years of age, it was defined as early onset, and if it started after 10 years of age, it was defined as late onset[12]. Those with a VIDA score of <0 were classified as stable, and those with a VIDA score of >1 were classified as active vitiligo[13].

The patient and control groups underwent clinical and laboratory evaluation (e.g., fasting blood glucose, TSH, free T4, antithyroglobulin antibody, antithyroid peroxidase antibody, vitamin B12, and hemogram) to rule out autoimmune, allergic, and/or chronic inflammatory systemic and/or skin diseases.

Measurement of granulysin and cathepsin-L in serum

Peripheral venous blood samples placed in a gel biochemistry tube after fasting for at least 8 h were stored at room temperature for 20 min to coagulate and then centrifuged at 3,000 rpm for 10 min. After centrifugation, serum was separated, filled into Eppendorf tubes, and stored at –80°C until the day of the study.

Serum samples brought to room temperature before the study day were analyzed separately using the enzyme-linked immunosorbent assay (ELISA) method.

For the measurement of granulysin in serum, the BT LAB brand ELISA kit for human granulysin was used with a reference range of 0.5–100 ng/mL; for the measurement of cathepsin-L in serum, the BT LAB brand ELISA kit for human cathepsin-L was used with a reference range of 0.1–40 ng/mL.

Statistical analysis

Descriptive analyses were performed for the general characteristics of the study groups. Categorical data were presented as numbers (n) and percentages (%). When numerical data were normally distributed, they were expressed as mean±standard deviation, and when they were not normally distributed, they were expressed as median (min–max). The association between continuous variables was determined with the Pearson correlation test; the agreement of variables with normal distribution was assessed with the Kolmogorov-Smirnov test. For the comparison of continuous variables by groups, the independent-sample t-test and the one-way analysis of variance (ANOVA) were used as parametric tests; the Kruskal-Wallis test and the Mann-Whitney U test were used as non-parametric tests. Comparison of categorical variables by groups was performed with the chi-square test and Fisher's exact chi-square test.

All analyses were performed with the Statistical Package for Social Sciences program (SPSS Inc., Chicago, IL) version 20.0. Statistical significance was accepted as $p<0.05$ in all comparisons.

RESULTS

A total of 46 vitiligo patients and 46 healthy volunteers were included in the study. There were 24 women (52.2%) and 22 men (47.8) in the patient group and 23 women (50%) and 23 men (50%) in the control group. The mean ages of the patient and control groups were 31.22±10.91 and 32.72±9.47 years, respectively (p=0.483), and the age and gender distributions of the patient and control groups were similar (p=0.835). The family history of vitiligo was significantly higher in the patient group than in the control group (p=0.003).

When vitiligo was classified according to clinical subtypes, diffuse vitiligo was present in 27 patients (58.7%), acral/acrofacial vitiligo in 12 patients (26.1%), and focal vitiligo in 7 patients (15.2%). Three of the patients (6.5%) had early-onset vitiligo, and 43 (93.5%) had late-onset vitiligo. The mean disease duration was 5.51±5.01 years (0.08–19 years), and the disease onset age was 25.84±12.12 years (6–55.75 years).

The clinical characteristics of vitiligo patients are shown in Table 1.

The median VASI score of the patients was 1 (0.1–46.15), VIDA score was -1 in 10.9%, 0 in 21.7%, +1 in 2.2%, +2 in 17.4%, +3 in 15.2%, and +4 in 32.6%.

Serum levels of granulysin and cathepsin-L were statistically significantly higher in the patient group than in the control group (p=0.048 and p=0.024, respectively) (Table 2).

There was no statistically significant correlation between serum granulysin and cathepsin-L levels and age, BMI, disease onset age, duration, and severity in the patient group.

No statistically significant association was found between serum granulysin and cathepsin-L levels and gender, Fitzpatrick skin type, concomitant halo nevus, leukotrichia, mucosal involvement, presence of Koebner, disease activity, and vitiligo subtype (p>0.05).

A very high positive correlation was found between serum granulysin and cathepsin-L levels in vitiligo patients (Table 3).

Table 1. Clinical characteristics of vitiligo patients.

Characteristic		n (%)
Vitiligo subtype	Diffuse	27 (58.7)
	Acral/acrofacial	12 (26.1)
	Focal	7 (15.2)
Disease onset age	Early onset (<10 years)	3 (6.5)
	Late onset (>10 years)	43 (93.5)
Disease duration	<15 years	43 (93.5)
	>15 years	3 (6.5)
Disease activity status	Active	31 (67.4)
	Stable	15 (32.6)
Halo nevus	(+)	2 (4.3)
	(-)	44 (95.7)
Leukotrichia	(+)	25 (54.3)
	(-)	21 (45.7)
Koebnerization	(+)	10 (21.7)
	(-)	36 (78.3)
Mucosal involvement	(+)	9 (19.6)
	(-)	37 (80.4)

+: present; -: absent.

Table 2. Serum granulysin and cathepsin-L levels of the patient and control groups.

	Patient (median±SD)	Control (median±SD)	p
Serum granulysin level (ng/mL)	45.53±31.42	34.26±21.53	**0.048**
Serum cathepsin-L level (ng/mL)	13.98±10.84	9.61±6.92	**0.024**

Statistically significant p-values are denoted in bold.

Table 3. Correlation between serum granulysin and cathepsin-L levels in the patient group.

		Serum cathepsin-L level (ng/mL)
Serum granulysin level (ng/mL)	r	0.975
	p	**0.000**

Statistically significant p-value is denoted in bold.

DISCUSSION

Vitiligo is a disease whose treatment and clinical follow-up are difficult because its etiopathogenesis is not clear[14]. There is a need for molecules and parameters that will help clinicians overcome these difficulties and provide clues to disease severity, activity status, and clinical course. There are a very limited number of recent studies on the possible role of granulysin and cathepsin-L in the etiopathogenesis of vitiligo and as indicators of disease severity and activity[15,16]. To the best of our knowledge, this study is the first to simultaneously investigate the possible role of these two molecules in the pathogenesis of vitiligo as indicators of cellular immunity and cytotoxicity.

Vitiligo is a chronic, acquired, systemic disease that affects melanocytes in the body, particularly in the basal layer of the epidermis, and takes an unpredictable clinical course[1,2]. Although the pathogenesis is not fully understood, the autoimmune theory is the most widely accepted theory on the subject[17]. The efficacy of biological and targeted therapies used in vitiligo patients in recent years supports the autoimmune theory[18,19].

Both cellular immunity and humoral immunity play a role in the pathogenesis of vitiligo, an autoimmune disease. Recent studies support the view that specific cytotoxic T cells (CD8+ T cells) are responsible for the destruction of melanocytes by releasing mainly type 1 cytokines such as TNF α and IFN-γ[20,21]. CXC chemokines such as CXCL 9, CXCL10, and CXCL11, which are induced by IFN-γ, are those most strongly associated with the recruitment of CTLs in the epidermis. These chemokines act on melanocytes by binding to the CXCR3 receptor, and in recent years, studies in mouse models have shown the importance of the IFN-γ-CXCL10-CXCR3 axis in melanocyte destruction in vitiligo[22,23].

Cytotoxicity is an essential component of the cell-mediated immune system and is a multifactorial process. NK cells and CD8+ T cells are the two major cytotoxic cell populations. NK and CD8+ T cells initiate targeted cell death by inducing apoptosis. There are many cytolytic molecules stored in granules that initiate apoptosis, including perforin, granzyme, granulysin, and cathepsin-L[24-26]. The granulysin level in serum is considered an important indicator for the assessment of cytotoxic immunity[7].

In a recent study, Mohammed et al.[15] investigated granulysin levels in vitiligo and found that serum granulysin levels are higher in vitiligo patients than in healthy volunteers, similar to this study, and that the molecular level is not associated with disease severity and activity. In another recent study, granulysin levels were found to be higher in the patient group than in healthy volunteers, and unlike this study, a positive correlation was found between the molecule and disease severity and activity[16].

Oba et al.[27] from our country, who examined serum granulysin levels before and after treatment with tofacitinib in patients with alopecia areata, found that the serum granulysin levels decreased significantly after treatment compared with baseline and that this molecule level was correlated with the immunological activity of alopecia areata. Similar to alopecia areata patients, the serum granulysin level in this study was also statistically significantly higher in the patient group than in the control group. However, no correlation was found between granulysin level, disease activity, and severity.

Cathepsins are lysosomal proteases that play a role in many important biological processes, such as proteolytic processing of proenzymes, antigen presentation, inflammation, cell proliferation, differentiation, and apoptosis[28]. Cathepsin-L, which belongs to this group and plays a role in various phases of the innate and adaptive immune response, also has important functions in the regulation of epidermal homeostasis and the hair cycle[9,29,30]. Cathepsin L2, which has a very similar protein sequence to cathepsin-L, has been reported to be expressed at higher levels in keratinocytes from lightly pigmented skin than in darkly pigmented skin[31].

CD 8+ T cells contribute significantly to cell death and tissue destruction in inflammatory skin diseases such as psoriasis and lichen planus[32]. In an animal experiment conducted by Yamada et al.[33], to determine the effect of inhibiting cathepsin-L on T cell-mediated autoimmunity, they found that inhibition of cathepsin-L prevented the cytotoxic activity of CD8+ T cells in pancreatic islets in mice with autoimmune type 1 diabetes. They suggested that cathepsin-L may be a new target molecule that can be used in the treatment of type 1 diabetes.

In this study, although cathepsin-L levels were significantly higher in vitiligo patients than in the control group, the molecule concentration was not associated with disease activity and severity. However, it would be useful to investigate this issue in a prospective and large series of studies.

CONCLUSION

The etiopathogenesis of vitiligo is still not clearly understood. Although granulysin and cathepsin-L are molecules that play a role in the etiopathogenesis of vitiligo, they may not be useful for the assessment of disease severity and activity. New molecules are needed to assess the activity, severity, and potential course of vitiligo disease.

ETHICAL APPROVAL

Ethical approval (20-CREC-297) was obtained from the clinical research ethics committee.

PATIENT CONSENT STATEMENT

All patients and healthy volunteers included in the study gave their verbal and written informed consent.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231115

Preoperative cancer antigen-125 levels as a predictor of recurrence in early-stage endometrial cancer

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SUMMARY

OBJECTIVE: Endometrial cancer is the most common gynecological cancer in developed countries, with a majority of cases being low-grade endometrioid endometrial cancer. Identifying risk factors for disease recurrence and poor prognosis is critical. This study aimed to assess the correlation between preoperative cancer antigen-125 levels and disease recurrence in early-stage endometrioid endometrial cancer patients.

METHODS: The study was a retrospective analysis of 217 patients diagnosed with endometrioid endometrial cancer who underwent surgical treatment at a university-affiliated tertiary hospital between 2016 and 2022. Patients were divided into two groups based on their preoperative cancer antigen-125 levels and compared with clinicopathological findings and disease recurrence. Disease-free survival rates were calculated, and logistic regression analysis was performed to determine independent factors affecting disease-free survival.

RESULTS: The mean age of patients was 61.59±0.75 years, and the mean follow-up time was 36.95±1.18 months. The mean cancer antigen-125 level was 27.80±37.81 IU/mL. The recurrence rate was significantly higher in the group with elevated cancer antigen-125 levels (p=0.025). Disease-free survival was lower in patients with elevated cancer antigen-125 compared with those with normal levels (p=0.005). Logistic regression analysis revealed that elevated cancer antigen-125 levels were associated with disease recurrence (OR: 3.43, 95%CI 1.13–10.37, p=0.029).

CONCLUSION: The findings of this study suggest that preoperative cancer antigen-125 levels can be used as a predictor of disease recurrence in early-stage endometrioid endometrial cancer patients. cancer antigen-125 levels may be a useful tool for risk stratification and patient management in endometrial cancer.

KEYWORDS: Endometrial cancer. Endometrioid adenocarcinoma. Prognosis. CA-125 antigen. Recurrence.

INTRODUCTION

Endometrial cancer (EC) is the most common gynecological cancer in developed countries[1]. The most common type of EC is endometrioid EC (EEC), which accounts for 75–80% of cases. Most EECs are low grade (grade 1–2), diagnosed at an early stage, and have a good prognosis[2], but up to 7% of patients may still be at risk of disease-related mortality[3]. Considering that the majority of patients are in the low-grade EEC group, the number of disease-related deaths can be considered quite high. Additionally, recurrence in early-stage EC can be as high as 15–20%[4]. Therefore, it is crucial to identify patients at risk of poor prognosis before surgery.

Patients' age, tumor size, tumor grade, histological type, and lymphovascular space involvement (LVSI) have been identified as risk factors for poor prognosis in ECs[5,6]. Preoperative cancer antigen-125 (CA-125) levels have also been linked to disease recurrence[7]. While an elevation in serum CA-125 levels has been found to be correlated with advanced-stage EECs, its role in early-stage EECs is still a topic of debate[8-10].

In this study, our goal was to assess the correlation between preoperative CA-125 levels and disease recurrence in early-stage EEC patients.

METHODS

Patients diagnosed with EC who underwent surgical treatment at a university-affiliated tertiary hospital between January 2016 and March 2022 were analyzed retrospectively after obtaining approval from the local ethics committee (2011-KAEK-25 2022-11/06). This study was conducted in accordance with the Declaration of Helsinki. The sociodemographic characteristics, preoperative CA-125 levels, surgery reports, histopathology results, and postoperative follow-up data of the patients were reviewed from electronic/archival files. A total of 217 patients were examined. Patients with non-endometrioid type adenocarcinomas (n=11), high-stage endometrioid cancers (n=13), no preoperative CA-125 levels (n=32), other concurrent cancers, pelvic endometriosis or adenomyosis or adnexal mass (n=9),

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 04, 2023. Accepted on January 19, 2024.

follow-up examinations at another center (n=18), previous chemo-radiotherapy (n=9), or incomplete data (n=27) were excluded from the study. The final study population (n=167) included the International Federation of Gynecology and Obstetrics (FIGO) stage 1–2 EEC diagnosed and operated for the first time at our hospital.

Patients were divided into two groups based on their preoperative CA-125 levels: those with normal levels (<35 IU/mL) and those with elevated levels (≥35 IU/mL), and were compared against clinicopathological findings and disease recurrence.

All the patients underwent surgical staging according to FIGO classification[11], which included total hysterectomy and bilateral salpingo-oophorectomy. Selective systemic pelvic-paraaortic lymphadenectomy was performed based on intraoperative frozen section findings using Mayo-Clinic criteria[12]. All the specimens were evaluated by gynecologic pathologists in our institution. The final histopathology reports included information on histological grade and type, myometrial invasion (MI), cervical invasion, LVSI, and lymph node metastasis status. The administration of adjuvant therapy was determined by a team of experts from multiple disciplines[13]. Recurrence was diagnosed by clinicians using physical examination and imaging reports. Disease-free survival (DFS) was defined as the time from surgery to the first recurrence of the disease.

Statistical analysis was conducted using SPSS version 23 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to determine the normality of the variables. Non-parametric continuous data were compared using the Mann-Whitney U test, and categorical data were analyzed using the chi-square test. The Kaplan-Meier survival analysis was used to calculate DFS in patients based on their preoperative CA-125 levels. Logistic regression analysis was performed to identify independent factors associated with disease recurrence. A $p<0.05$ was considered statistically significant.

RESULTS

The mean age of patients was 61.59±0.75 years, with a range of 36–86 years. The mean BMI was 35.97±0.31 kg/m^2. The mean follow-up time was 36.95±1.18 months, with a range of 12–66 months. Complete surgical staging, including pelvic-paraaortic lymphadenectomy, was performed on 88.0% (n=147) of patients, while the remaining 12.0% (n=20) did not undergo lymphadenectomy. The mean number of lymph nodes removed was 49.37±0.94, with a range of 27–95 nodes. The mean preoperative CA-125 level was 27.80±37.81 IU/mL, with a range of 0.5–291.

In this study, 167 patients were evaluated, of which 125 (74.9%) had normal preoperative CA-125 values and 42 (25.1%) had elevated CA-125 levels. The demographic and clinical characteristics of the groups are presented in Table 1. The groups were comparable in terms of age, BMI, follow-up periods, and menopausal status (Table 1). No significant differences were observed between the groups with regard to tumor grade, MI, LVSI, and tumor stage (Table 1). Disease recurrence was

Table 1. Baseline characteristics of patients according to preoperative cancer antigen-125 level.

	CA-125 normal (n=125)	CA-125 elevated (n=42)	p
Age (years)*	61.23±10.09	62.69±8.97	0.502
BMI (kg/m^2)*	36.15±4.28	35.45±3.39	0.667
Gravida	3 (0–8)	3 (0–9)	0.129
Follow-up (m)	39 (12–66)	32 (12–66)	0.156
CA-125 (IU/mL)*	13.04±5.90	71.74±55.15	<0.001
Menopause status			
Premenopause	107 (85.6)	37 (88.1)	0.800
Postmenopause	18 (14.4)	5 (11.9)	
Histological grade			
G1	66 (52.8)	18 (42.9)	0.208
G2	43 (34.4)	16 (38.1)	
G3	16 (12.8)	8 (19.0)	
Tumor size			
≤2 cm	18 (14.4)	7 (16.7)	0.803
>2 cm	107 (85.6)	35 (83.3)	
MI			
<50%	83 (66.4)	23 (54.8)	0.197
≥50%	42 (33.6)	19 (45.2)	
Cervical stromal invasion positivity	4 (3.2)	2 (4.8)	0.642
LVSI positive	11 (8.8)	3 (7.1)	0.738
FIGO stage			
I	121 (96.8)	40 (95.2)	0.642
II	4 (3.2)	2 (4.8)	
Recurrence			
Yes	10 (8.0)	9 (21.4)	0.025
No	115 (92.0)	33 (78.6)	

Values are given median (min–max) or number (%), unless otherwise specified. Mann-Whitney U test or chi-square test was performed. $p<0.05$ was significant. *Values are given as mean±SD. Y: years; BMI: body mass index; m: months; G: grade; MI: myometrial invasion; LVSI: lymphovascular space involvement; FIGO: International Federation of Gynecology and Obstetrics.

significantly higher in the elevated CA-125 group compared with the normal CA-125 group (21.4 vs. 8.0%, p=0.025) (Table 1).

According to Kaplan-Meier analysis, DFS was significantly lower in the elevated CA-125 group compared with the normal CA-125 group (p=0.005) (Figure 1).

A logistic regression analysis was performed to identify factors associated with disease recurrence. The model included patient age, BMI, tumor grade, tumor size, LVSI, and elevated CA-125 levels. The results showed that this model was found to be significantly associated with disease recurrence (p<0.001, R^2=0.22). LVSI positivity and elevated CA-125 levels were found to be significant independent prognosticators for disease recurrence (OR:8.64, 95%CI 2.18–34.19, p=0.002 and OR:3.43, 95%CI 1.13–10.37, p=0.029, respectively).

DISCUSSION

In this study, we found that patients with preoperative CA-125 level ≥35 IU/mL had significantly higher rates of disease recurrence in EECs. The DFS in stage 1–2 EECs was found to be linked to preoperative CA-125 levels. Additionally, we determined that both an elevated preoperative CA-125 level and LVSI positivity were significant independent risk factors for disease recurrence in early-stage EECs.

The CA-125 antigen is a large transmembrane glycoprotein found in the cells of the pericardium, pleura, peritoneum, fallopian tube, and endometrial and endocervical tissues[14]. It is mostly used to monitor epithelial ovarian cancer[15]. Although there is evidence suggesting a possible association between CA-125 and histological grade, stage, lymph node metastases, MI, and cervical involvement in EC, the clinical utility of CA-125 as a marker for EC has yet to be established[13].

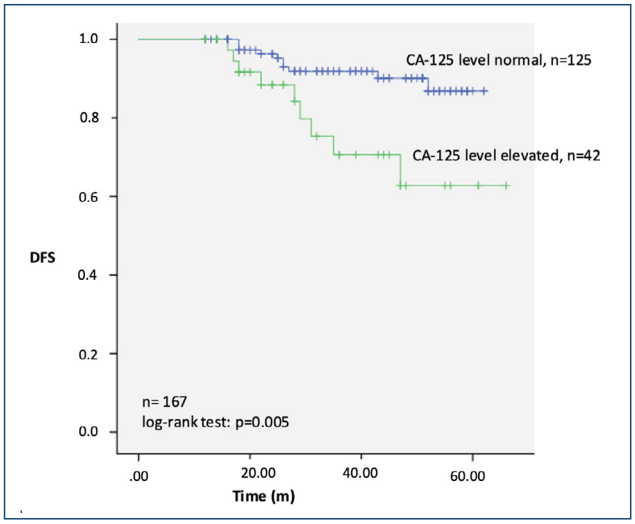

Figure 1. Kaplan-Meier survival analyses for disease-free survival in patients according to preoperative serum cancer antigen-125 levels. DFS: disease-free survival; m: months.

Most studies of CA-125 and EC included patients with advanced-stage disease[8-10]. Therefore, CA-125, as an epithelial surface antigen, can be expected to be elevated in patients with advanced-stage disease and may be associated with disease recurrence. Limited studies on low-risk and early-stage EC patients have produced conflicting results, leading to a lack of clarity in the findings.

In a multicenter retrospective study, Kim et al. found a significant association between elevated CA-125 levels and poor survival rates in patients with FIGO stage 1–2 EC[16]. In a prospective study with low-grade EC patients (n=240), disease recurrence was significantly higher in patients with elevated preoperative CA-125 levels compared with those with normal levels (19.4% vs. 7.9%, p=0.028)[7]. Logistic regression analysis identified age, tumor grade, LVSI positivity, and CA-125 levels as significant factors affecting DFS[7]. However, another study failed to find a relationship between preoperative serum CA-125 levels and disease recurrence in EC[17]. This study focused on early-stage EC patients with endometrioid histology and found an association between CA-125 levels and disease recurrence in this group of patients.

Studies investigating the role of CA-125 levels in predicting EC prognosis have reported varying thresholds[16-18]. Chen et al. established a cut-off level of 40 IU/mL for predicting disease relapse in stage 1 EC[18]. In another study, the cut-off values for CA-125 were determined to be between 15.3 and 22.9 IU/L for factors such as MI, cervical invasion, lymph node metastasis, LVSI, and disease recurrence[19]. We performed receiver operating characteristic (ROC) analysis to determine the CA-125 threshold for predicting disease recurrence and found that levels above 20.05 IU/mL had a specificity of 76.9% and a sensitivity of 68.4% for detecting recurrence risk (AUC: 0.714, 95%CI 0.59–0.83, p=0.002). However, this cut-off value was non-significant for other factors such as tumor grade, tumor size, MI, cervical invasion, and LVSI. Thus, we used the cut-off as 35 IU/mL in our study.

In EC patients, LVSI positivity is known to be an independent risk factor for disease recurrence[5,20]. However, a study by Bendifallah et al. failed to show a statistically significant relationship between LVSI and disease recurrence in low-risk EC patients (n=213), where only 10.4% were positive for LVSI[21]. The authors attributed the lack of significance to the low incidence of LVSI in the low-risk patient subset[21]. In this study, LVSI positivity was present in 16% of subjects, and we found a significant association between LVSI positivity and disease recurrence in early-stage EC.

In addition to LVSI, tumor size and MI depth are also considered risk factors for poor prognosis in EC[13,22]. However, the optimal tumor size for determining the risk of recurrence in low-risk EC is still unclear[23-25]. In a retrospective survival analysis of 720 patients, Ureyen et al. did not find a statistically significant difference in disease-free survival rates between patients with tumor size ≥35 vs. <35 mm (96.6 vs. 100%; p=0.102)[23]. In contrast, a multicenter study of 302 low-risk EC patients reported a significant difference in recurrence rates between patients with tumor size ≥35 vs. <35 mm (1 vs. 8%, p=0.006)[24]. Yet another study that used a cutoff of 2 cm for tumor size found no difference in recurrence-free survival rates of the stage 1 EC patients (HR 0.702, 95%CI 0.302–1.629, p=0.41)[25]. In our study, we did not find any association between tumor size >2 cm and disease recurrence or CA-125 levels. In the field of EC, MI is considered a crucial factor in determining a patient's risk profile[13]. In a prospective study, Kim et al. found a significant association between high levels of CA-125 and a higher rate of MI>50%[7]. Our results revealed that patients with elevated CA-125 tended to have higher rates of MI>50%, but the difference was not statistically significant.

Various molecular changes, including genetic mutations, can play a significant role in influencing the prognosis of EC. Specifically, the current staging system for EC places a specific emphasis on certain genetic mutations, highlighting their importance among the myriad molecular alterations that impact the prognosis of EC[26]. Ongoing research in this field is shedding light on potential risk factors. For instance, Giordana et al. have suggested that polyps characterized by the hyperexpression of MKI67 and BCL2 may pose a potential risk for EC[27]. Additionally, in a study involving women with polycystic ovary syndrome (PCOS), it has been discussed that the increased risk of endometrial hyperplasia and malignancy in PCOS may be linked to decreased CASP3 (Caspase-3) activity in these patients[28]. Further exploration of these molecular signatures holds the potential to deepen our understanding of the underlying mechanisms and facilitate the development of targeted preventive strategies in the context of EC.

This study has limitations including retrospective design and single-center data, as well as the absence of follow-up CA-125 levels. Additionally, not performing LND in all patients could result in an underestimation of the stage of EC, which is another limitation. Despite these limitations, the relatively large number of patients, only early-stage diseases being studied, exclusion of adnexal masses as they may cause elevation of CA-125, and similar demographic data between groups are the strengths of this study.

CONCLUSION

Preoperative elevation of CA-125 levels may predict a poor prognosis and decreased DFS in patients with early-stage EC. Therefore, preoperative evaluation of CA-125 can be used as an additional tool, alongside MI or tumor size, to determine the risk in these patients. However, further prospective studies are needed to validate these findings.

ETHICAL APPROVAL

The study was approved by the Local Ethics Committee (2011-KAEK-25 2022-11/06). This study was conducted in accordance with the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

AE: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft. **EK:** Conceptualization, Investigation, Project administration, Resources. **ZA:** Data curation, Investigation. **SB:** Data curation, Investigation. **NKE:** Methodology, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231116

Increased risk of bladder cancer recurrence due to bacillus Calmette-Guérin shortage in Brazil

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SUMMARY

OBJECTIVE: Our study aimed to evaluate the impact of bacillus Calmette-Guérin shortage on recurrence and progression in patients with non-muscle invasive bladder cancer in a Brazilian cohort.

METHODS: We retrospectively reviewed the clinicopathological data of 409 patients who had their first transurethral resection of the bladder tumor for intermediate or high-risk non-muscle invasive bladder cancer between June 2014 and May 2021 in a tertiary public hospital in Brazil. Patients included had non-muscle-invasive urothelial carcinoma of the bladder resected completely for the first time, regardless of bacillus Calmette-Guérin use. Low-risk disease patients were excluded from the analysis. Demographic, clinicopathological, and bacillus Calmette-Guérin use data were collected from our database. Recurrence and progression data were obtained from patient records or through telephone interviews. Recurrence-free survival and progression-free survival were calculated from the date of transurethral resection of the bladder tumor until the events of recurrence, progression, last office visit, or phone interview.

RESULTS: Within a median follow-up period of 26.7 months, 168 (41.1%) patients experienced a recurrence in a median time of 27 months (95%CI 16.1–38). Bacillus Calmette-Guérin was administered to 57 (13.9%) individuals after transurethral resection of the bladder tumor. Patients with ≥3 lesions ($p<0.001$), those with lesions >3 cm ($p=0.02$), and those without bacillus Calmette-Guérin treatment ($p<0.001$) had shorter recurrence-free survival. According to a Cox multivariate regression model, bacillus Calmette-Guérin use was independently associated with a reduced recurrence rate, with an HR of 0.43 (95%CI 0.25–0.72). Out of the patients studied, 26 (6.4%) experienced progression. T1 stage ($p<0.001$) and high-grade ($p<0.001$) were associated with shorter progression-free survival. Bacillus Calmette-Guérin did not influence bladder cancer progression. In the Cox multivariate analysis, high-risk disease was independently associated with progression ($p<0.001$).

CONCLUSION: Our study confirms that non-muscle invasive bladder cancer exhibits a high recurrence rate. The use of adjuvant bacillus Calmette-Guérin in intermediate and high-risk patients significantly reduces this rate. Furthermore, the bacillus Calmette-Guérin shortage could have negatively impacted these patients.

KEYWORDS: Non-muscle invasive bladder neoplasms. Transurethral resection of bladder. BCG vaccine. Recurrence. Disease progression.

INTRODUCTION

Bladder cancer (BC) is a common urological neoplasm, accounting for more than 10,000 new cases in Brazil in 2020[1]. Fortunately, approximately 75% of patients will present with non-muscle-invasive bladder cancer (NMIBC)[2], which is primarily treated with transurethral resection of the bladder tumor (TURBT)[3].

Although the surgical treatment is minimally invasive and highly effective, up to 78% of patients will experience recurrence, and 40% will progress to muscle-invasive bladder cancer (MIBC) within 5 years[4,5]. To stratify the patients accordingly, the European Association of Urology proposed a new classification of patients based on their risk of progression in 2021[3]. Patients with intermediate, high, and even very high risk of progression should receive intravesical immunotherapy with bacillus Calmette-Guérin (BCG) because it reduces the risk of recurrence and progression when compared with TURBT alone or in combination with intravesical chemotherapy[6-8]. However, patients have suffered from BCG shortages in recent years worldwide, impacting not only recurrence and progression rates but on a wider scale also significant economic impact[9,10].

To assess the impact of the BCG shortage on clinical outcomes in a Brazilian cohort of patients with NMIBC, we evaluated patients submitted to their first TURBT in a tertiary public hospital. This evaluation focused on recurrence and progression in relation to the use of adjuvant BCG.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 17, 2023. Accepted on January 19, 2024.

METHODS

Patients who underwent their first complete TURBT for papillary NMIBC at our institution from June 2014 to May 2021 were selected for analysis. They were followed up, and data were collected until December 2021. The exclusion criteria were CIS only with no visible papillary tumor, primary histologies other than urothelial carcinoma (although other subtypes were allowed as long as the urothelial type was the most common in the specimen), incomplete resections, patients with previous treatment for BC or other neoplasms, and MIBC at first TURBT. According to EAU risk groups[5], only patients with intermediate or high-risk diseases were included in the final analysis, regardless of adjuvant BCG use. The flowchart is demonstrated in Figure 1.

Data were retrospectively collected and retrieved following approval by the local ethical committee (approval #5.194.005, date of approval: January 06, 2022). All patients provided informed consent to participate in this study. The collected data were age at surgery, sex, smoking history, presence of hydronephrosis in the pre-operative imaging, data of the first TURBT such as the number of lesions, size of the most extensive lesion, presence of muscular propria, and carcinoma in situ (CIS), final pathology grade according to WHO 2016 and stage, and BCG use. Information on recurrence, progression, metastasis, or death was obtained from patient records or through telephone interviews.

Recurrence was defined when any of the following events occurred: diagnosis of NMIBC or MIBC, metastasis, or death from BC. Progression was defined as MIBC diagnosed on a TURBT, metastasis, or death from BC. Low-risk patients were followed with cystoscopy 3 months after the resection and yearly thereafter, having had ultrasound exams performed in between. Patients with T1 disease and high grade without the presence of muscular propria were submitted to re-TUR in 4–6 weeks. Intermediate and high-risk patients were followed up with cystoscopy every 6 months and with an ultrasound and cytology yearly. When available, intravesical BCG instillations were offered to all patients with intermediate and high-risk patients according to EAU guidelines on NMIBC[3].

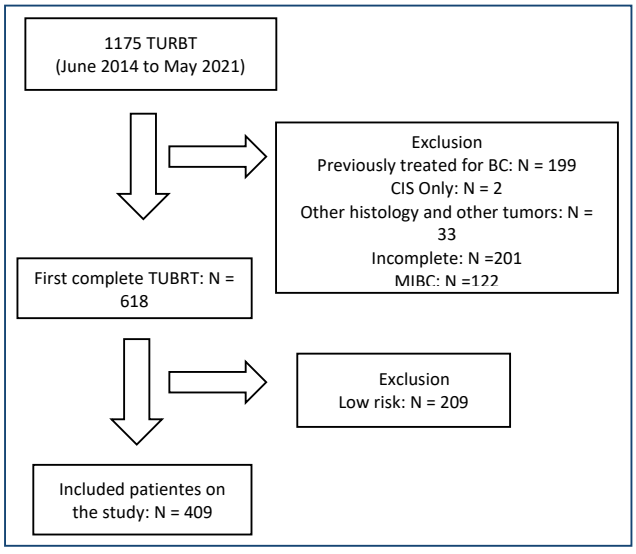

Figure 1. Flowchart of included patients.

Parametric data are presented as mean and standard deviation (SD) or median and interquartile range (IQR) for those with normal or non-normal distribution, respectively. Recurrence-free survival (RFS) was calculated from the date of TURBT until first local or distant recurrence, and progression-free survival (PFS) was calculated until progression, metastasis, or death event. The data were collected from records from the last office visit, or through telephone interviews. The log-rank test was used to correlate the clinicopathological variables and BCG use with RFS and PFS using the SPSS v.28.0. A $p<0.05$ was considered statistically significant. To identify independent factors associated with recurrence and progression, a Cox regression model was used to estimate the hazard ratio (HR) and the 95% confidence interval (CI) values. All significant variables were tested in a univariate Cox regression model, and those which showed to be associated with the studied outcome were included in the multivariate model to confirm their independent association.

RESULTS

From June 2014 to May 2021, 1175 patients underwent TURBT at our institution. Of these, 618 patients underwent their first complete resection for BC with papillary tumors, and 409 of these, who met the criteria for BCG treatment, were included in the final analysis. The median age at surgery was 69.4 years (IQR 14.9), and the median follow-up period was 26.7 months (IQR 25.7), as shown in Table 1. Most were men and current or former smokers (Table 1). Only 26 (6.4%) patients presented hydronephrosis on pre-procedural imaging.

Intraoperative findings during TURBT and the pathological results are summarized in Table 1. According to the new EAU risk classification for NMIBC, 241 patients (58.9%) were classified as intermediate risk and 168 (41.1%) as high or very high risk of progression. The clinical and pathological characteristics of these patients are detailed in Table 1.

Bacillus Calmette-Guérin usage was low across the cohort, with only 13.9% of patients receiving at least one cycle of

Table 1. Clinicopathological characteristics of the patients submitted to transurethral resection of the bladder tumor for non-muscle invasive bladder cancer (n=409).

Follow-up in months—median (IQR)	26.7 (25.7)
Age at surgery in years—median (IQR)	69.4 (14.9)
Men (%)	317 (77.5)
Smoking history (%)	300 (73.3)
Hydronephrosis (%)	26 (6.4)
Mean number of lesions (IQR)	1.0 (2.0)
1–3 (%)	331 (81.9)
>3 (%)	73 (18.1)
Mean size of the biggest lesion (IQR)	4.0 (2.0)
<3 cm (%)	84 (20.5)
≥3 cm (%)	327 (79.5)
Grade	
Low (%)	162 (39.6)
High (%)	247 (60.4)
Stage	
Ta (%)	296 (72.4)
T1 (%)	113 (27.6)
BCG	
No (%)	352 (86.1)
Induction (%)	45 (11.0)
Induction + maintenance (%)	12 (2.9)
Group risk (EAU)	
Intermediate	241 (58.9)
High/very high	168 (41.1)

TURBT: transurethral resection of bladder cancer; NMIBC: non-muscle invasive bladder cancer; n: number of patients; IQR: interquartile; BCG: bacillus Calmette-Guérin; EAU: European Association of Urology.

induction of BCG. Only 2.9% of patients received BCG maintenance therapy (Table 1).

Recurrence was observed in 188 patients (46.0%) during follow-up, with a median time to recurrence of 27.3 months. Gender, hydronephrosis, stage, grade, and presence of detrusor muscle, or CIS, did not correlate with RFS. Patients with more than three lesions, those with lesions larger than 3 cm, and those who did not receive BCG treatment exhibited significantly shorter RFS. When stratified by no use, induction, and induction plus maintenance, the patients who received BCG maintenance had the longest RFS (Figure 2). A univariate Cox regression analysis showed that having more than three lesions, lesions with ≥3 cm, and non-use of BCG were associated with recurrence. Further analysis with a multivariate Cox regression model controlling for these variables indicated that patients with more than three lesions or those who did not receive BCG therapy had a higher risk of BC recurrence independently (Table 2). BCG significantly reduced the recurrence rate by 57% (p=0.001).

A total of 26 (6.4%) patients progressed during the follow-up period, with a mean time of 83.7 months and a median time not reached. PFS was not influenced by gender, hydronephrosis, the number or size of the lesions, the presence of detrusor muscle, or CIS at TUR. BCG use also had no influence on PFS. Notably, those who never smoked had a shorter PFS compared with patients who were current or former smokers (p=0.040). High grade (p=0.001) and T1 stage (p<0.001) were also associated with shorter PFS. When grouped by the 2022 EAU risk classification, patients in the high-risk group had a significantly shorter PFS than those in the intermediate risk (p<0.001). The univariate Cox analysis confirmed these

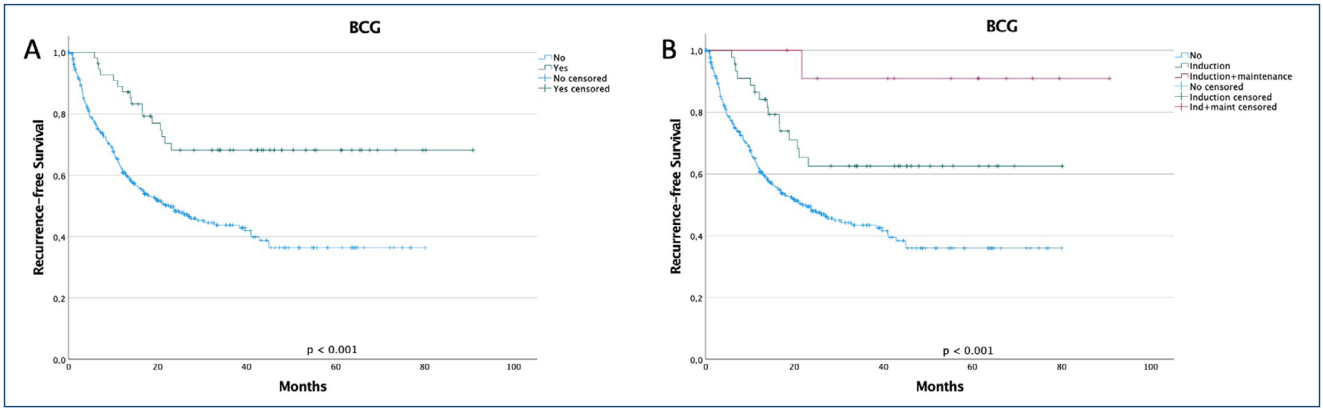

Figure 2. Kaplan-Meier curve for recurrence-free survival according to the use of bacillus Calmette-Guérin for patients submitted to transurethral resection of intermediate and high-risk non-muscle invasive bladder cancer (n=409). (A) shows the patients stratified for use or no use of bacillus Calmette-Guérin. In (B), patients were stratified into patients who have not received bacillus Calmette-Guérin, those who received only induction, and those who received induction plus maintenance. Bacillus Calmette-Guérin use had the longest RFS, especially if maintenance was used (log-rank test; p<0.001).

Table 2. Cox regression univariate and multivariate analysis of recurrence, and progression rate of the patients submitted to transurethral resection of the bladder tumor for non-muscle invasive bladder cancer (n=409).

Recurrence risk	Univariate analysis		Multivariate analysis	
	HR (95%CI)	p	HR (95%CI)	p
Gender (male vs. female)	1.23 (0.88–1.71)	0.229		
Smoking history (yes vs. no)	1.12 (0.78–1.60)	0.538		
Hydronephrosis (yes vs. no)	1.25 (0.74–2.12)	0.410		
Number of lesions (>3 vs. 1–3)	1.78 (1.27–2.48)	**<0.001**	1.80 (1.29–2.52)*	**<0.001**
Size of lesions (≥3 vs. <3 cm)	1.58 (1.07–2.32)	**0.021**	1.37 (0.93–2.04)*	0.115
Grade (high vs. low)	1.03 (0.77–1.37)	0.854		
Stage (T1 vs. Ta)	0.84 (0.60–1.17)	0.301		
BCG (yes vs. no)	0.39 (0.23–0.65)	**<0.001**	0.43 (0.25–0.72)*	**0.001**
EAU risk group (high vs. intermediate)	1.14 (0.85–1.53)	0.372		
Progression risk	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Gender (male vs. female)	1.05 (0.42–2.61)	0.922		
Smoking history (yes vs. no)	0.44 (0.20–0.99)	**0.046**	0.48 (0.21–1.07)**	0.072
Hydronephrosis (yes vs. no)	0.53 (0.07–3.95)	0.540		
Number of lesions (>3 vs. 1–3)	1.75 (0.73–4.21)	0.207		
Grade (high vs. low)	5.74 (1.72–19.14)	**0.004**	3.41 (0.96–12.10)**	0.058
Stage (T1 vs. Ta)	4.58 (2.08–10.09)	**<0.001**	2.83 (1.22–6.54)**	**0.015**
BCG (yes vs. no)	0.57 (0.17–1.91)	0.363		
EAU risk group (high vs. intermediate)	5.74 (2.31–14.32)	**<0.001**	5.35 (2.13–13.39)***	**<0.001**

TURBT: transurethral resection of bladder cancer; NMIBC: non-muscle invasive bladder cancer; HR: hazard ratio; CI: confidence interval; BCG: bacillus Calmette-Guérin; EAU: European Association of Urology. *Controlled for the number of lesions, lesion size, and BCG use. **Controlled for smoking history, grade, and stage. ***Controlled for EAU risk group and smoking history. Statistically significant values are indicated in bold.

characteristics as associated with PFS. In the multivariate Cox regression analysis, controlled for smoking status, grade, and T stage, only the T1 stage maintained an independently significant association with progression, as shown in Table 2. The T1 stage increased the progression by a factor of 2.83 (p=0.015; Table 2). When the patients were grouped according to EAU risk groups, both high-risk classification and smoking history retained their independent association with progression (Table 2).

DISCUSSION

In our study, we report the outcomes of recurrence and progression in a large cohort of patients with intermediate or high-risk papillary NMIBC and the potential impact that BCG shortage on these results, particularly in terms of recurrence. We found that adhering to guidelines for BCG usage could reduce recurrence rates by 60%. Throughout the study period, Brazil has experienced several BCG shortages due to production issues with its sole manufacturer[10]. Regrettably, our patients were unable to import BCG due to prohibitive costs. Furthermore, our hospital did not offer alternatives such as gemcitabine and other intravesical chemotherapies. It is noteworthy that although all patients with high-risk diseases were advised to undergo radical cystectomy, only a small proportion opted for this procedure.

Intravesical BCG therapy is a well-established treatment for NMIBC, particularly recommended for patients with an intermediate and high-risk chance of recurrence and progression[3]. Meta-analyses have demonstrated that BCG use can reduce recurrence by 28–56% compared with mitomycin C plus TUR or TUR-only treatments, though maintenance therapy is crucial to achieve these results[6,7]. Our data align with these findings, showing a 60% reduction in recurrence when BCG therapy was administered. When stratified by induction with or without maintenance, the lowest risk of recurrence was observed with BCG maintenance. Regrettably, only 13% of our patients received at least induction, and <3% of our patients received BCG maintenance, which may explain the nearly 50% recurrence rate with a median time of 27 months in our cohort. Typically, the expected recurrence rate for NMIBC is around

32–47%[4,11], mostly during the first 2 years of follow-up, aligning with our observations.

Other variables associated with recurrence in our study were the presence of more than three lesions in the bladder and their size. These are well-known factors for recurrence, as previously demonstrated by Sylvester et al. in 2006[4]. Among these factors, and even after controlling for BCG use, the number of lesions identified at TURBT was independently associated with shorter RFS.

Bacillus Calmette-Guérin therapy can also reduce progression by 28%, which is a critical outcome because the progression to MIBC significantly impacts mortality[8]. However, this benefit seems to be limited to patients receiving maintenance therapy[8]. In our cohort, we did not observe any significant reduction in progression with BCG treatment. This could be attributed to the low use of BCG (13.9%) by our patients, particularly maintenance therapy (2.9%), and the low risk of progression (6.4%) during follow-up. Progression rates are expected to be around 11–13%, with follow-up around 46–69 months[4,11]. The shorter duration of follow-up in our study might explain the lower observed progression rate and the absence of a significant association with BCG use. However, we were able to confirm two important risk factors associated with progression: high grade and T1 stage. These are well-recognized prognostic factors for progression, as demonstrated by Sylvester in 2006[4] and reaffirmed in 2021[5].

Other groups worldwide have also demonstrated the impact of BCG shortage on the recurrence and progression rates with findings similar to ours. In South Korea, Lee et al. reported that BCG shortage and the presence of multiple tumors in the bladder were independently associated with recurrence in high-risk patients[12]. In 2023, Perez-Aizpurua et al. confirmed that reducing only a few doses of BCG led to increased recurrence rates of patients with high-risk disease[13]. Beyond clinical outcomes, the economic impact of the BCG shortage warrants attention. For instance, in Saudi Arabia, patients incurred costs of approximately EUR 1745 per patient[14] due to the need to travel for the six-dose induction. In France, BCG shortage was associated with a higher recurrence rate and an increased cost of EUR 783 per patient[9]. Collectively, our findings and those from the literature highlight the importance of pursuing the BCG adjuvant treatment and might suggest that in the event of a BCG shortage, patients with high-risk disease should be prioritized for this treatment.

Our study has some limitations, including its retrospective nature, the lack of information about the number of doses of BCG received by each patient, and the low rate of patients receiving any form of adjuvant intravesical treatment. Despite these limitations, the study has notable strengths: it is the first to address this issue in a Brazilian cohort, all patients were treated according to consistent guidelines, and it includes a large patient population.

CONCLUSION

Patients with intermediate- and high-risk NMIBC experience high recurrence rates, and the shortage of BCG might negatively impact these patients. If used according to guidelines, BCG could have reduced the recurrence rate by 60%.

AUTHORS' CONTRIBUTIONS

CBM: Conceptualization, Methodology, Project administration, Writing – original draft. **KKREH:** Conceptualization, Data curation, Formal Analysis, Methodology. **BCD:** Data curation, Investigation. **MAWY:** Data curation, Visualization. **VDC:** Data curation, Methodology, Supervision. **JFAC:** Project administration, Supervision.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231118

Evaluating pregnancy termination decisions for fetal anomalies: a retrospective study in a tertiary referral center

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SUMMARY

OBJECTIVE: The aim of this study was to examine the factors that influence pregnancy termination due to fetal anomalies, regardless of gestational age, within the legal framework of Turkey.

METHODS: This retrospective study was conducted between January 2021 and July 2023 at a tertiary perinatology center to analyze patients undergoing pregnancy termination. The process involved multidisciplinary evaluations and informed consent, resulting in 326 pregnancy terminations, categorized by gestational timing.

RESULTS: Of the 326 patients studied, 219 opted for terminations. Gestational week at diagnosis significantly influenced the decision to terminate, with fetal anomalies being the primary indication. Chromosomal abnormalities accounted for 15.9% of the cases, while structural anomalies and maternal disorders accounted for 84.1% and structural malformations accounted for 84.1% of the cases. Late terminations (≥23 weeks) accounted for 30% of cases and required complex procedures.

CONCLUSION: The findings of this study indicate that maternal demographic factors have a limited impact on termination decisions. Early diagnosis of fetal anomalies is crucial for informed decision-making and emotional support, and the psychological consequences of late termination highlight the need for maternal support. Obstetricians play a vital role in facilitating early intervention. This study underscores the complex medical, ethical, and psychological aspects of pregnancy termination due to fetal anomalies. It emphasizes the importance of a holistic approach, considering medical, ethical, and psychological factors and the crucial role of healthcare professionals in supporting families during this challenging process.

KEYWORDS: Congenital anomalies. Induced abortion. Maternal health. Fetus. Prenatal diagnoses.

INTRODUCTION

In recent years, significant transformations have occurred in the medical field with the widespread adoption of ultrasound technology in gynecology and obstetrics and advancements in diagnostic devices. Advances in laboratory technologies, particularly in the early stages of pregnancy, have facilitated the early detection of fetal structural and genetic anomalies. In conjunction with establishing universal prenatal screening policies, these advancements have enabled timely management decisions at earlier gestational weeks. When confronted with severe or fatal abnormalities devoid of viable intrauterine treatments, families are often offered the option of terminating pregnancy. Numerous factors, including gestational age, anomaly severity, systemic involvement, and chromosomal abnormalities, influence decisions regarding termination[1].

Within the framework of official laws and specific conditions defined in our country, the choice of pregnancy termination in response to fetal anomalies is extended to parents. The legal gestational age for permissible termination varies across countries. While some countries do not impose a gestational week restriction for pregnancy termination because of fetal anomalies, others prohibit this practice[2-5].

In our country, the "Family Planning Law," enacted on May 24, 1983, allows elective termination until the 10th week of gestation[2]. After this period, termination can be performed without gestational week limitations if the pregnancy risks the woman's health and has a high likelihood of severe disability or an incurable fatal disease in the fetus. Notably, there was no upper gestational age limit for pregnancy termination. When maternal or fetal grounds warrant termination, the decision requires concurrence from a panel of at least three specialists.

The primary objective of this study was to comprehensively investigate and assess pregnancies in our clinic, which provided the opportunity for pregnancy termination due to fetal anomalies, regardless of the gestational week.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 22, 2024. Accepted on February 04, 2024.

METHODS

This retrospective study was conducted in the Department of Obstetrics and Gynecology of a leading tertiary referral center for perinatology. Patients who underwent pregnancy termination for fetal anomalies and maternal medical conditions between January 2021 and July 2023 were analyzed. The study strictly adhered to the ethical principles of the Declaration of Helsinki.

Perinatologists used the Arietta 850 USG system (Hitachi, Japan) for fetal ultrasonographic evaluations and preliminary diagnoses. In cases where fetal anomalies were confirmed, families were reevaluated by a multidisciplinary committee of experts in medical genetics, pediatric surgery, pediatric nephrology, pediatric cardiology, pediatric neurology, and neurosurgery. These evaluations were conducted within the genetics or cardiology council of the perinatology clinic, where families are informed of their diagnoses and prognoses.

Following comprehensive deliberation and obtaining informed consent from the cases reviewed by the committee, 326 cases were presented with the option of pregnancy termination, regardless of gestational week. Pregnancy termination plans were subsequently developed for the patients who consented to the procedure.

The cases were classified based on gestational timing, with pregnancies exceeding 23 weeks as "late termination" and those below 23 weeks as "early termination." Detailed discussions occurred with expectant mothers concerning pregnancy termination methods and the associated risks. The preferred termination method at our hospital involves medical termination using vaginal misoprostol (Cytotec; 200 µg tablet, Ali Arif, Istanbul), with or without oxytocin induction. In cases of "late termination" (≥23 weeks), fetal demise was ensured through intracardiac administration of potassium chloride under ultrasound guidance.

The International Classification of Diseases: Version 10 (ICD-10) was used to categorize the termination indications in this study. Hospital database records were used to retrieve each case's medical and demographic data. Prenatal karyotype analysis was recommended for all terminations except in instances dictated by maternal indications. After the termination of pregnancy, a fetal autopsy was performed in cases that accepted the autopsy, and the families were given comprehensive information about the procedures to be performed. Termination of pregnancy was initiated after obtaining signed consent from all parents.

The study was conducted with the approval of the Ethics Committee of Basaksehir Cam and Sakura City Hospital (KAEK/27.09.2023.433).

Statistical analysis

Statistical analysis was performed using the chi-squared test or Fisher's exact test for categorical variables, and univariate analysis was conducted using the Student's t-test or Mann–Whitney U test for factors that may correlate with the outcome being terminated. Descriptive statistics are presented as percentages, means±standard deviations, and medians (min–max). The results were analyzed using IBM SPSS Statistics for Windows, Version 26.0, and statistical significance was set at $p<0.05$.

RESULTS

Table 1 summarizes the demographic characteristics of the 326 patients offered the option to terminate the pregnancy. Of these patients, 107 chose not to terminate the pregnancy, whereas 219 opted for termination and underwent the procedure.

Table 2 compares the characteristics of the declined termination (n=107) and termination acceptance groups (n=219).

In total, 219 pregnancies were terminated, including 215 singleton pregnancies and four twin pregnancies. The mean age of the 219 patients who underwent termination was 28.84±7.56. The average gestational week at diagnosis was 19.43±4.77, and the mean gestational week at the time of termination was 19.97±4.74 for those who underwent termination. In contrast, the mean gestational age at diagnosis in the non-termination group was 26±6.71. Notably, only the gestational week for termination showed a significant difference between the two groups. The median gestational age in the non-terminated group was significantly higher than that in the terminated group ($p<0.001$) (see Table 2).

Of the 219 pregnancy terminations, 70% (153 cases) were categorized as early terminations (<23 weeks) because of fetal anomalies or maternal indications. Karyotype analysis was conducted in 21.9% (48 cases), revealing chromosomal or genetic anomalies in 35 cases. Trisomy 21 was the most

Table 1. The demographic characteristics of 326 patients offered the option to terminate the pregnancy.

Characteristics	Mean±SD	Median (min–max)
Age	28.6±7.5	28 (1–46)
Gravidity	2.4±1.7	2 (0–13)
Parity	1.0±1.2	1 (0–9)
Miscarriage	0.5±0.9	0 (0–5)
Number of children	1.0±1.2	1 (0–9)
GA at diagnosis (week)	21.6±6.3	21 (2–37)
GA at TOP (week)	20.0±4.7	20.5 (11–33)

GA: gestational age; TOP: termination of pregnancy; SD: standard deviation.

Table 2. Comparison between cases that declined and those that accepted pregnancy termination.

	Declined termination (n=107)		Accepted termination (n=219)		p-value
Age	28.23±7.35	28 (2–46)	28.84±7.56	28.5 (1–45)	0.490
Gravidity	2.25±1.47	2 (0–7)	2.54±1.84	2 (0–13)	0.302
Parity	0.9±1.14	0 (0–5)	1.04±1.24	1 (0–9)	0.271
Miscarriage	0.37±0.77	0 (0–4)	0.5±0.92	0 (0–5)	0.292
Number of children	0.87±1.14	0 (0–5)	1.03±1.24	1 (0–9)	0.183
GA at diagnosis (week)	26±6.71	27 (2–37)	19.43±4.77	20 (10–33)	**<0.001**
GA at TOP (week)	–	–	19.97±4.74	20.5 (11–33)	NA

GA: gestational age; TOP: termination of pregnancy. NA indicates data not applicable or not available. Data are presented as the mean with standard deviation and the median with minimum and maximum. Significances are presented in bold.

common chromosomal anomaly, followed by trisomy 18 and 13. Distinct genetic disorders were also identified, including nail patella syndrome, Meckel–Gruber syndrome, and deletions in smn1. Postmortem autopsy was performed in 2.2% (5 cases) of the cases, confirming concordance with the prenatal findings.

Termination indications primarily comprised structural anomalies and maternal disorders, accounting for 84.1% of cases. Central nervous system (CNS) anomalies, primarily neural tube defects, constituted the majority (50.6%) of terminations. Chromosomal abnormalities were the second most prevalent cause (16%), followed by genitourinary system anomalies (10%). The isolated abdominal wall or gastrointestinal anomalies did not lead to termination.

The gestational weeks ranged from the 11th week due to maternal interstitial lung disease to the 33rd week in patients with trisomy 21. The misoprostol protocol was primarily used for terminations before the 23rd gestational week, whereas those beyond the 23rd week underwent a feticide procedure followed by the misoprostol protocol. However, exceptions were noted in five cases that required hysterotomy. No uterine ruptures or hysterectomies were performed.

The study included four twin pregnancies: one terminated due to conjoined twins (thoracopagus) and the others due to dichorionic diamniotic twins. Structural anomalies prompted the termination of a single fetus in these cases. The distribution of terminations based on the specific indications is presented in Table 3.

DISCUSSION

This study presents a comprehensive review of pregnancy termination indications and methods by trimester and offers valuable insights. Demographic analysis revealed no significant differences between the termination and continuation groups regarding age, pregnancy, or living children. Maternal age, reproductive history, and pregnancy count minimally affected the termination decisions.

This study aimed to explore variables influencing pregnancy termination decisions. Among the 326 participants, 219 terminated pregnancies, while 107 continued pregnancies. Those declining terminations had higher gestational age, emphasizing their role in decision-making; the likelihood of termination decreased as gestational age increased.

Our study confirmed that fetal structural malformations were the primary reason for termination[6]. CNS and genitourinary anomalies were the predominant anomalies. Chromosomal/genetic factors constituted 16% of the reasons for termination.

Karyotype analysis, performed on 35 patients, identified 16% chromosomal/genetic anomalies, including trisomy 21, 18, and 13, and genetic disorders. Karyotyping aids families in informed decision-making. The fetal autopsy rate in our study was 2.5%, which is lower than the rates reported in the literature[7,8]. This decrease was attributed to healthcare professionals' and families' misunderstandings of the procedure, cultural and religious beliefs, emotional difficulties, and accessibility issues. The emergence of new diagnostic methods may further reduce the need for autopsy. Therefore, it is essential to address this issue by increasing awareness, education, and communication.

Early detection of fetal anomalies is crucial for making informed decisions regarding pregnancy termination, optimizing diagnosis and medical procedures, and offering support to families. In our study, we found that 70% of early terminations (<23 weeks) and 30% of late terminations (≥23 weeks) were performed because of severe fetal anomalies or maternal indications requiring feticide.

Turkey has witnessed a decline in late pregnancy terminations from 46.2 to 30% over the past 16 years, primarily owing to improved screening and healthcare services[9]. However, the 30% late termination rate raises concerns about the effectiveness of 11–14-week examinations.

Table 3. Distribution of pregnancy terminations according to fetal and maternal indications.

	Number of cases	Percentage of total (%)
Central nervous system	111	50.7
Neural tube defects	49	22.4
Anencephaly	31	14.2
Hydrocephaly	7	3.2
Encephalocele	11	5.0
Agenesis of corpus callosum	9	4.1
Other	4	1.8
Multiple anomalies	10	4.6
Hydrops fetalis	7	3.2
Limb body wall complex	3	1.4
Chromosomal anomalies–genetic diseases	35	16.0
Trisomy 21	22	10.0
Trisomy 18	7	3.2
Trisomy 13	3	1.4
Other	3	1.4
Genitourinary system anomalies	21	9.6
Renal agenesis	11	5.0
Multicystic dysplastic kidney	10	4.6
Skeletal system	8	3.6
Cardiovascular system anomalies	5	2.2
Hypoplastic left heart syndrome	3	1.37
Pulmonary atresia–hypoplastic right ventricle	2	0.91
Head and neck anomalies	4	1.8
Maternal disorders	6	2.8
Breast cancer	4	1.8
Maternal severe heart disease	1	0.5
Maternal lung disease	1	0.5
Other (anhydramnios, conjoined twin)	19	8.7
Total	219	100

Termination of pregnancy has been noted to have profound psychological effects, with research showing that individuals who undergo this procedure are more likely to show symptoms of post-traumatic stress disorder (PTSD) and experience depression, especially when the gestational age is advanced[10-15]. Healthcare professionals must prioritize understanding the psychological dimensions of this process, offering support, and promoting maternal mental health. Although medical advances may reduce the need for termination, early anomaly identification remains crucial. Genetic screening may shift the indications for termination from structural to genetic issues and from late to early termination. Legal flexibility at an advanced gestational age is essential.

However, this study has limitations, including its retrospective design, single-center focus, and potential cultural and ethical influences.

CONCLUSION

This study provides valuable insights into pregnancy termination, emphasizing the need for a holistic approach that considers medical, ethical, and psychological aspects and the critical role of early diagnosis. An immediate focus should be on timely anomaly management.

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

The procedures followed the ethical guidelines of the responsible committee on human experimentation and the 1964 Declaration of Helsinki and its amendments, and informed consent was obtained from all participants.

ETHICS APPROVAL

The study was conducted with the approval of the Ethics Committee of Basaksehir Cam and Sakura City Hospital (KAEK/27.09.2023.433).

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231282

Antibiotic stewardship and nosocomial infection prevention in critically ill patients: a quality improvement program

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate the impact of the implementation of a bundle of interventions through a "Program for Antibiotic Management and Nosocomial Infection Prevention" in the intensive care unit on antibiotic and devices use and healthcare-associated infections.

METHODS: This was a quasi-experimental study of consecutive series of cases in periods before and after the establishment of protocols and checklists for the use of antibiotics as well as other measures to prevent healthcare-associated infection as part of a quality improvement program. Antimicrobial consumption was assessed by the defined daily dose.

RESULTS: A total of 1,056 and 1,323 admissions in the pre-intervention and post-intervention phases, respectively, were evaluated. The defined daily dose per 100 patient-day decreased from 89±8 to 77±11 (p=0.100), with a decrease in carbapenems, glycopeptides, polymyxins, penicillins, and cephalosporins. The rates of ventilator and central venous catheter use decreased from 52.8 to 44.1% and from 76 to 70%, respectively. The rates of healthcare-associated infection decreased from 19.2 to 15.5%.

CONCLUSION: Quality improvement actions focused primarily on antimicrobial management and prevention of healthcare-associated infection are feasible and have the potential to decrease antibiotic use and healthcare-associated infection rates.

KEYWORDS: Antibiotic stewardship. Nosocomial infection. Nosocomial pneumonia. Quality improvement. Procalcitonin.

INTRODUCTION

Infections caused by multidrug-resistant pathogens (MDRPs) are a growing problem in intensive care units (ICUs) due to the complexity of patients, high consumption of antimicrobials, and inappropriate use of these drugs[1,2]. In the study "Extended Study on Prevalence of Infection in Intensive Care III" (EPIC III) with 15,165 patients admitted to the ICU, 54% of patients had at least one suspected infection, 70% received at least one ATB, and the mortality rate was 30%[3]. The most notable finding of EPIC III was how little has changed in terms of the prevalence of infection and associated mortality over three decades[4].

Important steps for more rational use of antibiotics (ATBs) are the rapid, adequate, and optimized initiation of empirical treatment, use of therapeutic regimens that allow maximum bactericidal effect, with rapid reduction of bacterial load, and early de-escalation and discontinuation of antimicrobial therapy. According to the guidelines of the Survival Sepsis Campaign (SSC), de-escalation or interruption of treatment should be performed as soon as possible[5]. The SSC expert panel also suggested the use of procalcitonin (PCT) along with clinical evaluation to decide when to discontinue antimicrobials.

Antimicrobial Stewardship Programs can lead to significant reductions in ATB use and in the costs in healthcare facilities[6]. In addition, the use of protocols and checklists has been shown to be effective in improving processes and outcomes, including the reduction of nosocomial infections[7,8]. Programs to reduce ICU-acquired infections ("Zero Bacteremia" and "Zero ventilator-associated pneumonia") in more than 200 ICUs in Spain led to a significant reduction in infection rates and ATB use in the participating units[9,10]. The aim of this study was to evaluate the impact of a set of actions focused on ATB management and infection prevention in ICUs on ATB use and healthcare-associated infections (HAIs), particularly respiratory tract infections.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 21, 2023. Accepted on January 19, 2024.

METHODS

This quasi-experimental study was conducted to evaluate the impact of a multifaceted intervention ("Program for Antibiotic Management and Nosocomial Infection Prevention") in the Intensive Care Center of a tertiary teaching hospital (2 units with 40 clinical-surgical ICU beds). The study was approved by the Ethics Committee (CAAE: 12539119.4.0000.5415). Data on the rates of ATB, device use, and HAI of all patients hospitalized between August 1 and December 31, 2018 (post-intervention group) were recorded by a trained team. The same data were retrieved from the records (pre-intervention group) which consisted of patients hospitalized between August 1 and December 31, 2017. The primary objective of the study was to evaluate the impact of the implementation of this program on ATB and device use. The secondary objective was to evaluate the impact of the program on the occurrence of HAI.

The rising rate of HAIs was considered critical in the analysis of the study team in the first half of 2018 (from January 1 to July 31). During this period, monthly meetings were held to discuss and implement the "Program for Antibiotic Stewardship and Nosocomial Infection Prevention" in the ICU. The Program consisted of a set of sequential interventions that included: setting up a working group, analysis of ICU problems using the Ishikawa diagram, prioritization of problems using the gravity, urgency, and tendency (GUT) matrix, use of the Plan, Do, Check and Adjust (PDCA) method, as well as training and establishment of protocols and checklists[11]. The following actions were deemed as priorities and implemented by the working group (Figure 1):

1. We ensured the support of senior institutional leadership and appointed leaders for the working group for each ICU (intensive care and infection control physicians, nurses, and pharmacists) to ensure adherence to the program. The complete team was composed of four intensivists (SML, CFM, GBL, JDS, and MRRGJ), one infectologist (MMB), three nurses (IFS, DAVC, and LTK), two microbiologists (MCLN and MTGA), one clinical pathologist (MGLO), two pharmacists (JVG and HTAO), and one internist medical student (NSC).
2. Protocols were established and trained for the use of ATB considering dosage (loading and maintenance), prolonged infusion time for beta-lactams and carbapenems, duration of treatment, as well as recommendations for the use of combined therapy in specific cases.
3. A checklist to identify patients at high risk for MDRP infections was implemented, in addition to actively monitoring colonization with weekly rectal swab cultures.
4. Guidelines for de-escalation and termination of treatments guided by clinical response, cultures, and daily PCT were determined[12,13]. PCT values were used as a recommendation for the discontinuation of ATB in the post-intervention phase, with discontinuation strongly encouraged if PCT ≤0.25 μg/l and encouraged if there was a decrease of ≥80% of the peak level or ≥0.25 and ≤0.5 μg/l.
5. The measures to prevent HAI consisted in reviewing of the protocols and training of the team on hand hygiene, cleaning of the rooms and equipment, and bathing and oral hygiene of patients, as well as daily assessment of the possibility of removing catheters, probes, and drains. Adherence rates were analyzed through checklists of these measures with periodic disclosures of HAI rates through meetings and dashboard use.

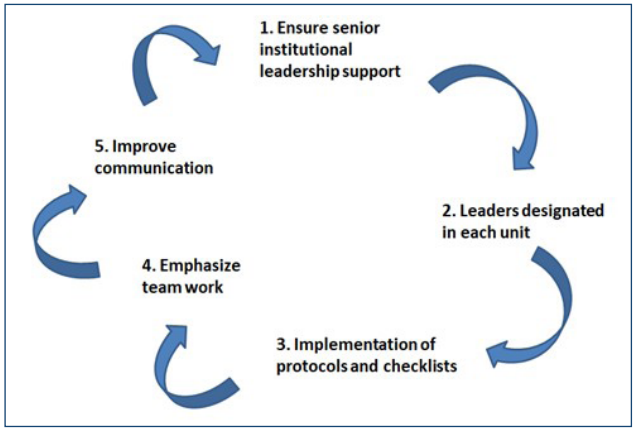

Figure 1. Main steps of the antibiotic stewardship and nosocomial infection prevention program.

Antimicrobial use was evaluated by the defined daily dose (DDD), which is the average daily maintenance dose of the antimicrobial (in grams) taken by a 70-kg adult for the main therapeutic indication of that drug. The values of the total amount of the drugs taken were obtained through the drug dispensing system of the hospital's pharmacy, considering the antimicrobials used by all patients during ICU stay. The formula for calculating the DDD was $\frac{A \times 100}{B \times P}$, where A is the total use of the antimicrobial (in grams), in the period of time considered, B is the standard daily dose of the antimicrobial (in grams) calculated for 70-kg adults without renal failure [defined by the World Health Organization (WHO)], and p is patient-day, in the period of time considered[14].

The indicators of device use and HAI were provided by the Hospital Infection Control Commission (CCIH) of the institution for the two study periods. The diagnostic criteria follow those set by the Centers for Disease Control and Prevention (CDC).

Statistical analysis

This was a pragmatic study with a convenience sample whose size calculation was not performed. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) software. The means and variances of the phases were evaluated by Student's t-test or the Mann-Whitney test, depending on the distribution of the data. The frequencies of the qualitative and categorical variables of the subgroups were assessed using the chi-square test or Fisher's exact test. Statistical significance was set at $p<0.05$. The relative risks (RR) as the ratio of risk of an event were calculated in both groups.

RESULTS

In the first period of evaluation (pre-intervention) 1,056 patients were admitted to ICUs. In the second period (post-intervention) 1,323 patients were admitted. ICU and hospital stay decreased from 7.3±7.4 to 5.9±6.7 days and from 16.7±15.5 to 13.0±12.6 days ($p<0.05$ for both), respectively (Table 1). The hospital standardized mortality ratio (SMR) was 1.28 in the pre-phase and 1.14 in the post-phase.

The DDD per 100 PD decreased from 89±8 to 77±11 ($p=0.100$). The reduction was significant in the cephalosporin group (15.4±3.8 DDD per 100 PD to 10.5±1.4 DDD per 100 PD; $p=0.045$) (Table 2). There was a decreasing trend in all groups, especially for polymyxins ($p=0.07$), with the exception of aminoglycosides for which the trend was to increase, although they were less used in both periods.

The rates of ventilator and central venous catheter use fell from 52.8 to 44.1% (RR 0.86, 95%CI 0.79–0.93, $p<0.005$) and from 76 to 70% (RR 0.92, 95%CI 0.87–0.97, $p<0.001$), respectively (Table 3). The rates of HAI decreased from 19.2% in the pre-intervention phase to 15.5% (RR 0.81, 95%CI 0.67–0.97, $p<0.005$) in the post-intervention phase and the incidence density of VAP per 1,000 MV-day decreased from 20.3 to 14.3%. Clinical incidence density for blood stream infections (BSI/1000 CC-day) was zero for both periods.

Table 1. Epidemiological and clinical profile of patients hospitalized in the pre- and post-intervention phases of the study.

	Pre-intervention	Post-intervention
Number of patients	1,056	1,323
Clinic (%)	48.7	54.6
Elective surgery (%)	18.2	20.4
Emergency surgery (%)	33.1	22.5
Age (years)	54±19	59±18
Sex male/female (%)	59/41	55/45
SAPS 3	54.6±22	51.5±21
Charlson comorbidity index	1.5±2.0	1.5±1.9
ICU occupancy rate (%)	88.4	88.3
Readmission rate < 48 h (%)	2.6	1.6
SMR (hospital)	1.28	1.14
ICU stay (days)	7.3±7.4	5.9±6.7*
Length of hospital stay (days)	16.7±15.5	13.0±12.6*
Death ICU (%)	28.4	24.6*

M/F: male/female; SAPS 3: simplified acute physiology score 3; ICU: intensive care unit; SMR: standardized mortality rate. *$p<0.05$ vs. pre-intervention.

DISCUSSION

After the implementation of the program, the DDD per 100 PD decreased from 85.0±8.0 to 72±9.6, and the reduction was more significant in the use of cephalosporin. Other authors have shown similar results. A quality improvement program in neonatal ICUs was successful in reducing the rate of ATB use through a rigorous ATB management education process[15]. A large study including 77 ICUs in nine Latin American countries evaluated the impact of an ATB management program on the adequacy of antimicrobial prescriptions and HAI[16]. The ICUs with ≥75th percentile in the final scores of adherence to the program had a greater reduction in the use of ATBs (143.4 vs. 159.4 DDD per 100 PD compared with the 25th percentile). In Saudi Arabia, an ATB management program conducted between 2015 and 2016 reduced the use of ATBs by 28.4%, which was a higher reduction than that obtained in our study (15%), but the observation time was longer[17].

The use of biomarkers such as PCT or C-reactive protein has the potential to improve the therapeutic management of patients with infections and sepsis. In our ICUs, serum PCT measurement was previously in use to aid in the diagnosis of infection and sepsis in association with clinical judgment based on signs, symptoms, laboratory tests, cultures, and radiological exams in the phase PRE of the study. The use of PCT to discontinue antimicrobial therapy was only standardized after the intervention. It is possible that there was an impact of its use in the duration of ATB therapies; however, this study does not allow definitive conclusions of a cause–effect relationship due to its pragmatic design. Randomized controlled trials have suggested that the use of PCT to guide discontinuation of ATB therapy might decrease the use of ATBs by around 1 day[18-20]. The SSC 2021 expert panel suggested its use combined with clinical evaluation to decide when to discontinue antimicrobials based on data from 14 randomized controlled trials in patients with sepsis and from two studies that included critically ill patients in general[5].

Table 2. Consumption of the main classes of antibiotics according to the defined daily dose/100 patient-day in the pre- and post-intervention phases of the study.

	Pre-intervention	Post-intervention	95%CI	p-value
Carbapenems	29.3±4.8	27.8±3.9	-5.05; 8.07	0.603
Glycopeptides	18.4±.3	17.5±3.0	-2.79; 4.64	0.549
Polymyxins	14.3±2.4	10.8±2.9	-0.52; 7.45	0.079
Penicillins	7.6±2.2	5.4±1.3	-0.65; 4.99	0.109
Cephalosporins	15.4±3.8	10.5±1.4	0.16; 9.60	0.045
Aminoglycosides*	4.1±1.6	5.3±2.0	-3.90; 1.50	0.335
All	89.0±8.5	77.3±11.0	-2.90; 26.50	0.100

Results are presented as mean ± standard deviation. Student's t-test was used for differences. *Combined therapy with the addition of an aminoglycoside (amikacin and gentamicin; 3 days) was recommended by the protocol for patients at high risk of MDRP, septic shock, and neutropenia (2,5), and this led to a slight increase in the use of this class of ATB.

Table 3. Indicators of the annual report of the Hospital Infection Control Commission in the pre- and post-intervention phases of the study.

Variable	Pre-intervention	Post-intervention	p-value
Devices			
Mechanical ventilation-day	3,829	3,489	0.034
Central venous catheter-day	5,504	5,577	0.183
Use of central venous catheter (%)	75.8	70.5	0.000
Healthcare-associated infection			
Number of patients with HAI/month	166	145	0.026
HAI/patient	1.12	1.09	0.872
Infection rate per 1,000 patient-day (%)	2.03	1.83	0.088
Patients with pneumonia	105	61	0.000
Incidence density VAP/1,000 MV-day (%)	20.3	14.3	0.037

Data are presented as numbers or (%) when indicated. Student's t-test was used for differences. VAP incidence density is the number of ventilator-associated pneumonias (VAP) in the month/number of ventilator-day in the month × 1,000. Incidence density of bloodstream infection (BSI) is BSI month/number of central catheter-day at month × 1,000 (clinical diagnosis).

The use of daily checklists was important to reduce the use of devices and may have impacted the rate of HAI and therefore DDD. However, the pragmatic nature of this study does not allow us to determine the individual effect of these measures. In view of the complexity of the scenario in critically ill patients, multiple interventions are often complementary and necessary, as it is more likely that using bundles, a set of interventions rather than isolated interventions works better. In addition, it has been demonstrated by other authors that even simple education programs can lead to optimization in the use of ATBs[18].

The main strength of this study is the careful planning, execution, and evaluation of an integral patient safety and educational program involving wide technical support from a multidisciplinary team. However, we should highlight some limitations. The results achieved were possibly related to the program implemented. However, the pragmatic nature of before-after studies with such a design (non-random allocation) does not allow us to state that these results were definitely due to the intervention as other variables could not be controlled and is rather hypothesis generating and should be confirmed in large randomized trials. Nevertheless, before-and-after studies are feasible and can be used as pilots for future randomized studies, and it has been demonstrated by other authors that even simple education programs can lead to optimization in the use of ATBs[18]. Using total daily dose (TDD) could have been a more reliable to access AB use. However, DDD is often used to assess trends in the use of these drugs and make comparisons between population groups. Finally, it may not be possible to generalize the findings of this study, but we believe that they could be applied to large tertiary or university hospitals where the consumption of carbapenems, polymyxins, and glycopeptides is high in the ICUs.

Antimicrobial resistance is a global priority. Currently, with the overuse of ATBs exacerbated by the COVID-19 pandemic, combating antimicrobial resistance is an even greater challenge, and initiatives to optimize the proper use of ATBs are urgent. Whether programs like this can effectively lead to more restricted use of ATBs and reduction in rates of ATB resistance and costs should be the subject of further multicenter randomized studies.

In conclusion, quality improvement programs with a set of actions focused on ATB stewardship and infection prevention in ICUs may decrease the duration of ATB treatments and the use of invasive devices with the potential to decrease HAI rates.

ACKNOWLEDGMENTS

The authors would like to thank Jorge Fares, Mario Roberto Resende Guimaraes Junior, Maria Gabriela de Lucca Oliveira, Margarete Teresa Gottardo de Almeida, and Helga Tamara Agostinho Oler for support of the study.

AUTHORS' CONTRIBUTIONS

NSC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **LFM:** Investigation, Validation, Visualization. **GDL:** Investigation, Validation, Visualization. **JDS:** Investigation, Validation, Visualization. **IFS:** Investigation, Validation, Visualization. **DAVC:** Investigation, Validation, Visualization. **LTK:** Investigation, Validation, Visualization. **MMB:** Conceptualization, Investigation, Formal Analysis, Project administration, Validation, Visualization. **MCLN:** Conceptualization, Investigation, Formal Analysis, Project administration, Validation, Visualization. **JVG:** Conceptualization, Investigation, Formal Analysis, Project administration, Validation, Visualization. **SML:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231317

Physician's perceptions regarding the pharmaceutical industry: a Brazilian national study

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OBJECTIVE: The objective of this study was to investigate the newly graduated physicians' attitudes and perceptions regarding the medical relationship with the pharmaceutical industry and identify the sociodemographic patterns related to such thinking.

METHODS: A structured questionnaire was administered to 4,601 participants selected from a pool of 16,323 physicians who were registered with one of the 27 Regional Medical Councils of Brazil in 2015. Answers were analyzed using two stratification variables: type of medical school (public vs. private) and the sex of the respondents.

RESULTS: Out of the participants, 61.8% believed that industry funding could support medical conferences and education, and 48.4% felt that small gifts and conference travel funding were acceptable. Conversely, 64.7% disagreed with industry-sponsored social events. Views on whether pharmaceutical representatives' visits influenced prescriptions were divided. Statistically significant differences were observed between genders and medical school types, with men and private school graduates being more accepting of certain industry interactions.

CONCLUSION: The study highlights the nuanced attitudes of new doctors toward industry relationships, indicating the need for clearer ethical guidelines and education in medical schools to align practice with evolving societal values.

KEYWORDS: Medical education. Pharmaceutical industry. Physicians. Medical ethics. Conflict of interest.

INTRODUCTION

In recent years, in the light of progressively fewer boundaries between medicine and the pharmaceutical industry, there has been increasing concern regarding the interactions between the drug industry and physicians[1,2]. Studies from many countries show that exposure to drug companies influences physicians' prescription choices and may affect evidence-based medical practice, prescribing costs, and patient safety[2,3].

Despite the rapidly evolving pharmaceutical industry in developing countries and the great impact that their marketing strategies and interactions with physicians may have on medical practice[4], literature assessing the doctor–industry relationship in Brazil is quite sparse[5,6].

To the best of our knowledge, this is the first Brazilian study with national proportions not only to investigate the newly graduated physicians' attitudes and perceptions regarding the medical relationship with the pharmaceutical industry but also to identify the sociodemographic patterns related to such thinking.

METHODS

This paper is part of the research "Profile and Perceptions of New Graduates in Brazil," a 104-structured multiple-choice questionnaire grouped into 11 thematic groups aimed at addressing the demographic profile of new qualified physicians registered with one of the 27 Regional Boards of Medicine (CRMs) in Brazil. This survey was conducted between September 2014 and August 2015. This study focuses on the relationship between the pharmaceutical industry and recently graduated doctors, and it builds upon prior investigations conducted utilizing the same survey tool. Among the established questions, 12 were related to the socioeconomic conditions of each participant and 5 were linked to the viewpoints of the graduates regarding the pharmaceutical industry.

The study involved 4,601 volunteer participants from a pool of 16,203 recent medical school graduates. The process of survey development, inclusion and exclusion guidelines,

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: this study was supported by Agreement N. 0075/2015, FMUSP, Fundação Faculdade de Medicina (FFM), Conselho Regional de Medicina do Estado de São Paulo (Cremesp), and Conselho Federal de Medicina (CFM). This study was partially funded by Lemann Foundation, IPEPO Vision Institute, and CAPES Foundation (GuRG).
Received on September 26, 2023. Accepted on January 19, 2024.

validation procedures, and distribution methods have been detailed in previous publications[5-7].

The sampling process included the use of the following stratification variables: the type of medical school, the sex of the respondents, and the Brazilian region of the medical school. The number of participants varied across questions and within each subgroup. A stratified sampling approach was used to correct the representativeness of these subgroups in the population results, avoiding the bias that could arise from the voluntary adherence sampling method. The correction factor was defined by the fraction of the target population within each subgroup. The 95% confidence intervals for frequencies were computed by bootstrapping. All the analyses were performed in the IBM SPSS Statistics software version 25 (IBM Corp., Armonk, NY)[5-7].

RESULTS

A comprehensive exploration of the socio-demographic attributes of the participants was previously delineated by Scheffer et al.[6], providing an in-depth depiction of the graduates' profiles. The opinions of the fresh medical graduates regarding the medical relationship with the pharmaceutical industry are summarized in Table 1.

The physicians' relationship with the pharmaceutical industry was a controversial topic among medical graduates. Among them, 61.8% agreed that medical conferences, publications, and continuing medical education programs can be financed by the industry, and 48.4% agreed that "the doctor can receive small gifts and conference travel funding." Almost 65% disagreed that it is correct for the industry to sponsor parties, barbecues, and cocktails for students and residents; however, 16.2% of respondents believe that this practice is correct. The graduates were divided regarding the statement "the visit of the representative influences the doctor's prescription"—42.6% of respondents agreed with the statement, while 44.2% disagreed. Approximately 73% judged that the physician should be prohibited from linking a medical prescription to the receipt of material advantages or financial support, although 11.6% agreed with this practice.

The participants' judgment about the relationship with the pharmaceutical industry varied significantly according to the

Table 1. Fresh graduate physicians' opinions about the medical relationship with the pharmaceutical industry.

	I agree			Disagree			I prefer not to answer		
	n	Freq. %	95%CI	n	Freq. %	95%CI	n	Freq. %	95%CI
Medical conferences, publications, and continuing education programs can be financed by the industry.	1,988	61.8	58.5–65.0	724	21.5	19.3–23.8	527	16.7	14.8–18.9
The doctor can receive gifts of small value and travel funding for conferences.	1,559	48.4	44.2–52.6	1,063	32	29.6–34.5	614	19.6	16.9–22.5
The industry representative visit influences the doctor's prescription practices.	1,447	42.6	38.1–47.2	1,391	44.2	41.1–47.4	397	13.2	11.7–14.9
It is correct for the industry to finance "cervejadas," barbecues, and cocktails for students and residents.	525	16.2	12.6–20.6	2,094	64.7	60.0–69.2	617	19.1	17.2–21.2
The physician should be prohibited from linking medical prescription practices to the receipt of material benefits or financial support.	2,369	72.9	70.4–75.2	385	11.6	10.4–12.9	478	15.5	13.6–17.7

The percentages were obtained through weighing of individuals, so the direct division of cells by the totals in this table will yield incorrect results and therefore are discouraged.

variables sex (Table 2) and type of medical school (Table 3). Men agreed more often than women regarding the ethical adequacy of industry-funded publications and continuing medical education programs (66.0 vs. 59.3%, p=0.019), receiving small gifts and conference travel funding (53.9 vs. 45%, p=0.037), and having pharmaceutical-company sponsored parties, barbecues,

Table 2. Opinions of fresh graduates on the relationship between physicians and the pharmaceutical industry significantly differences according to gender.

	Gender								
	Male			Female			Total		
	Freq. %	95%CI	n	Freq. %	95%CI	n	Freq. %	95%CI	n
[1]Medical conferences, publications, and continuing education programs can be financed by the industry.									
I agree	66	64.8–67.8	961	59.3	57.8–60.8	1,027	61.4	58.5–65.0	884
Disagree	19.5	16.1–23.4	307	22.7	20.9–24.5	417	22.4	19.3–23.8	2,023
I prefer not to answer	14.5	12.7–17.2	210	18.1	17.0–19.2	317	16.3	14.8–18.9	770
[2]The doctor can receive gifts of small value and travel funding for conferences.									
I agree	53.9	52.3–55.6	778	45	43.4–46.7	781	48.4	44.2–52.6	1,559
Disagree	29.9	25.9–34.2	466	33.3	31.9–34.8	597	32	29.6–34.5	1,063
I prefer not to answer	16.2	13.4–19.4	235	21.6	20.4–23.0	379	19.6	16.9–22.5	614
[3]It is correct for the industry to finance "cervejadas," barbecues, and cocktails for students and residents.									
I agree	21.6	17.7–26.2	317	12.9	11.4–14.5	208	16.2	12.6–20.6	525
Disagree	59.8	56.4–63.0	905	67.7	66.2–69.2	1,189	64.7	60.0–69.2	2,094
I prefer not to answer	18.6	14.1–24.2	256	19.4	17.8–21.2	361	19.1	17.2–21.2	617

[1]p=0.019, [2]p=0.037, [3]p=0.002.

Table 3. Fresh graduate physicians' significantly different opinions on the relationship between physicians and the pharmaceutical industry stratified by the type of medical school.

	Type of medical school								
	Public			Private			Total		
	Freq. %	95%CI	n	Freq. %	95%CI	n	Freq. %	95%CI	n
[1]The doctor can receive gifts of small value and travel funding for conferences.									
I agree	50.3	47.0–53.6	725	47.7	42.4–53.1	834	48.4	44.2–52.6	1,559
Disagree	34.4	33.3–35.6	498	31.2	28.2–34.3	565	32	29.6–34.5	1,063
I prefer not to answer	15.3	12.1–19.1	226	21.1	18.8–23.5	388	19.6	16.9–22.5	614
[2]The industry representative visit influences the doctor's prescription practices.									
I agree	51.5	48.8–54.1	728	39.5	38.4–40.7	719	42.6	38.1–47.2	1,447
Disagree	38.2	35.2–41.3	570	46.3	45.4–47.1	821	44.2	41.1–47.4	1,391
I prefer not to answer	10.3	8.8–12.1	150	14.2	13.6–14.9	247	13.2	11.7–14.9	397
[2]The physician should be prohibited from linking medical prescription practices to the receipt of material benefits or financial support.									
I agree	77.9	75.2–80.4	1,110	71.1	69.3–72.9	1,259	72.9	70.4–75.2	2,369
Disagree	10.9	9.2–12.8	166	11.8	10.3–13.6	219	11.6	10.4–12.9	385
I prefer not to answer	11.2	9.6–13.1	171	17	16.6–17.5	307	15.5	13.6–17.7	478

[1]p=0.037, [2]p<0.001.

and cocktails (21.6 vs. 12.9%, p=0.002). Public school graduates more often consider that the visit of industry representatives influences the doctor's prescription than private school graduates (51.5 vs. 39.5%), who in turn disagree more with this statement (46.3 vs. 38.2%), p<0.001.

DISCUSSION

It is widely known that the pharmaceutical industry has a huge economic impact worldwide, generating billions of dollars in revenue annually[1,8]. As part of the strategy to further increase their sales and profit, a considerable amount of money is spent on marketing to physicians, which includes pharmaceutical sales representative visits, sponsorship of conferences and other continuing medical education programs, drug promotional offers, free samples, and gifts[1,4].

Research has shown that physician–industry interactions may result in a prescribing behavior that deviates from evidence-based guidelines and, therefore, from the patient's best interest and safety[1-3]. This can be exemplified in the prescription of drugs without clear benefits over the other options, the request for more expensive drugs, the decrease in the use of generic drugs, and the prescription of medications based on the availability of drug samples or in the relationship with pharmaceutical representatives[1-3]. It has been exposed that the frequency of visits to a physician by industry representatives is linked to an increase in the physician's inclination to prescribe the representative's product[9]. Moreover, the non-rational prescribing behavior can also affect patient trust in physicians[3].

Considering the current scenario in which over 50% of the medical consultations result in drug prescription[10], and in the light of the previously cited negative influence of drug company marketing strategies on physicians' prescription choices[1,2,4], many medical organizations worldwide, such as the American Medical Association and the American Medical Student Association, have developed recommendations toward the interaction between physicians or medical students and the industry[4].

In Brazil, there is no specific legislation on this topic, unlike in the United States, which has the "Sunshine Act"[11]. Notwithstanding, there are some ongoing legislative projects in the Chamber of Deputies[12-15]. The Code of Medical Ethics in Brazil states that doctors are prohibited from practicing medicine with ties to or dependence on industries of any nature[16]. Additionally, the Association of Research-Based Pharmaceutical Industry in Brazil also has a code of conduct that discusses aspects of the medical–industry relationship, with the aim to guide ethical decisions and promote a culture of compliance[17].

In the literature, physician exposure to drug companies was found to be widespread worldwide. Previous studies from Brazil[8,10,18] and other countries such as Turkey[2], Japan[3], the United States[1], and Germany[19] have shown that doctors and medical students frequently interact with the pharmaceutical industry, and a high percentage of them report having received small gifts, having attended drug company-sponsored events or meals, and even having scientific publication fees sponsored[18].

A percentage of 61.8% of respondents believe the industry can sponsor medical conferences, publications, and continuing medical education. However, the literature presents conflicting results. In a previous Brazilian study, medical students viewed industry funding for conferences, research, and publications as potentially unethical[8]. On the contrary, in a study from the United States, 89% of medical students agreed that most industry-sponsored grand rounds are helpful and educational, and only 11% disagreed.[1] A Pakistani study also showed that 81% of medical students supported pharmaceutical sponsorship of educational events[4], and a Japanese study echoed this positive sentiment toward industry-backed seminars[3].

Our study found that 48.4% of participants believed that doctors could accept minor gifts and conference travel funding from the industry. This sentiment aligns with global findings. For instance, in the United States, over 80% of medical students felt entitled to gifts from drug companies, with nearly 70% believing these would not influence their practices[1]. Similarly, a Japanese survey revealed that 67% of medical students saw no issue with small gifts, though only 10% believed that such gifts or meals could sway their practices[3]. A German study supported these views, with 45.6% finding minor gifts acceptable due to their perceived minimal impact and 25% considering them influential on prescribing behavior[19].

In developing nations, medical students often hold divided opinions about accepting industry gifts. A Pakistani study found over 40% of the students were neutral regarding considering it unacceptable for a physician to receive a gift from a drug company in any form, with approximately 30% considering it acceptable and 27% considering it unacceptable[4]. Similarly, a Turkish study showed that 33% believed medical students should always decline industry gifts[2]. In the literature, it has been shown that receiving a gift or compensation may alter the physician's attitude toward the person who gave the gift[9].

This study revealed that 65% of participants disagreed with industry-sponsored social gatherings for students and residents, while 16.2% supported it. A higher acceptance rate was found in a US. study, in which 30% of the medical students considered it appropriate and 31.6% were neutral regarding social outings being sponsored by drug companies.

Considering industry-funded meals, 77% found it appropriate[1]. In Japan, only around 9% of medical students in clinical years considered it inappropriate to have industry-sponsored meals, with 40.4 and 25.5% totally agreeing and somewhat agreeing with it, respectively. Additionally, 65% of the students believed industry-sponsored lunches would not impact their clinical practice[3].

Regarding the influence of pharmaceutical representative visits on prescription writing, 42.6% of the respondents agreed that doctors are influenced by it, and 44.2% disagreed. These results are supported by a previous Brazilian study, which found mixed perceptions among medical students—43.2% felt unaffected by representative visits, while 42% agreed that doctors are often influenced[10]. Another Brazilian study also highlighted the belief that industry marketing strategies could affect prescription writing[8].

International studies from the United States[1], Germany[19], and Pakistan[4] indicated that only a minority of medical students expected their future prescriptions to be influenced by pharmaceutical gifts or incentives. In contrast, a Turkish study found about 70% of final-year students believed drug company interactions impact physicians' prescribing preferences[2]. Interestingly, past research revealed a common belief among medical students and doctors that colleagues are more susceptible to industry influence than themselves[1,10,19].

Our findings exposed that 72.9% of the respondents believe that physicians should not receive benefits or compensation for prescribing specific drugs, aligning with the Brazilian Code of Medical Ethics[16]. However, 11.6% of the respondents disagreed with it, expressing an opinion contrary to the current ethical regulation. A Pakistani study yielded similar results: 70% of medical students oppose doctors receiving financial incentives from drug companies for prescriptions[4].

Considering that the first interaction between medical students and drug companies starts early in their training in medical school, during a time that shapes their professional conduct and future prescribing behavior[2,4,8], it would be crucial for the universities and academic regulatory agencies to implement policies regulating the medical student–pharmaceutical industry interaction[2,4,18]. Future medical school curriculum reforms to include wide discussion and formal guidance on the topic, as well as courses that stimulate rational prescribing behaviors and evidence-based medicine and that reinforce conflict of interest policies would also be beneficial[2,4,18].

As medical students also learn from the attitudes and examples set by physicians, doctors who work in medical schools should also receive training[18]. Ensuring continuous monitoring and adaptation of regulations are important steps in promoting ethical conduct and professionalism in the relationship between physicians and the pharmaceutical industry.

Furthermore, this study revealed statistically significant gender-based disparities in views on the physician–industry relationship: men were more favorable than women toward industry sponsorship of publications, medical education programs, gifts, parties, cocktails, and travel funding. In the literature, no previous Brazilian study had analyzed this variable. In contrast, a Japanese study found no significant gender differences among medical students in their stance on accepting gifts from the pharmaceutical industry[3]. Future studies may explore possible variations in behaviors and ethical-professional attitudes according to gender but should also consider that certain types of practice and medical specialties, predominantly male, may be more exposed to direct relationships with the industry.

Our study revealed significant disparities based on the legal status of participants' medical schools. Public school graduates in Brazil more often believed that industry visits affect prescriptions (51.5 vs. 39.5% for private school graduates). This is the first Brazilian study delving into this, with no previous national research comparing physician or student interactions with the pharmaceutical industry in private versus public settings. A Pakistani study found that private school students were more skeptical of pharmaceutical company information and more comfortable accepting expensive gifts[4]. A Japanese survey also noted private school students were more receptive to accepting textbooks and sponsored lunches from drug firms than their public school peers[3].

Our results might reflect the different levels of exposure to pharmaceutical marketing between the private and public settings and possible differences in the medical school curriculum of private and public schools. The socioeconomic backgrounds of students might also contribute to this outcome[4]. However, it is noteworthy that the opinion on physician–industry relationship was not significantly associated with family income in our study. Participants with family income greater or equal to 10 minimum wages had similar opinions to those with less than 10 minimum wages.

LIMITATIONS

Despite encompassing a significant participant pool from all regions in Brazil, this study has some limitations. As a cross-sectional study, it lacks temporal insights regarding changes in student's opinions over time, and as we can only measure correlations, conclusions regarding causal relationships cannot be reached. Furthermore, there is an 8-year interval between data collection and publication of the results. However, it is useful

to register these findings in the literature to enable comparisons with future studies. Further prospective or intervention studies involving medical students and doctors at different career stages and from different specialties are suggested for a more comprehensive understanding of the factors affecting their opinions toward the pharmaceutical industry. This could help institutions frame ethical policies on physician–industry interactions.

CONCLUSION

Despite the presence of regulations and medical ethical codes serving as benchmarks to delineate boundaries to industry operation, our study indicates the level of permissiveness recent graduates might exhibit toward gifts and incentives from this industry. This demonstrates that while laws might reflect the change in social values and norms over time, they do not necessarily reflect the totality of people's attitudes and personal opinions.

Universities may consider implementing policies regulating medical student interactions with pharmaceutical companies and incorporate training in evidence-based medicine and conflict of interest into the curriculum, beginning in the first years of medical school, which is when the first connection between medical students and the industry usually occurs, shaping their professional demeanor and future prescribing habits. Continuous education for medical school physicians—who act as role models—and regular policy updates are also crucial to maintain professionalism in the industry relationship. New investigations should be carried out, perhaps even with qualitative studies, as well as a more focused approach in medical schools regarding the effectiveness of new processes and curriculum modifications.

AVAILABILITY OF DATA AND MATERIALS

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

ETHICS APPROVAL

The study was approved by the University of Sao Paulo Medical School's Research Ethics Committee under the number 797.424.

CONSENT TO PARTICIPATE

Consent of participants was implied upon voluntary completion of the questionnaire.

AUTHORS' CONTRIBUTIONS

GiRG: Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **GuRG:** Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **RMS:** Formal Analysis, Writing – original draft, Writing – review & editing. **AGAG:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **AJFC:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **MCS:** Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231333

The effects of leptin and cannabinoid CB1 receptor agonist/antagonist in cerebral tissues of epileptic rats

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SUMMARY

OBJECTIVE: In this study, the effects of leptin, cannabinoid-1 (CB1) receptor agonist ACEA and antagonist AM251, and the interactions between leptin and CB1 receptor agonist/antagonist on oxidant and antioxidant enzymes in the cerebrum, cerebellum, and pedunculus cerebri tissue samples were investigated in the penicillin-induced epileptic model.

METHODS: Male Wistar albino rats (n=56) were included in this study. In anesthetized animals, 500 IU penicillin-G potassium was injected into the cortex to induce epileptiform activity. Leptin (1 μg), ACEA (7.5 μg), AM251 (0.25 μg), and the combinations of the leptin+ACEA and leptin+AM251 were administered intracerebroventricularly (i.c.v.) after penicillin injections. Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels were measured in the cerebral tissue samples and plasma with the ELISA method.

RESULTS: MDA levels increased, while SOD and GPx levels decreased after penicillin injection in the cerebrum and cerebellum. The efficacy of penicillin on SOD, MDA and GPx levels was further enhanced after leptin or AM251 injections. Whereas, ACEA decreased the MDA levels and increased GPx levels compared with the penicillin group. Administration of AM251+leptin did not change any oxidation parameter compared with the AM251. Furthermore, co-administration of ACEA and leptin significantly increased oxidative stress compared with the ACEA-treated group by increasing MDA and decreasing GPx levels.

CONCLUSION: It was concluded that leptin reversed the effect of ACEA on oxidative stress. Co-administration of AM251 and leptin did not change oxidative stress compared with the AM251-treated group suggesting AM251 and leptin affect oxidative stress using the same pathways.

KEYWORDS: Epilepsy, Penicillin, Leptin, Cannabinoids, ACEA, AM251

INTRODUCTION

Epilepsy is a disorder characterized by the consequences of cognitive, psychological, neurobiological and social status and a predisposition to produce continuous epileptic seizures. The limitations and difficulties of epilepsy research have led researchers to epileptic animal models. In vivo and in vitro studies have been performed with many substances (pentylenetetrazole, bicuculline, picrotoxin, penicillin, etc.) to reveal the molecular mechanism of seizure activity in experimental epilepsy models[1-3].

Reactive oxygen radicals (ROS) including malondialdehyde (MDA) and superoxide dismutase (SOD) are thought to play important roles in epilepsy formation and neuronal death following seizures[4]. Many studies using penicillin, kainate, pilocarpine and PTZ to induce epileptic seizures have shown that oxidative stress may be involved in the pathophysiology of epilepsy[5].

The main role of leptin in the body is to regulate food intake and energy metabolism with a negative feedback effect on the cerebrum (especially the hypothalamus) and to prevent the development of obesity[6]. Leptin has also been found to increase nerve cell excitability in various studies[3,7]. Leptin has been shown to increase neuronal excitability by increasing NMDA receptor and synaptic transmission in rat hippocampal cell culture and cerebrum slices by increasing intracellular calcium[8]. In another rat study, leptin increased action potentials in electrophysiological recordings from proopiomelanocortin-type neurons[7].

Cannabinoids are chemical substances obtained from a plant called Cannabis sativa. Cannabinoids, whose anticonvulsant effects have been known for centuries, are known to produce anticonvulsant effects via CB1 receptors in many experimental epilepsy models[3,9,10]. Recent studies on the functions of leptin suggest that there may be a relationship between leptin and cannabinoids[11,12]. In obese Zucker rats, it was revealed that leptin regulates the eating function via cannabinoid CB1 receptors in the subcortical and limbic areas of the cerebrum[11]. In addition,

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Received on January 09, 2024. Accepted on January 12, 2024.

CB1 receptor mRNA levels showed steady increases in obese Zucker rats. This indicates that the signaling of leptin on CB1 receptor mRNA is impaired[11].

In the literature, there is no study showing the effects of combined administration of leptin and CB1 receptor agonist/antagonist on oxidative stress parameters. Therefore, we aimed to investigate these combined effects.

METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Ondokuz Mayis University (2009/61) and were conducted in accordance with the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals as per the US National Institutes of Health (NIH Publications No. 8023, revised 1978). In the experiments, 56 male Albino Wistar were divided into 8 groups with 7 animals in each group. Rats were purchased from the Animal House of Ondokuz Mayis University, Samsun, Türkiye. The rats were maintained in a temperature (22 ± 10C) and humidity-controlled (55% ± 5) room on a 12-hour light/dark cycle. All animals were given free access to standard rat food and tap water ad libitum.

Experimental Groups

Experimental procedures for ECoG recordings and injections are described in detail in the study Arslan et al.[3] and the tissues were used in this study. In anesthetized animals, 500 IU Penicillin-G potassium was injected into the cortex and the control group was given sterile physiological saline (SF). The most effective dose of leptin on epileptiform activity was determined based on the data of previous study[3]. CB1 receptor agonist ACEA 7.5 µg (i.c.v.) and CB1 receptor antagonist AM251 0.25 µg (i.c.v.) were used to investigate the effects of the cannabinoid system on epileptic activity[3]. Then, cannabinoid receptor agonist and antagonist were combined with leptin to examine the interaction between these pathways.

Biochemical Analyses

After the ECoG recordings[3], intracardiac 6-8 ml of blood was collected from the rats and the blood was transferred into EDTA tubes and biochemistry tubes without anticoagulant. The blood was centrifuged and the plasma was separated. After reperfusion with saline (0.9% NaCl), cerebrum, cerebellum and pedunculus cerebri tissues were removed. The tissues were frozen with liquid nitrogen and stored at -80°C until the day of our study.

MDA, SOD and GPx levels were measured in the plasma and the tissue samples. Protein determination in tissue was assessed by a commercial enzyme-linked immunosorbent assay kit (Biotek, USA) using the Lowry method[13]. Plasma and tissue malondialdehyde-lipid peroxidation levels were measured spectrophotometrically by the thiobarbituric acid-reactive substrates (TBARS) method. Plasma and tissue glutathione peroxidase levels and plasma SOD levels were measured by the ELISA method following the manufacturer's kit instructions (CAYMAN Chemical, Ann Arbor, MI, USA). Results were calculated per mg.protein.

Statistical analysis

The data obtained from the study were analyzed in SPSS 17.0 package program. Shapiro-Wilk test was performed whether the data was normally distributed. After it was determined that the data did not fit the normal distribution, Kruskal-Wallis test was used for intergroup comparisons. Statistical significance level was accepted as $p<0.05$. Descriptive characteristics of the data were expressed as mean and median (minimum-maximum).

RESULTS

MDA, which is an indicator of oxidative damage, and GPx and SOD, which are antioxidant enzymes, were measured and statistically evaluated in the cerebrum, cerebellum, pedunculus cerebri tissues and plasma samples.

MDA levels in the cerebral tissues and plasma

In the cerebrum tissue; MDA levels were significantly higher in the penicillin group compared with the control group ($p<0.01$), higher in the penicillin group compared with the leptin group ($p<0.05$), higher in the penicillin+leptin group compared with the penicillin group ($p<0.01$), higher in the penicillin+AM251 group compared with the penicillin group ($p<0.01$), lower in penicillin+ACEA group compared with the penicillin group ($p<0.01$), and higher in the penicillin+ACEA+leptin group compared with the penicillin+ACEA group ($p<0.01$) (Table 1).

MDA levels in the cerebellum tissue were significantly higher in the penicillin group compared with the control group ($p<0.05$), higher in the penicillin group compared with the leptin group ($p<0.05$), higher in the penicillin+leptin group compared with the penicillin group ($p<0. 05$), higher in the penicillin+AM251 group compared with the penicillin group ($p<0.01$), lower in penicillin+ACEA group compared with the penicillin group ($p<0.05$), and higher in the penicillin+ACEA+leptin group compared with the penicillin+ACEA group ($p<0.01$) (Table 1).

No significant difference was found in MDA levels among any group in the pedunculus cerebri tissue and plasma ($p>0.05$).

SOD levels in the cerebral tissues and plasma

In cerebrum tissue; SOD levels were significantly lower in the penicillin group compared with the control group (p<0.01), lower in the penicillin group compared with the leptin group (p<0.01), lower in the penicillin+leptin group compared with the penicillin group (p<0.05), lower in the penicillin+AM251 group compared with the penicillin group (p<0.01) (Table 2).

In the cerebellum tissues; SOD levels were significantly lower in the penicillin group compared with the control group (p<0.01), lower in the penicillin group compared with the leptin group (p<0.05), lower in the penicillin+AM251 group compared with the penicillin group (p<0.05)

There were no significant differences among any groups in SOD levels in the pedunculus cerebri tissue and plasma.

GPx levels in the cerebral tissues and plasma

In cerebrum tissue, GPx levels were significantly lower in the penicillin group compared with the control group (p<0.01), lower in the penicillin group compared with the leptin group (p<0.05), lower in the penicillin+leptin group compared with the penicillin group (p<0.05), higher in the penicillin+ACEA group compared with the penicillin group (p<0.05), and significantly lower in penicillin+ACEA+leptin group compared with the penicillin+ACEA group (p<0.01) (Table 3).

Table 1. Tissue malondialdehyde levels of the study groups

Groups	MDA Cerebrum (μmol/g)	MDA Cerebellum (μmol/g)	MDA Ped. Cerebri (μmol/g)
Control (SF)	2.3 (1.6-4.3)	3.8 (3.2-6.8)	2.6 (1.6-2.8)
Penicillin	4.5 (2.8-11.7)[a]	5.4 (4.1-13.5)[aa]	2.6 (2.3-4.3)
Leptin	3.5 (1.8-4.0)[b]	4.6 (3.3-5.7)[bb]	2.7 (2.1-3.4)
Penicillin + leptin	8.7 (6.8-15.6)[c]	8.8 (6.0-13.6)[cc]	2.6 (2.3-4.8)
Penicillin + AM251	7.7 (7.2-9.1)[d]	9.3 (8.3-9.9)[dd]	2.8 (2.5-27.5)
Penicillin + ACEA	2.5 (1.8-3.2)[e]	4.2 (3.1-5.4)	3.3 (2.5-3.5)
Penicillin + AM251 + leptin	9.8 (5.9-11.0)	11.0 (6.2-13.8)	2.6 (1.8-3.3)
Penicillin + ACEA + leptin	5.6 (4.0-7.1)[f]	7.7 (4.5-9.8)[ff]	3.0 (1.6-15.6)

Kruskal-Wallis test was used. Values are given as median (min-max).
[a]p< 0.01, [aa]p< 0.05; between control and penicillin groups; [b]p< 0.05, [bb]p< 0.05; between penicillin and leptin groups; [c]p< 0.01, [cc]p< 0.05; between penicillin and penicillin+leptin groups; [d]p< 0.01, [dd]p< 0.01; between penicillin and penicillin+AM251 groups; [e]p< 0.01; between penicillin and penicillin+ACEA groups; [f]p< 0.01, [ff]p< 0.01; between penicillin+ACEA and penicillin+ACEA+leptin groups.

Table 2. Tissue superoxide dismutase levels of the study groups.

Groups	SOD Cerebrum (U/mg)	SOD Cerebellum (U/mg)	SOD Ped. Cerebri (U/mg)
Control (SF)	324 (287-433)	186 (135-214)	156 (68-292)
Penicillin	182 (143-209)[a]	102 (47-153)[aa]	158 (96-224)
Leptin	282 (254-392)[b]	153 (109-199)[bb]	163 (64-309)
Penicillin + leptin	74 (49-93)[c]	71 (44-90)	122 (111-248)
Penicillin + AM251	82 (27-101)[d]	58 (30-67)[dd]	131 (38-202)
Penicillin + ACEA	169 (86-347)	110 (47-413)	161 (69-309)
Penicillin + AM251 + leptin	38 (26-98)	61 (49-74)	148 (103-201)
Penicillin + ACEA + leptin	122 (56-308)	68 (29-155)	115 (68-258)

Kruskal-Wallis test was used. Values are given as median (min-max).
[a]p< 0.01, [aa]p< 0.01; between control and penicillin groups; [b]p< 0.01, [bb]p< 0.05; between penicillin and leptin groups; [c]p< 0.05; between penicillin and penicillin+leptin groups; [d]p< 0.01, [dd]p< 0.05; between penicillin and penicillin+AM251 groups.

Table 3. Tissue glutathione peroxidase levels of the study groups

Groups	GPx Cerebrum (nmol/mg)	GPx Cerebellum (nmol/mg)	GPx Ped. Cerebri (nmol/mg)
Control (SF)	11.4 (9.4-15.5)	13.1 (8.5-23.7)	8.0 (4.2-18.3)
Penicillin	6.3 (4.4-7.6)[a]	6.6 (2.5-15.9)	8.6 (2.6-16.8)
Leptin	8.7 (6.0-15.3)[b]	12.2 (5.9-17.9)	8.3 (3.1-12.5)
Penicillin + leptin	4.2 (3.0-5.4)[c]	7.7 (2.4-9.0)	6.5 (2.2-17.6)
Penicillin + AM251	4.4 (2.5-8.8)	6.9 (2.7-13.3)	7.8 (1.9-9.6)
Penicillin + ACEA	8.9 (4.6-13.8)[d]	6.8 (2.4-17.0)	8.2 (2.3-14.9)
Penicillin + AM251 + leptin	2.0 (0.6-12.3)	6.2 (3.1-7.5)	6.2 (2.9-15.4)
Penicillin + ACEA + leptin	3.6 (0.6-12.5)[e]	6.2 (4.0-12.0)	7.0 (2.6-13.4)

Kruskal-Wallis test was used. Values are given as median (min-max).
[a]p< 0.01; between control and penicillin groups; [b]p< 0.01; between penicillin and leptin groups; [c]p< 0.05; between penicillin and penicillin+leptin groups; [d]p< 0.05; between penicillin and penicillin+ACEA groups; [e]p< 0.01; between penicillin+ACEA and penicillin+ACEA+leptin groups.

No significant difference was found in GPx levels among any group in the pedunculus cerebri tissue and plasma (p>0.05).

DISCUSSION

Animal models of epilepsy are used to understand the pathophysiology of seizures and to develop new therapies to treat epilepsy[3,14]. Penicillin is one of the most widely used drugs for inducing experimental seizures. With the administration of intracortical penicillin, γ-aminobutyric acid (GABA) type-A receptors are inhibited and this suppresses the chlorine entry inside the neurons resulting in focal seizure focus which is recorded from the surface of the cortex via ECoG[2,3].

Free radical production causes the accumulation of excitatory neurotransmitter glutamic acid and a decrease in GABA, an inhibitory neurotransmitter, in the cerebrum[14]. Free oxygen radicals are thought to play important roles in the formation of epilepsy and neuronal death following seizures[5,15]. Obay et al. declared that in the cerebrum, MDA levels were increased, while SOD and GSH levels were decreased after pentylenetetrazole-induced seizures in rats[15]. Parallel to this study, we found an increase in MDA levels and a decrease in GPx and SOD levels observed in the cerebrum and cerebellum tissues of rats after penicillin-induced epileptiform activity.

Leptin has been found to increase nerve cell excitability in some studies[3,7]. Arslan et al. reported that leptin increased epileptiform activity in the experimental epilepsy model induced by penicillin in rats[3]. Moreover, leptin increases excitability by increasing intracellular calcium and synaptic transmission[7]. Kutlu et al. reported that lipid peroxidase and glutathione levels were decreased in the cerebrum as a result of leptin administration[16]. Furthermore, the external application of leptin did not change MDA levels in a glial cell culture study[17]. We determined that the leptin administration to the non-epileptic animals did not have a significant effect on oxidative stress. However, leptin significantly increased the MDA levels in the cerebrum and cerebellum tissues of the rats compared to the penicillin group and significantly decreased the SOD and GPx levels suggesting that leptin may show its proconvulsant effect through oxidative stress probably by increasing intracellular Ca^{+2} levels.

Cannabinoids CB1 and CB2 receptors are G protein-coupled receptors and belong to different families of cell membrane-bound proteins. CB1 receptors inhibit presynaptic N and P/Q type Ca^{+2} channels and activate inflow rectifying K^{+} channels[18]. CB1 receptors are found extensively in the cerebrum, especially in the cerebral cortex, hippocampus, basal ganglia, and cerebellum[19]. Studies so far have shown that cannabinoids exert their behavioral and neuronal effects through cerebrum CB1 receptor activation[20]. On the other hand, cannabinoids have been known to exert anticonvulsant effects through CB1 receptors[2,3,21,22]. In the epileptic model created with pentylenetetrazole, ACEA was found to have an antiepileptic effect[9] and inhibited the proconvulsant effect of toxoplasmosis[22].

Synthetic cannabinoids are full agonists that bind to CB1 and CB2 receptors with a higher potency and affinity[23]. Since cannabinoids are known to exert their anticonvulsant effects on the CB1 receptor, selective CB1 receptor agonists and antagonists were used in the present study. For this reason, ACEA was preferred as the CB1 agonist and AM251 as the antagonist. Systemically administered cannabinoids undergo various

pharmacokinetic interactions until they reach the cerebrum[20]. Therefore, we preferred to administer cannabinoids by i.c.v. route. Di Giacomo et al. showed that cannabidiol and cannabigerol have neuroprotective and antioxidant effects in rat astrocytes and isolated cortexes[24]. Marsicano et al. showed that cannabinoids have strong antioxidant effects in cerebellar granule cell cultures by inhibiting cell excitability with increasing K^+ permeability and decreasing Ca^{+2} permeability through the CB1 receptor[25].

In the present study, ACEA administration to the penicillin-injected rats decreased the MDA levels and increased SOD and GPx levels in the cerebrum and cerebellum tissues compared with the penicillin group. When AM251 was administered after penicillin, AM251 enhanced the activity of penicillin on MDA, SOD and GPx levels. Based on the data obtained, it is thought that ACEA, at a dose of 7.5 µg, acts on CB1 receptors presynaptically and reduces intracellular Ca^{+2} levels, preventing oxidative stress and thus suppressing the epileptiform activity. Thus, AM251 is thought to have the opposite effect of ACEA by increasing the intracellular Ca^{+2} level, increasing oxidative stress, and thus increasing the epileptiform activity induced by penicillin.

Recent studies have suggested that there may be a relationship between leptin and cannabinoids [10-12]. Co-administration of ACEA and leptin significantly increased oxidative stress compared with the alone ACEA after penicillin injection. So, it was concluded that leptin reversed the effect of ACEA on oxidative stress. Combined administration of AM251 and leptin on our epileptic model no significant change was found in terms of oxidative stress compared to the AM251-administered group. From a pharmacological point of view, AM251 and leptin, which have proconvulsant effects when administered separately, are expected to significantly enhance oxidative stress when administered together. However, no significant differences were found. We suggested that AM251 and leptin have identical effects on oxidative stress by using similar pathways on Ca^{+2}. Although our study revealed that leptin and cannabinoids exert their effects on epilepsy through the oxidative system, further molecular and biochemical studies are needed to further elucidate the molecular mechanisms. Findings underlying these interactions may lead to the development of new therapeutic strategies for the treatment of epilepsy.

CONFLICT OF INTEREST

There are no competing interests.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author on a reasonable account.

ETHICS

This study was approved by the Institutional Animal Care and Use Committee of the Ondokuz Mayis University (Approval number: 2009/61).

AUTHORS' CONTRIBUTIONS

MK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing. **UD:** Data curation, Formal Analysis, Investigation, Methodology. **GA:** Formal Analysis, Supervision, Writing, Revising. **EA:** Formal Analysis, Supervision, Revising. **MA:** Supervision, Revising. **NK:** Conceptualization, Data curation, Formal Analysis, Investigation.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231337

Expression of brain-derived neurotrophic factor and formation of migrasome increases in the glioma cells induced by the adipokinetic hormone

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SUMMARY

OBJECTIVE: It has been previously shown that brain-derived neurotrophic factor is linked with various types of cancer. Brain-derived neurotrophic factor is found to be highly expressed in multiple human cancers and associated with tumor growth, invasion, and metastasis. Adipokinetic hormones are functionally related to the vertebrate glucagon, as they have similar functionalities that manage the nutrient-dependent secretion of these two hormones. Migrasomes are new organelles that contain numerous small vesicles, which aid in transmitting signals between the migrating cells. Therefore, the aim of this study was to investigate the effects of Anax imperator adipokinetic hormone on brain-derived neurotrophic factor expression and ultrastructure of cells in the C6 glioma cell line.

METHODS: The rat C6 glioma cells were treated with concentrations of 5 and 10 Anax imperator adipokinetic hormone for 24 h. The effects of the Anax imperator adipokinetic hormone on the migrasome formation and brain-derived neurotrophic factor expression were analyzed using immunocytochemistry and transmission electron microscope.

RESULTS: The rat C6 glioma cells of the 5 and 10 μM Anax imperator adipokinetic hormone groups showed significantly high expressions of brain-derived neurotrophic factor and migrasomes numbers, compared with the control group.

CONCLUSION: A positive correlation was found between the brain-derived neurotrophic factor expression level and the formation of migrasome, which indicates that the increased expression of brain-derived neurotrophic factor and the number of migrasomes may be involved to metastasis of the rat C6 glioma cell line induced by the Anax imperator adipokinetic hormone. Therefore, the expression of brain-derived neurotrophic factor and migrasome formation may be promising targets for preventing tumor proliferation, invasion, and metastasis in glioma.

KEYWORDS: Adipokinetic hormone. Brain-derived neurotrophic factor. Migrasome. Migration. Glioma.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most aggressive primary brain cancer in adults. Despite extensive research has been performed for better understanding of the GBM biology, it remains an incurable and deadly disease[1].

The increased expression of BDNF is closely associated with enhanced tumor invasion and metastasis. Previous studies have demonstrated that BDNF plays a role in growth, invasion, and metastasis in several types of tumors, such as gastric cancer, lung cancer, hepatocellular carcinoma, and ovarian and prostate cancers. The BDNF binds to tropomyosin-related kinase B (TrkB) receptor and triggers the production of pro-migratory, pro-survival, and anti-apoptotic proteins[2,3].

The adipokinetic hormones (AKHs) play a crucial role in the energy mechanism of insects. They are responsible for regulating the mobilization of carbohydrates and lipids from the fat in insects[4]. The AKHs are functionally related to the vertebrate glucagon. The AKH-producing cells and pancreatic alpha cells share numerous similar mechanisms that manage the secretion of AKH and glucagon, respectively. The AKH release causes hyperglycemia through binding to the AKH receptor[5]. Hyperglycemia has been shown to induce proliferation in various cancer cells. Previous research has indicated a relationship between migration of cancer cells and glucose levels. The cancer cells increase glucose uptake to provide energy support to the proliferation and metastasis[6-8].

In 2015, researchers discovered a new cellular mechanism and organelle, called migracytosis and migrasome[9], which are

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: Scientific Research Projects Coordination Unit of Istanbul University (project number TYL-2019-34745).
Received on January 13, 2024. Accepted on January 28, 2024.

a unique type of extracellular vesicles that help transmit signals between the migrating cells. The migrasomes are released into the environment and can be taken up by recipient cells. The migrasomes have been suggested to mediate intercellular communications, potentially causing physiological and pathological effects. The formation of migrasomes increases during cell migration[10], which makes it a potential marker for detecting metastasis.

The aim of this study was to investigate the effects of Ani-AKH on BDNF expression and ultrastructure of cells in the rat C6 glioma cell line using immunocytochemistry and transmission electron microscope.

METHODS

Cell culture and treatments

The rat C6 glioma cell line used in this experiment was obtained from the American Type Culture Collection (ATCC-CRL2199). The cells were cultured for 24 h in a Dulbecco's modified Eagle's F12 cell culture medium with fetal bovine serum, penicillin, and streptomycin in addition to Ani-AKH. The cells were then seeded at a density of $1x10^5$ cells per well into 6-well plates with coverslips. Of note, 5 and 10 µM of Ani-AKH were added to the cells and incubated for 24 h. The numbers of living and dead cells were evaluated using trypan blue in the presence or absence of Ani-AKH (5 and 10 µM). No significant differences were observed between the number of living and dead cells in the Ani-AKH and control groups.

Transmission electron microscopy

The culture cells were fixed with 2.5% glutaraldehyde in phosphate-buffered saline and then post-fixed with osmium-containing potassium ferrocyanide. The cells were then dehydrated in the graded ethanol and embedded in resin. After that, the ultrathin sections were cut and grids were stained with uranyl acetate and lead citrate. Finally, the samples were examined with a Jeol JEM 1011 transmission electron microscope (TEM).

The brain-derived neurotrophic factor immunocytochemistry and immunofluorescence staining

The cells were evaluated using rabbit polyclonal anti-BDNF primary antibody (Santa Cruz Biotechnology, sc-20981, 1:50 dilution), ImmunoCruz ABC detection kit, and anti-goat IgG-fluorescein isothiocyanate (FITC) secondary antibody (Santa Cruz Biotechnology, K1715, 1:50 dilution). The coverslips were visualized using the Olympus BX61 fluorescence microscope with the DP72 Olympus camera system at 40x objective. Five digital images were randomly selected and analyzed using the Aperio ImageScope software. The BDNF positive pixel count algorithm was determined from five random views within the images. Comparisons between the groups were evaluated using a one-way ANOVA and Tukey post hoc tests by the KaleidaGraph 4.0 software. $p<0.05$ was considered significant.

RESULTS

In the control group, C6 glioma cells had an oval shape with occasionally slightly indented euchromatin nucleus and a relatively smooth cell membrane with small and short filopodia and little blebs. The endoplasmic reticulum and mitochondria were distinguishable and small vacuoles appeared in some cells. However, the migrasome structures were not displayed in the C6 glioma cells of the control group. The alterations induced in C6 glioma cells of the Ani-AKH-10 group were similar to those observed in C6 glioma cells of the Ani-AKH-5 group. Both AKH groups were found to multiply cleft nucleus that was divided into several lobes of irregular sizes. Filopodia were noticed in their cell membranes, and their cytoplasm was rich in vacuoles. The C6 glioma cells of the Ani-AKH-5 and Ani-AKH-10 groups showed the membrane-bound vesicular structures with cytoplasmic extensions observed around the C6 glioma cells using TEM. These membrane structures, called migrasomes, contained numerous smaller vesicles and elongated toward other cells. Ani-AKH-5 and Ani-AKH-10 groups induced the migrasome formation without cytotoxicity (Figure 1).

The BDNF staining intensity of Ani-AKH-5 and Ani-AKH-10 groups were significantly higher than the control group ($p<0.0001$). The BDNF immunofluorescence staining results were consistent with that seen in the immunocytochemistry staining results. The immunofluorescence positive staining for BDNF was detected throughout the cytoplasm from the perinuclear region of rat C6 glioma cells (data not shown).

DISCUSSION

Glioblastoma multiforme is the most lethal tumor of the central nervous system with limited treatment options. Standard GBM therapy includes neurosurgery, radiotherapy, and chemotherapy. However, despite these treatments, GBM remains a lethal tumor with a poor prognosis, with a median overall survival estimated between 15 and 17 months, and a 5% survival rate at 5 years[11,12]. The GBM cells communicate with

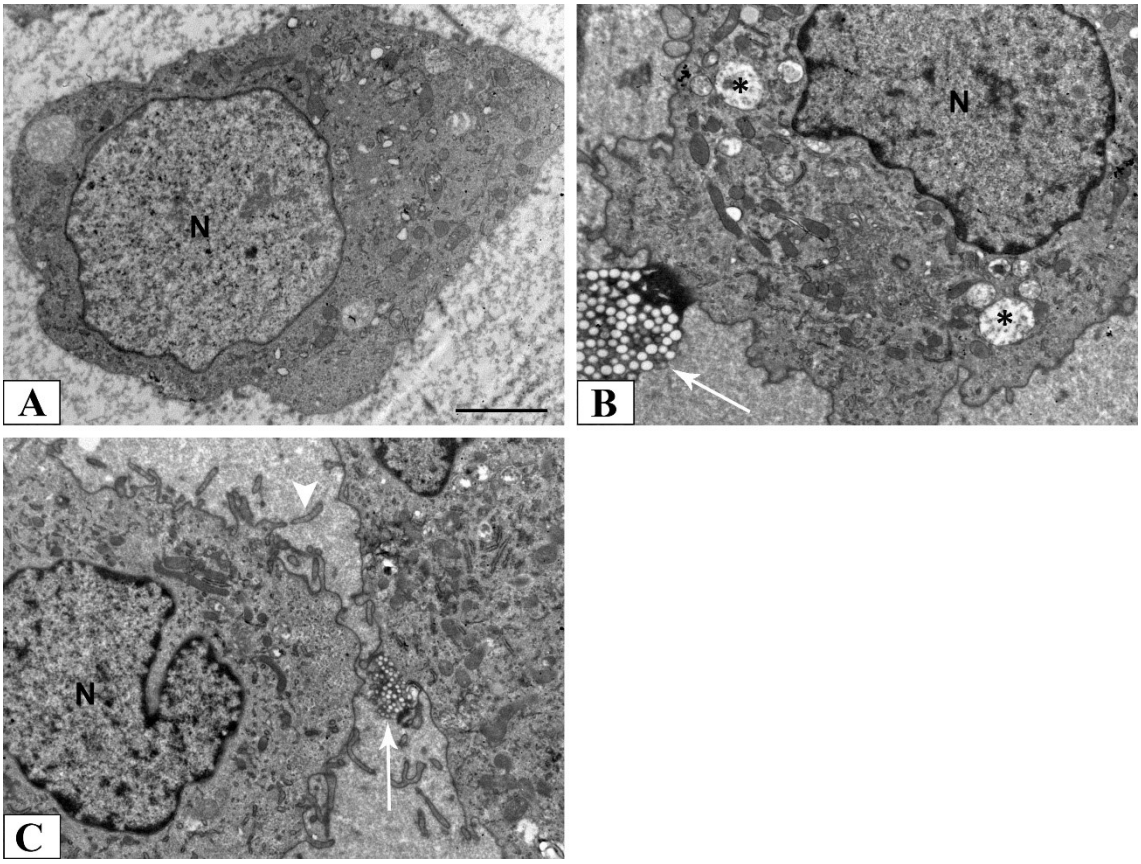

Figure 1. Transmission electron microscopic views of groups: control (A), Anax imperator adipokinetic hormone-5 (B), and Anax imperator adipokinetic hormone-10 (C). Visualization of migrasomes by transmission electron microscope [(B) and (C)]. The white arrow shows migrasomes connected with the cell body. N: nucleus; asterisk: vacuole; arrow: migrasome; arrowhead: filopodium. Scale bar=2 µm.

the tumor microenvironment for their own benefit. The various communication ways in GBM cells lead to affect tumor growth, metastasis, and angiogenesis. Therefore, GBM cells make use of various communication ways to interact with the surrounding cells[13,14].

Migrasome plays an important role in various fields including cell-to-cell communication, and occurrence, progress, and diagnosis of various diseases. The information transfer among cells may be the primary function of migrasomes[15]. Migrasomes are generated in a variety of cells, including metastatic tumor cells. In the cancer cells, production of migrasomes is significantly higher compared to normal cells, which increases their migration ability. Migrasome production depends on cell migration, and migrasome-mediated intercellular information transmission is extremely special because it only exists in migrating cells[16]. The regulation and functions of migrasome formation is not well understood[17]. Previous studies have suggested that migrasomes may have an important effect on the occurrence and development of cancer and might be new targets for cancer treatment. As a new target for disease treatment, the intervention of migrasomes may save patients' lives and improve their prognosis. Blocking the migration-promoting effect of migrasomes through various factors may be a new strategy for anti-metastasis therapy. The studies on the relationship between migrasomes and diseases are still ongoing[18-20]. While one cell migrates away from its current place, it leaves migrasomes behind and then another cell takes these migrasomes[9]. During this study, it was often observed that migrasome left behind by one cell is taken up by another cell[21]. The migrasome-mediated communication between GBM cells and the tumor microenvironment may be associated with tumor progression and recurrence. One of the functions of migrasomes is probably cell–cell communication.

The rat C6 glioma cells were examined using TEM to control whether migrasomes are present. The migrasomes were observed as plasma membrane-bound vesicular structures in the extracellular space around the rat C6 glioma cells. These migrasomes were oval-shaped and contain various small vesicles.

The AKH is a neuropeptide hormone, which is a member of the gonadotropin-releasing hormone superfamily. The AKH-producing cells and human pancreatic alpha cells command many similar mechanisms controlling the secretion of AKH and glucagon[5,22]. While a previous study showed the stimulating effect of glucagon on the growth of human colorectal adenocarcinoma cells, the mechanism by which glucagon stimulates colon cancer cell proliferation remains uncertain[23]. Cancer cells require high glucose uptake to support cell survival, growth, and metastasis. The present study showed that Ani-AKH induced the formation of migrasome in the Ani-AKH-5 and Ani-AKH-10 groups.

Recently, the BDNF has been shown to be overexpressed in various types of cancer and associated with their poor prognosis in promoting tumorigenesis and progression[24]. The effect of BDNF on cellular functions is induced by receptor TrkB. The BDNF activates the AKT pathway in order to maintain cell survival[25]. Thus, the BDNF inhibits cell apoptosis and facilitates cell proliferation in various human cancers. In this study, the rat C6 glioma cells induced by Ani-AKH showed high expression of BDNF and formation of migrasomes in the Ani-AKH-5 and Ani-AKH-10 groups compared to the control group.

CONCLUSION

Glioblastoma multiforme is the most lethal tumor of the central nervous system. Intercellular communication represent an essential feature for proliferation and metastasis[1]. Further comprehension of the complex mechanics taking place between migrasome formation, expression of BDNF, and metastasis could have a great beneficial influence on the therapeutic results of patients with GBM and may ensure the generation of new treatment strategies.

This study revealed that the C6 glioma cells induced by the Ani-AKH increased migrasome formation and expression of BDNF, and thus also increases the relationship between BDNF and migration. The C6 glioma cells have an increased BDNF expression. Increases of BDNF expression may have an impact on its ability to produce migrasomes. Our research has also provided a way for identification and to study the mechanism of migrasome formation. The findings from this study also suggest the possible use of the new therapeutic targets such as BDNF and formation of migrasome in the glioma treatment. Hence, there is a need to search for new strategies to decrease the BDNF level and formation of migrasome as a target to prevent metastases and discover therapy for various cancers such as glioma.

Only few clinical studies exist on migrasomes. The clinical use of migrasome formation and BDNF expression inhibitors in combination with standard chemo- or radiotherapy or other signal transduction pathway inhibitors may make a significant contribution to the treatment of GBM.

AUTHORS' CONTRIBUTIONS

SK: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, writing – review & editing. **SD:** Data curation, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, writing – review & editing. **CEY:** Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **OM:** Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231376

Determinants of anemia among pregnant women attending a tertiary hospital, Mogadishu, Somalia: unmatched case-control study

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SUMMARY

OBJECTIVE: The objective of this study was to identify the factors associated with anemia among pregnant women attending a tertiary referral hospital in Mogadishu, Somalia.

METHODS: An unmatched case-control study was conducted on pregnant women who visited the antenatal clinics of a tertiary referral hospital between March and July 2021. The study recruited pregnant women who had a hemoglobin level of <11 g/dL into the anemic group, while those with hemoglobin levels ≥11 g/dL were included in the non-anemic group. Demographics, clinical, obstetrics, nutrition-related, hygiene- and sanitation-related, and parasitic infection-related data were collected.

RESULTS: A total of 449 pregnant women (399 anemic and 50 non-anemic) participated in the study. A total of 224 (56.7%) in the anemic group and 31 (62.0%) in the non-anemic group did not consume any dark green, leafy vegetables such as spinach, bukurey, cagaar, and koomboow (p=0.040). Notably, 255 (63.9%) in the anemic group and 21 (42.0%) in the non-anemic group had a middle-upper arm circumference <23 cm. More than half of anemic [335 (84%)] and non-anemic [46 (92.0%)] were classified under low dietary diversity score. Majority of the study participants, 288 (72.4%) of the anemic and 39 (78%) of the non-anemic groups, used pit toilets in dwellings, and 70.2% (134/191) of the anemic and 64.4% (246/382) of the non-anemic groups disposed of solid waste in open fields.

CONCLUSION: This study demonstrated that women who consumed green vegetables such as spinach, bukurey, cagaar, and koomboow in their diet had middle-upper arm circumference less than 23 cm, and those with low dietary diversity significantly developed anemia during pregnancy.

KEYWORDS: Risk factors. Anemia. Pregnant women. Diet. Antenatal care. Somalia.

INTRODUCTION

Anemia is a public health concern that affects nearly one-third of the global population, with higher rates among young, impoverished, and pregnant women[1]. It is a significant cause of maternal and fetal morbidity and mortality, especially in low-resourced countries[2].

The primary cause of anemia is iron deficiency, although other deficiencies, diseases, and inherited blood disorders may also contribute. Risk factors for anemia can be individual, household, community, district, regional, or national[3]. Globally, anemia results in over 115,000 maternal and 591,000 perinatal deaths annually, accounting for 20% of maternal deaths worldwide[4]. Developing countries have higher rates of anemia among pregnant women, with Africa and Southeast Asia having the highest rates[5]. In Somalia, maternal anemia is highly prevalent, with a rate of 49.9%, especially in rural areas[6].

The World Health Organization (WHO) aims to reduce anemia in reproductive-aged women by 50% by 2025[7]. Maternal nutrition is a key priority in the Health Sector Transformation Plan (HSTP), and the prevalence of anemia in reproductive-aged women is one of the outcome measures of HSTP targets[8]. However, there are currently no reported studies on anemia among pregnant women in Somalia. Therefore, in resource-limited settings like Somalia, it is critical to obtain recent and accurate data on anemia severity and associated factors in order to meet goals and prioritize those most at risk.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 06, 2024. Accepted on January 19, 2024.

This study aimed to identify the factors that contribute to anemia in pregnant women who sought care at a tertiary referral hospital in Somalia, where there is less evidence available on this issue. By identifying the risk factors associated with anemia, healthcare providers can prioritize interventions that will benefit the most vulnerable women and reduce the burden of anemia-related morbidity and mortality.

METHODS

An institution-based unmatched case-control study was carried out on pregnant women who visited the antenatal clinics of Mogadishu Somalia Turkish Training and Research Hospital between March and July 2021. The research received approval from the Ethical Committee of the hospital and adhered to the tenets of the Declaration of Helsinki (Approval number: 17.02.2021-MSTH/5462). Pregnant women were provided information on the voluntary nature of the study, and written consent was obtained.

Pregnant women who suffered from acute or chronic disease, were receiving anemia treatment, were receiving deworming medication within the past 4 weeks, and those who could not provide information were excluded.

Anemia in pregnancy was determined following the guidelines provided by the WHO[9]. Pregnant women who had a hemoglobin level of <11 g/dL were included in the anemia group, while those with hemoglobin levels ≥11 g/dL were included in the non-anemic group. Hemoglobin levels were measured using a Sysmex XN 9000 (Roche Diagnostics Indianapolis, IN) analyzer. The blood samples were collected, processed, and analyzed by trained and experienced laboratory technicians and healthcare professionals with research experience. Data were collected using a questionnaire based on the existing literature and adapted to this study. Demographics, clinical, obstetrics, nutritional, hygiene- and sanitation-related, and parasitic infection-related data were gathered through face-to-face interviews with trained research assistants. To ensure the quality of data, data collectors were supervised by the principal investigator. All filled questionnaires were checked on a daily basis for completeness and consistency.

The study used the dietary diversity questionnaire (DDQ) to evaluate the usual dietary intakes of pregnant women in the last 6 months. Minimum Dietary Diversity for Women (MDD-W) was assessed by 24-h dietary recall. Dietary diversity was classified into low (<3), medium[4-6], and high (<7)[10]. The nutritional status of pregnant women was assessed by measuring mid-upper arm circumference (MUAC) with a tape measure by the trained research assistant. A MUAC <23 cm was indicative of malnutrition[11].

Statistical analysis

Data were analyzed through Statistical Package for Social Sciences (SPSS) v.20.0. Descriptive statistics were utilized to calculate frequencies and percentages, with $p \leq 0.05$ considered significant. Statistical analysis was done using the chi-square test, Fisher's exact test, or Mann-Whitney test, as appropriate.

RESULTS

Socioeconomic characteristics

The study involved 449 pregnant women, out of which 399 had anemia and 50 served as the non-anemic group. The mean age in the anemic and non-anemic groups were 25 (±6.0) and 25.0 (±5) years, respectively. The vast majority of participants resided in urban areas, with 93.9% in the anemic group and 98% in the non-anemic group. Notably, 3% of the anemic group was from rural areas. Regarding occupational status, most participants were housewives, with 317 (79.4%) in the anemic group and 38 (76%) in the non-anemic group. Family size was greater than nine for over half of the participants, with 50.4% in the anemic group and 51.0% in the non-anemic group. A total of 216 (55.2%) participants in the anemic group and 35 (70.0%) non-anemic group had a monthly income ranging between 300 and 500 USD (Table 1).

Anthropometric characteristics

The majority of the pregnant women involved in the study were malnourished, with 255 (63.9%) in the anemic group and 21 (42.0%) in the non-anemic group having a MUAC of less than 23 cm (Table 2).

Maternal dietary factors

More than half of the anemic [335 (84%)] and non-anemic groups [46(92.0%)] had a low dietary diversity score, whereas 45 (11.4%) and 16 (4.0%) of the anemic group and 3 (6%) and 1 (2.0%) of the non-anemic group had medium and high dietary diversity scores, respectively (Table 2).

Maternal obstetric factors

The pregnancy interval for most study participants was less than 2 years, with 320 (97.9%) of the anemic group and 41 (100%) of the non-anemic group. The anemic group with menstrual bleeding more than 8 days per cycle was 29 (7.3%), while it was 5 (10%) in the non-anemic group. Those who attended antenatal visits less than four times during their pregnancies were 17 (4.3%) of the anemic women and 1 (2.0%) of the non-anemic group (Table 2).

Hygiene- and sanitation-related factors

A total of 288 (72.4%) of the anemic group and 39 (78%) of the non-anemic group used pit toilets in dwellings. Notably, 70% (134/191) of the anemic group and 64.4% (246/382) of the non-anemic group stated that they disposed of solid waste in open fields. A total of 158 (98.8%) of the anemic group and 23 (100%) of the non-anemic group had access to protected piped water as a source of drinking water (Table 3).

Nutrient intake

Examination of the nutrition intake showed that 224 (56.7%) of the anemic group and 31 (62.0%) non-anemic group did not consume any dark green, leafy vegetables such as spinach, bukurey, cagaar, and koomboow (p=0.040). A total of 181 (45.6%) anemic and 25 (50.0%) non-anemic participants did not eat fresh or dried fish or shellfish, and 208 (52.3%) of the anemic and 20 (40%) of the non-anemic groups did not

Table 1. Baseline characteristics (n=449).

Variables		Anemic group (n=399)		Non-anemic group (n=50)		p-value
		n	%	n	%	
Age (years)	Mean±SD/median (range)	25±6/25 (25)		25±5/25 (24)		0.959
Age (years)	15–19	66	16.6	5	10.0	0.395
	20–24	111	27.9	20	40.0	
	25–29	123	30.9	13	26.0	
	30–34	66	16.6	9	18.0	
	35–40	32	8.0	3	6.0	
Marital status	Married	368	93.2	45	93.8	0.201
	Widowed	5	1.3	2	4.2	
	Divorced	22	5.6	1	2.1	
Residence	IDP	11	2.8	1	2.0	0.599
	Rural	13	3.3	0	.0	
	Urban	372	93.9	49	98.0	
Family size	<9	192	49.6	24	49.0	0.933
	≥9	195	50.4	25	51.0	
Occupation of respondent	Daily laborer	30	7.5	3	6.0	0.780
	Government employee	17	4.3	3	6.0	
	Housewife	317	79.4	38	76.0	
	Private employee	11	2.8	2	4.0	
	Student	24	6.0	4	8.0	
Occupation of spouse	Daily laborer	188	47.2	29	59.2	0.451
	Government employee	85	21.4	12	24.5	
	Merchant	48	12.1	3	6.1	
	Private employee	41	10.3	4	8.2	
	Farmer/pastoralist	7	1.8	0	0	
	Unemployment	29	7.3	1	2.0	
Income	Poor (<100 USD)	15	3.8	0	0	0.084
	Middle (100–300 USD)	160	40.9	15	30.0	
	High (300–500 USD)	216	55.2	35	70.0	
Educational status	No formal school	137	34.5	17	34.0	1.000
	Illiterate	8	2.0	1	2.0	
	Primary school education (Grades 1–9)	130	32.7	16	32.0	
	Secondary school education (Grades 10–12)	86	21.7	11	22.0	
	Tertiary (college/university)	36	9.1	5	10.0	

Table 2. Health conditions and anthropometric characteristics of pregnant women attending antenatal care in Mogadishu Somalia Training and Research Hospital, 2021 (n=449).

Variables		Anemic group (n=399)		Non-anemic group (n=50)		p-value
		n	%	n	%	
Interval between pregnancies	<2 years	320	97.9	41	100.0	1.000
	≥2 years	7	2.1	0	0	
Antenatal follow-up	<4	382	95.7	49	98.0	
	≥4	17	4.3	1	2.0	
Tetanus vaccination	No	138	34.8	19	38.0	0.867
	Incomplete	252	63.6	30	60.0	
	Complete	6	1.5	1	2.0	
Iron supplementation	No	134	33.6	16	32.0	0.823
	Yes	265	66.4	34	68.0	
Folic acid supplementation	No	173	43.5	20	40.0	0.641
	Yes	225	56.5	30	60.0	
Treatment for intestinal worms	No	374	94.2	47	95.9	1.000
	Yes	23	5.8	2	4.1	
Treatment for malaria infection	No	382	96.2	48	96.0	1.000
	Yes	15	3.8	2	4.0	
Smoking	No	395	100.0	50	100.0	na
	Yes	0	.0	0	.0	
Menstrual period (days)	<8	366	92.7	45	90.0	0.568
	≥8	29	7.3	5	10.0	
MUAC	<23 cm	255	63.9	21	42.0	0.003*
	≥23 cm	144	36.1	29	58.0	
Dietary diversity score	Low	335	84.6	46	92.0	0.376
	Medium	45	11.4	3	6.0	
	High	16	4.0	1	2.0	

MUAC: mid-upper arm circumference; CS: cesarean section; NSD: normal spontaneous vaginal delivery; *p<0.05.

Table 3. Hygiene- and sanitation-related factors of anemia in pregnant women attended antenatal care in Mogadishu Somalia Training and Research Hospital, 2021 (n=449).

Variables		Anemic group		Non-anemic group	
		n	%	n	%
Source of drinking water	Protected piped water into dwelling	159	98.8	23	100.0
	Unprotected piped water into dwelling	2	1.2	0	0
Toilet facility	Composed latrines	2	0.5	0	0
	Flush toilet in dwelling	105	26.4	11	22.0
	No facility/bush/field	3	0.8	0	0
	Pit toilet in dwelling	288	72.4	39	78.0

consume any meat, such as beef, lamb, goat, chicken, or duck at all. Totally, 159 (39.8%) of those with anemia and 20 (40.0%) of the non-anemic group did not consume injera, muufo, soor, bread, rice, noodles, spaghetti, porridge, or other foods made from grains such as oats, maize, and barley. Notably, 203 (50.9%) of the anemic group and 29 (58.0%) of the non-anemic group never consumed any foods made from beans, peas, lentils, or nuts. A total of 209 (52.6%) of the anemic group and 18 (36.0%) of the non-anemic group did not consume yogurt, cheese, or other food made from milk.

DISCUSSION

Anemia is a major global public health issue, particularly during pregnancy in developing nations such as Somalia. This study aimed to identify the factors contributing to anemia among pregnant women who were admitted to the obstetrics unit in Mogadishu, Somalia.

The study found that anemia was more prevalent among pregnant women who consumed dark green, leafy vegetables such as spinach, bukurey, cagaar, and koomboow compared with those who did not consume green leafy vegetables. This finding is in contrast to previous studies in Dessie, northern and eastern Ethiopia, which identified low intake of green vegetables as a contributing factor to anemia[12-14]. Food taboos during pregnancy, socioeconomic factors, climatic conditions, and inadequate dietary diversity may contribute to anemia in Somalia[3,15].

This study found that a MUAC of less than 23 cm was significantly associated with anemia during pregnancy. MUAC is a common metric for assessing nutritional status, and a circumference of 23 cm serves as a threshold for determining the level of nourishment for women, with circumferences below this value being indicative of undernourishment. The result of this study aligns with prior research conducted in various regions, including eastern and western Ethiopia, Kenya, Nepal, and India[12,16,17]. These studies further demonstrate that undernourished pregnant women are more likely to develop anemia. The nutritional disadvantage of pregnancy might manifest itself later in pregnancy, as mothers are unable to provide the nutritional needs of the growing fetus, which may lead to negative consequences for both the mother and the fetus.

Dietary diversity is used as an indicator of the nutritional quality of pregnant women. This study found that a majority of pregnant women had a low dietary diversity score, which could be attributed to restrictive dietary behavior in Somalia where women are expected to avoid some foods during pregnancy. This, in turn, may contribute to anemia[18]. It is advisable to diversify the diet during pregnancy to meet the high nutritional needs.

Limitations

The study had some limitations. First, it is a facility-based study, so it is difficult to generalize the findings to the other settings of Somalia or to other countries. Second, the cross-sectional design makes it difficult to draw causal inferences. Third, 24-h recall data might be subject to some recall bias. Finally, there might be social desirability bias in self-reports of dietary intake and household income. Despite having these limitations, to the best of our knowledge, this is the first comprehensive research on anemia among pregnant women to be reported from Mogadishu, Somalia.

CONCLUSION

This study demonstrated that consumption of dark green, leafy vegetables such as spinach, bukurey, cagaar, and koomboow, low dietary diversity, and a MUAC less than 23 cm were found to be significant determinants of anemia among pregnant women in Somalia. Identifying and addressing the determinants of anemia and effective interventions are crucial to combat anemia in pregnancy.

ETHICS

Ethical approval for this study was provided by the Somalia Mogadishu–Turkey Recep Tayyip Erdogan Training and Research Hospital Ethics Committee (Approval number: 17.02.2021-MSTH/5462). The database management is in accordance with privacy legislation, and this study is in accordance with the ethical principle of the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

MMO: Data curation, Writing – original draft, Writing – review & editing. **EK:** Conceptualization, Writing – original draft, Writing – review & editing. **GB:** Conceptualization, Writing – original draft, Writing – review & editing. **HHE:** Writing – original draft. **ŞK:** Formal Analysis, Writing – original draft, Writing – review & editing. **OO:** Writing – original draft.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231382

Genetic variants in miR-146a and miR-196a2 in endometriosis: a Brazilian study

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SUMMARY

OBJECTIVE: The aim of this study was to determine the allelic and genotypic frequencies of the polymorphisms, rs2910164 miR-146a and rs11614913 miR-196a2, by investigating their association with endometriosis.

METHODS: This is a case–control study performed with approximately 120 women. The polymorphisms were determined by real-time polymerase chain reaction. For the statistical analysis, the chi-square and logistic regression tests were used.

RESULTS: There were no significant differences in the genotype and allele frequencies of rs2910164 and rs11614913 between cases and controls. The frequencies in both polymorphisms are in accordance with Hardy-Weinberg equilibrium regarding miR-146a (patients: χ^2=1.64, p=0.20; controls: χ^2=0.25, p=0.62) and miR-196a2 (patients: χ^2=0.58, p=0.44; controls: χ^2=2.78, p=0.10). No relationship was observed between rs2910164 and rs11614913 and endometriosis in the inheritance models analyzed.

CONCLUSION: In this study, our results show that the studied polymorphisms are not implicated in the development of endometriosis.

KEYWORDS: Endometriosis. MicroRNAs. Real-time polymerase chain reaction. Biomarkers. Polymorphism, genetic. Diagnosis.

INTRODUCTION

Endometriosis is a gynecological condition defined by the presence of endometrial tissue in extrauterine locations. The clinical presentation is extremely variable with symptoms including dysmenorrhea, dyspareunia, pelvic pain, dyschezia, dysuria, changes in bowel habits, and often infertility[1]. The disease has a negative effect on quality of life and may cause psychological disorders and lower productivity at work[2,3].

Currently, videolaparoscopy and subsequent anatomopathological analysis are still present as the gold standard procedure for the definitive diagnosis of endometriosis, resulting in delayed diagnosis of 8–12 years. Therefore, the search for noninvasive diagnostic test options has been the subject of intensive investigation in the scientific literature[4,5]. In this sense, genetic polymorphism deserves to be highlighted, especially single nucleotide polymorphisms (SNPs), such as in regions that encode microRNAs, a class of noncoding small RNAs responsible for the post-transcriptional regulation of gene expression and that may be implicated in the pathophysiology of endometriosis[6,7]. A recent study suggests that four miRNAs could be included as a prognostic marker in endometriosis[7]. However, future clinical studies should evaluate the efficacy of these miRNAs in endometriosis diagnosis and treatment[8].

It is also worth noting that miRNAs can control inflammatory responses, cell proliferation, angiogenesis, and tissue remodeling, which are common biological processes in endometriosis[9]. A recent study evaluated the prevalence of miRNA variants, such as miR-146a rs2910164, miR-149 rs2292832, miR-196a2 rs11614913, and miR-499 rs3746444, in endometriosis[10]. Some studies have investigated the association of rs2910164 and rs11614913 in female reproductive disorders such as polycystic ovary syndrome[11-14], preeclampsia[15,16], ovarian cancer[17], and idiopathic recurrent pregnancy loss[18]. However, less is known about the effect of these miRNAs polymorphisms in endometriosis[10,19], which highlights the need for further research.

Hence, the objective of the present study was to determine the allelic and genotypic frequencies of the polymorphisms rs2910164 miR-146a and rs11614913 miR-196a2 and investigate their association with endometriosis.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: Fundação de Amparo à Pesquisa do Estado de Minas Gerais—FAPEMIG— Demanda Universal 2021 (Processo APQ 01545-21).
Received on January 29, 2024. Accepted on February 04, 2024.

METHODS

This is a case–control study approved by the Research Ethics Committee of the Federal University of Triângulo Mineiro (UFTM), protocol 1628. All participants who signed the informed consent form were from the Gynecology and Obstetrics outpatient clinic at UFTM, Brazil. The presence (case group) or absence (control group) of the disease was confirmed through videolaparoscopy or laparotomy. The women with endometriosis (case group) had histological confirmation of endometriosis and classification of the disease according to the Revised American Society for Reproductive Medicine. The control group included endometriosis-free patients who underwent surgery for tubal sterilization, chronic pelvic pain, and infertility. Participants' inclusion in the study occurred in the period from 2012 to 2016.

It is worth noting that the gold standard for diagnosing endometriosis is done through a surgical procedure called laparoscopy. As it is an invasive method, it is only justified for women with clinical complaints associated with endometriosis. Therefore, to minimize the confounding clinical effects of the disease, samples from the control group were collected only after the histopathological report was issued following laparoscopy surgery, ruling out endometriosis.

Genomic DNA was extracted by a salting-out method[20] from the peripheral blood drawn by venipuncture from the participants. Genotyping of the rs2910164 polymorphism was performed in 122 women (47 with endometriosis and 75 without the disease). A total of 115 (46 case group and 69 control group) were analyzed for the polymorphism miR-196a2 rs11614913.

The polymorphisms were determined by real-time PCR with the method of allelic discrimination on the SNP Genotyping Assay (Applied Biosystems). Primers and probes for the detection of rs2910164 miR-146a and rs11614913 miR196a2 polymorphisms are available from the manufacturer (Assay ID—C_15946974_10 and C_31185852_10, respectively). The reactions were carried out in 96-well plates, in the equipment StepOne™ Real-Time PCR System. Genotypes were determined from the results of amplification products observed as amplification curves recognized from the marking for each probe (VIC/FAM).

The statistical analysis used the chi-square test (χ^2) to compare allele and genotype frequencies between the groups and to assay Hardy-Weinberg equilibrium. These analyses were carried out by the BioEstat program. Analyses were also performed assuming recessive, codominant, and dominant models of inheritance by the SNPStats program and odds ratio (OR), their 95%CI ranges by logistic regression adjusted for age, with $p<0.05$ being considered statistically significant.

RESULTS

Allele frequencies and genotype of miR-146a rs2910164 and miR-196a2 rs11614913 polymorphisms are shown in Table 1. There were no significant differences in the genotype and allele frequencies of rs2910164 and rs11614913 between cases and controls.

The frequencies in both polymorphisms are in accordance with Hardy-Weinberg equilibrium regarding miR-146a (patients: $\chi^2=1.64$, p=0.20; controls: $\chi^2=0.25$, p=0.62) and miR-196a2 (patients: $\chi^2=0.58$, p=0.44; controls: $\chi^2=2.78$, p=0.10).

The genotype of patients and controls were adjusted for age according to the inheritance models and no relationship was observed between rs2910164 and rs11614913 and endometriosis in the models analyzed (Table 2).

The discrepancies in relation to the number of cases and controls presented in Table 1 are due to non-real-time amplification of some samples or the samples are over. In Table 2, there is a lack of information about age.

DISCUSSION

The polymorphisms analyzed in miRNAs were not associated with endometriosis in the studied population. However, the literature explores the possible therapeutic strategies of miRNAs for the diagnosis and treatment of several human diseases, such as cancer[21], diabetes mellitus[22], and neurodegenerative conditions[23].

Several regulatory mechanisms control the expression, activity, and bioavailability of miRNAs, among which the

Table 1. Frequency distribution of miR-146a and miR-196a2 genotypes and alleles in endometriosis patients and controls.

rs2910164 polymorphism	Patients (n=47)	Controls (n=75)	p
Genotypes	n (%)	n (%)	0.68
GG	26 (55.3)	41 (54.7)	
GC	20 (42.5)	30 (40)	
CC	1 (2.2)	4 (5.3)	
Alleles			0.87
G	0.77	0.75	
C	0.23	0.25	
rs11614913 polymorphism	**(n=46)**	**(n=69)**	**p**
Genotypes	n (%)	n (%)	0.26
CC	14 (30.5)	26 (37.7)	
CT	25 (54.3)	27 (39.1)	
TT	7 (15.2)	16 (23.2)	
Alleles			1.00
C	0.58	0.57	
T	0.42	0.43	

Table 2. Association of co-dominant, dominant, and recessive of miR-146a and miR-196a2 genotypes in endometriosis patients and controls.

rs2910164 polymorphism	Patients (n=43)	Controls (n=62)	OR (95%CI)	p
Codominant	n (%)	n (%)		0.94
GG	24 (55.8)	33 (53.2)	1.00	
GC	18 (41.9)	27 (43.5)	1.09 (0.49–2.42)	
CC	1 (2.3)	2 (3.3)	1.45 (0.12–16.98)	
Dominant				0.79
GG	24 (55.8)	33 (53.2)	1.00	
GC-CC	19 (44.2)	29 (46.8)	1.11 (0.51–2.43)	
Recessive				0.78
GG-GC	42 (97.7)	60 (96.8)	1.00	
CC	1 (2.3)	2 (3.2)	1.40 (0.12–15.94)	
rs11614913 polymorphism	**(n=43)**	**(n=62)**	**OR (95%CI)**	**p**
Codominant	n (%)	n (%)		0.32
CC	13 (30.2)	24 (38.7)	1.00	
CT	23 (53.5)	24 (38.7)	0.57 (0.23–1.08)	
TT	7 (16.3)	14 (22.6)	1.08 (0.35–3.36)	
Dominant				0.37
CC	13 (30.2)	24 (38.7)	1.00	
CT-TT	30 (69.8)	38 (61.3)	0.69 (0.30–1.57)	
Recessive				0.42
CC-CT	36 (83.7)	48 (77.4)	1.00	
TT	7 (16.3)	14 (22.6)	1.50 (0.55–4.10)	

genetic polymorphisms can alter the expression pattern in genes involved in the development of specific pathologies[24]. A recent review showed that miR-126, miR-143, and miR-146b polymorphisms have been associated with risk of endometriosis; thus, understanding the role of these transcripts is a possible way to develop novel diagnostic tests and therapeutic targets for this disorder[9].

In the present study, the polymorphisms rs2910164 and rs11614913 located on chromosomes 5 and 12, respectively, were analyzed. The base exchange observed in the miR-146a polymorphism was the substitution of C to G, decreasing the production of miR-146a[15], while in the miR-196a2 polymorphism there was an exchange of C for T[13]. These polymorphisms were investigated in several gynecological conditions, with quite different results (Table 3).

According to Table 3, eight previous studies investigated miR-146a and six analyzed miR-196a2 polymorphism, among which one and three, respectively, found no association with endometriosis, in agreement with our results. Table 3 presents 11 studies on the miR146a polymorphisms, mainly, and miR196a2, seven of which were conducted in Iran. In all these studies, such polymorphisms were associated with the investigated conditions.

Positive results were found mainly in studies conducted in Iran (Table 3). The ethnicity effect might be related to differences

Table 3. Summary of the mains results of previous studies that investigated rs2910164 and rs11614913 polymorphisms in female reproductive disorders.

Study	Country	Subjects	Gynecological condition analyzed	Polymorphism(s)	Association with the gynecological condition analyzed
Chang et al.[19]	Taiwan	218 cases–202 controls	Endometriosis	miR-196a2 rs11614913	miR-196a2=yes
Farsimadan et al.[10]	Iran	260 cases–260 controls	Endometriosis	miR-146a rs2910164	miR-146a=yes
				miR-196a2 rs11614913	miR-196a2=no
Hosseini et al.[11]	Iran	205 cases–205 controls	Polycystic ovary syndrome	miR-146a rs2910164	miR-146a=yes
Ebrahimi et al.[12]	Iran	180 cases–192 controls	Polycystic ovary syndrome	miR-146a rs2910164	miR-146a=yes
Li et al.[13]	Iran	385 cases–385 controls	Polycystic ovary syndrome	miR-146a rs2910164	miR-146a=yes
				miR-196a2 rs11614913	miR-196a2=yes
Soyman et al.[14]	Turkey	50 cases–50 controls	Polycystic ovary syndrome	miR-146a rs2910164	miR-146a=yes
Salimi et al.[15]	Iran		Preeclampsia	miR-146a rs2910164	miR-146a:
		Blood: 219 cases–242 controls			Blood=yes
		Placental: 111 cases–119 controls			Placental=yes
Asadi-Tarani et al.[16]	Iran		Preeclampsia	miR-196a2 rs11614913	miR-196a2:
		Blood: 315 cases–317 controls			Blood=no
		Placental: 103 cases–133 controls			Placental=yes
Lukács et al.[17]	Hungary	75 cases–75 controls	Ovarian cancer	miR-146a rs2910164	miR-146a=no
				miR-196a2 rs11614913	miR-196a2=no
Alipour et al.[18]	Iran	120 cases–90 controls	Idiopathic recurrent	miR-146a rs2910164	miR-146a=yes
			pregnancy loss	miR-196a2 rs11614913	miR-196a2=yes
Present study	Brazil	47/46 cases	Endometriosis	miR-146a rs2910164	miR-146a=no
		75/69 controls		miR-196a2 rs11614913	miR-196a2=no

in susceptibility to these polymorphisms. However, the present study did not collect information on ethnicity or skin color, and all individuals were from the same region of Brazil. The analysis of the mode of inheritance (Table 2) did not reveal differences between the groups in its distribution.

The studies in Table 3 used blood as a standard biological sample and techniques of polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and quantitative real-time polymerase chain reaction (RT-qPCR) to investigate these two polymorphisms. In this sense, we believe that such variables would not interfere with the results obtained.

Only two studies investigated these polymorphisms in endometriosis[10,19], with different results for the miR-196a2. Genome-wide association studies have revealed 23 genome-wide significant loci that are associated with the risk of endometriosis, particularly on chromosome 12, where the miR-196a2 polymorphism is located[4]. Our results and number of individuals investigated were similar to those of Lukács et al[17].

A systematic review concludes that no particular miRNA or miRNA combination has been validated for improved diagnosis of endometriosis to date. This may have reflected the heterogeneity of the disease and resultant differences in tissue composition[25].

It was not possible to analyze the clinical data with the molecular ones, but it is noteworthy that, in this retrospective study, there was a predominance of more advanced stages of endometriosis. Further studies on different regions and ethnic groups seem necessary to assess the effects of the changes in these polymorphisms with the etiology of endometriosis.

CONCLUSION

In this study, our results show that the studied polymorphisms are not implicated in the development of endometriosis.

AUTHORS' CONTRIBUTIONS

GCO: Data curation, Formal Analysis, Project administration, Writing – original draft. **MTRC:** Data curation, Formal Analysis, Project administration, Writing – original draft. **MFPL:** Data curation, Formal Analysis. **MKOG:** Data curation, Formal Analysis. **ABTM:** Data curation, Formal Analysis, Project administration, Writing – original draft.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231401

Determination of microbiota awareness levels in women planning pregnancy

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SUMMARY

OBJECTIVE: It was recently discovered that the microbiota has a significant impact on pregnancy, gynecological, and neonatal health. However, studies indicate that people struggle to understand topics, such as microbiota, microbiome, probiotics, and prebiotics, or comprehend them inaccurately or incompletely. Understanding the human microbiota and probiotics that can regulate the microbiota helps women develop daily habits for both healthy nutrition and health protection. The aim of this study was to assess the microbiota awareness levels of women who are planning pregnancy.

METHODS: A cross-sectional descriptive study was carried out on 417 women who were planning pregnancy. Face-to-face interviews and questionnaires were used to collect research data. A microbiota awareness scale was used as a data collection tool.

RESULTS: The study found a statistically significant difference in the subdimension scores related to microbiota awareness, general information, product knowledge, chronic disease, and probiotic and prebiotic knowledge based on the educational status of the participants. The study concluded that the participants had a confusion about microbiota awareness, general information, product information, chronic disease, and probiotic and prebiotic subdimensions. Furthermore, it was found that the participants had only a partial understanding of the relationship between microbiota and diseases.

CONCLUSION: It is recommended that training programs focusing on the relationship between microbiota and health in women, such as "microbiota and its importance in women's health" and "microbiota and disease relationship," be organized and women would be encouraged to participate in these training programs.

KEYWORDS: Microbiota. Awareness. Women. Pregnancy.

INTRODUCTION

The term "microbiota" is derived from ancient Greek words "micro" (μικρος, small) and "biota" (βιοτα), which refers to the living organisms of an ecosystem or a specific area[1]. Microbiota refers to all microorganisms present in different parts of our body, while microbiome refers to their genetic materials[2,3]. Our "last organ" is known as the human microbiome[4]. In recent years, researchers have begun to understand the effects of microbiota on pregnancy, the postnatal period, infant health, obesity, allergic diseases, gastrointestinal system, and urogenital infections such as vulvovaginal infection (VVI) and bacterial vaginosis (BV) infections[2-5].

The mode of delivery plays a significant role in determining the microbiota makeup of newborns. While babies delivered vaginally are exposed to the mother's vaginal and intestinal microorganisms, babies delivered through cesarean section are exposed to the mother's skin and environmental microorganisms. Various factors such as prenatal probiotic use, gestational week at birth, frequency of vaginal examination, birth environment, birth weight, length of hospital stay after birth, baby's diet, economic level, number of siblings, geographical location, climate, culture, gender, country's development level, and perinatal stress factors all influence newborn gut microbial diversity and colonization[6].

Pregnancy is a crucial period in a woman's life that involves various immunological and metabolic changes. The most important factors that influence the formation of a healthy microbiota in a child are the transfer of microbiota from mother to newborn during birth and the continuation of these processes with breastfeeding[7]. Given the importance of microbiota in pregnancy and women's, and children's health, knowing the microbiota of women planning pregnancy and concepts such as probiotics that can modulate their microbiota helps them develop daily habits for both healthy nutrition and health protection. However, there is a lack of studies aimed at determining the level of awareness about microbiota in individuals. Therefore, this study aims to determine the level of awareness about microbiota among women planning pregnancy, which can help in education planning based on their needs for this purpose.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 04, 2024. Accepted on January 08, 2024.

METHODS

This study was conducted at Kirklareli Training and Research Hospital between February 2022 and July 2022. The population for this study included 417 female participants aged 18 and higher with no communication problems and who applied to the hospital during the research period. The participants agreed to take part in the study and signed the informed consent form.

Data collection tools

The data collection tool is separated into two sections. The first section includes questions related to the individuals' sociodemographic traits, while the second section consists of the microbiome awareness scale form comprising 28 items[8].

Evaluation of data

The SPSS (Statistical Package for Social Sciences) 25.0 program was used to analyze the study data. The independent sample t-test and one-way analysis of variance were also used. Statistical significance was accepted as $p<0.05$.

Ethical aspect

The study was conducted with permissions of the Kirklareli University Health Sciences Institute Ethics Committee (Number: PR0366R01) and the Kirklareli Provincial Health Directorate (Number: 2, February 8, 2022). After the purpose of the study was explained to the participants, written and verbal agreements were obtained. Additionally, permission to use the scale was obtained from the authors who created the scale used in the study. All practices were carried out in accordance with the 1964 Helsinki Declaration.

RESULTS

When the highest distribution rates are evaluated according to the sociodemographic characteristics of the participants, it was determined that 25.4% of the participants' age was 25–30 years and 24.2% of the educational status was associate's degree. It was revealed that 75.8% of the participants stated they had regular and adequate eating habits, while 76% had no chronic diseases. When the smoking status of the participants was investigated, it was found that 56.4% answered that they had never smoked (Table 1).

There was a statistically significant differences in the subdimension scores such as microbiota awareness, product knowledge, and probiotic and prebiotic knowledge based on the participants' age ($p<0.05$). Participants under the age of 25 years had lower scores in all three subdimensions. Similarly, there was a statistically significant difference in scores observed for subdimensions such as microbiota awareness, general information, product knowledge, chronic disease, and probiotic and prebiotic knowledge based on the participants' educational status ($p<0.05$). It was observed that participants with postgraduate degrees had higher microbiota awareness scores, while those with chronic diseases had higher scores in product knowledge subdimension than those without chronic diseases (Table 2).

Table 1. Sociodemographic characteristics of the participants.

Variables		n	%
Age (years)	Under 25	51	12.2
	25–30	106	25.4
	31–35	77	18.5
	36–40	89	21.3
	41+	94	22.5
Educational status	Not literate/literate	25	6.0
	Primary school	54	12.9
	Secondary school	70	16.8
	High school	99	23.7
	Associate's degree	101	24.2
	Bachelor's degree	46	11.0
	Postgraduate degree	22	5.3
Location	Provincial center	304	72.9
	District center	91	21.8
	Village	22	5.3
Number of people living in the household (including you)	2	72	17.3
	3	135	32.4
	4	133	31.9
	5	53	12.7
	6+	24	5.8
Number of pregnancy	0	64	15.3
	1	113	27.1
	2	138	33.1
	3	65	15.6
	4+	37	8.9
Number of living children	0	82	19.7
	1	133	31.9
	2	148	35.5
	3+	54	12.9
Do you think that you have regular and adequate eating habits?	Yes	316	75.8
	No	101	24.2
Do you have any chronic disease?	Yes	100	24.0
	No	317	76.0
Total		417	100.0

Table 2. Comparison of microbiota awareness scale and subdimension scores according to the sociodemographic characteristics of the participants.

Variables		Microbiota awareness (total)	General information	Product knowledge	Chronic disease	Probiotic and prebiotic knowledge
		X̄±SD	X̄±SD	X̄±SD	X̄±SD	X̄±SD
Age (years)	Under 25	63.14±11.18	21.67±4.52	8.88±3.1	15.84±3.32	16.75±3.48
	25–30	66.80±11.87	22.74±4.65	9.68±3.43	16.31±3.12	18.08±4.14
	31–35	70.31±10.16	23.73±4.28	10.69±3.47	17.03±2.66	18.87±3.2
	36–40	69.38±10.36	23.34±3.95	10.61±3.69	16.88±2.63	18.56±3.44
	41+	69.72±11.36	23.33±4.78	11.07±3.36	16.44±2.58	18.88±3.61
	F test	**4.502**	1.987	**4.631**	1.838	**3.614**
	p	**0.001**	0.096	**0.001**	0.121	**0.007**
	Bonferroni	**3,4,5>1**	–	**3,4,5>1**	–	**3,4,5>1**
Educational status	Not literate/literate	62.88±8.71	21.52±3.95	8.56±3.14	16.00±3.01	16.8±2.68
	Primary school	61.26±10.38	21.19±3.98	8.22±2.6	15.52±2.91	16.33±3.76
	Secondary school	65.99±9.65	21.97±4.13	9.9±3.44	16.24±2.49	17.87±2.98
	High school	67.72±10.69	22.66±4.56	10.24±3.16	16.53±2.63	18.29±3.42
	Associate's degree	70.12±11.64	24.01±4.79	10.74±3.51	16.35±2.98	19.02±4.1
	Bachelor's degree	75.30±8.67	25.2±2.68	12.15±3.48	18.00±2.80	19.96±3.29
	Postgraduate degree	77.05±10.57	25.68±4.89	12.64±3.86	18.41±2.54	20.32±3.31
	F test	**12.438**	**7.242**	**9.415**	**5.392**	**7.328**
	p	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
	Bonferroni	**6,7>1,2,3,4 5>1,2 4>2**	**6,7>1,2,3,4 5>2**	**6,7>1,2,3,4 4,5>2**	**6,7>2,3,5**	**6>1,2,3 7>1,2 4,5>2**

p<0.05; t-test: independent sample t-test; F test: one-way analysis of variance (ANOVA).
X±SD: average (X) and standard deviation (SD).

The answers given by participants to the propositions in the microbiota awareness scale are as stated in Table 3.

DISCUSSION

The vaginal microbiota, which is part of the female microbiome, has crucial functions. It is made up of a balanced host of *Lactobacillus* bacteria, anaerobic bacteria, and *Candida* yeast. However, in some situations, these microorganisms exhibit dysbiosis, which can lead to vaginal infections[2]. *Lactobacillus* bacteria, which dominate the vaginal microbiota of healthy women, maintain vaginal pH and inhibit pathogen growth by secreting numerous antimicrobial compounds. Therefore, the dominance of these probiotic bacteria, which protect the vaginal microbiota through various mechanisms, is essential for protecting against recurrent vulvovaginal infection (RVVI), including recurrent vulvovaginal candidiasis (RVVC) and BV infections[9]. Recent studies have clearly demonstrated the importance of microbiota in reproductive system infections, fertilization, pregnancy, postpartum period, and newborn health[5-7]. The newborn's health, which is closely related to women's health, will be preserved, as will the continuity of healthy individuals. When women's health is considered holistically, a woman's overall health will contribute to the pregnancy processes. Because the changes in the pregnancy process make women more sensitive, it is critical to explain the changes that will occur in their bodies and raise their awareness of healthy living. Pregnancy involves numerous processes that contribute to the neonatal microbiota[7].

Recent studies have highlighted the importance of monitoring individuals' awareness of microbiota and probiotics that alter microbiota[8,10-17]. Researchers noted that people who are aware of the importance of microbiota in their health are more likely to choose probiotic-containing diets[12,13,15]. Similarly, it has been found that people understand that antibiotics lower the amount of beneficial bacteria in the microbiome and that the need to use antibiotics when necessary[18].

Table 3. Microbiota awareness scale.

Statements*	I strongly disagree		I do not agree		I'm undecided		I agree		Absolutely I agree	
	n	%	n	%	n	%	n	%	n	%
1	21	5.0	16	3.8	31	7.4	226	54.2	123	29.5
2	13	3.1	22	5.3	113	27.1	201	48.2	68	16.3
3	19	4.6	38	9.1	93	22.3	180	43.2	87	20.9
4	20	4.8	26	6.2	86	20.6	180	43.2	105	25.2
5	11	2.6	32	7.7	129	30.9	180	43.2	65	15.6
6	24	5.8	18	4.3	41	9.8	202	48.4	132	31.7
7	22	5.3	28	6.7	98	23.5	193	46.3	76	18.2
8	13	3.1	33	7.9	209	50.1	138	33.1	24	5.8
9	20	4.8	27	6.5	81	19.4	206	49.4	83	19.9
10	18	4.3	36	8.6	173	41.5	157	37.6	33	7.9
11	13	3.1	32	7.7	132	31.7	185	44.4	55	13.2
12	17	4.1	26	6.2	198	47.5	146	35.0	30	7.2
13	23	5.5	19	4.6	47	11.3	168	40.3	160	38.4
14	8	1.9	25	6.0	239	57.3	111	26.6	34	8.2
15	18	4.3	19	4.6	106	25.4	191	45.8	83	19.9
16	26	6.2	44	10.6	205	49.2	110	26.4	32	7.7

*Statements: (1) The human body contains a large number of microorganisms. (2) The gut microbiota begins to form when the baby is in the womb. (3) I know what prebiotic products are. (4) The use of antibiotics adversely affects the intestinal microbiota. (5) Disruptions in the intestinal microbiota cause obesity. (6) Diet is one of the important factors affecting the intestinal microbiota. (7) I know what probiotic products are. (8) Changes in the microbiota are associated with bowel cancer. (9) Probiotics should be consumed regularly. (10) Disruptions in the intestinal microbiota cause diabetes. (11) I believe that using probiotics can help with diarrhea. (12) An increase in the number of harmful bacteria in the intestines can cause nonalcoholic fatty liver disease. (13) Breastfeeding positively affects the intestinal microbiota of the baby. (14) Changes in the gut microbiota are associated with celiac disease. (15) I believe that using probiotics can help with constipation. (16) There is a link between gut microbiota and depression and Alzheimer's.

Individuals' awareness of the microbiota–health relationship is also related to their recognition and understanding of microbes. As a consequence, individuals' basic descriptions of microbes should be maintained. Individuals may benefit from this awareness by incorporating components that affect the microbiota into their daily routine, such as nutrition, good hygiene habits, and regular exercise[14].

When studies on microbiota knowledge are analyzed, it is notable that the majority of the studies assessed the individuals' awareness of the concepts of probiotic, prebiotic, as well as their probiotic and prebiotic food consumption status[10-19] and directly measured individuals' microbiome awareness[8,13]. It can be noted that the main themes examined are persons of all ages and professional groupings, such as healthcare professionals[14,17]. The same topics were explored utilizing mothers[16], postmenopausal women[11], and educational level factors[10-19].

The microbiome awareness of women planning pregnancy was assessed in this descriptive cross-sectional study. The findings of 417 female participants were included in this study, and the answers given to the questions in the microbiota and awareness scale were discussed by comparing the demographic characteristics of the participants and the scale's subdimensions. Researchers evaluated that the prevalence of probiotic use was higher in female participants with higher education levels[10]. Similarly, in this study, based on the participants' educational status, there was a statistically significant difference in subdimension scores such as microbiota awareness, general information, product knowledge, chronic disease, and probiotic and prebiotic knowledge. The current rates exemplify the awareness among participants that probiotics have important health functions. The effective use of probiotic prebiotics, especially in constipation and diarrhea problems, can be attributed to the fact that they were previously recommended to the participants by physicians. In a study that assessed the probiotic knowledge level and consumption status of postmenopausal women[11], they enrolled 150 women with ages ranging from 50 to 70 years. Probiotic users (58.9%) were found to be more educated, more aware of gut health, and to have healthier lifestyle habits overall. Another study concluded that participants' education levels and probiotic knowledge levels were related[12].

The level of microbial awareness is affected by educational status. As a result, prospective moms should receive appropriate knowledge and supportive treatment, particularly during the pre-pregnancy phase. Participants in the study were included in the microbiota awareness scale. Finally, the study concluded that the participants had a confusion regarding general information, product information, chronic disease, and probiotic and prebiotic subdimensions, and that they could not establish the relationship between microbiota and diseases such as Alzheimer's disease, celiac disease, obesity, diabetes, and bowel cancer. Similarly, several studies have highlighted that people lack understanding about the relationship between microbiome and diseases[8,11,13,16].

CONCLUSION

In this study, it was concluded that the participants experienced confusion regarding general information, product information, chronic disease, and probiotic and prebiotic subdimensions in the microbiota awareness scale, and that they could not fully establish the relationship between microbiota and diseases. In this context, the society should be sensitized on diseases that they are aware of, as well as daily nutritional habits that support the microbiota, so that participants can establish the relationship between microbiota and diseases and can help to eradicate these inadequacies. As a result, it is recommended that training programs targeting the relationship between microbiota and health in women planning pregnancy, such as "breast milk microbiota and infant nutrition," "microbiota and its importance in women's health," and "microbiota and disease relationship," be organized and encouraged to participate in.

AVAILABILITY OF DATA AND MATERIALS

Data used and analyzed in the current study are available from the corresponding author upon reasonable request.

ACKNOWLEDGEMENTS

We are grateful to all participants in the study, as well as the Kirklareli Provincial Health Directorate, for enabling the study to take place.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231430

Hospital admissions for chronic liver diseases: a temporal study in the South Region of Brazil

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SUMMARY

OBJECTIVE: The aim of the study was to compare the epidemiology and clinical profiles of hospital admissions in a single Brazilian Hepatology Unit from the period 2014–2017 to 2019–2022.

METHODS: A retrospective analysis of hospital database from the abovementioned periods was done. The study included patients over the age of 18 years who were hospitalized due to complications of diseases such as viral hepatitis, alcoholic disease, nonalcoholic fatty liver disease, and autoimmune liver and drug-induced hepatitis.

RESULTS: In both study periods, middle-aged males were predominant and were younger than females. In the first period (2014–2017), hepatitis C (33.5%) was the most prevalent cause of admission, followed by alcoholic liver disease (31.7%). In the second period (2019–2022), nonalcoholic fatty liver disease (38%) and alcoholic liver disease (27.6%) were the most frequent causes of admission. No changes were observed in the proportion of alcoholic liver disease or drug-induced hepatitis in both study periods. The prevalence of viral hepatitis decreased in both genders, with hepatitis C decreasing from 32.4 to 9.7% for males and 35.4 to 10.8% for females, and OR=0.2; 95%CI 0.1–0.3 for both males and females. Similarly, the prevalence of hepatitis B decreased from 19.1 to 8.1% and OR=0.3; 95%CI 0.2–0.5 for males and 8.2 to 3.7% and OR=0.4; 95%CI 0.1–0.9 for females. The prevalence of autoimmune liver diseases increased only in males, from 2.1 to 5.9% and OR=2.9; 95%CI 1.2–6.6.

CONCLUSION: Over the past 4 years, there has been a shift in hospital admission profile at a Brazilian Hepatology Unit, with a decrease in viral hepatitis and an increase in autoimmune diseases and nonalcoholic fatty liver disease. Males were more affected at younger ages than females. Furthermore, ascites was the most prevalent cause of complications in both periods analyzed.

KEYWORDS: Hepatitis. Patient admission. Liver failure.

INTRODUCTION

Chronic liver disease has become widespread globally[1,2], becoming the third leading cause of premature death in the United Kingdom. It is frequently diagnosed at a late stage when medical interventions are less effective, resulting in cirrhosis that may be complicated by ascites, gastroesophageal varices, encephalopathy, and hepatocellular carcinoma[3].

The profile of these diseases has changed considerably in the last decade[2]. Although the prevalence of chronic viral hepatitis has decreased due to effective curative regimens for hepatitis C, vaccine campaign, and safe and tolerable medications to suppress hepatitis B[4], the prevalence of nonalcoholic fatty liver disease (NAFLD) has significantly increased and is becoming one of the most common chronic liver diseases[2]. The worldwide increase in the prevalence of NAFLD can be explained by the increase in the prevalence of obesity, type 2 diabetes, and weight-related metabolic comorbidities[2].

Findings from a survey of 230,406 adult Americans indicated that 1.846 had chronic liver diseases implying that they are less likely to be employed, have higher health care expenses, and have impairment in all aspects of health-related quality of life[5]. This highlights the importance of preventive measures and public approaches for early detection and treatment of these diseases. Furthermore, as most of risk factors for liver diseases are modifiable, strategies to treat these diseases can be developed by understanding their prevalence and how they vary over time. An investigation of the causes and epidemiology of chronic liver diseases requiring hospital admission may be beneficial in developing strategies to manage these diseases.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 26, 2024. Accepted on February 04, 2024.

The aim of this study was to assess the epidemiology and clinical profile changes of chronic liver diseases, comparing two periods, in a Brazilian sample.

METHODS

This study was approved by the Institutional Research Ethics Committee under protocol number 2.115.702 and CAAE number 68121917.1.0000.0093. All participants signed an informed consent form.

This was a retrospective study conducted at the Hepatology Service of the Nossa Senhora das Graças Hospital (HNSG) in Curitiba, PR, Brazil, and utilized the service database. A list of all patients admitted to the Hepatology service between January 2014 and December 2017 and January 2019 and December 2022 was obtained from the HNSG. The authors used the hospital's electronic system to access all data of patients.

We included patients over the age of 18 years and had been diagnosed with viral hepatitis (types B or C), alcoholic disease, NAFLD, autoimmune liver diseases, and drug-induced hepatitis. The patients were hospitalized due to complications of the diseases such as ascites, gastrointestinal bleeding, hepatocarcinoma, encephalopathy, and renal dysfunction. Patients admitted to the hospital due to liver complications other than the ones previously mentioned were excluded from the study.

Epidemiological data such as gender and age, causes of chronic liver diseases, and complications that led to hospital admission were collected. To assess and predict the prognosis of patients with chronic decompensated liver disease, the Model End-Stage Liver Disease (MELD) was used, which provides a score based on laboratory parameters, such as serum creatinine, total bilirubin, and INR (international normalized ratio)[6].

The study retrospectively evaluated and compared two time periods: January 2014 to December 2017 and January 2019 to December 2022. The authors decided to exclude data from 2018 to create a temporal span that separates the two study periods solely for comparison purposes.

Statistical analysis

Nominal data was reported in percentages, whereas numerical data was reported as either mean and standard deviation (SD) or median and interquartile range (IQR), depending on the data distribution. To compare nominal data such as causes of chronic liver diseases, patients' gender, and number of transplants, the chi-squared and Fisher's exact tests were used. To compare numerical data such as patients' age and MELD, the unpaired t-test and Mann-Whitney U-test were used. All tests were performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA; www.graphpad.com). The adopted significance level was 5%.

RESULTS

A total of 1,310 patients were included in the study, out of which 435 were admitted between 2014 and 2017 and 875 between 2019 and 2022.

The total number of admissions increased in the second period (2019–2022), but the proportion of males and females remained the same (p=0.25).

The mean age of both male and female patients admitted during the 2014–2017 period was 61.6 with SD of 11.5 years, while the mean age of patients admitted during the 2019–2022 period was 63.2 (12.3) years (p=0.0006), indicating that patients were older during the latter period compared to the former. An age comparison by gender showed that no difference was found in females' ages between the two periods [63.9 (13.4) years in the first period vs. 64.5 (14.0) years in the second, p=0.33]. However, males were older in the second period compared to the first one [mean age of 60.3 (10.1) years vs. 62.4 (10.9) years, p=0.0002]. Females were older than males, p<0.0001 in both study periods.

Table 1 shows the primary causes of chronic liver diseases that required hospitalization as well as their distribution according to gender. Alcoholic liver disease was more prevalent in males during the two study periods, but its prevalence did not change from the first (2014–2017) to the second period (2019–2022). NAFLD was more prevalent in females over the two study periods, and its prevalence increased in both genders in the second period. Hepatitis B was more prevalent among males in the two times periods, and its prevalence decreased in the second period in both genders. Hepatitis C had an equal distribution across both genders and its prevalence decreased from period 1 to period 2 in both genders. Autoimmune hepatitis was more prevalent in females during both study periods, but its prevalence increased only in males from period 1 to period 2. Drug-induced hepatitis was evenly distributed in both genders, and its prevalence remained unchanged throughout the study period.

The median MELD value was 13 (IQR=10–18) in patients admitted during the 2014–2018 period and 15 (IQR=11–20) in those admitted during the 2019–2022 period (p<0.0001).

Table 2 shows that ascites was the most common cause of hospitalization in both study periods, with an increase in the second period compared to the first. Regarding gastrointestinal bleeding and hepatocellular carcinoma cases, a significant reduction was observed in the second period as compared to the first one.

Table 1. Most prevalent causes of hospital admissions in the Hepatology Unit by gender between 2014 and 2017 compared to 2019 and 2022.

	2014–2017				2019–2022				2014–2017 vs. 2019–2022: p-value and OR (95%CI)	
	Total	Male	Female	p (*) and OR 95%CI	Total	Male	Female	p (*) and OR (95%CI)	Male	Female
Alcohol	138 (31.7%)	111 (40.0%)	12 (7.5%)	<0.0001 OR=8.1 (4.4–15.8)	242 (27.6%)	209 (39.8%)	33 (9.4%)	<0.001 OR=2.7 (2.3–3.3)	0.94	0.50
NAFLD	91 (20.9%)	30 (10.8%)	61 (38.6%)	0.0001 OR=0.1 (0.1–0.3)	391 (44.6%)	200 (38.0%)	191 (54.5%)	<0.001 OR=0.5 (0.3–0.6)	<0.001; OR=5.6 (3.3–7.6)	<0.001 OR=1.9 (1.3–2.7)
Hepatitis B	66 (15.1%)	53 (19.1%)	13 (8.2%)	0.002 OR=2.6 (1.3–5.0)	56 (6.4%)	43 (8.1%)	13 (3.7%)	<0.001 OR=2.3 (1.2–4.3)	<0.001 OR=0.3 (0.2–0.5)	0.04 OR=0.4 (0.1–0.9)
Hepatitis C	146 (33.5%)	90 (32.4%)	56 (35.4%)	0.53	89 (10.1%)	51 (9.7%)	38 (10.8%)	0.30	<0.001 OR=0.2 (0.1–0.3)	<0.001 OR=0.2 (0.1–0.3)
Autoimmune diseases	27 (6.2%)	6 (2.1%)	21 (13.2%)	<0.0001 OR=0.1 (0.1–0.3)	89 (10.1%)	31 (5.9%)	58 (16.5%)	<0.001 OR=0.3 (0.1–0.4)	0.01 OR=2.9 (1.2–6.6)	0.34
Drug-induced	9 (2.0%)	6 (2.1%)	3 (1.8%)	0.99	8 (0.9%)	5 (0.9%)	3 (0.8%)	0.99	0.2	0.38
Total	435	277	158	–	875	525	350	–	–	–

(*) p-values refer to comparison between males and females; NAFLD: nonalcoholic fatty liver disease; OR: odds ratio; CI: confidence interval.

Table 2. Complications caused by chronic hepatic diseases among patients admitted between 2014 and 2017 (n=435) compared to 2019 and 2022 (n=875).

	2014–2017 (n=435)		2019–2022 (n=875)		p-value	OR–95%CI
	n	%	n	%		
Ascites	175	40.2	442	50.5	0.0004	1.5 (1.2–1.9)
Hepatic encephalopathy	145	33.3	231	26.4	0.09	1.4 (1.1–1.8)
Digestive hemorrhage	132	30.3	173	19.8	<0.0001	1.8 (1.4–2.3)
Hepatocellular carcinoma	139	32.0	73	8.3	<0.0001	5.2 (3.8–7.0)
Spontaneous bacterial peritonitis	33	7.6	9	1.0	<0.0001	7.9 (3.7–26.7)

OR: odds ratio; CI: confidence interval.

DISCUSSION

The results from the present study showed that hepatitis C followed by alcoholic liver disease were the most common hepatic-related causes of hospital admission in the period of 2014–2017, and alcoholic liver disease and NAFLD were the most common ones during the 2019–2022 study period. It was also found that the number of hospital admissions secondary to viral hepatitis and admissions associated with hepatic carcinoma decreased in the second study period, while the number of diagnoses for autoimmune liver diseases increased. The prevalence of alcoholic liver disease did not change between the two study periods. Moreover, males were not only more prevalent, but they were also more affected by chronic liver disease at a younger age than females.

Alcoholic liver diseases are one of the most common causes of chronic liver disease worldwide, and it is the most common cause of cirrhosis in Europe[7]. A literature review by Rehm et al.[8] in 2009 showed that alcohol was responsible for 3.8% of all deaths worldwide annually and for 4.6% of global disability-adjusted life-years. The treatment for alcoholic liver disease is challenging and the main therapeutic goals are abstinence and nutritional support[7]. Besides alcohol ingestion, genetic and environmental risk factors have a significant role in liver injury[7], and identifying risk factors is crucial to provide adequate

treatment and avoid the consequences of the established disease. Despite being a fully preventable disease, no changes in its prevalence were observed during the study periods, showing an urgent need for improvements in public health policy programs to manage this issue.

On the contrary, viral hepatitis associated with chronic liver disease has decreased substantially during both study periods. Considerable advances in antiviral therapy and access to effective vaccines are some of the measures associated with the change in the epidemiological scenario of these diseases[9]. Hepatitis B vaccination, introduced in 1980, has significantly reduced the prevalence of this infection[10]. In Brazil, the hepatitis B vaccine is available through the public health system for all non-vaccinated adults, and it is administered ordinarily in children aged 2, 4, and 6 months[11]. Although there is no vaccine for hepatitis C, efficient antiviral treatments are currently available[12]. However, since these infections can be asymptomatic or oligosymptomatic[10], they can go undiagnosed until the chronic consequences appear. Therefore, active surveillance is necessary to ensure early diagnosis. The World Alliance against Viral Hepatitis and the World Health Organization (WHO) are presently endorsing strategies to remove viral hepatitis as a public health concern, with the goal of reducing new cases by 90% and deaths from viral hepatitis by 65% by 2030[13].

Nonalcoholic fatty liver disease was one of the conditions in which the prevalence increased in the second study period. This is a condition closely related to obesity, insulin resistance, type 2 diabetes, and enhanced cardiovascular risk besides cirrhosis and hepatocarcinoma[13]. NAFLD is now considered as the hepatic manifestation of metabolic syndrome[13]. The rising prevalence of NAFLD has been associated with unhealthy diets, lack of physical activity, and obesity. Hence, lifestyle changes should be targeted to prevent NAFLD.

A growing prevalence of autoimmune liver diseases was also observed in the present study. These diseases are more common in females[14,15], and our findings are consistent with previous studies. However, this increase was only observed in males. The rising prevalence of this disease may be explained by two possible factors: the overall increase in autoimmunity, which has recently been observed, and the increasing rates of obesity. Obesity is associated with autoimmunity through an increase in pro-inflammatory cytokines (IL-6, TNF alpha, and IL-17) and more than 50 adipokines as well as changes in the expression of the apoptotic inhibitor of macrophages[16-18]. An additional explanation is that the medical community has increased awareness of this disease in the past 5 years[19].

The median MELD value in this study was 13 in the first quadrennium and 15 in the second, demonstrating a significant increase between the two time periods. Three laboratory parameters were used to calculate the MELD value: serum creatinine, total bilirubin, and INR. The final score has been used to predict the prognosis, in addition to being part of the liver allocation policy for transplants[20,21]. According to Glisic et al., the median value of MELD was 16.4, and this scale had a significant correlation with the presence of esophageal varices, therefore being relevant for screening patients with portal hypertension[22]. Thus, it is understood that the use of MELD may be relevant among patients with chronic decompensated liver disease, and its median value increased between the two study periods.

Regarding the complications observed, the prevalence of digestive bleeding and hepatocellular carcinoma were significantly reduced between the study periods, while hepatic encephalopathy remained unchanged in both periods. Baiges and Hernandez-Gea reported a prevalence of 25–35% of digestive bleeding among patients with cirrhosis[23]. It is worth recalling the high mortality rate associated with digestive bleeding, with a 24% risk of death in the first 6 weeks, even with gold standard therapy[24]. Despite the observed reduction, digestive bleeding remained one of the main complications in both periods.

There was an extreme reduction in the number of individuals admitted with hepatocellular carcinoma. This is the most prevalent type of cancer worldwide and its incidence is closely associated with advanced liver disease[25]. In Brazil, the estimated incidence of liver cancer for 2023–2025 period is 4.95/100,000 habitants, but its prevalence varies according to the Brazilian region, being more prevalent in the North, and less prevalent in the South where this study was conducted[25]. Although there are various causes of cirrhosis, the most common is viral hepatitis whose decrease may explain the observed shift in numbers[25].

A limitation of this study is that the data were collected from the hospital database, and only the most frequent causes of hospitalization for chronic hepatitis were requested. Furthermore, since the Brazilian Health System does not provide integration between primary and tertiary health systems, we did not have data about patients' follow-up. The study was conducted in a single center, which serves patients from both the public and private health systems. The data collected refers to the population of Southern Brazil, which has unique ethnic and environmental characteristics. Nevertheless, this study highlights findings from a real-life scenario of a Brazilian Hepatology Unit.

Additional studies on chronic liver diseases in the Brazilian population are recommended, addressing regional variations and establishing a national database that allows the Ministry of Health in developing improved prevention strategies for

these diseases, particularly NAFLD, whose prevalence has significantly increased worldwide.

This study examines the changes in hospital admission profiles at a Brazilian Hepatology Unit in the past 4 years, with a decrease in diseases associated with viral hepatitis and an increase in autoimmune diseases and NAFLD. Males were more affected at younger ages than females. Ascites was the most prevalent cause of complications leading to hospitalizations in both periods analyzed.

ETHICS APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Committee of Ethics in Research from Institution under protocol number 2.115.702.

TRANSPARENCY DECLARATION

The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

AUTHORS' CONTRIBUTIONS

BMI: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **LSC:** Data curation, Investigation, Methodology, Writing – review & editing. **CAPI:** Data curation, Investigation, Methodology, Formal Analysis, Writing – review & editing. **APJ:** Data curation, Writing – review & editing. **TLS:** Investigation, Methodology, Formal Analysis, Writing – review & editing. **RN:** Conceptualization, Formal Analysis, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231445

The impact of the coronavirus disease 2019 pandemic on the clinical presentation of tubal ectopic pregnancies: a retrospective cohort study

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SUMMARY

OBJECTIVE: We aimed to assess the impact of the coronavirus disease 2019 pandemic on the clinical presentation of tubal ectopic pregnancies.

METHODS: This retrospective cohort study was conducted at a tertiary center and included 76 cases of tubal ectopic pregnancies. The study period was divided into two groups: the pre-coronavirus disease group (January 2018 to February 2020, Group 1; n=47, 61.8%) and the coronavirus disease group (March 2020 to February 2022, Group 2; n=29, 38.2%). Subgroup analysis was also performed for tubal ruptured ectopic pregnancies as Group 1 (n=15, 62.5%) and Group 2 (n=9, 37.5%).

RESULTS: No statistically significant differences were observed between the pre-coronavirus disease and coronavirus disease groups in terms of demographic characteristics. Although the serum beta-human chorionic gonadotropin level was found to be higher in Group 2, the difference was not statistically significant (p=0.7). The groups appeared to be similar in treatment management, duration of hospitalization, and blood transfusion needs (p=0.3, p=0.6, and p=0.5, respectively). Additionally, no significant difference was observed between the groups in the evaluation of ruptured ectopic pregnancies (p=0.5). In the subgroup analysis of tubal ruptured ectopic pregnancies, no significant difference was observed.

CONCLUSION: To the best of our knowledge, there are few studies evaluating the effect of the pandemic on tubal ectopic pregnancies in the literature. Although we did not report statistically significant differences between groups in our study, given the potential prolonged duration of the pandemic, healthcare professionals should actively prompt their patients to seek necessary medical assistance.

KEYWORDS: COVID-19. Ectopic pregnancy. Pandemic.

INTRODUCTION

Ectopic pregnancy (EP) is defined as the occurrence of implantation outside the uterine cavity[1]. EPs account for 1–2% of all pregnancies, with nearly 95% of cases occurring in the fallopian tubes[1,2]. In the first trimester, tubal EPs can be diagnosed using serum beta-human chorionic gonadotropin (β-hCG) levels and transvaginal ultrasonography, allowing the detection of about 85% of asymptomatic cases[2]. Early gestational week tubal EPs are often managed with the methotrexate (MTX) regimen or expectant management. However, later gestational week tubal EPs require surgical intervention and cannot be treated expectantly or with MTX[3]. Delay or failure to diagnose tubal EPs promptly can lead to gynecological emergencies with abnormal vital signs, hypovolemic shock, and maternal hemorrhage[4]. Tubal ruptured ectopic pregnancies (rEPs) require immediate surgical intervention and account for about 15% of all EP cases[3]. Tubal rEPs are responsible for three-quarters of maternal deaths in the first trimester. Unfortunately, they are also accountable for about one-sixth of all pregnancy-related deaths[5]. Current surgical indications for EPs include failed MTX treatment, EPs with embryonic cardiac motion (ECM) on ultrasonography, recurrent EP in the same tube, and contraindications to medical or expectant treatment[4].

In late 2019, the coronavirus disease 2019 (COVID-19) pandemic originated in China and subsequently spread globally. In our country, the first case was reported in March 2020, leading to various restrictions to control transmission. However, these restrictions did not apply to gynecological, obstetric, or other medical emergencies. Despite this, some patients avoided seeking medical care during the pandemic due to concerns about COVID-19 transmission. Consequently, non-COVID-19 medical visits to hospitals significantly decreased during this period[6]. The incidence of molar pregnancy and the number of urgent surgical interventions for tubal rEP have increased during the pandemic[6,7]. This increase is thought to be due to delayed diagnosis. There are few studies evaluating the effect of the pandemic on tubal EPs in the literature[7-9]. However, there is no study in the literature evaluating the effect of the pandemic

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 30, 2023. Accepted on January 19, 2024.

on tubal rEPs. In this study, first, we aimed to assess the impact of the COVID-19 pandemic on the clinical presentation of tubal EPs. Second, our objective was to evaluate tubal rEPs.

METHODS

This was a retrospective cohort study conducted at a tertiary center. Institutional ethical approval was provided. Patients had signed informed consent forms, permitting their medical data to be utilized for scientific research, provided that their personal identifiers remain confidential. The study was conducted in accordance with the Helsinki Declaration Principles. Between January 2018 and February 2022, 85 EP patients were diagnosed, followed up, and treated in our clinic. Nine nontubal location EPs (pregnancy of unknown location; n=4, cervical; n=1, cesarean scar; n=1, cornual; n=3) were excluded from the study. The remaining tubal EPs (n=76) were included in the study. March 11, 2020, when the first case of COVID-19 was identified in our country, served as the dividing point between the groups. The cases were classified as pre-COVID (between January 2018 and February 2020, Group 1) and COVID (between March 2020 and February 2022, Group 2). Initially, tubal EPs, followed by tubal rEPs, were analyzed between the groups. The diagnosis and treatment of EPs were managed in accordance with the current literature[10]. The treatment protocol of our institute is as follows: (1) expectant management: stable vital signs, initial serum β-hCG <1,000 IU/L, and 15–20% decrease within 48 h; (2) MTX treatment: stable vital signs, serum β-hCG <5,000 IU/L, non-severe symptoms, and tubal mass size <35 mm, received a single IM dose (50 mg/m^2) of MTX on day 1. On day 7, if serum β-hCG level dropped ≥15% from day 4, the protocol was ceased. Additional MTX doses were administered if needed. (3) Elective surgical procedure: stable vital signs, serum β-hCG >5,000 IU/L, and ECM or tubal mass size ≥35 mm. (4) Urgent surgical procedure: abnormal vital signs, severe symptoms, and significant free fluid on ultrasonography.

Demographic characteristics, laboratory data, ultrasonographic findings, clinical symptoms, treatment methods, intraoperative findings, and follow-up data were obtained from hospital records. On the ultrasonography examination, the amount of free fluid filling the pelvis was accepted as increased. Intraoperative increased intrabdominal free fluid was defined as hemoperitoneum >1,000 mL. A drop in hemoglobin >2 g/dL or abnormal vital signs necessitated a blood transfusion.

Analyses were performed with SPSS version 26.0 (IBM Inc., Chicago, IL, USA). Normality analysis was performed according to the Kolmogorov-Smirnov test. Non-normally distributed parameters were analyzed with the Mann-Whitney U test. Quantitative data were presented as median (minimum–maximum), and qualitative data were presented as numbers and percentages (%). Chi-square and Fisher precision tests were used for the analysis of categorical data. The p-value considered statistically significant was <0.05.

RESULTS

Between January 2018 and February 2022, 85 EPs were diagnosed, followed up, and treated in our clinic. Nine nontubal location EPs (pregnancy of unknown location; n=4, cervical; n=1, cesarean scar; n=1, cornual; n=3) were excluded from the study. These nine EPs were in the COVID period group. The remaining tubal EPs (n=76) were analyzed. Between January 2018 and February 2022, 89.4% (76/85) of all EPs were tubal EPs. The rate of tubal EPs was 100% (47/47) in the pre-COVID period and 76% (29/38) in the COVID period. The cases were classified as pre-COVID (Group 1; n=47, 61.8%) and COVID (Group 2; n=29, 38.2%). Then, tubal rEPs (n=24) were analyzed between the groups. Tubal rEPs accounted for 31.5% (24/76) of the study cohort.

Table 1 presents the demographic characteristics of the study groups. The median age of study participants was 32.5 years. The median age of Group 1 was 32 years and Group 2 was 34 years (p=0.9). The median gravida and parity of the study participants were 2 and 1, respectively. The median gravida of Group 1 was 2 and Group 2 was 2 (p=0.9). The median parity of Group 1 was 0 and Group 2 was 1 (p=0.2). The groups did not exhibit a statistically significant difference in terms of abortion rates (48.9 vs. 34.5%, p=0.2). There was no significant association between the groups regarding previous EP occurrences (19.1 vs. 10.3%, p=0.2). The incidence of previous cesarean section, intrauterine device use, and smoking habit did not exhibit statistically significant differences between the two groups. No patient has received oral contraceptive pill medication. Although the previous gynecological operation rate of Group 2 was higher, this difference was not statistically significant (10.6 vs. 27.6%, p=0.05). There was no significant relationship between the groups in terms of either assisted conception or previous ultrasound (8.5 vs. 17.2%, p=0.2 and 70.2 vs. 69%, p=0.5, respectively). Both groups had statistically similar median gestational ages (6 vs. 6, p=0.4).

The clinical, laboratory, and ultrasonography findings of the groups are listed in Table 2. The groups were statistically similar in terms of abdominal pain and vaginal bleeding (76.6 vs. 58.6%, p=0.08 and 85.1 vs. 72.4%, p=0.1, respectively). The serum β-hCG level of Group 2 was found to be higher (2,261 vs. 2,450 IU/mL, p=0.7). The levels of hemoglobin

Table 1. Demographic characteristics of groups.[a,b]

	Group 1 n=47 (61.8%)	Group 2 n=29 (38.2%)	All cases n=76 (100%)	p-value
Maternal age (years)	32 (22–41)	34 (21–45)	32.5 (21–45)	0.9
Gravida	2 (1–10)	2 (1–6)	2 (1–10)	0.9
Parity	0 (0–3)	1 (0–4)	1 (0–4)	0.2
Previous abortion	23/47 (48.9%)	10/29 (34.5%)	33/76 (43.4%)	0.2
Previous ectopic pregnancy	9/47 (19.1%)	3/29 (10.3%)	12/76 (15.8%)	0.2
Previous C/S	13/47 (27.7%)	5/29 (17.2%)	18/76 (23.7%)	0.2
IUD	1/47 (2.1%)	0/29 (0%)	1/76 (1.3%)	0.6
OCP	0/47 (0%)	0/29 (0%)	0/76 (0%)	–
Smoking habits	4/47 (8.5%)	1/29 (3.4%)	5/76 (5.5%)	0.3
Previous gynecological operation	5/47 (10.6%)	8/29 (27.6%)	13/76 (17.1%)	0.05
Assisted reproduction	4/47 (8.5%)	5/29 (17.2%)	9/76 (11.8%)	0.2
Gestation age[c]	6 (4–9)	6 (5–9)	6 (4–9)	0.4
Prior ultrasound	33/47 (70.2%)	20/29 (69%)	53/76 (69.7%)	0.5

C/S: cesarean section, IUD: intrauterine device, OCP: oral contraceptive pills. [a]Values are given as numbers (percentage, %) unless stated otherwise. [b]Values are given as median (minimum–maximum) unless stated otherwise. [c]Based on menstrual dates.

Table 2. Clinical, laboratory, and ultrasonography findings of groups.[a,b]

	Group 1 n=47 (61.8%)	Group 2 n=29 (38.2%)	All cases n=76 (100%)	p-value
A) Clinical findings				
Abdominal pain	36/47 (76.6%)	17/29 (58.6%)	53/76 (69.7%)	0.08
Vaginal bleeding	40/47 (85.1%)	21/29 (72.4%)	61/76 (80.3%)	0.1
B) Laboratory at presentation				
β-hCG level (IU/mL)	2,261 (57–53,000)	2,450 (75–26,980)	2,301 (57–53,000)	0.7
Hemoglobin (g/dL)	11.9 (8.1–14.4)	11.8 (9.4–14.6)	11.8 (8.1–14.6)	0.7
C) Ultrasonography				
Embryonic cardiac motion	4/47 (8.5%)	0/29 (0%)	4/76 (5.3%)	0.1
Increased intraabdominal free fluid[c]	15/47 (31.9%)	9/29 (31%)	24/76 (31.6%)	0.5
D) Ruptured ectopic pregnancy	15/47 (31.9%)	9/29 (31%)	24/76 (31.6%)	0.5
E) Treatment management				0.3
Expectant	4/47 (8.5%)	4/29 (13.5%)	8/76 (10.5%)	0.4
Methotrexate	19/47 (40.4%)	9/29 (31%)	28/76 (36.8%)	0.4
Additional methotrexate dose requirements	5/47 (10.6%)	3/29 (10.3%)	8/76 (10.5%)	0.9
Laparoscopic salpingectomy	12/47 (25.5%)	12/29 (41.4%)	24/76 (31.6%)	0.1
Laparatomic salpingectomy	7/47 (14.9%)	1/29 (3.4%)	8/76 (10.5%)	0.1
Methotrexate failure	1/19 (5.3%)	2/9 (22.5%)	3/28 (10.7%)	0.1
F) Hospitalization (days)	2 (1–7)	1 (1–14)	2 (1–14)	0.6
G) Blood transfusion needs	7/47 (14.9%)	5/29 (17.2%)	12/76 (15.8%)	0.5

[a]Values are given as numbers (percentage, %) unless stated otherwise. [b]Values are given as median (minimum–maximum) unless stated otherwise. [c]Estimated by the physician during the ultrasound examination.

were similar in both groups (11.9 vs. 11.8 g/dL, p=0.7). Regarding ECM, it was detected in 8.5% of the patients in Group 1, while no ECM was detected in Group 2 (p=0.1). The presence of increased intraabdominal free fluid showed no statistically significant difference between the two groups (31.9 vs. 31%, p=0.5). In the evaluation of the rEPs, no differences were observed between the groups (31.9 vs. 31%, p=0.5). The treatment management was statistically similar between the two groups (p=0.3). Expectant treatment is more prevalent in Group 2 (8.5 vs. 13.5%, p=0.4). MTX and additional MTX dose requirements are higher in Group 1 (40.4 vs. 31%; p=0.4 and 10.6 vs. 10.3%; p=0.9, respectively). Laparoscopic salpingectomy was higher in Group 2, while laparatomic salpingectomy was higher in Group 1 (25.5 vs. 41.4%; p=0.1 and 14.9 vs. 3.4%; p=0.1, respectively). MTX failure was detected in one patient in Group 1 and two patients in Group 2 (5.3 vs. 22.2%; p=0.1). In these cases, tubal rupture occurred and urgent laparoscopic salpingectomy was performed. The groups showed no statistically significant difference in terms of hospitalization (2.2 vs. 2.6%, p=0.6) and the need for blood transfusion (14.9 vs. 17.2%, p=0.5).

Table 3 shows the comparison of the groups who underwent urgent surgery for tubal rEPs. Laparoscopic salpingectomy was performed in eight patients (53.3%) in Group 1 and seven patients (89%) in Group 2. Laparatomic salpingectomy was performed in seven patients (46.7%) in Group 1 and one patient (11%) in Group 2. The difference in surgical management between the groups was not statistically significant (p=0.08). The observed difference in serum β-hCG levels between Group 1 and Group 2 was not statistically significant (3,621 vs. 8,888 IU/mL, p=0.2). Higher preoperative hemoglobin levels were observed in Group 2 (11.8 vs. 12.6 g/dL, p=0.2), while postoperative hemoglobin levels were lower in Group 1 (8.3 vs. 9 g/dL, p=0.2). The postoperatively evaluated hemoglobin decrease was higher in Group 2 (1.3 vs. 2.8 g/dL, p=0.08). Increased abdominal free fluid on ultrasonography and intraoperative free fluid was detected in all cases of both groups. The groups were statistically similar in terms of hospitalization and blood transfusion need (3 vs. 3, p=0.8; 46.6 vs. 44.4%, p=0.6, respectively).

DISCUSSION

In this retrospective cohort study, our primary objective was to assess the impact of the COVID-19 pandemic on the clinical presentation of tubal EPs. The secondary objective was to evaluate tubal rEPs. The study findings revealed that the pandemic did not have a statistically significant effect on the management and incidence of tubal EPs and rEPs.

Approximately 95% of EPs are localized in the tuba uterina[3]. In our study, between January 2018 and February 2022, 89.4% of all EPs were tubal. Aiob et al. reported tubal EPs were 89.8% of all EPs in the pre-COVID period[8]. This incidence was 89.4% in the COVID period[8]. We found that the rate of tubal EPs was 100% in the pre-COVID period and 76% in the COVID period.

Table 3. Comparison of groups who underwent urgent surgery for ruptured tubal ectopic pregnancies.[a,b]

	Group 1 n=15 (62.5%)	Group 2 n=9 (37.5%)	All cases n=24 (100%)	p-value
A) Surgery management				0.08
Laparoscopic salpingectomy	8/15 (53.3%)	8/9 (89%)	16/24 (66.6%)	
Laparatomic salpingectomy	7/15 (46.7%)	1/9 (11%)	8/24 (33.3%)	
B) Laboratory findings				
β - hCG level (IU/mL)	3,621 (57–53,000)	8,888 (152–26,980)	4,186 (57–53,000)	0.2
Preoperative hemoglobin (g/dL)	11.8 (8.1–13.4)	12.6 (9.4–13.2)	12 (8.1–13.4)	0.2
Postoperative hemoglobin (g/dL)	8.3 (6.1–12.2)	9 (6.6–12.1)	8.7 (6.1–12.2)	0.2
Hemoglobin drop (g/dL)	1.3 (0.6–5.7)	2.8 (0.6–5.7)	2.1 (0.6–5.7)	0.08
C) Ultrasonography findings				
Increased abdominal free fluid[c]	15/15 (100%)	9/9 (100%)	24/24 (100%)	–
D) Intraoperative free fluid[d]	15/15 (100%)	9/9 (100%)	24/24 (100%)	–
E) Hospitalization (days)	3 (2–4)	3 (2–14)	3 (2–14)	0.8
F) Blood transfusion needs	7/15 (46.6%)	4/9 (44.4%)	11/24 (45.8)	0.6

[a]Values are given as numbers (percentage, %) unless stated otherwise. [b]Values are given as median (minimum–maximum) unless stated otherwise. [c]Estimated by the physician during the ultrasound examination. [d]Intraoperative observation by the surgeon of free fluid filling at least the Douglas pouch.

These results align with the existing literature and indicate that tubal EPs remain the most common type, with a slight insignificant decrease observed during the COVID period.

In the reviewed studies, no statistically significant differences were observed between the prepandemic and pandemic groups concerning demographic characteristics, including maternal age, gravida, parity, and previous cesarean section[7-9]. Our study findings were consistent with these reports.

Barg et al. investigated the effect of the pandemic on EPs[7]. They have found no difference between the groups in gestational week[7]. On the contrary, Aiob et al. reported that the gestational week of EPs was approximately 1 week higher in the pandemic period[8]. This difference was statistically significant. In our study, the gestational weeks of both groups were similar.

Throughout our research, we observed a relatively higher level of serum β-hCG at the initial visit in Group 2. Nevertheless, it is noteworthy to mention that this disparity was not statistically significant. These findings are in accordance with other studies that have also reported higher serum β-hCG levels in EPs during the pandemic period[7-9]. Furthermore, Barg et al. made a noteworthy observation regarding the serum β-hCG levels in the COVID group, which were found to be twice as high as those in the pre-COVID group[7]. The researchers reported that this difference was statistically significant.

In their study, Aiob et al. compared the complaints of EPs between the prepandemic and pandemic periods[8]. They found that abdominal pain exhibited a statistically significant difference between the prepandemic and pandemic groups, with the pandemic group having a 1.6 times higher prevalence of abdominal pain. Similarly, the pandemic group showed a higher rate of vaginal bleeding symptoms. However, this finding was not statistically significant. On the contrary, our study yielded different results, indicating that these symptoms were actually lower in the COVID group. Despite these differences in symptom prevalence, the overall comparison between the two groups showed no statistically significant differences, suggesting that the COVID period did not significantly influence the occurrence of these specific symptoms in our cohort of patients with tubal EPs.

Barg et al. have revealed notable differences in the management and outcomes of tubal EPs between the pre-COVID and COVID periods[7]. Expectant management was twofold more common in the pre-COVID period, whereas MTX treatment was administered at similar rates between the groups. However, MTX failure was threefold higher in the COVID group. Additionally, the COVID group showed a 1.3-fold increase in the number of elective and urgent laparoscopic surgeries. Tubal rEPs were also three times more common in the COVID group. All of these differences in treatment approaches and surgical interventions were statistically significant.

Aiob et al. reported that there was no statistically significant difference between the groups concerning expectant and MTX management[8]. This indicates that both the pandemic and prepandemic groups received similar treatment approaches for their condition, with no significant variation in management strategies between the two groups according to their study findings[8]. Although ruptured EPs were more common in the pandemic group, they occurred at a lower rate. Nonsurgical management was similar in both groups. In the pandemic group, the count of urgent laparoscopies was found to be twice as high as that in the prepandemic group. Conversely, the prepandemic group had three times more elective laparoscopies than the pandemic group. These findings indicate a notable difference in the urgency and timing of laparoscopic procedures between the two groups during the study period. These differences were found to be statistically significant. In our study, we did not observe any significant difference between the groups in the evaluation of rEPs. The findings indicate that both groups showed similar rEP patterns, suggesting no distinct impact of the COVID period on rEPs in comparison with the pre-COVID period. Additionally, the treatment management was similar between the two groups. Expectant management was higher in Group 2. MTX treatment and additional MTX dose requirements were higher in Group 1. Laparoscopic salpingectomy was higher in Group 2, while laparatomic salpingectomy was higher in Group 1. MTX failure was 4.2 times more likely in Group 2, which was higher than the rate reported in the literature. However, the difference was not statistically significant. In these cases, tubal rupture had occurred and urgent laparoscopic salpingectomy was performed. These findings highlight the importance of appropriate and timely interventions in managing tubal EPs, particularly when faced with potential treatment challenges during the COVID period. Dvash et al. have reported that tubal rEPs were twofold higher in the pandemic group[9]. This difference was statistically significant. In their analysis, the researchers found that no rEPs were detected in women who became pregnant after undergoing assisted reproduction. The researchers proposed another factor contributing to the absence of rupture in such patients: frequent medical observation and high patient awareness. This vigilance and proactive approach facilitated early detection and streamlined treatment protocols, potentially mitigating the risk of rupture and improving patient outcomes. Serum β-hCG levels of the pandemic group were twice that of the prepandemic group. However, there was no statistically significant difference between the groups. Both groups were similar in terms of non-surgical and surgical management.

Interestingly, the duration of hospitalization was longer and the amount of intraabdominal free fluid observed preoperatively was higher in the prepandemic group. In our study, the incidences of rEP were similar between groups. Although serum β-hCG levels were higher in Group 2, there was no statistical difference between the groups. Both groups were similar in terms of assisted reproduction. The tubal rupture status of these patients was not evaluated separately. The duration of hospitalization was longer in Group 2. Group 2 had higher preoperative hemoglobin levels and lower postoperative hemoglobin levels. Postoperatively evaluated hemoglobin drop was higher in Group 2. The groups were similar in terms of blood transfusion needs. However, these data were not statistically different. As reported in tubal rEP analysis, laparoscopic salpingectomy was performed in eight patients in Group 1 and seven patients in Group 2. Laparatomic salpingectomy was performed in seven patients in Group 1 and one patient in Group 2. There was no statistically significant difference between the groups in terms of surgical management. In our clinic, we prefer the laparoscopic method as the current approach. Exceptionally, hemodynamically unstable patients with diffuse intraabdominal free fluid on ultrasonography may undergo urgent laparotomy.

The retrospective design is a limitation of our study. Vaccinations during the pandemic period were not examined. Another limitation of our study is the small population size. The strength of our study is that it includes data from a single-center tertiary hospital. In addition, especially during the pandemic period, our clinic served as a reference center.

In conclusion, to the best of our knowledge, there are few studies evaluating the effect of the pandemic on tubal EPs in the literature. Unlike other studies, we performed a detailed subgroup analysis specifically focusing on tubal rEPs. Performing a detailed subgroup analysis, specifically focusing on tubal rEPs, can indeed yield more precise and valuable insights into the impact of the pandemic on this specific subgroup of patients.

COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed were in accordance with the ethical standards of the institutional committee (registration number 2022/04-20) and with the Helsinki Declaration and its later amendments or comparable ethical standards.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231464

Hospital cohort study on survival predictors for intubated coronavirus disease 2019 patients

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SUMMARY

OBJECTIVE: The objective of this study was to assess the predictors of survival among patients with coronavirus disease 2019 who underwent tracheal intubation, as part of a hospital cohort study.

METHODS: This retrospective cohort study in the Rio do Sul County Hospital, Santa Catarina, Brazil, from April 2020 to May 2021, focused on patients aged 18 years or older intubated for coronavirus disease 2019. We assessed the 90-day survival of intubated patients by estimating the hazard ratio using a Cox proportional hazards regression model.

RESULTS: The study included 132 participants, with an average age of approximately 60 years. Tracheal intubation was successfully accomplished in 97% of cases within two attempts. The overall mortality rate was 62.9%. Notably, mortality rates were significantly higher in patients aged over 60 years (hazard ratio=2.57; 95%CI 1.54–4.29; p<0.001), those with blood oxygen saturation below 85% (hazard ratio=1.92; 95%CI 1.03–3.57; p=0.04), instances where tracheal intubation was carried out using a conventional laryngoscope (hazard ratio=2.59; 95%CI 1.22–5.48; p=0.013), and when performed by emergency physicians (hazard ratio=3.96; 95%CI 1.51–10.4; p=0.005).

CONCLUSION: Our analysis unveiled that the risk of death in intubated coronavirus disease 2019 patients is four times higher when an emergency physician, as opposed to an anesthesiologist, leads the tracheal intubation team.

KEYWORDS: Airway management. COVID-19. Critical care. Intubation. Pneumonia. Respiratory distress syndrome.

INTRODUCTION

During the 2020–2021 coronavirus disease 2019 (COVID-19) pandemic, the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in severe respiratory disease. Many affected areas lacked essential resources and infrastructure, leading to increased patient mortality. The initial World Health Organization data revealed hospitalization rates of 10–15%, with 39% requiring intensive care and 23% needing tracheal intubation and mechanical ventilation[1]. Despite standard protocols, global mortality of patients on mechanical ventilation ranged from 30 to 80%[2,3].

Coronavirus disease 2019 patients who needed tracheal intubation were severely ill and at risk of cardiovascular collapse[4]. Tracheal intubation was often performed rapidly, without prior face mask ventilation to prevent contamination, leading to quick desaturation and worsened conditions. These challenges complicated COVID-19 patient tracheal intubation[5,6].

Worldwide healthcare had to adapt protocols for dealing with COVID-19. Specialized tracheal intubation teams were formed, including the Catarina Intubation Team in the Regional Hospital of Rio do Sul County, Brazil, in March 2020. The goal of this hospital cohort study was to assess the predictors of survival among COVID-19 patients who underwent tracheal intubation.

METHODS

This single-center cohort study occurred at High Itajai River Valley Hospital in Rio do Sul County, Brazil. The Research Ethics Committee of the University for the Development of the High Itajai River Valley granted an exemption from the requirement for patient consent forms for this observational study, which relied on data from information systems. The study received approval on May 28, 2021, with final approval n. 4741418. Eligible participants were adults (≥18 years) with positive COVID-19 tests

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 02, 2023. Accepted on December 16, 2023.

requiring tracheal intubation from April 2020 to May 2021, excluding COVID-negative patients and those transferred to other hospitals. Survival analysis extended to 90 days from the tracheal intubation date. Our intubation team comprised 17 volunteers, with shifts covering all days of the week, including anesthesiologists, intensivists, anesthesiology residents, and emergency physicians.

Airway management adhered to a COVID-19-specific tracheal intubation protocol, guided by national and international guidelines[5-7]. All members underwent practical training in protocol implementation, airway management, and personal protective equipment use. The team followed institutional procedures, with a group established for debriefing and error correction. Both conventional laryngoscopes and videolaryngoscopes (McGRATH™ MAC—Aircraft Medical LTD., Edinburgh, UK) were employed for tracheal intubation using prepared kits with essential materials, medications, and personal protective equipment. These kits were strategically placed in hospital wards, the emergency department, and the intensive care unit (ICU) COVID-19 sector. Outcomes were documented in medical records and on a form completed by team members. Information on length of stay and survival was extracted from patients' medical records.

To estimate hazard ratios (HR), Cox proportional hazard regression models were used for statistical analysis. In the initial phase, the model was applied to each category of explanatory factors, including demographic data (block 1), comorbidities (block 2), symptoms (block 3), and factors related to tracheal intubation (block 4). The model then adjusted each patient's length of hospital stay based on the outcome (death or discharge) in relation to these factors. The predicted outcome provided an estimate of the risk of death in relation to the duration of hospitalization, accounting for each factor. A 5% significance level was set for two-tailed Wald tests, and statistically significant factors were incorporated into a final multiple regression model.

We scrutinized the statistically significant variables while considering sample adequacy and the statistical power provided for hypothesis testing. To calculate the required number of deaths, we used the formula suggested by Schoenfeld[8], which considers the study's specifics:

$$necessary\ deaths = \frac{(z_\beta + z_{1-\alpha})^2}{(Prop_E Prop_{NE} log_e^2 \Delta)}$$

where, to achieve a power of 80% at a one-tailed significance level of 5% (Z_β =0.841 and $Z_{1-\alpha}$=1.645), it becomes essential to determine the minimum number of observed deaths for each group of exposed individuals ($Prop_E$), unexposed individuals ($Prop_{NE}$), and log-natural scale regression coefficient ($log_e^2 \Delta$).

Complementary analyses included sensitivity, specificity, and accuracy for difficult airway prediction vs. confirmation using chi-square testing (p<0.05).

RESULTS

A cohort of 132 participants with an average age of around 60 years was recruited for the study. The majority were male, and the predominant ethnicity was Caucasian. Prevalent comorbidities included systemic arterial hypertension, diabetes mellitus, obesity, and heart disease. Participants exhibited various symptoms, with cough, dyspnea, and fever being the most frequently reported (Table 1).

Table 1. Coronavirus disease 2019 patient data, Rio do Sul County, Brazil, April 2020 to May 2021.

Demographic data, comorbidities, and symptoms	Mean (SD), median (IQR), or n (%) (n=132)
Age	Mean 59 (SD 13)
Sex	
Male	79 (59.8%)
Female	53 (40.2%)
Race	
White	126 (95.5%)
Other	6 (4.5%)
Comorbidities	117 (88.6%)
Obesity	44 (33.3%)
Systemic arterial hypertension	78 (59.1%)
Diabetes mellitus	46 (34.8%)
Thyroid disease	9 (6.8%)
Chronic obstructive pulmonary disease	8 (6.1%)
Asthma	7 (5.3%)
Heart disease	17 (12.9%)
Nephropathy	5 (3.8%)
Neurological disease	10 (7.6%)
Psychiatric disease	11 (8.3%)
Cancer	8 (6.1%)
Other	41 (31.3%)
Symptoms	
Dyspnea	88 (66.7%)
Cough	100 (75.8%)
Fever	62 (47%)
Coryza	25 (18.9%)
Odynophagia	13 (9.8%)
Fatigue	57 (43.2%)
Myalgia	48 (36.4%)
Smell or taste disorder	16 (12.1%)
Chest pain	3 (2.3%)
Headache	28 (21.2%)
Gastrointestinal disorder	19 (14.4%)
Other	5 (3.8%)
O_2 saturation (SaO_2)	
<85%	36 (27.3%)
85–90%	53 (40.2%)
>90%	43 (32.6%)
Days of symptoms	Median 11 (IQR 7)

All 132 patients experienced respiratory failure requiring tracheal intubation. Successful intubation rates were 91.7% on the first attempt and 97% on the second attempt. Predictions of difficult airways were made in 14.4% and confirmed in 8.3%, with moderate sensitivity (55%) and high specificity (89%). The prediction's accuracy was 86% (p=0.0004), highlighting its reliability in anticipating difficult airways. However, the study's unfortunate high mortality rate was evident in the 90-day follow-up, with 83 deaths (62.9%) and 49 discharges indicating recovery (27.1%). For further insights, Table 2 details the factors associated with tracheal intubation.

In our analysis of adjusted Cox proportional hazards regression models, we observed a fourfold increase in the risk of death when tracheal intubation was performed by emergency physicians (HR=3.96, 95%CI 1.51–10.4; p=0.005), with similar findings for conventional laryngoscope use (HR=2.59, 95%CI 1.22–5.48; p=0.013), patient age over 60 years (HR=2.57, 95%CI 1.54–4.29; p<0.001), and severe hypoxemia (HR=1.92, 95%CI 1.03–3.57; p=0.04). These results maintain statistical power due to sufficient sample size (Table 3).

Table 2. Tracheal intubation associated data, Rio do Sul County, Brazil, April 2020 to May 2021.

Factors related to tracheal intubation	n (%)
Intubation team leader	
Anesthesiologist	93 (70.5%)
Intensivist	9 (6.8%)
Emergency physician	9 (6.8%)
Anesthesiology resident	21 (15.9%)
Intubation environment	
Emergency department	85 (64.4%)
Intensive care unit	47 (35.6%)
Number attempt count	
1	121 (91.7%)
2	7 (5.3%)
3	3 (2.3%)
4 or more	1 (0.8%)
Support staff	
Physician	62 (47%)
Nurse	64 (48.5%)
Nursing technician	4 (3%)
Physiotherapist	2 (1.5%)
Pharmaceuticals used in intubation	
Ketamine + rocuronium	108 (81.8%)
Etomidate + alfentanil + rocuronium	18 (13.6%)
Etomidate + rocuronium	6 (4.5%)
Laryngoscope variety	
Videolaryngoscope	20 (15.2%)
Conventional laryngoscope	112 (84.8%)

Discussion

In this context, our performance met expectations, showcasing a high success rate within one or two attempts for tracheal intubations. The research reveals a moderate sensitivity and noteworthy specificity, underscoring the substantial accuracy attainable through difficult airway prediction. The study emphasizes advanced age and severe hypoxemia as independent and cumulative mortality factors in COVID-19 patients. Moreover, our findings suggest reduced survival rates among patients intubated by emergency physicians.

The surge in COVID-19-related respiratory failure has strained global healthcare providers. Ensuring successful, efficient endotracheal intubation is crucial, with specialized teams offering better outcomes. Patient survival depends on factors such as age and pathology severity, while equipment and clinician skill also influence results. Intubation teams have demonstrated proficiency in COVID-19 patient intubations, achieving initial success rates between 85 and 92%, which increased from 97 to 98% upon the second attempt[9-11]. In their study on COVID-19 patients, Zheng et al. reported intubation success rates using videolaryngoscopes ranging from 89 to 94% on the first attempt and 80 to 100% on the second attempt[12]. Lower success rates were observed with conventional laryngoscope, at 70% on the first attempt and 83% on the second attempt[12]. Conversely, Wong et al. observed no variation in intubation success rates on the first attempt between the two equipment types[13].

The American Society of Anesthesiologists' guidelines advise conducting a pre-airway management risk assessment for difficult airway situations[14]. In a meta-analysis of 50,760 patients, the prediction of a difficult airway demonstrated low to moderate sensitivity (20–62%) and moderate to high specificity (82–97)[15]. Similarly, Norskow et al. observed a notably higher specificity compared to sensitivity in difficult airway prediction[16].

Several studies have consistently reported increased mortality among elderly COVID-19 patients with severe hypoxemia[17-21]. Increased mortality among older patients can be linked to age-associated immune responses, culminating in reduced effectiveness and heightened inflammation[19,21]. Hypoxemia is intricately tied to an inflammatory response. Patients afflicted by severe hypoxemia in the context of COVID-19 exhibit elevated levels of proinflammatory cytokines, lung injury, and acute respiratory distress syndrome[20,21]. Tang et al. observed higher mortality with intubations by emergency physicians compared with anesthesiologists[22].

Table 3. Factors linked to coronavirus disease 2019 mortality post-intubation as derived from Cox proportional hazards models with adjustments, Rio do Sul County, Brazil, April 2020 to May 2021.

	Adjusted hazard ratios (95%CI)	p (Wald's test)	Adequate sample size—80% power and 95% significance level
Intubation environment			
Intensive care unit			
Emergency department	1.74 (0.99–3.07)	0.055	No
Intubation team leader			
Anesthesiologist			
Intensivist	0.97 (0.33–2.89)	0.963	
Anesthesiology resident	0.93 (0.45–1.95)	0.852	
Emergency physicians	3.96 (1.51–10.4)	0.005	Yes
Laryngoscope variety			
Videolaryngoscope			
Conventional laryngoscope	2.59 (1.22–5.48)	0.013	Yes
Neurological disease	1.96 (0.88–4.41)	0.102	No
Age			
≤60 years			
>60 years	2.57 (1.54–4.29)	<0.001	Yes
O_2 saturation			
>95%			
85–90%	0.94 (0.51–1.75)	0.857	
<85%	1.92 (1.03–3.57)	0.04	Yes

This study has notable limitations, including its observational nature, absence of a comparator group, and being conducted at a single center.

CONCLUSION

In our study, mortality was four times higher when tracheal intubation was conducted by emergency physicians compared with cases where an anesthesiologist served as the intubation team leader. This adjustment accounts for confounding factors, including intubation location, comorbidities, patient age, and disease severity.

AUTHORS' CONTRIBUTIONS

FJD: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft. **PSSD:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft. **LCS:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Visualization, Writing – original draft. **EKB:** Conceptualization, Investigation, Methodology, Visualization, Writing – original draft. **ESS:** Conceptualization, Investigation, Methodology, Visualization, Writing – original draft. **MJCG:** Conceptualization, Investigation, Methodology, Visualization, Writing – original draft. **GJS:** Conceptualization, Investigation, Methodology, Visualization, Writing – original draft. **FRR:** Conceptualization, Formal Analysis, Investigation, Software, Writing – original draft. **GZL:** Conceptualization, Formal Analysis, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231499

Evaluation of the relationship between end-tidal carbon dioxide level and heart failure classification

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SUMMARY

OBJECTIVE: Heart failure is a disease with cardiac dysfunction, and its morbidity and mortality are associated with the degree of dysfunction. The New York Heart Association classifies the heart failure stages based on the severity of symptoms and physical activity. End-tidal carbon dioxide refers to the level of carbon dioxide that a person exhales with each breath. End-tidal carbon dioxide levels can be used in many clinical conditions such as heart failure, asthma, and chronic obstructive pulmonary disease. The aim of the study was to reveal the relationship between end-tidal carbon dioxide levels and the New York Heart Association classification of heart failure stages.

METHODS: This study was conducted at Kahramanmaraş Sütçü İmam University Faculty of Medicine Adult Emergency Department between 01/03/2019 and 01/09/2019. A total of 80 patients who presented to the emergency department with a history of heart failure or were diagnosed with heart failure during admission were grouped according to the New York Heart Association classification of heart failure stages. The laboratory parameters, ejection fraction values, and end-tidal carbon dioxide levels of the patients were measured and recorded in the study forms.

RESULTS: End-tidal carbon dioxide levels and ejection fraction values were found to be significantly lower in the stage 4 group compared to the other groups. Furthermore, pro-B-type natriuretic peptide (BNP) values were found to be significantly higher in stage 4 group compared to the other groups.

CONCLUSION: It was concluded that end-tidal carbon dioxide levels could be used together with pro-BNP and ejection fraction values in determining the severity of heart failure.

KEYWORDS: Emergency medicine. Heart failure. Capnography.

INTRODUCTION

Heart failure (HF) is clinically defined as a clinical syndrome in which patients experience typical symptoms such as dyspnea, swelling of the legs, fatigue, and lung rales. Additionally, the cardiac apex beat is displaced, and there is an increase in jugular venous distension during inspection[1].

There are different classifications of heart failure, such as systolic/diastolic heart failure, acute/chronic heart failure, right/left heart failure, and high/low output heart failure[2]. The most commonly used classification system is the one established by the New York Heart Association (NYHA), which evaluates the effect of HF on the patient's physical capacity[1].

The change in carbon dioxide (CO_2) level over time can be evaluated by CO_2 waveform or capnography. End-tidal carbon dioxide ($EtCO_2$) is the level of CO_2 that a person exhales with each breath[3] and can be used to determine the prognosis of HF patients, along with the NYHA classification and left ventricular ejection fraction (EF)[4].

The aim of this study was to reveal the relationship between the level of $EtCO_2$, which is considered to be effective in the clinical course and prognosis of HF, and the NYHA classification of heart failure.

METHODS

In this study, patients who presented to the adult emergency department between March 01, 2019 and September 01, 2019 with a history of HF or a diagnosis of HF at admission were grouped according to NYHA stages. A total of 80 patients, including 20 patients with NYHA stage 1, 20 patients with stage 2, 20 patients with stage 3, and 20 patients with stage 4, were included in the study. Ethical approval was obtained from the Ministry of Health of the Republic of Turkey and the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with resolution number 23 in session 2019/03 on February 20, 2019.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on November 01, 2023. Accepted on January 05, 2024.

Written informed consent was obtained from all patients included in our study.

Voluntary patients older than 18 years of age who had a diagnosis of HF or were newly diagnosed with HF were included in the study. Those with pathologies that may affect $EtCO_2$ values such as sepsis and septic shock, hemorrhagic shock, pulmonary embolism, pneumothorax, renal failure, acute cerebrovascular accident, chronic obstructive pulmonary disease (COPD), asthma, drug intoxication, and metabolic acidosis–alkalosis were excluded from the study. Patients' demographic data, medical history, and sampling dates were recorded on the previously prepared study forms.

N-terminus pro-B-type natriuretic peptide (NT pro-BNP) analysis was evaluated with the AQT 90 flex (Radiometer Medical Aps, Bronshoj, Denmark) device. $EtCO_2$ levels of the patients were measured by the sidestream method using the Capnostream® 20 (Oridion, Jerusalem, Israel) monitor and disposable nasal cannula. The echocardiographic examinations of the patients included in the study were performed by cardiology clinic physicians using the GE Vivid S5 (General Electric Ving Med Systems, Horten, Norway) brand device. The EF values were calculated using the modified Simpson method.

In the evaluation of the data, the conformity of the variables to the normal distribution was examined by the Shapiro-Wilk test. Group comparisons of normally distributed variables were analyzed by one-way analysis of variance. Tukey HSD, one of the multiple comparison tests (post hoc), was used. Group comparisons in non-normally distributed variables were analyzed using the Kruskal-Wallis H test. Multiple comparisons were analyzed by the Dunn-Sidak test and Bonferroni test. The relationship between group distributions in categorical variables was examined by chi-square test and exact test. Statistical significance was considered $p<0.05$. The data were evaluated in the IBM SPSS version 22 program.

RESULTS

A total of 80 patients who had a diagnosis of heart failure or were newly diagnosed with heart failure, consisting of 20 patients each from stage 1, stage 2, stage 3, and stage 4 according to the NYHA classification, were included in our study. Blood tests were taken from each patient included in the study, echocardiography was performed by the cardiology department, and the $EtCO_2$ values were measured. Patients consisted of 52 (65%) males and 28 (35%) females.

The mean age was found to be 62.75 years for the patients in the stage 1 group, 68.90 years for the patients in the stage 2 group, 70.90 years for the patients in the stage 3 group, and 73.50 years for the patients in the stage 4 group. The mean age of the stage 4 group was significantly higher compared to the stage 1 group, which was found to be statistically significant ($p<0.05$). When the medical histories of the patients were examined, 28 (35%) of them had diabetes mellitus, 55 (68.75%) had hypertension, and 56 (70%) had coronary artery disease. No significant difference was found between the groups in terms of hypertension, coronary artery disease (CAD), diabetes mellitus (DM), and malignancy. The distribution of age and comorbidities by stages is presented in Table 1.

When the NT pro-BNP values were examined, the pro-BNP values in the stage 2 and stage 4 groups were found to be significantly higher compared to the stage 1 and stage 3 groups ($p=0.001$).

When the EF (%) of the patients included in the study was evaluated, the mean EF (%) values of the patients within the group were found to be 40.75% in the stage 1 group, 34.50% in the stage 2 group, 33.15% in the stage 3 group, and 30.25% in the stage 4 group. The EF values were found to be statistically significantly lower in the stage 4 group compared to the stage 1 group ($p=0.013$).

When the groups were examined in terms of $EtCO_2$ values, the mean $EtCO_2$ values of the patients within the group

Table 1. Distribution of age and comorbidities by stages.

			Stage				p
			1	2	3	4	
Age		Mean±SD	62.75±13.31[4]	68.90±7.88	70.90±11.49	73.50±14.76[1]	0.043*
Diabetes mellitus	No	n (%)	13 (65,0)	11 (55,0)	13 (65,0)	15 (75,0)	0.624
	Yes	n (%)	7 (35,0)	9 (45,0)	7 (35,0)	5 (25,0)	
Hypertension	No	n (%)	10 (50,0)	4 (20,0)	6 (30,0)	5 (25,0)	0.185
	Yes	n (%)	10 (50,0)	16 (80,0)	14 (70,0)	15 (75,0)	
Coronary artery disease	No	n (%)	2 (10,0)	9 (45,0)	7 (35,0)	6 (30,0)	0.103
	Yes	n (%)	18 (90,0)	11 (55,0)	13 (65,0)	14 (70,0)	

*Statistically significant p-value.

were found to be 32.45 in the stage 1 group, 31.30 in the stage 2 group, 33.15 in the stage 3 group, and 25.75 in the stage 4 group. The $EtCO_2$ values were found to be significantly lower in patients in the stage 4 group compared to the other groups ($p<0.001$).

Patients' mean EF and $EtCO_2$ values and median NT pro-BNP values by stages are presented in Table 2.

DISCUSSION

The level of $EtCO_2$ provides important information about the current clinical and respiratory status of the patient[5]. It is used in many clinical situations, which are cardiac arrest, confirmation of endotracheal intubation tube location, procedural sedation, trauma, sepsis, metabolic acidosis, pulmonary embolism, heart failure, respiratory distress, and convulsions[6].

In the study of Glöckner et al., when the gender distribution of the patients was examined, the rate of male patients was found to be 68.0%[7]; however, this rate was found to be 61.2%[8] as a result of a study conducted by Roger et al. Notably, 65% of the patients included in our study were male. This rate we found in our study is similar to other studies in the literature.

Considering the comorbidities of the patients included in the study, it was determined that while 35% of them had DM, 68.75% had HT, and 70% had CAD. In a study conducted by Roger et al., it was determined that 88 and 76% of the patients had HT and CAD, respectively[8]. In most of the studies in the literature, HT and CAD were found in the first place among comorbidities. Our study is also similar to other studies in the literature. In our study, when comorbidities were examined by stages, no statistically significant difference was found between the groups in terms of HT, DM, and CAD. Similar to our study, in the study conducted by Williams et al.[9], no statistically significant difference was found between the groups in terms of HT, DM, and CAD by stages.

Natriuretic peptides (NP) play an important role in diagnosing HF. Especially low levels of NP are very valuable to exclude the diagnosis of HF[10]. In our study, we examined the pro-BNP values of our patients by stages and found that the median values were the lowest at 1,305 pg/mL in the stage 1 group and the highest at 6,930 pg/mL in the stage 4 group. The difference between the groups was statistically significant. In the study conducted by Baggish et al., the pro-BNP median values by stages were 3,512 pg/mL in the stage 2 group, 5,610 pg/mL in the stage 3 group, and 6,196 pg/mL in the stage 4 group, and the difference between the stages was also found to be significant[11]. Similarly, in a study conducted by Arat-Özkan et al., it was found that pro-BNP levels increased as the stage progressed, which was statistically significant[12]. In our study, the fact that the pro-BNP values were the highest in the stage 4 group and the lowest in the stage 1 group was similar to the literature.

The EF (%) of the patients in our study were measured and compared by stages. The mean EF (%) values were found to be 40.75 in the stage 1 group, 34.50 in the stage 2 group, 32.75 in the stage 3 group, and 30.25 in the stage 4 group, and the difference between the groups was statistically significant. In a study conducted by Hu Ying et al., it was determined that EF decreased as the stage progressed, and the difference between the stages was found to be significant[13]. In most of the data in the literature, it was determined that EF values decreased as the NYHA stage progressed, and a similar result was found in our study.

It has been reported that the evaluation of $EtCO_2$ measurement with capnography can be used together with NYHA staging in the prognosis of HF4. The $EtCO_2$ values of the patients included in our study were measured, and when we examined them by stages, they were found to be significantly lower in the stage 4 group compared to the other groups. The results were found to be similar between stage 1, 2, and 3 groups. In a study conducted by Seguchi et al., it was determined that $EtCO_2$ values decreased as the stage progressed, which was statistically significant; similar to our study, the lowest values were found in the stage 4 group in that study[14]. In the study conducted by Matsumoto et al., the $EtCO_2$ levels were compared between the NYHA stage 1, 2, and 3 groups and the control group consisting of healthy subjects. A significant decrease was found in $EtCO_2$ levels as the NYHA stage progressed, and the $EtCO_2$ values of the control group were found to be significantly higher compared to the group

Table 2. Distribution of laboratory, ejection fraction, and end-tidal carbon dioxide values by stages.

		Stage 1	Stage 2	Stage 3	Stage 4	p
NT pro-BNP	Median	1,305.00[2,4]	5,775.00[1,3,4]	2,412.50[2,4]	6,930.00[1,3]	0.001*
EF	Mean	40.75[4]	34.50	32.75	30.25[1]	0.013*
$EtCO_2$	Mean	32.45[4]	31.30[4]	33.15[4]	25.75[1,2,3]	<0.001*

*Statistically significant p-value. The superscript numbers indicate New York Heart Association Functional Classification Stages.

consisting of patients[15]. In the study conducted by Tanabe et al., patients in NYHA stage 1, 2, and 3 groups were compared, and it was determined that the $EtCO_2$ levels decreased as the stage progressed[16]. Unlike the literature, no significant difference was found between stage 1, 2, and 3 groups in our study. Furthermore, patients with pathologies such as sepsis and septic shock, hemorrhagic shock, pulmonary embolism, pneumothorax, renal failure, acute cerebrovascular accident, COPD, asthma drug intoxication, and metabolic acidosis–alkalosis that may affect $EtCO_2$ values were excluded from our study. Thus, this is the first study in the literature.

CONCLUSION

The incidence of heart failure increases in direct proportion to the prolongation of life expectancy due to advancing medical treatment possibilities, which increases the admission of patients with HF to emergency departments.

In this study, the $EtCO_2$ values measured in patients with heart failure were found to be significantly lower in NYHA stage 4 patients. We believe that the measurement of $EtCO_2$ values in patients with HF can be used to determine the severity of HF.

ETHICS APPROVAL

Ethical approval was obtained from the Ministry of Health of the Republic of Turkey and the Ethics Committee of Kahramanmaraş Sütçü İmam University Faculty of Medicine with resolution number 23 in session 2019/03 on 20.02.2019.

AUTHORS' CONTRIBUTIONS

MT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **HH:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AİK:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MSG:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CÖ:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MKG:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AÇA:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231548

A randomized clinical trial of transdermal (gel) versus oral estrogen for endometrial preparation in frozen embryo transfer cycle

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SUMMARY

OBJECTIVE: The aim of this study was to compare endometrial thickness with the use of transdermal estrogen (gel) versus oral estrogen (pills) for endometrial preparation in the frozen embryo transfer cycle and serum estrogen concentrations during the preparation cycle, side effects, and chemical and clinical pregnancy rates.

METHODS: This was a prospective, randomized controlled trial of women undergoing endometrial preparation for cryopreserved blastocyst transfer. A total of 88 women were randomized, of which 82 completed the study protocol. Of this group, 44 received 6 mg/day of estradiol valerate orally (pills group) and 38 received 4.5 mg/day of estradiol hemihydrate transdermally (gel group). Endometrial thickness was measured using transvaginal ultrasound between the 7 and 10th day of the cycle. Serum estradiol concentrations were measured on the day of initiating the cycle, on control transvaginal ultrasounds, and on the day of embryo transfer. Side effects were documented at each study visit. $p<0.05$ were adopted as statistically significant. The groups were compared using Student's t-test for continuous variables and chi-square or Fisher's exact test for categorical variables.

RESULTS: There were no significant group differences ($p>0.05$) in endometrial thickness, biochemical and clinical pregnancy rates, miscarriage rate, blood estradiol concentrations, duration of estradiol administration, or cycle cancellation rates.

CONCLUSION: Endometrial preparation with transdermal estrogen yielded similar reproductive outcomes to oral estrogen with fewer side effects.

KEYWORDS: Embryo transfer. Estrogens. Cutaneous administration. Oral administration. Endometrium.

INTRODUCTION

Over the past decade, the proportion of frozen embryo transfers has increased substantially[1]. Despite its proven efficacy and indications, the ideal protocol for endometrial preparation (EP) for thawed embryo transfer remains the subject of debate[2-8].

When transdermal estrogen is used, the metabolism of the first passage through the liver does not take place, with consequent lower stimulation of hepatic proteins and coagulation factors, and a neutral metabolic profile, which is potentially more favorable in terms of cardiovascular risk and thromboembolic events[9-12].

In this context, the results of studies comparing the efficacy of different routes for estrogen in freeze–thaw embryo transfer cycles have been conflicting[8,13-16].

The lack of investigations on this topic prompted the present study comparing two regimens of hormone replacement for EP in thawed embryo transfer: estrogen gel and estrogen pills.

METHODS

We conducted a prospective randomized clinical trial of women undergoing treatment for cryopreserved embryo transfer to compare two EP protocols: estrogen gel and estrogen pills.

This was a single-center study conducted at a private reproductive center between June 2020 and October 2021. The study was approved by the Ethics Committee for Research in Humans of the Santa Casa of Sao Paulo School of Medical Science (Process number 23023219.2.0000.5479) in accordance with good clinical practice guidelines. Patients signed the informed consent form agreeing with the assisted reproduction treatment procedures according to local ethics regulations. It was also registered at the Registro Brasileiro de Ensaios Clínicos (ReBEC—Brazilian Clinical Trials Registry) under UTN (Universal Trial Number): A36950145802.

The study's inclusion criteria were as follows: women aged ≥21 and ≤38 years when undergoing embryo transfer using

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 11, 2023. Accepted on January 24, 2024.

their own embryos, or those aged ≥21 and ≤50 years when undergoing embryo transfer using egg or embryo donation; women who had a body mass index (BMI) ≥18 and ≤35 kg/m^2; and women who had one or two cryopreserved embryos from the 5 or 6th day.

However, patients were excluded from the study if one or more of the following were present: women with serum progesterone concentrations ≥1 ng/mL on the day of study commencement; a history of thrombosis; abnormal liver function; anatomical endometrial abnormality; BMI<18 and >35 kg/m^2; and history of gastroplasty.

Study protocol

Screening: Anamnesis, physical examination, and specific complementary tests were performed for high-complexity assisted reproduction, according to Brazilian Health Regulatory Agency (ANVISA).

Timepoint 0 (T0): After a basal ultrasonography, analysis of laboratory tests, application of the inclusion and exclusion criteria, and signing of the Free and Informed Consent Form, treatment was started on the 2nd and 3rd day of menstruation. At this initial stage, blood levels of progesterone, estradiol, ALT, and AST were determined and participants randomly allocated into the two study intervention groups at a ratio of 1:1 using randomly generated numbers (http://www.randomization.com):

Gel group—Transdermal route: two 0.75 mg pumps of estradiol hemihydrate every 8 h (Oestrogel® Besins Healthcare, Belgium); pills group—oral route: 2 mg of estradiol valerate every 8 h (Primogyna® Bayer, Germany).

Timepoint 1 (T1): Transvaginal ultrasound scan was performed between the 7 and 10th day of EP. If the endometrial thickness was ≥7 mm, then embryo transfer was scheduled, and women started taking vaginal micronized progesterone 600 mg/day (Utrogestan®, Besins Healthcare, Belgium) for 5–6 days before transfer.

Timepoint 2 (T2): In cases where the endometrium failed to exhibit satisfactory thickness, the estrogen dose was increased to 6 or 8 mg/day in the Gel and Oral groups, respectively. Women whose endometrium failed to reach the thickness or pattern required, even after increasing estrogen dose, were excluded from the study.

Timepoint 3 (T3—FET): Embryo transfer was performed. Following completion of transfer, participants were asked to do a pregnancy test (beta-hCG quantitative) after 10 days.

Timepoint 4 (T4): At 4–5 weeks after embryo transfer, in the event of a positive pregnancy test, a transvaginal obstetric ultrasound scan was performed.

All adverse symptoms were recorded at each of the study visits.

Serum estradiol concentration was measured in all women at baseline, at control transvaginal ultrasounds, and on the day of embryo transfer. Progesterone level was analyzed at baseline and at control ultrasounds. Transaminase levels were determined at the start of treatment and on the day of embryo transfer. All tests were carried out by the same laboratory.

Chemical pregnancy was defined as the presence of beta-hCG ≥ 25 UI/L at 10 days after embryo transfer. Clinical pregnancy was determined as the presence of a gestational sac on transvaginal ultrasound at 4–5 weeks after embryo transfer. Miscarriage was defined as a nonviable intrauterine pregnancy prior to 20 weeks gestation.

Statistical study

Student's t-test was employed for calculating sample size, adopting a 5% level of significance and a test power of 80%. Data was drawn from a pilot study, which found a mean endometrial thickness of 7.35 mm and a standard deviation (SD) of 1.0. Differences of 10% above the mean were allowed for, giving a sample size of 30 participants per group.

For descriptive analysis of data, clinical and demographic characteristics were expressed as mean and SD for continuous variables, and as frequencies and percentages for categorical variables.

Regarding statistical tests, Student's t-test was used for continuous variables and the chi-square or Fisher exact test for categorical variables.

All statistical analyses were performed using the SPSS 21 software (IBM software) and p-values of <0.05 were considered statistically significant.

RESULTS

A total of 88 women were included in the study. Overall, 93.1% (82/88) completed the study protocol, comprising 38 women in the transdermal estrogen group (gel group) and 44 women in the oral estrogen group (pills group).

Six women were excluded from the gel group: two for administering medication incorrectly; one for reporting difficulty applying the gel and deciding to take oral estrogen; and three for having progesterone level>1 ng/mL on day of treatment commencement. All participants in the pills group were included (Figure 1).

The gel and pills groups had similar characteristics for BMI, infertility time, egg/embryo donation, number of embryos transferred, and embryo quality (see Table 1).

The mean thickness of the endometrium thickness on the ultrasound performed between the 7 and 10th day of EP (T1) and was similar between both groups: 7.87±1.74 mm in the

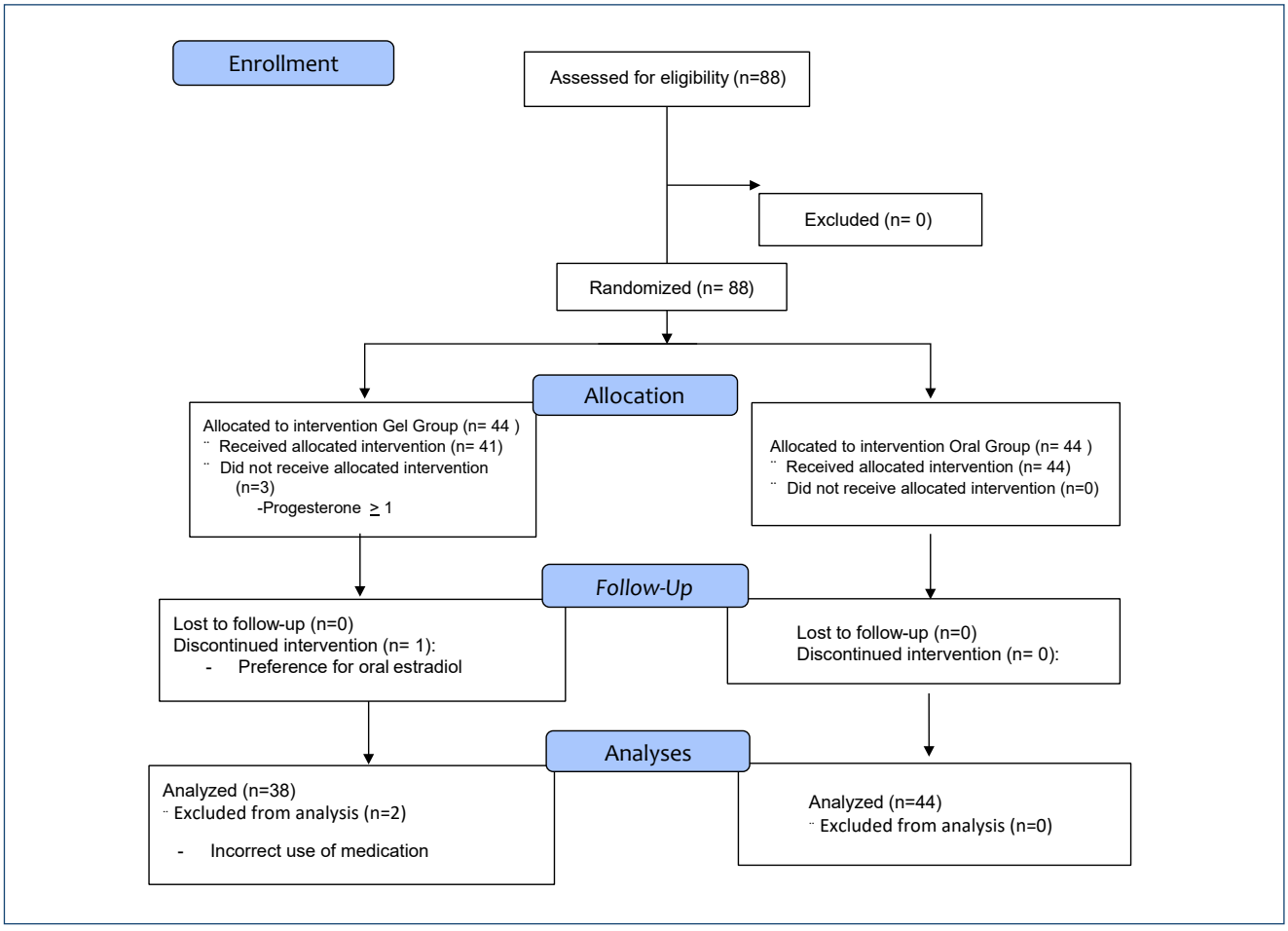

Figure 1. CONSORT flowchart of the trial.

gel group versus 8.45±2.04 mm in the pills group (p=0.175) (Table 2—T1).

There were no significant group differences on laboratory tests performed 7–10 days into EP (Table 2).

Adverse effects were probed at 7–10 days of medication use. Women using oral estrogen (pills group) reported a higher rate of adverse effects compared to women using transdermal estrogen (gel group) (34.1 vs. 10.5%, p=0.0017). The most frequent adverse symptom in the pills group was headache, occurring in 18.2% of cases versus 2.6% in the gel group (p=0.033) (Table 2—T1).

Of the 82 cycles initially selected for the study, 20 women had endometrium thickness<7.0 mm after 7–10 days of estrogen use in both groups. As per the study protocol, estrogen dose was increased in these women and follow-up assessment performed after 5 days. No significant group difference in endometrial thickness/aspect or serum progesterone and estradiol concentration was evident (Table 2—T2).

After increasing the estrogen dose, 15 women attained endometrial thickness ≥7.0 mm and 5 had treatment cancellation due to insufficient endometrial thickness for embryo transfer. Of these cancellations, three were from the gel group and two from the pills group.

On the day of embryo transfer, the laboratory test results of the two intervention groups were compared. No significant group differences were detected for TGO, TGP, or estradiol (Table 2—FET).

Comparison of clinical outcomes showed no significant group differences, with results proving similar irrespective of route of estrogen administration (Table 3).

Regarding pregnancy rates, chemical pregnancy was 51.4 versus 66.7% (p=0.261) and clinical pregnancy 31.4 versus 38.1% (p=0.476) in the gel and pills groups, respectively.

Similarly, there were no significant differences between the gel and pills groups for abortion rates (38.8 vs. 42.8%) (p=0.070), live births per cycle transferred (25.7 vs. 38.09%) (p=0.275), duration of EP (14.86±2.37 vs. 14.43±1.56 days) (p=0.344), or cycle cancellation rate (7.9 vs. 4.5%) (p=0.527).

Table 1. Basic and demographic characteristics of patients in two groups.

	Gel group	Pills group	p
	n=38	n=44	
	n (%)	n (%)	
Age (years)*	37.15±5.27◊	37.25±5.57◊	0.939
BMI (kg/m²)*	25.46±3.9◊	26.48±3.73◊	0.223
Duration of infertility (years)*	6.78±4.83◊	6.52±3.68◊	0.791
Cause of infertility**			
Tuboperitoneal	14 (36.8%)	8 (18.1%)	0.057
Ovulatory + male factor	3 (7.8%)	7 (15.9%)	0.326
Male factor	6 (15.7%)	10 (22.7%)	0.429
Unexplained	3 (7.8%)	4 (9.0%)	1
Same sex couple/solo parent	5 (13.15%)	3 (6.8%)	0.462
Tubal + male factor	1 (2.63%)	3 (6.8%)	0.620
Ovulatory + male factor	4 (10.5%)	5 (11.3%)	1
Tubal + ovulatory factor	2 (5.26%)	4 (9.0%)	0.679
Egg and embryo donation**			
Own eggs	27 (71.1%)	30 (68.2%)	0.485
Egg or embryo donation	11 (28.9%)	14 (31.8%)	
No. of embryos transferred**			
1	10 (28.6%)	31 (73.8%)	0.815
2	25 (71.4%)	11 (26.2)	
At least a top-quality embryo transferred**	6/35▫ (17.1%)	6/42▫ (14.3%)	0.977

N: no. of participants; %: percentage; *Student's t-test; **chi-square test; ◊mean (±SD): standard deviation; p<0.05; ▫only cycles with embryo transfer.

DISCUSSION

The aim of the present study was to further the knowledge on hormone replacement regimens for EP in frozen–thawed embryo transfer.

Unlike most previous investigations assessing transdermal estrogen patches, the present study administered transdermal estrogen through the use of gel[15,17].

The results of the present study on endometrial thickness measured at 7–-10 days of EP revealed no significant group difference between oral estrogen (pills group) and transdermal estrogen (gel group).

These findings corroborate the studies by Tehraninejad et al.[15] and Garimella et al.[17]. These studies found no difference in endometrial thickness between the oral and transdermal estrogen groups.

In contrast to the present study and other investigations cited, Ferrer-Molina et al.[14], in a prospective randomized study, found greater endometrial thickness in the transdermal estrogen (estradiol hemihydrate patches) group compared with the oral estrogen (estradiol valerate) group, with no apparent repercussions on the rate of pregnancy, miscarriages, or live births.

Thus, no significant group differences in pregnancy, miscarriage, or live birth rates were evident. These results are similar to those reported by Davar et al.[13], whose prospective randomized study compared EP using transdermal estradiol 17-B patches against oral estradiol valerate, and also reflect the findings of Garimella et al.[17].

Conversely, Tehraninejad et al.[15] found a lower rate of miscarriage and a higher rate of ongoing pregnancy and live births in the group using transdermal estrogen gel than the group taking oral estrogen in pill form. According to the opinion of the authors, these effects might be explained by the high concentration of estradiol in the orally administered group or by the more physiological fluctuation in estrogen level associated with the transdermal route compared to oral administration. However, the authors emphasized the need for further trials to confirm their results.

For blood estradiol levels, no difference between the present study intervention groups was found at 7–10 days of EP. However, Garimella et al.[17] and Tehraninejad et al.[15] found significantly higher estradiol levels in women undergoing EP using oral estrogen pills than those who administered transdermal gel. The disparate result of the present study, differing from the findings of most of the cited publications, does not affect the rates of pregnancy, miscarriage, or live births.

Similar to the studies by Garimella et al.[17] and Tehraninejad et al.[15], no difference in EP cycle cancellation rate was found between the intervention groups.

Regarding adverse effects, rates were lower for the transdermal route (gel group) (10.5%) than the oral route (pills group) (34.1%) (p=0.017), with the most reported symptom of headache occurring in 2.6% versus 18.2%, respectively (p=0.033). Corroborating the present findings, Garimella et al.[17] reported that a higher number of women had side effects in the oral group than gel group, where the most frequent adverse reaction reported in the oral group was gastrointestinal effects (30 vs. 1.4% p<0.01) and headache (17.3 vs. 3.6% p<0.01).

Contradicting these results, Ferrer-Molina et al.[14] found that oral treatment was perceived as more comfortable than transdermal, a finding attributed to the high humidity of the city in which the study was carried out, claiming that many women had complaints regarding detachment of patches and skin reactions at the site of application.

Thus, the present study makes several contributions to clinical practice, with the absence of significant differences in endometrial thickness, rates of live births, pregnancy, or miscarriage between the intervention groups demonstrating similar efficacy. Moreover, the administration of estrogen gel has

Table 2. Comparison of groups in relation to laboratory and ultrasound tests at T0, T1, T2, and frozen embryo transfer.

		Gel group	Pills group	p
		n=38	n=44	
		Mean±SD	Mean±SD	
T0	AST (U/L)*	17.57±4.10	21.58±9.20	0.016
	ALT (U/L)*	17.89±6.95	20.27±12.10	0.289
	P4 (ng/mL)*	0.25±0.15	0.33±0.25	0.086
	E2 (pg/mL)*	41.28±32.82	42.90±21.31	0.793
T1	ET (mm)*	7.87±1.74	8.45±2.04	0.175
	P4 (pg/mL)*	0.22±0.21	0.25±0.16	0.569
	E2 (ng/mL)*	275.79±193.40	224.57±83.24	0.116
	Endometrium trilinear**	32/38 (84.2%)◊	42/44 (95.5%)◊	0.087
	Adverse effects***	4 (10.5%)◊	15 (34.1%)◊	0.017
	Headache***	1 (2.6%)◊	8 (18.2%)◊	0.033
	Gastrointestinal***	0	5 (11.4%)◊	0.058
	Cramps***	1 (2.6%)◊	1 (2.3%)◊	1
	Paresthesia***	1 (2.6%)◊	1 (2.3%)◊	1
	Others***	1 (2.6%)◊	3 (6.8%)◊	0.365
		n=12	n=8	
T2	ET (mm)*	7.68±1.79	7.35±1.00	0.64
	P4 (ng/mL)*	0.15±0.11	0.19±0.27	0.722
	E2 (pg/mL)*	327.94±161.22	302.33±99.72	0.736
	EE trilinear***	10 (83.3%)◊	8 (100.0%)◊	0.224
		n=35	n=42	
FET	AST (U/L)*	25.36±13.05	28.15±21.16	0.593
	ALT (U/L)*	22.21±13.68	19.03±9.62	0.264
	E2 (pg/mL)*	226.24±138.44	224.35±91.50	0.945

N: no. of participants; SD: standard deviation; P4: progesterone; E2: estradiol; %: percentage; ET: endometrial thickness; ◊n (%). T0: Initial time. T1: visit 1 of the study performed between the 7 and 10th day of endometrial preparation. T2: visit 2 of the study. FET: frozen embryo transfer. *Student's t-test; **chi-square test; ***Fisher's exact test; p<0.05.

Table 3. Comparison of clinical outcomes of study groups.

	Gel group	Pills group	p
	n=38	n=44	
	n (%)	n (%)	
Duration of endometrial preparation (days)*	14.86±2.37◊	14.43±1,56◊	0.344
Cycle cancellation rate**	3/38 (7.9%)	2/44 (4.5%)	0.527
Biochemical pregnancy**	18/35▫ (51.4%)	28/42▫ (66.7%)	0.261
Clinical pregnancy**	11/35▫ (31.4%)	16/42▫ (38.1%)	0.542
Multiple pregnancy**	3/11Δ (27.3%)	3/16Δ (18.8%)	0.601
Miscarriage rate**	7/18 (38.8%)	12/28 (42.8%)	0.070
Live births per cycle transferred**	11 /35▫ (31.4%)	16/42▫ (38.1%)	0.542

N: no. of participants; %: percentage; ◊Mean (±SD): standard deviation; ▫only cycles with embryo transfer, Δ positive clinical pregnancy only. *Student's t-test; **chi-square test; p<0.05.

the added benefit of a lower rate of side effects. This study has special relevance in that there is a dearth of studies in the literature comparing the efficacy of estrogen gel versus oral estrogen in FET cycles.

Nonetheless, this study has some limitations. The analysis of the results was originally performed to consider endometrial thickness, but the rate of live births may be of greater clinical interest.

Finally, the results of this study are strengthened by its selection of the appropriate sample size for the primary objective and by the fact that both groups were homogeneous for baseline clinical and laboratory characteristics. Therefore, taken together, these results suggest that EP using estrogen gel can be offered as a first line for EP once it is associated with fewer side effects and has similar reproduction outcomes compared with oral estrogen.

AUTHORS' CONTRIBUTIONS

MOCC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft. **SMRR:** Supervision, Validation, Writing – review & editing. **CGG:** Data curation. **LOT:** Data curation. **RSR:** Data curation. **SG:** Conceptualization, Resources, Supervision, Validation. **NEB:** Conceptualization, Resources, Supervision, Validation. **CEB:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Validation.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231626

Comparative analysis of the effectiveness of coarctation surgery between neonates and infants

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SUMMARY

OBJECTIVE: This study aimed to compare the effectiveness of resection and extended end-to-end anastomosis between neonate and infant patients with coarctation.

METHODS: This study was designed retrospectively and included 41 neonate (<30 days) and infant (30 days to 1 year) patients who were operated on using the resection and extended end-to-end anastomosis technique for aortic coarctation. Preoperative aortic annulus diameters and Z scores, all aortic arch diameters and Z scores, the presence of hypoplastic aortic segment, and the presence of prematurity were reviewed in both groups. Subsequently, we investigated whether these parameters were statistically related to the residual gradient in the operation area, whether there was a need for early re-intervention, and what was the incidence of mortality in the early postoperative period. In addition, the aortic arch Z scores of the patients at 6 months postoperatively were examined.

RESULTS: While the mean age (p<0.001), body weight (p<0.001), and proximal arch Z score (p=0.029) were found to be significantly lower in the neonate group than in the infant group, the total length of the intensive care unit stay (p=0.013) and the total length of hospital stay (p=0.017) were found to be significantly higher. In addition, significant enlargement was detected in the proximal arch, distal arch, and isthmus segments in both patient groups.

CONCLUSION: The resection and extended end-to-end anastomosis is an equally effective technique that can provide a marked decrease in gradient in the coarctation area and a significant enlargement of the aortic arch segments in the early period after coarctation repair in both neonate and infant patients.

KEYWORDS: Coarctation. Newborn. Infant.

INTRODUCTION

Aortic coarctation is a congenital anomaly characterized by discrete or diffuse stenosis in the juxtaductal region of the descending aorta. It constitutes approximately 10% of all cases of congenital heart diseases[1].

The resection and extended end-to-end anastomosis (REEA) technique is a very useful procedure that provides resection of the coarctated region and expands the hypoplastic distal arch and isthmus. However, as the aorta of neonate patients with hypoplastic arch is very small in diameter and prone to rupture, effective utilization of this technique requires extensive experience. Therefore, to minimize the difficulty of the operation, some clinics postpone surgeries for hemodynamically stable neonates until the infancy period to allow the cardiac structures to grow.

Our aim in this study was to compare the coarctation patients who had undergone coarctation surgery using the REEA technique, in the neonatal and infant period, in terms of early surgical results in the postoperative period. These included the need for re-intervention, development of early recoarctation, growth of the aortic arch segments, and in-hospital mortality. Thus, it was investigated whether the timing of the operation had an effect on the postoperative results.

METHODS

This was a retrospective observational study that included pediatric patients who were operated on with a left posterolateral thoracotomy using the REEA technique, between March 2019 and December 2022, in Ankara Bilkent City Hospital Pediatric Cardiovascular Surgery department. The study was approved by the institutional ethics committee of the same institution with approval number E2-23-3744. The patients were classified into two groups: neonates (<30 days) and infants (30 days to 12 months). All patients were further examined to determine whether they had isolated coarctation or coarctation accompanied by hypoplastic aortic arch. An exclusion criterion in the

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 11, 2023. Accepted on December 29, 2023.

study was the presence of additional major cardiac anomaly, which was hemodynamically significant and required additional surgical intervention.

Preoperative demographic and echocardiographic data of the patients were collected. For this purpose, the data reporting the patients' body weight, age, presence of prematurity, presence of genetic syndrome, diameter, and Z score of aortic annuli as well as Z scores of proximal arch, distal arch, and isthmus were all gathered. Cardiovascular structures with a Z score below -2 were defined as hypoplastic.

In accordance with the common consensus of cardiology and cardiovascular surgery clinics, endovascular balloon dilatation was applied to the patients who required urgent intervention in the neonatal period due to hemodynamic instability. Other patients diagnosed in our clinic or referred to our institution were scheduled for surgery as soon as possible without any intentional delay.

Both arm-leg systolic blood pressure gradient (ALSG) and maximum systolic echocardiographic gradients in the repaired coarctation and/or hypoplastic aortic arch region of the patients were recorded within the first week after surgery. A simultaneous systolic gradient above 20 mm Hg in both of them was considered diagnostic in terms of recurrent coarctation. These measurements were repeated at the sixth-month follow-up visit of the patients following their discharge. In addition, aortic annulus, proximal arch, distal arch, and isthmus Z scores were also calculated and compared with preoperative values.

Statistical analysis

Mean and standard deviation were calculated in descriptive statistics of continuous variables. In the definition of categorical variables, frequency (n) and percentage (%) values were used. Normality assumptions of the variables were examined with the Shapiro-Wilk test. The Mann-Whitney U test was used to compare continuous variables between the two groups, and the chi-square/Fisher's exact analysis was used to determine the relationships between categorical variables. The IBM SPSS.25 program was used in all analyses, and the $p<0.05$ value was accepted as the significance level.

RESULTS

A total of 41 patients, including 22 (53.7%) neonates and 19 (46.3%) infants, were included in the study. A comparison of demographic and clinical data of neonate and infant patients is shown in Table 1.

The mean age of the neonate group (20.86±9.69 days vs 112.89±47.53 days) ($p<0.001$), body weight (3.33±0.57 kg vs 5.32±1.28 kg) ($p<0.001$), and proximal arch Z score (-3.94±1.49 vs -2.99±1.42) ($p=0.029$) were significantly lower than the infant group, while the duration of intensive care unit stay (2.55±0.69 days vs 2.14±0.97 days) ($p=0.013$) and total hospitalization time (14.23±5.03 days vs 11.24±6.34 days) ($p=0.017$) was found to be significantly higher. On the contrary, no significant relationship was found between the neonate and infant groups in terms of the presence of genetic anomalies and prematurity ($p=0.32$ and $p=0.52$).

While hypoplasia (Z score<-2.0) was detected in at least one segment of the aortic arch in 95.5% of neonate patients, this rate was 73.3% in infant patients ($p=0.08$).

In the postoperative period, three neonates (13.6%) and one infant (5.3%) died. The underlying cause of death in all patients was multiorgan failure secondary to sepsis. No significant stenosis was observed in the operative area in the postoperative echocardiographic examinations of these patients.

An interventional procedure was performed in three patients (13.6%) in the neonate group and in one patient (5.3%) in the infant group due to the development of recoarctation within the first 6 months postoperatively.

As shown in Table 2 in both neonate and infant groups, patients with an ALSG and preoperative systolic max ECHO gradient above 20 mm Hg in the coarctation area had significant decreases in the postoperative measurements. However, it was observed that these values persisted above 20 mm Hg in two of the neonates and in three of the infant patients ($p<0.001$). In two of these patients, surgical reintervention was immediately performed after the first coarctation repair due to the presence of high proximal blood pressure, insufficient distal perfusion, and hemodynamic instability in the intensive care unit. Both of these reoperated patients were neonates and had a high systolic max ECHO gradient (>70 mm Hg) at the anastomosis region, accompanied by significant diastolic extension in the descending aorta and diminished lower extremity pulses in the postoperative evaluation. In the reoperation procedure, an aortoplasty using a bovine pericardial patch was performed on the previous operative area. The systolic gradients of these patients regressed below 20 mm Hg right after the second operation, and their follow-up was uneventful. The remaining one neonate and three infant patients whose ALSG and maximum systolic anastomosis gradients were above 20 mm Hg were not intervened due to a lack of distal perfusion disturbances and any diastolic extension in the descending aorta. In two of these infant patients, the gradients rapidly decreased below 20 mm Hg in the following postoperative days. On the contrary, in one neonate and one infant patient, signs of left ventricular hypertrophy developed, and high anastomosis gradients with

Table 1. Demographic and clinical data.

Parameters	Neonate (<30 days)	Infant (>30 days)	p
n (%)	22 (53.7)	19 (46.3)	
Age (days)*	20.86±9.69	112.89±47.53	**<0.001**
Weight (kg)*	3.33±0.57	5.32±1.28	**<0.001**
Gender n (%)**			0.436
Male	19 (86.4)	14 (73.7)	
Female	3 (13.6)	5 (26.3)	
Genetic anomaly (%)			0.321
Yes	21 (95.5)	16 (84.2)	
No	1 (4.5)	3 (15.8)	
Prematurity n (%)***			0.524
No	17 (77.3)	13 (68.4)	
Yes	5 (22.7)	6 (31.6)	
Preoperative cardiac Z scores			
Aortic annulus Z score*	-1.99±1.50	-1.22±2.12	0.284
Proximal arc Z score*	-3.94±1.49	-2.99±1.42	**0.029**
Distal arc Z score*	-3.91±1.73	-3.43±1.51	0.472
Istmus Z score*	-4.68±2.52	-3.39±1.52	0.094
Hypoplastic arcus n (%)**			0.080
No	1 (4.5)	5 (26.3)	
Yes	21 (95.5)	14 (73.7)	
Length of stay at intensive care unit (days)*	2.55±0.69	2.14±0.97	**0.013**
Total length of hospitalization (days)*	14.23±5.03	11.24±6.34	**0.017**
Mortality n (%)*			0.610
No	19 (86.4)	18 (94.7)	
Yes	3 (13.6)	1 (5.3)	
Postoperative re-intervention requirement n (%)*			0.610
No	19 (86.4)	18 (94.7)	
Yes	3 (13.6)	1 (5.3)	

*Mann-Whitney U test; **Fisher's exact test; ***Chi-square test.

diastolic extension in the descending aorta persisted. In line with the institutional first-line treatment strategy in recoarctation, balloon angioplasty was performed in the third and fourth months after the operation, respectively. In these patients, ALSG and the maximum systolic anastomosis gradient was successfully declined below 20 mm Hg in the recoarctation area and the diastolic tail in the descending aorta disappeared. In both groups, once the pre- and early ALSG and postoperative maximum systolic ECHO gradients were compared, significant gradient decrease was detected (p<0.001 vs p<0.001). On the contrary, no significant difference was found between the ALSG and postoperative early and sixth month systolic max ECHO gradients for both groups (p=1.0 vs p=1.0).

As shown in Table 3, Z scores of the aortic annulus (p=0.002), proximal arch (p<0.001), distal arch (p=0.001), and isthmus (p=0.001) in the neonate group were observed to be significantly increased when compared with those in the sixth month postoperatively. Similarly, Z scores of the same structures in the infant group have also significantly increased (p<0.001). However, no significant change was detected in the infant group in terms of annulus Z score between preoperative and postoperative 6-month periods (p>0.05).

Table 2. Comparison of arm-leg blood pressure systolic gradient and systolic max echo gradients obtained at different times in neonates and infants.

Neonate	ALSG and postoperative early systolic max echo gradient (mm Hg)*		p
ALSG and preoperative Sistolik max echo gradient (mm Hg)*	<20 mm Hg (n, %)	≥20 mm Hg (n, %)	**<0.001**
< 20 mm Hg (n, %)	1 (5.0)	0 (0.0)	
≥ 20 mm Hg (n, %)	19 (95.0)	2 (100.0)	
	ALSG and postoperative sixth-month systolic max echo gradient (mm Hg)*		1.00
ALSG and postoperative early systolic max echo gradient (mm Hg)*	<20 mm Hg (n, %)	≥20 mm Hg (n, %)	
<20 mm Hg (n, %)	19 (90.5)	1 (100.0)	
≥20 mm Hg (n, %)	2 (9.5)	0 (0.0)	
Infant	**ALSG and postoperative early systolic max echo gradient (mm Hg)***		**p**
ALSG and preoperative systolic max echo gradient (mm Hg)*	<20 mm Hg (n, %)	≥20 mm Hg (n, %)	**<0.001**
<20 mm Hg (n, %)	1 (6.3)	0 (0.0)	
≥20 mm Hg (n, %)	15 (93.8)	3 (100.0)	
	ALSG and postoperative sixth-month systolic max echo gradient (mm Hg)*		
ALSG and postoperative early systolic max echo gradient (mm Hg)*	<20 mm Hg (n, %)	≥20 mm Hg (n, %)	1.00
<20 mm Hg (n, %)	15 (88.2)	1 (50.0)	
≥20 mm Hg (n, %)	2 (11.8)	1 (50.0)	

*Fisher's exact test. ALSG: arm-leg blood pressure systolic gradient.

Table 3. Comparison of preoperative and postoperative sixth-month Z score parameters in neonatal and ınfant groups.

	Preoperative Z score	Postoperative sixth-month Z score	p
Neonate			
Aortic annulus	-1.99±1.50	-1.00±0.93	**0.002**
Proximal arc	-3.94±1.49	-1.27±1.07	**<0.001**
Distal arc	-3.91±1.73	-1.63±1.61	**0.001**
Istmus	-4.68±2.52	-2.08±1.96	**0.001**
Infant			
Aortic annulus	-1.22±2.12	-0.26±1.10	0.094
Proximal arc	-2.99±1.42	-1.04±1.09	**<0.001**
Distal arc	-3.43±1.51	-0.53±1.21	**<0.001**
Istmus	-3.39±1.52	-1.19±0.47	**<0.001**

Statistically significant p-value are indicated in bold.

DISCUSSION

According to the contemporary literature, the REEA technique through left posterolateral thoracotomy is accepted as one of the most preferred surgical approaches in coarctation, whether isolated or accompanied by a hypoplastic arch. However, there is an ongoing debate about the surgical method to be applied in cases where aortic coarctation is accompanied by hypoplasia of the proximal arch of the aorta. Many researchers state that if the hypoplastic proximal arch is not intervened on in the first surgical operation, recoarctation may develop in the medium and long term, re-intervention may be required, and additional morbidities, such as persistent hypertension, may develop. Mery et al., performed arch reconstruction and coarctation repair using the "aortic arch advancement" technique with median sternotomy, CPB, and antegrade selective perfusion support, on 275 neonate and infant patients under the age of 1 year with a proximal arch Z score average of -5.06. Perioperatively, eight patients (3%) died. The surviving patients were followed for an average of 6 years, and the need for re-intervention was observed in eight of these patients (3%)[2]. Other researchers have published similar results[3]. On the contrary, some researchers argue that unless the proximal arch Z score is below -4.5, additional intervention is unnecessary and the proximal arch will develop progressively with the high blood flow provided after the removal of the coarct segment with the REEA technique and the enlargement of the distal arch and isthmus. In this study, we found that in cases where the proximal arch was hypoplasic and only the distal arch and

isthmus were enlarged by applying the REEA technique, in the postoperative sixth-month imaging, there was no significant gradient in the operated area, the Z scores of all aortic arch segments developed significantly, and the need for re-intervention was not observed.

In the literature, many risk factors that may cause reintervention after coarctation repair have been reported. These can be listed as operating age, operating weight, presence of prematurity, underdevelopment of left heart structures, association of hypoplastic arch, and surgical technique applied[4-6]. It has been shown that surgical repair performed in the neonatal period has a positive effect on the development of left heart structures such as the left ventricular outflow tract, mitral and aortic valve annulus dimensions, and aortic arch in the long term and gives more favorable results than repairs performed in the infant period, in terms of preventing persistent hypertension[1]. Therefore, in many clinics around the world, coarctation repair is performed as soon as possible after diagnosis. However, some researchers suggest that surgical repair of coarctation in neonates should be postponed to infancy because of its own disadvantages. These can be listed as follows: coarctation repair in neonates is technically more difficult than infant repair, as the aortic arch and descending aorta are much smaller in size than infants and are more prone to rupture. In neonates and, in particular, in low-birth-weight patients, it is very critical to perform the anastomosis once and flawlessly in order to avoid complications such as acute bleeding after anastomosis or recoarctation in the chronic period. In addition, the ductus arteriosus is more fragile in neonates and complete resection of ductal tissue remnants from the entire aortic segment is more challenging[7]. However, despite all these difficulties, the early and long-term results of aortic coarctation repairs performed in the neonatal period are very successful because of improved surgical techniques and surgical instruments. In this study, the operative age and operative weight of the neonate patients were found to be significantly lower than the infants. However, it was found that the gradient in the coarctation region decreased significantly in the early period after surgical repair in both groups, and this situation persisted significantly at the postoperative sixth month. In both groups, it was observed that in addition to coarctation in the aortic arch, the hypoplastic arch also accompanied prominently, and there was no significant difference between the two groups in this regard. Moreover, the need for re-intervention after the surgery was similarly low in both age groups.

According to a study conducted with the data of coarctation patients treated in 52 hospitals in the United States, the presence of prematurity and low birth weight may lead to both prolonged hospitalization and increased in-hospital mortality. In addition, each 30-day delay in hospitalization of the patient reduces the postoperative hospital stay of the patient by 2.6 days[8]. IJsselhof et al., compared neonate and infant patients who underwent coarctation repair and found that neonate patients have required significantly longer intensive care and total hospitalization, compared with infants. It has been stated that the reason for this situation may be the more severe form of coarctation seen in neonate patients, and therefore their recovery period may be longer[1]. In our opinion, this situation is due to the prematurity of the heart and lung structures of the neonates compared with the infant patients, and accordingly, they require non-invasive respiratory support longer in the intensive care unit after extubation. Despite the lack of statistical significance, in our study, a higher mortality was observed in neonates compared with infant patients, and these data were similar to other series in the literature.

CONCLUSION

The REEA technique provides equally successful results in neonate and infant patients with coarctation, with a low rate of re-intervention and significant reduction of postoperative systolic maximum gradient at the coarctation area. It was observed that the hypoplastic aortic arch segments repaired using this technique enlarged rapidly in the early postoperative period in both groups. Although the total length of intensive care unit stay and total length of hospital stay were significantly longer in neonate patients than in infants, the REEA technique enables coarctation repair with low mortality in both age groups[9].

AUTHORS' CONTRIBUTIONS

MY: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing– review & editing. **BST:** Conceptualization, Formal Analysis, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **ANE:** Data curation, Formal Analysis, Investigation, Project administration, Resources, Writing – original draft, Writing – review & editing. **YÖŞ:** Conceptualization, Formal Analysis, Methodology, Validation, Visualization, Writing – review & editing. **AA:** Formal Analysis, Methodology, Validation, Project administration, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231683

Are monocyte-to-HDL and C-reactive protein-to-albumin ratios useful for the diagnosis and follow-up of Takayasu arteritis?

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SUMMARY

OBJECTIVE: In this study, we aimed to investigate the role of erythrocyte sedimentation rate, C-reactive protein, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, monocyte/lymphocyte ratio, red blood cell distribution width, mean platelet volume, monocyte/HDL ratio, and C-reactive protein/albumin ratio in the diagnosis and treatment follow-up of active and remission Takayasu arteritis patients compared with healthy control group.

METHODS: This is a retrospective case-control study in which 56 Takayasu arteritis patients and 40 age- and sex-matched healthy control were included. The blood values of Takayasu arteritis patients were analyzed during their active period and post-treatment remission periods, after comparing them with the healthy control. Furthermore, all parameters were evaluated by receiver operating characteristic analysis.

RESULTS: Erythrocyte sedimentation rate, C-reactive protein, neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, monocyte/lymphocyte ratio, monocyte/HDL ratio, and C-reactive protein/albumin ratio values were significantly higher in active Takayasu arteritis patients compared with healthy control and remission Takayasu arteritis groups. In the receiver operating characteristic analysis performed in active Takayasu arteritis and Takayasu arteritis patients in remission, C-reactive protein had the highest power to indicate disease activity, followed by C-reactive protein/albumin ratio, erythrocyte sedimentation rate, and monocyte/HDL ratio. When Takayasu arteritis in remission was compared with the healthy control, a significant difference was found between erythrocyte sedimentation rate, C-reactive protein, red blood cell distribution width, and C-reactive protein/albumin ratio, while no significant difference was found between monocyte/HDL ratio values.

CONCLUSION: C-reactive protein/albumin ratio and red blood cell distribution width can be used in the diagnosis of Takayasu arteritis, and C-reactive protein/albumin ratio, red blood cell distribution width, and monocyte/HDL ratio measurements can be used in the follow-up. As C-reactive protein/albumin ratio is more powerful than C-reactive protein in differentiating the Takayasu arteritis group from the healthy control group, evaluation of C-reactive protein/albumin ratio together with albumin instead of evaluation of C-reactive protein alone when diagnosing the disease may help us to obtain more accurate results in daily practice.

KEYWORDS: Takayasu arteritis. Inflammation mediators. Acute-phase proteins.

MEDICAL SUBJECT HEADINGS

1. Arteritis, Takayasu
2. Acute phase proteins
3. Vasculitis

INTRODUCTION

Takayasu arteritis (TA) is a chronic inflammatory disease of unknown etiology that mainly targets the aorta and its main branches[1]. Acute phase reactants (APRs) and imaging methods are helpful in the diagnosis[2]. Disease activity indices largely utilize C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) for both clinical and acute phase responses. However, CRP and ESR are not always elevated in acute TA disease. High concentrations of APRs are usually observed during active TA, but some studies have observed normal APRs in 10–30% of patients. This leads to the search for markers to be used both at the time of diagnosis and in treatment follow-up[3,4].

Acute phase reactants are proteins that show a 25% increase (positive APR) or decrease (negative APR) in serum concentration in response to inflammation[5]. Neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), monocyte/lymphocyte ratio (MLR), red blood cell distribution width (RDW), mean platelet volume (MPV), monocyte/HDL ratio (MHR) and CRP/albumin ratio (CAR) are the parameters currently investigated.

In this study, we aimed to investigate the roles of NLR, PLR, MLR, RDW, MPV, RDW, and MPV, especially CAR and MHR in the diagnosis, disease activity, and follow-up of TA, which we think may be an alternative to APRs such as ESR and CRP, which are currently used but are not definitive indicators of disease activity and inflammation.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 21, 2024. Accepted on January 28, 2024.

METHODS

This is a retrospective case-control study. It included 56 patients with newly diagnosed TA between January 2007 and 2023 according to the classification criteria determined by the American College of Rheumatology (ACR) in 1990. Forty healthy individuals of similar age and sex were also included[6]. Activation of the patients was analyzed by Kerr score, and patients with Kerr score >2 were included. These patients were considered active TA[4], who were all evaluated with blood results during remission periods after treatment. In patients with previously active disease, remission was defined as more than 6 months of stable disease without the development of new vascular lesions[7]. Patients under the age of 18 years and patients with active infection, malnutrition, malignancy, pregnancy, proteinuria, chronic liver disease, chronic renal failure, autoimmune disease, hematological disease, or lymphoproliferative history were excluded. This study protocol was approved by the Human Research Ethics Committee of Çukurova University (No. 137/2023).

The hospital's electronic medical record system was utilized to collect demographic information, clinical characteristics, pharmacological history, laboratory results, and imaging findings of the aorta and its branches. The analyses were conducted on ESR, CRP, NLR, PLR, MLR, RDW, MPV, MHR, and CAR values of the TA patients during the active and remission phases. The hematological parameters were evaluated by taking peripheral blood samples from the patients in a tube with EDTA. It was obtained that ESR for the patients aged under 50 years was 0–15 mm/h, ESR for women was 0–20 mm/h, ESR for men aged over 50 years was 0–20 mm/h, ESR for women was 0–30 mm/h, CRP was 0–5 mg/L, RDW was 11.8–13.4%, MPV was 6.5–11.6 fL, and albumin was 3.4–5.4 g/dL and they were considered normal laboratory values. NLR was obtained by dividing the number of neutrophils into lymphocytes, PLR by dividing the platelet into lymphocytes, MLR by dividing the monocyte to lymphocyte, MHR by dividing the number of monocytes by HDL concentration, and CAR by dividing CRP by albumin. In age and gender-matched healthy control (HC) group, ESR, CRP, NLR, PLR, MLR, RDW, MPV, MHR, and CAR values were accessed from the hospital electronic system. Laboratory parameters of active TA, TA in remission, and HC group were evaluated.

Receiver operating characteristic (ROC) analysis of inflammation markers between active TA and TA in remission groups was performed. The most powerful parameters in showing disease activation were CRP, CAR, ESR, and MHR, respectively ($p<0.05$, for all). For CAR, sensitivity was 80%, specificity was 95%, and area under the curve (AUC) (95%CI) was 0.916 (0.863–0.969), and for MHR, sensitivity was 64%, specificity was 85%, and AUC (95%CI) was 0.808 (0.729–0.887).

Statistical analysis

The statistical analyses were conducted using the SPSS for Windows 25.0 software. The normality of the variables was assessed through both visual and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). A p-value greater than 0.05 in the Kolmogorov-Smirnov test indicated that the data followed a normal distribution. When normal distribution was not determined, the patient and control groups were compared using the Mann-Whitney U test. The median values were taken. Wilcoxon signed-rank test was used to compare the values of a single dependent group in two-time intervals when there was no normal distribution. The chi-square test was used for the group comparisons of the qualitative variables. The AUC, sensitivity, specificity, and cutoff values were compared using the ROC curve. The Spearman correlation test was used for the correlation evaluation, and the correlation coefficient was taken as rho. The correlation coefficient<0.25 means no relationship or very weak relationship, 0.25–0.5 means weak to moderate relationship, 0.5–0.75 means strong relationship, and>0.75 means very strong relationship. Statistically, $p<0.05$ was considered significant.

RESULTS

A total of 56 patients with the TA (87.5% female, at the mean age of 40.5±10.08 years) and 40 age-sex matched healthy in the control group (82.5% female, at the mean age of 38.03±7.85 years) were included in the study. No statistically significant difference was found in the age and gender distribution of the patient and HC group (p=0.149 and p=0.499, respectively) (Table 1).

Table 1. Demographic data of Takayasu arteritis and healthy control group.

		Takayasu arteritis (n=56)	Healty control group (n=40)	p-value
Gender	Female	49	33	0.499
	Male	7	7	
Age (years±SD)		40.5±10.08	38.03±7.859	0.149

Inflammation markers of active TA, healthy population, and TA in remission groups are summarized in Table 2. According to these results, ESR, CRP, NLR, PLR, MLR, RDW, MHR, and CAR values of active TA patients were statistically significantly higher than those of the healthy population (p≤0.001, p≤0.001, p≤0.001, p≤0.001, p=0.016, p≤0.001, p≤0.001, p=0.004, p≤0.001, p≤0.001, and p≤0.001, respectively). There was no statistically significant difference between the MPV results of both groups (p=0.241). In the active TA and remission TA groups, ESR, CRP, NLR, PLR, MLR, MPV, MHR, and CAR values measured in the remission TA group were statistically significantly lower than those of active TA (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p=0.030, p=0.001, p=0.027, p<0.001, p<0.001, and p<0.001, respectively). No significant change was found in RDW values (p=0.056) (Table 2). When the TA group in remission was compared with the HC group, ESR, CRP, RDW, and CAR were statistically significant, while no significant difference was found between NLR, PLR, MPV, and MHR (p=0.04, p=0.02, p=0.038, p<0.05, p=0.545, p=0.696, p=0.666, and p=0.687, respectively).

The ROC analysis was performed to address the question of the predictive ability of inflammation markers by evaluating the active TA group and the HC group. In this analysis, the dependent variable is the presence of TA. The cutoff, sensitivity, and specificity values and AUC results obtained from this analysis are given in Table 3. According to these results, CAR was the most powerful marker among the markers evaluated to indicate inflammation. This was followed by ESR, CRP, MLR, NLR, PLR, RDW, MHR, and PLR, respectively. MPV value was not found to be a reliable marker of inflammation (p=0.275).

According to the correlation analysis, ESR was strongly correlated with CRP and CAR (r=0.642 and r=0.576, respectively) and weakly to moderately correlated with RDW and MHR (r=0.459 and r=0.336, respectively). CRP was very strongly correlated with CAR (r=0.828), strongly correlated with ESR and RDW (r=0.624 and r=0.512, respectively), and weakly to moderately correlated with MHR (r=0.307). CAR, which was found to be one of the strongest markers of inflammation by the ROC analysis, was found to be very strongly correlated with

Table 2. Evaluation of inflammation markers in active Takayasu arteritis compared with the healthy control group and Takayasu arteritis in remission.

	TA active (n=54) median (IQR)	Healthy control group (n=40) median (IQR)	p-value	Remission (n=54) median (IQR)	p-value
ESR	44 (28.5–62)	7 (4–9)	<0.001	10.5 (5.25–18.75)	<0.001
CRP	14.6 (8.750–40)	2.4 (1.2–3.350)	<0.001	2 (1.125–2.425)	<0.001
NLR	3.375 (2.235–5.4)	2.1 (1.31–3.36)	<0.001	2.2 (1.6025–3.03)	<0.001
PLR	156 (113.5–202.4)	125 (95.5–157)	0.016	127.4 (9.1–162.5)	0.030
MLR	0.37 (0.26–0.52)	0.21 (0.145–0.29)	<0.001	0.25 (0.18–0.35)	0.001
RDW	16.3 (14.4–17.550)	14.75 (13.3–16.1)	0.004	15.8 (13.9–17.3)	0.056
MPV	8.4 (7.6–8.9)	8.7 (8–9.4)	0.241	8.8 (8–9.75)	0.027
MHR	0.0175 (0.01–0.025)	0.0085 (0.005–0.02)	<0.001	0.009 (0.00625–0.011)	<0.001
CAR	3.775 (1.65–6.29)	0.03 (0.02–0.045)	<0.001	0.375 (0.2–0.51)	<0.001

CAR: CRP-to-albumin ratio; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MHR: monocyte-to-HDL ratio; MLR: monocyte-to-lymphocyte ratio; MPV: mean platelet volume; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; RDW: red cell distribution width; TA: Takayasu arteritis.

Table 3. Performance of inflammation markers to discriminate patients with active Takayasu arteritis and healthy controls.

	ESR	CRP	NLR	PLR	MLR	RDW	MHR	CAR
Cutoff	11.5	4.745	2.58	136.5	0.265	15.3	0.01275	0.1275
Sensitivity	91.1	85.7	67.9	57.1	73.2	62.5	64.3	64.3
Specificity	92.5	85	67.5	65	70	6.,5	65	35
AUC (95%CI)	0.948 (0.899–0.996)	0.915 (0.859–0.971)	0.730 (0.628–0.832)	0.647 (0.538–0.756)	0.772 (0.673–0.871)	0.692 (0.586–0.799)	0.683 (0.565–0.802)	0.952 (0.9–1)
p-value	<0.001	<0.001	<0.001	0.014	<0.001	0.01	0.002	<0.001

CAR: CRP-to-albumin ratio; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MHR: monocyte-to-HDL ratio; MLR: monocyte-to-lymphocyte ratio; MPV: mean platelet volume; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; RDW: red cell distribution width; TA: Takayasu arteritis.

CRP and ESR (r=0.828 and 0.576, respectively) and weakly to moderately correlated with RDW (r=0.376). MHR was weakly to moderately correlated with ESR, CRP, and RDW (r=0.336, r=0.307, and r=0.322, respectively) and strongly correlated with MLR (r=0.531).

DISCUSSION

In this study, we aimed to evaluate the inflammation markers of TA patients in active and remission periods and investigate the place of NLR, PLR, MLR, RDW, and MPV, especially MHR and CAR, which have been the subject of many studies recently, in disease diagnosis and follow-up of TA.

In our study, ESR, CRP, NLR, PLR, MLR, MHR, and CAR were significantly higher in the active TA group than in the HC group and the TA group in remission. In the TA group in remission, ESR, CRP, CAR, and RDW were significantly higher than the HC group. According to ROC analysis, the most powerful parameter in distinguishing TA from the HC group was CAR, followed by ESR, CRP, MLR, NLR, RDW, MHR, and PLR, respectively. CAR was strongly correlated with CRP, which was of course included in the formula, was strongly correlated with ESR, and was weakly to moderately correlated with RDW.

Erythrocyte sedimentation rate and CRP have been the best-known APRs for many years. Except for inflammation, ESR may increase in many inflammation-related conditions such as anemia, pregnancy, and old age[8]. CRP better represents many chronic inflammation-related conditions such as obesity, DM, HT, atherosclerosis, and the risk of cardiovascular heart disease due to atherosclerosis[8,9]. NLR, PLR, MLR, RDW, and MPV are also parameters that have been investigated in many studies on active infection and chronic inflammation[10,11].

In two previous studies, PLR and NLR levels were found to be higher in the TA patients with high disease activity compared with the healthy population, and these parameters were found to have a positive correlation with disease activation. They also reported that NLR and PLR predicted the TA similarly and that both PLR and NLR were correlated with ESR and CRP. Based on these findings, Pan et al. concluded that PLR and NLR could be used to reflect the inflammatory response and disease activity in the TA, and Li et al. concluded that PLR was more powerful than NLR in detecting the disease activation in their patients[12,13]. In our study, similarly, NLR was found to be significantly higher in the active TA compared with the HC group and in the active TA patients compared with the patients in remission. In PLR, there was no significant difference between the active TA and healthy population, but it decreased significantly when we compared the active period and remission in the same patient. When the formula of these markers was analyzed, it was observed that the difference was platelet-derived. In the study conducted by Pan et al. the blood samples were taken in a tube without anticoagulant and evaluated. In our study, a tube with EDTA was used. We know that EDTA can cause pseudothrombocytopenia[14]. Therefore, we think that the discordance in PLR value can be a negative effect of EDTA tube use. In the study conducted by Li et al. 180 (62.2%) active TA patients were included. In our study, this number was 56. We think that the difference in sample size caused this result[13].

The recent study on this subject was conducted by Seringec Akkececi et al. in 2019. It was found that ESR, CRP, NLR, PLR, MLR, RDW, and CAR were significantly higher in the active TA patients compared with the control and remission groups. It was reported that CAR had the highest correlation with the disease activity and showed a positive correlation with ESR, CRP, NLR, PLR, MLR, and RDW[7]. In our study, ESR, CRP, NLR, MLR, RDW, and CAR were found to be significantly higher in the patients with active TA compared with healthy subjects, and PLR was not found to be different. Similarly, ESR, CRP, NLR, PLR, MLR, MLR, and CAR decreased in the patients in remission. The results of our study are similar with the study by Seringec Akkececi et al. Unlike this study, we had the opportunity to evaluate MHR.

The limitations of our study include limited patient sample size, single-center design, and retrospective methodology. The majority of the patients included in our study were on steroid therapy after diagnosis. This may have caused changes in hematological parameters in the TA group in remission. To the best of our knowledge, an important aspect of this study is that it is the first research to demonstrate the importance of MHR in diagnosing and indicating disease activation in TA. Furthermore, rather than categorizing patients into two groups based on their activity level, we examined both the periods of active disease and remission within the same patient group. This approach allowed us to assess the parameters that could potentially be valuable in monitoring the treatment progress.

CONCLUSION

Monocyte/HDL ratio and CAR are usable markers both in the diagnosis of TA and to indicate active disease. Especially as CAR is a stronger marker than CRP in showing inflammation, evaluation of CRP together with albumin in daily practice reaches more accurate results in differentiating inflammation.

These markers will give us an advantage, particularly in patients with normal ESR and CRP, but clinical suspicion of activation. NLR, PLR, MLR, and RDW are inexpensive tests available in almost every laboratory and are promising in clinical practice, especially in TA patients with normal CRP and ESR values. In our opinion, especially CAR and MHR can be used as indicators of active inflammation in TA patients. Based on the result of our study that CAR is a more powerful marker than CRP in showing active inflammation, we think that the evaluation of albumin results together with CRP may give more accurate results in evaluating inflammation in daily practice. As clinical studies supporting these data increase, we think that its use in daily practice will become widespread.

AUTHORS' CONTRIBUTIONS

EAK: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **GV:** Conceptualization, Methodology, Supervision. **GK:** Conceptualization, Formal Analysis, Project administration. **İT:** Methodology, Project administration, Validation, Writing – review & editing. **HTEÖ:** Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231694

Value of soluble fms-like tyrosine kinase 1 for predicting acute pancreatitis severity: a systematic review and meta-analysis

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SUMMARY

OBJECTIVE: The objective of this study was to explore the relationship between serum soluble fms-like tyrosine kinase 1 and the severity of acute pancreatitis and its diagnostic utility.

METHODS: This study was carried out by searching Chinese and English literature from the establishment of the database to July 9, 2023, systematically, and assessing the quality and heterogeneity of the articles included.

RESULTS: Thirteen studies with a total of 986 patients were included. Patients with severe acute pancreatitis showed higher levels of soluble fms-like tyrosine kinase 1 compared with mild acute pancreatitis [weighted mean difference=76.64 pg/mL, 95% confidence interval (95%CI 50.39–102.89, p<0.001)]. Soluble fms-like tyrosine kinase 1 predicted pooled sensitivity, specificity, and area under the curve were 79%, 74%, and 0.85 for severe acute pancreatitis, with some heterogeneity (I^2>50% or p<0.05). In the subgroup analysis, cutoff >150 pg/mL was found to be a heterogeneous factor.

CONCLUSION: Soluble fms-like tyrosine kinase 1 is a reliable tool for identifying acute pancreatitis severity, but only as a screening tool.

KEYWORDS: Vascular endothelial growth factor receptor-1. Acute pancreatitis. Systematic review. Meta-analysis.

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease of varying severity. The majority of patients experience mild acute pancreatitis (MAP), which resolves on its own. However, as per the 2012 Revised Atlanta classification, approximately 30% of patients are categorized as moderately severe acute pancreatitis (MSAP), while around 10% are classified as severe acute pancreatitis (SAP), with some cases dying from AP[1-3]. The early pathological events of AP, whether systemic or local, are associated with endothelial activation and damage[4]. Consequently, persistent endothelial dysfunction and activation are early predictors of the likely severity of AP[5,6].

Soluble fms-like tyrosine kinase 1 (sFlt-1), also known as vascular endothelial growth factor (VEGF) receptor-1 (VEGFR-1), generated by alternative splicing of Flt-1 pre-mRNA, acts as a membrane receptor that binds VEGF and placental growth factor (PIGF). VEGF is a potent stimulator of endothelial activation and injury[4,6,7]. In AP patients, sFlt-1 was found to be an early marker of the severity of AP patients[6,8]. However, the relevant literature is still vague and needs further clarification and organization.

This meta-analysis aimed to assess the predictive capability of sFlt-1 in determining the severity of AP.

METHODS

Search strategy and study identification

A systematic search of publications up to July 9, 2023, in the Cochrane Library, PubMed, EMBASE, China Academic Journals Full-text Database, WANFANG, China Biology Medicine disc, and WEIPU databases was performed to evaluate serum sFlt-1 value as a marker of AP severity. We searched for "pancreatitis" and "Vascular Endothelial Growth Factor Receptor-1" based on "MeSH." We have no restrictions on language or date.

Our inclusion criteria were as follows: (1) a retrospective or prospective study; (2) age >18 years; and (3) serum sFlt-1 can diagnose AP severity that includes MSAP+SAP or SAP (the AP severity was defined by the authors of primary studies). Exclusion criteria were as follows: (1) case reports, reviews, editorials, and animal or in vitro studies; (2) studies without any form of severity stratification; (3) only the abstract was available; (4) duplicate study; and (5) inability to provide complete data required for analysis.

Data extraction and quality assessment

Three researchers (Wang SN, Tang T, and Zhou WM) selected documents by reviewing abstracts and titles. Data from each study were compiled as follows: first author (year of publication),

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 14, 2023. Accepted on December 16, 2023.

number of subjects (male/female), study design, age, time of blood sample, sFlt-1 measurement method, mean (SD) and cutoff value of sFlt-1, and sensitivity and specificity of the study. Methodological quality was evaluated independently by two reviewers (Wang SN and Zhou WM) using the QUADAS-2 by RevMan 5.3 (Cochrane Collaboration, Oxford, UK)[9]. All disagreements were settled by consensus among authors.

Statistical analysis

Systematic reviews and meta-analyses were performed based on the PRISMA checklist[10]. I^2 and p-values were used to evaluate heterogeneity. When I^2>50% or p<0.05, the random effects model was used for calculation and analysis, and subgroup analysis was performed to find the source of heterogeneity. The weighted mean difference (WMD) and 95% confidence interval (95%CI) of the included studies were calculated to evaluate the differences in sFlt-1 concentrations between different severity levels. If concentrations were reported as median and IQR in included studies, the corresponding mean and standard deviation were estimated and included in the analysis[11,12]. The pooled sensitivity, specificity, and area under the curve (AUC) of the included studies were calculated. Sensitivity analysis was performed to verify the stability of the results. Publication bias was detected using Deek's funnel plot or Egger's test. All statistical analyses were performed using Stata V.14.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Research characteristics

From the initial literature search, we identified and screened 260 references. Of these, 62 were excluded due to duplication. By reading the titles and abstracts, we further removed 181 articles that did not meet the requirements. Among the 17 articles obtained, after reading the full text, five articles were excluded due to failure to obtain complete data, duplicate data, or questionable data. Among them, Dumnicka et al.[5] reported the results on the first and second days after onset, which were regarded as two articles of this study. Finally, 13 studies were included in the paper[5,8,13-22]. Quality assessment was performed according to QUADAS-2.

Soluble fms-like tyrosine kinase 1 concentration correlates with acute pancreatitis severity

Random-effects results from 11 studies showed that patients with SAP showed higher levels of sFlt-1 compared with MAP (WMD=76.64 pg/mL, 95%CI 50.39–102.89, p<0.001) (Figure 1). In addition, 10 studies showed that patients with AP had higher

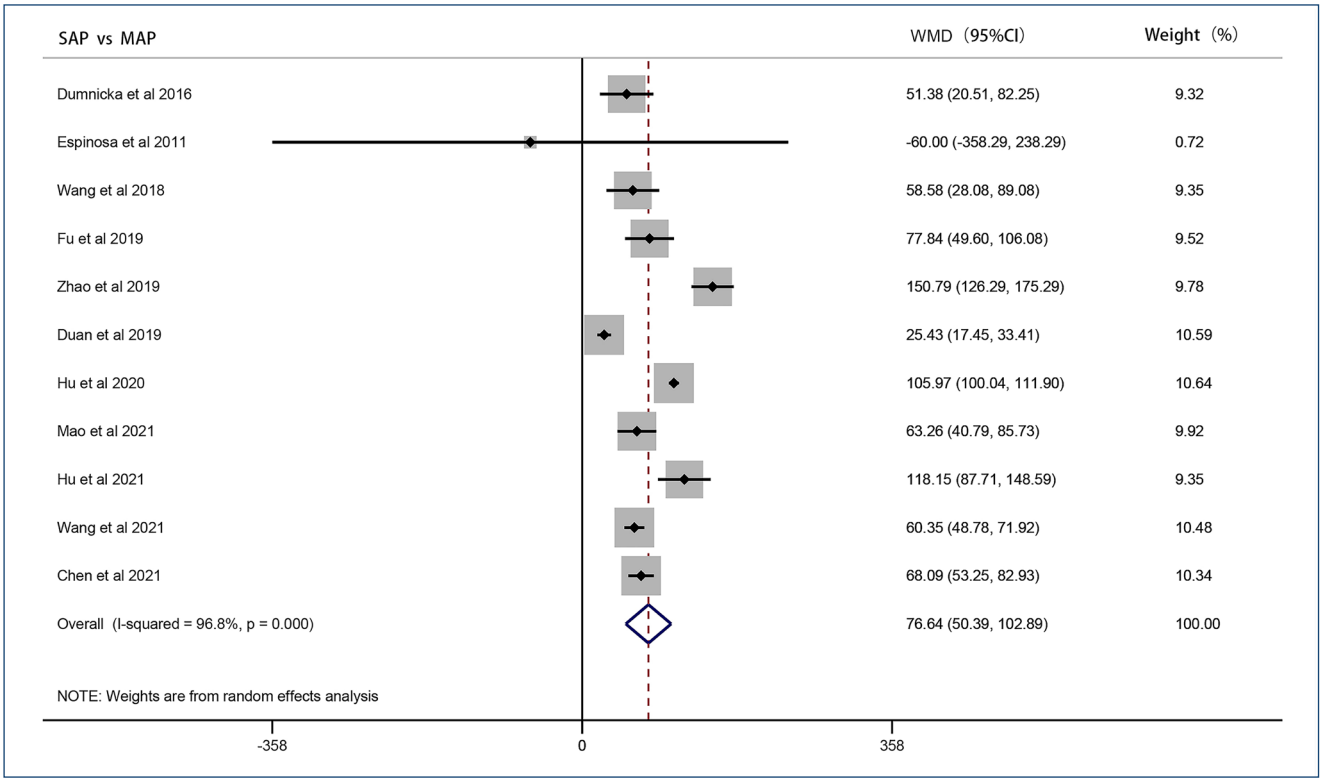

Figure 1. Forest plot of soluble fms-like tyrosine kinase 1 concentrations.

sFlt-1 levels compared with non-AP patients or healthy adults (WMD=74.80 pg/mL, 95%CI 36.40–113.19, p<0.001). Three studies reported sFlt-1 with a poor prognosis like multiple organ dysfunction and death, and we included them in the poor prognosis group. Results from the fixed-effect model showed significantly elevated sFlt-1 levels in patients with a poor prognosis (WMD=102.76 pg/mL, 95%CI 94.86–110.65, p=0.452).

Except for prognosis groups, significant heterogeneity existed in other groups. Sensitivity analysis showed that excluding any study in the SAP vs. MAP groups and AP vs. non-AP groups, the results did not change significantly. In the prognosis groups, the results changed significantly after excluding the studies of Hu et al. and Mao et al. No significant publication bias was found.

Diagnostic value of soluble fms-like tyrosine kinase 1 in acute pancreatitis

Among the included studies, five studies explored the diagnostic value of sFlt-1 for early AP and seven explored the diagnostic value of sFlt-1 for SAP. We performed separate diagnostic meta-analyses. The results showed that the pooled sensitivity, specificity, and AUC of sFlt-1 in predicting AP were 0.78 (95%CI 0.65–0.86), 0.74 (95%CI 0.65–0.81), 0.81 (95%CI 0.77–0.84), I^2>50%, p<0.05; and in predicting SAP, they were 0.79 (95%CI 0.70–0.86), 0.74 (95%CI 0.69–0.83), 0.85 (95%CI 0.81–0.88), I^2>40%, p<0.05 (Figure 2). In sensitivity analyses, the deletion of Hu and Wang's studies had a significant impact on the results.

To explore the influencing factors leading to heterogeneity, we performed a subgroup analysis (Table 1). Combined with the overall p-value of the subgroup, the subgroup analysis results of the diagnostic value of sFlt-1 for SAP showed that the "cutoff value (150 pg/mL)" may be an influencing factor of heterogeneity, while no significant influencing factors of heterogeneity were found in the analysis of AP.

The funnel plot of the diagnostic value of sFlt-1 for AP found significant bias, mainly from the study published by Duan, while SAP diagnostic value funnel plots did not show publication bias.

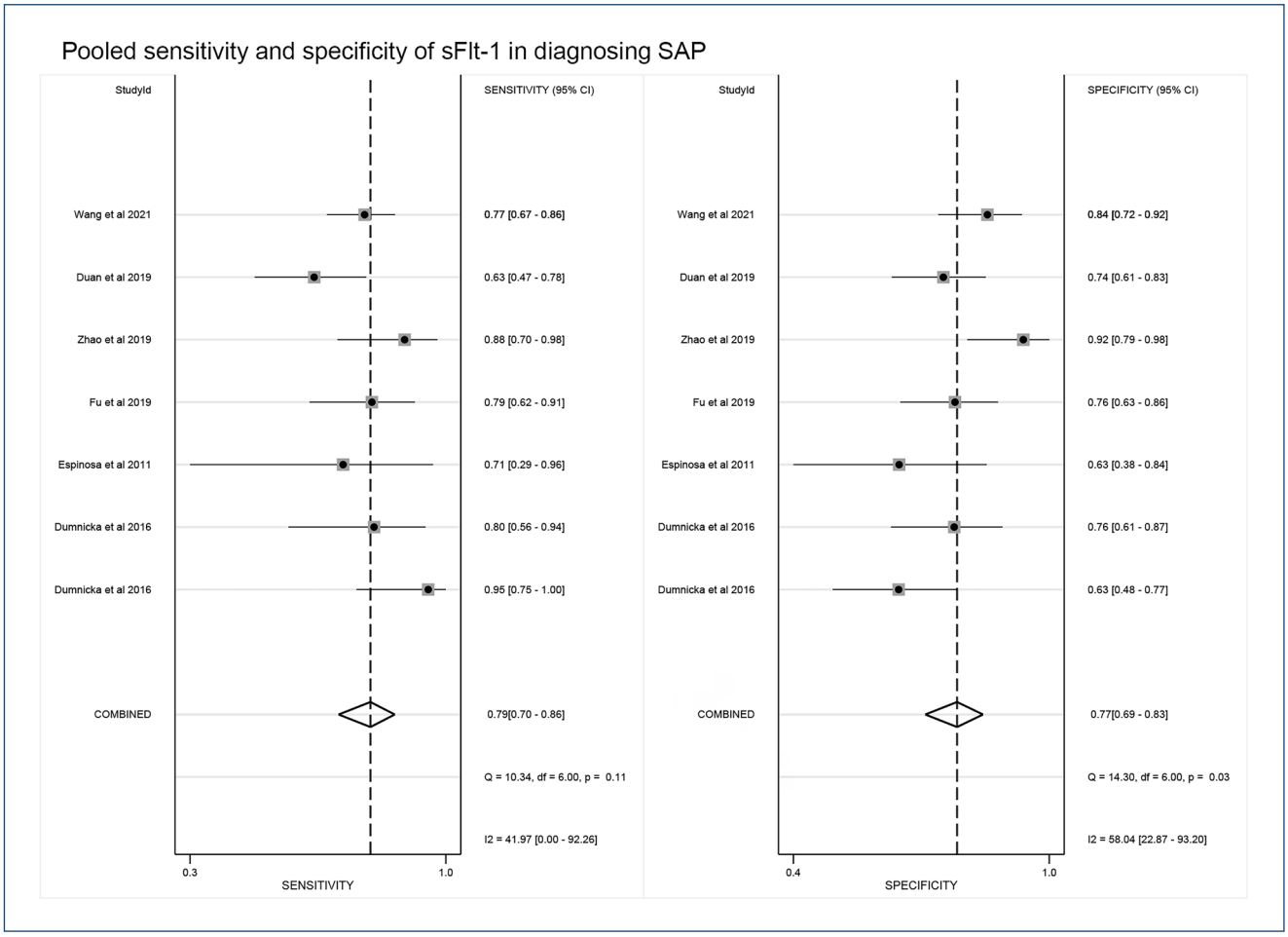

Figure 2. Pooled sensitivity and specificity of soluble fms-like tyrosine kinase 1 in diagnosing severe acute pancreatitis.

Table 1. The result of meta-regression and subgroup analysis.

		Category	Parameter	No. of studies	Sensitivity (95%)	P1	Specificity (95%)	P2	p-value	I^2
SAP	Definition of severity	Yes	Atlanta classification	3	0.85 [0.74–0.96]	0.51	0.68 [0.57–0.79]	0.00	0.04	70
		No	Others	4	0.77 [0.69–0.85]		0.81 [0.75–0.88]			
	Study design	Yes	Prospective	2	0.88 [0.77–0.99]	0.93	0.70 [0.57–0.82]	0.00	0.07	62
		NO	Retrospective	5	0.76 [0.68–0.83]		0.79 [0.73–0.86]			
	Geographical location	Yes	Asia	4	0.77 [0.69–0.85]	0.02	0.81 [0.75–0.88]	0.41	0.04	70
		No	Others	3	0.85 [0.74–0.96]		0.68 [0.57–0.79]			
	sFlt-1 sampling time	Yes	≤24 h	2	0.82 [0.75–0.89]	0.55	0.77 [0.70–0.85]	0.13	0.17	44
		NO	>24 h	2	0.69 [0.57–0.81]		0.75 [0.63–0.87]			
	Sample size	Yes	>100	2	0.72 [0.63–0.82]	0.00	0.79 [0.68–0.90]	0.15	0.10	57
		NO	≤ 100	5	0.84 [0.77–0.91]		0.75 [0.67–0.84]			
	Cutoff value	Yes	>150 pg/mL	3	0.81 [0.70–0.92]	0.39	0.83 [0.76–0.90]	0.21	0.00	84
		NO	≤150 pg/mL	3	0.78 [0.65–0.91]		0.71 [0.64–0.78]			
	Methods	Yes	ELISA	5	0.76 [0.68–0.83]	0.02	0.79 [0.73–0.86]	0.37	0.07	62
		NO	ECLIA	2	0.88 [0.77–0.99]		0.70 [0.57–0.82]			
AP	Definition of severity	Yes	Atlanta classification	1	0.81 [0.60–1.00]	0.99	0.62 [0.53–0.71]	0.00	0.04	68
		No	Others	4	0.76 [0.64–0.88]		0.79 [0.72–0.85]			
	Study design	Yes	Prospective	2	0.84 [0.73–0.95]	0.97	0.77 [0.64–0.89]	0.31	0.28	22
		NO	Retrospective	3	0.72 [0.58–0.86]		0.72 [0.62–0.82]			
	sFlt-1 sampling time	Yes	≤24 h	3	0.81 [0.70–0.92]	0.89	0.71 [0.61–0.81]	0.03	0.55	0
		NO	>24 h	2	0.71 [0.54–0.89]		0.78 [0.67–0.88]			
	Sample size	Yes	>100	1	0.51 [0.42–0.61]	0.00	0.79 [0.65–0.92]	0.48	0.00	82
		NO	≤100	4	0.82 [0.78–0.87]		0.73 [0.63–0.82]			
	Cutoff value	Yes	>150 pg/mL	3	0.83 [0.74–0.92]	0.86	0.68 [0.59–0.77]	0.00	0.05	66
		NO	≤150 pg/mL	2	0.66 [0.51–0.82]		0.81 [0.72–0.90]			

DISCUSSION

In this study, we determined that sFlt-1 is related to AP severity and is a moderate predictor of AP severity, with AUC, sensitivity, and specificity of 0.85, 79, and 74%, respectively. However, due to the small number of studies, there is no significant correlation between elevated sFlt-1 and the primary outcome of AP patients, and its value in assessing the prognosis of AP needs to be further explored. The above results indicate that sFlt-1 has a high false negative rate and false positive rate. Therefore, the use of sFlt-1 cannot be the gold standard for AP severity.

Soluble fms-like tyrosine kinase 1 is the soluble isoform of Flt-1 and has extremely high affinity with VEGF and PIGF, which will bind to them and hinder their angiogenic effects on VEGFR, and is considered a marker of endothelial dysfunction[14,23]. The early pathological events of AP are related to vascular disorders[4], involving the activation and dysfunction of endothelial cells[24]. Studies have found that VEGF is expressed increasingly in the inflamed pancreas, which is consistent with increased vascular permeability in early pancreatitis[4]. In some inflammatory diseases, circulating sFlt-1 levels are elevated and positively correlated with severity, suggesting adverse clinical events[25-27]. In summary, endothelial injury may also cause an increase in sFlt-1 in AP, which may reflect the severity of AP or organ dysfunction. This was confirmed in our meta-analysis.

As with any other laboratory marker, the accuracy of sFlt-1 appears to be dependent on cutoff values[28]. When the sFlt-1 value is >150 pg/mL, there is no difference in sensitivity and specificity between groups, but there is significant heterogeneity in the summary results ($p<0.01$), which may be the source of study heterogeneity. As we selected the same cutoff value for subgroup analysis, sFlt-1 was not found to be a source of heterogeneity in the analysis of the diagnostic value of sFlt-1

for AP. Therefore, we believe that for SAP, a higher cutoff value may be needed. Of course, the optimal cutoff value of sFlt-1 for both AP and SAP still needs to be discussed in future studies.

The expression of sFlt-1 is closely related to the timing of AP severity. There was no heterogeneity regardless of sampling time (p=0.19). Nearly every study measured sFlt-1 within 48 h of onset. However, when the sFlt-1 assay measured within 24 h was used to predict AP severity, increased sensitivity (82 vs. 69%) and similar specificity (77 vs. 75%) were found. Kolber et al. reported 95 AP patients admitted within 24 h of onset[29]. The sFlt-1 values in SAP and MSAP on the second day decreased compared with the first day. Likewise, Dumnicka et al. found that sFlt-1 predicted an AUC of 0.808 for MSAP+SAP in 66 patients. However, subsequent subgroup analysis of the onset time from 18 to 21 h found that the AUC increased (0.836)[5]. In addition, the AUC dropped to 0.791 on the second day of admission. The above results suggest that the peak expression of sFlt-1 may be 24 h after onset, but the specific time period is currently unclear and needs further research and exploration.

There are several other limitations to this study. First, heterogeneity was large in some analyses. Although the random effects model, sensitivity analysis, and subgroup analysis were used to correct the results and find sources of heterogeneity, the heterogeneity could not be completely removed. Second, the included articles are all in English or Chinese, and most of them are Chinese articles. Third, the dynamic nature of organ failure has been found to be critical for AP in recent years, as persistent organ failure carries a worse prognosis than transient organ failure[1,30]. Unfortunately, there are no original articles containing data on persistent organ failure, making it impossible to discuss this issue. Finally, the difference in cutoff values is observed among studies. Hence, we did not get a certain cutoff value, which could lead to the clinical application of the sFlt-1 being limited.

CONCLUSION

Serum sFlt-1 can only be used as a screening tool for the severity of AP. However, studies focusing on different determinations of sFlt-1, the timing of sFlt-1 measurement, and the consistency of definitions of AP severity may improve the accuracy of sFlt-1 in estimating AP severity.

AUTHORS' CONTRIBUTIONS

WMZ: Conceptualization, Data curation, Methodology, Resources, Supervision, Writing – review & editing. **SNW:** Data curation, Formal Analysis, Software, Visualization, Writing – original draft, Writing – review & editing. **TT:** Conceptualization, Data curation, Formal Analysis, Methodology.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20231727

Do you mind the role of spinal sensory block duration in a crucial endocrine disorder of diabetes mellitus? A prospective observational study

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SUMMARY

OBJECTIVE: Diabetes mellitus, per se, is a global health concern, which is often accompanied by complications such as diabetic neuropathy. This prospective observational study purposed to assess the durations of spinal sensory block and motor blocks in individuals with and without diabetes mellitus who had undergone spinal anesthesia.

METHODS: This study incorporated 80 cases, which were evenly divided into spinal sensory block without diabetes mellitus and spinal sensory block with diabetes mellitus. Various parameters were recorded at different time points, including heart rate, mean arterial blood pressure, SpO_2, and spinal block characteristics. Notable measures included maximum spinal sensory block onset time, time to reach the 10th thoracic vertebra (T10), maximal spinal sensory block, time for Bromage scores, and block regression while controlling for age-related variations.

RESULTS: Patients in the diabetic group exhibited extended block durations, with significant differences in heart rate noted at specific time points. Regarding the spinal block characteristics, the "maximum onset of SSB" and the "time to reach the T10" were more prolonged in the SSBwDM without significance. Maximum sensory spinal sensory block did not differ. However, some cases in the SSBwDM displayed blocks extending up to the T6. The times to achieve Bromage motor block scores 1–3 were shorter in SSBwDM and lost significance regarding age. Notably, the regression time was longer in SSBwDM, which held significance for both parameters.

CONCLUSION: Diabetic cases commonly encounter prolonged block durations post-subarachnoid intervention, potentially linked to nerve sensitivity, age-related changes, and glycemic control. As such, attenuated local doses for diabetic neuropathic cases may enhance early mobilization, attenuate thromboembolic events, and expedite gastrointestinal recovery.

KEYWORDS: Diabetes mellitus. Diabetic neuropathies. Anesthesia, spinal. Pathology. Surgery.

INTRODUCTION

Diabetes mellitus (DM), per se, is a chronic metabolic disorder characterized by persistent hyperglycemia, primarily resulting from impaired insulin secretion, resistance to insulin's peripheral actions, or a combination of both. The chronic elevation of blood sugar levels, in conjunction with other metabolic abnormalities, can inflict harm upon various organ systems, leading to the emergence of debilitating and life-threatening health complications, including microvascular issues such as retinopathy, nephropathy, and neuropathy, as well as macrovascular complications[1]. One of the significant complications of DM is neuropathy, a condition where nerves are damaged, leading to impairment of sensory, motor, and autonomic nerve functions. Peripheral nerve damage is particularly evident in individuals with DM and can result in neuropathy, which may substantially impact daily life activities and overall health. Diabetic neuropathy can manifest as symptoms such as pain, numbness, and tingling, significantly ruining quality of life[2-4].

In this context, the spinal approach offers advantages such as better hemodynamic control, lower risk of postoperative wound infection, reduced postoperative nausea and vomiting, and potentially faster recovery and mobilization. This study

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on December 14, 2023. Accepted on December 29, 2023.

investigates how subclinical peripheral nerve neuropathy in Type 2 DM responds to spinal blocks and aims to compare sensory and motor block durations after the spinal approach and the time elapsed until the first concerning Type 2 DM, whose outcomes could contribute to a better understanding of the use of spinal anesthesia in surgical procedures for diabetic cases.

METHODS

Study design

In this prospective study, individuals with Type 2 DM and those without DM who had been scheduled for elective procedures under spinal anesthesia were selected sequentially. The patient eligibility criteria included the age of 18–75 years, a height of 150–180 cm, and a body mass index (BMI) of less than 40 kg/m^2, while the exclusion criteria were pregnancy, significant vertebral column abnormalities, dehydration, neuropathy history, or contraindications for the spinal method. The primary outcome was the time to reach maximum spinal sensory block (SSB) to develop after the spinal one. A sample size of 40 participants in each was determined using an independent group t-test model with a 20% increase in SSB onset time for the SSBwDM, Cohen's d effect size of 0.596, 80% power, one-sided confidence interval, and a 5% type 1 error rate.

As such, the study initially included 94 cases, of which 11 were excluded due to unregulated blood sugar, 3 required general anesthesia due to inadequate block, and the remaining 80 were divided into SSB without DM (SSBwoDM) and SSB with DM (SSBwDM). Parameters including heart rate, mean arterial blood pressure, and peripheral oxygen saturation were assessed at seven time points. Demographic data, ASA physical classification, weight, height, BMI, and sex were also collected. The spinal approach was administered through the L3–L4 space, using 15 mg of 0.5% hyperbaric bupivacaine with a 24-G Sprotte needle. Monitoring was conducted from the arrival in the operating room and continued every 5 min for the first 30 post-spinal punctures and then every 15 min until both blocks resolved.

The level of sensory blockade was determined using a cotton swab soaked in alcohol, assessing sensitivity to cold. The motor block level was set concurrently using the Bromage Scale. Patients achieving an SSB below the T6 dermatome and with a Modified Aldrete score exceeding 8 were transferred to the post-anesthesia care unit, and block durations were recorded.

Statistical analysis

In analyzing the data obtained in the study, IBM-Statistical Package for Social Sciences (IBM-SPSS Inc., Chicago, IL, USA) version 22.0 was used. The normal distribution of the data was assessed using the Shapiro-Wilk test. The continuous variables were expressed as mean and standard deviation or median (25–75 percentile) depending on the distribution, and categorical variables were expressed as numbers and percentages. For the analysis of continuous variables, the independent samples t-test was applied when the parametric test assumptions were met; otherwise, the Mann-Whitney U test was used. The analysis of categorical variables used the chi-square test or Fisher's exact test. An analysis of variance (ANOVA) was employed for repeated measurements at different times among groups. An analysis of covariance (ANCOVA) was used to control for the age effect. The statistical significance level was set at $p<0.05$.

RESULTS

The statistical similarity was observed for weight, height, BMI, and sex when examining the demographic parameters. A significant difference ($p<0.001$) was observed between the ASA physical categorization score of 2 for all in SSBwDM and 70% for SSBwoDM. Furthermore, SSBwDM mean age was considerably greater ($p<0.001$) (Table 1). The assessment of

Table 1. The demographical and clinical characteristics of the participants.

Characteristics		SSBwoDM n=40	SSBwDM n=40	p-value
Age (years)		47±17	61±12	**<0.001**
Sex	Male	21 (52.5%)	17 (42.5%)	0.370
	Female	19 (47.5%)	23 (57.5%)	
ASA	1	12 (30%)	0 (0%)	**<0.001**
	2	28 (70%)	40 (100%)	
Weight (kg)		82±14	84±12	0.487
Height (cm)		170±8	168±9	0.303
BMI (kg/m^2)		28.3±5.2	29.6±3.9	0.212

Data are mean and standard deviation or number (%). BMI: body mass index, ASA: American Society of Anesthesiologists. Values of $p<0.05$ are marked in bold.

SSBwDM revealed that 32 cases (80%) were receiving oral antidiabetic (OAD) treatment, 3 (7.5%) were receiving only insulin, 9 (22.5%) were receiving both OAD and insulin, and 5 (12.5%) were not receiving any medication related to DM. The average HbA1c in SSBwDM was 7.4 (ranging 6.32–8.98). The SSBwDM mean heart rate values were consistently higher when the heart rate parameter (controlled for age) was evaluated. Still, this elevation was significant only for times t5, t6, and t7 (p=0.040, p=0.032, and p=0.003, respectively) (Table 2). Changes in heart rate mean over time (time-group interaction) were significant (p=0.018). The parameters of mean arterial blood pressure and SpO_2 (both controlled for age) were evaluated, and no difference at all times was recognized (p>0.05). Changes over time in mean arterial blood pressure and SpO_2 means (time-group interaction) were similar for both (p=0.382 and p=0.158, respectively). The SSB characteristics revealed that the parameters "maximum SSB onset time" and "time to reach T10 for SSB" were more prolonged in cases of SSBwDM. Still, this difference was insignificant (p=0.108 and p=0.366, respectively). Although there was no significant difference in the maximal SSB between them, 22.5% of SSBwDM had the SSB level at T6. In comparison, 12.5% of SSBwoDM had it and 30% of SSBwDM had the SSB level at T7, whereas 25% of SSBwoDM had it and 15% of SSBwDM had the SSB level at T10, and 2.5% in SSBwoDM had it. The times to achieve motor block Bromage scores 1–3 were shorter in SSBwDM. Still, when controlled for age, no significant difference between the groups for these three parameters was detected (p=0.081, p=0.248, and p=0.575, respectively). Herewith, the motor block and SSB regression duration were more prolonged in SSBwDM, which was significant for both parameters (p=0.014 and p<0.001, respectively) (Table 3).

Table 2. The heart rate values in groups according to time.

Time	SSBwoDM n=40	SSBwDM n=40	p-value	Adjusted (age) p-value
T1	76±12	76±11	0.744	0.828
T2	74±12	76±12	0.433	0.464
T3	72±12	73±13	0.741	0.913
T4	67±9	70±13	0.259	0.303
T5	64±9	69±12	**0.015**	**0.040**
T6	63±10	69±12	**0.017**	**0.032**
T7	61±9	69±11	**<0.001**	**0.003**

The data are mean and standard deviation. The values of p<0.05 are marked in bold.

DISCUSSION

The results of our study confirm that in patients with DM, the dermatomal block following the subarachnoid administration of 0.5% hyperbaric bupivacaine differs from that observed in non-diabetic cases, which is likely due to the increased sensitivity of diabetic nerves to local anesthetics, leading to a longer block duration. Diabetic polyneuropathy, per se, is the result of complex pathophysiological processes primarily triggered by chronic hyperglycemia. Diabetic neuropathy exhibits different responses to regional anesthesia, including a theoretically higher risk of nerve damage due to the initial increase in the nerve's electric stimulation threshold[5]. Kalichman and Calcutt reported no difference in block duration between

Table 3. The spinal block characteristics of the groups.

Characteristics		SSBwoDM n=40	SSBwDM n=40	p-value	Adjusted (age) p-value
Time to achieve maximum sensorial block level (s)		300 (185–365)	300 (245–400)	0.076	0.108
Time to achieve 10th thoracal vertebra sensorial block level (s)		120 (90–190)	125 (90–205)	0.597	0.366
Maximum sensorial block level (thoracal vertebra)	6	5 (12.5%)	9 (22.5%)	0.070	–
	7	10 (25%)	12 (30%)		
	8	23 (57.5%)	13 (32.5%)		
	9	1 (2.5%)	0 (0%)		
	10	1 (2.5%)	6 (15%)		
Time to achieve Bromage score 1 (s)		115 (90–180)	78 (60–120)	**0.009**	0.081
Time to achieve Bromage score 2 (s)		155 (120–240)	120 (95–180)	**0.048**	0.248
Time to achieve Bromage score 3 (s)		270 (205–360)	240 (180–300)	0.476	0.575
Time to regression motor block (min)		197.5 (180–240)	235 (210–240)	**0.007**	**0.014**
Time to regression sensorial block (min)		210 (190–240)	257.5 (230–270)	**<0.001**	**<0.001**

The data are median (25–75 percentiles) or number (%). The values of p<0.05 are marked in bold.

diabetic and control animals, while Kroin and Lirk demonstrated extended block durations in diabetic neuropathic animals[6-8]. Furthermore, Kroin et al. delved deeper into ascertaining whether neuropathy or hyperglycemia was responsible for the prolonged block durations, whose findings revealed that long-term glucose control, with concurrent neuropathy attenuation, restored average block duration, whereas acute glucose control or hyperglycemia management did not have the same effect[9]. The effects of diabetes on motor and sensory blocks have been studied in peripheral nerve blocks, but research on the impact of diabetes on spinal blocks is limited[10-16]. Therefore, to the best of our knowledge, this is the first study on this topic in the English-language literature.

One study showed that diabetic patients have more extensive cerebrospinal fluid (CSF) volumes in the brain regions compared with control subjects. This increase in CSF volume, particularly in hypertension and diabetes, did not significantly affect the block level during isobaric spinal anesthesia, so the changes in CSF volume and density in diabetic patients were not suggested to possess a significant clinical impact on spinal blocks[17]. While our study did not find statistical significance in maximum SSB onset time and the time to reach the T10 between both groups, other studies, like Echevarria's, found that the top block level duration and total regression time were more prolonged in diabetic ones. However, it is essential to note that their study used epinephrine and bupivacaine in their SSB, which is known to prolong block duration due to epinephrine's vasoconstrictive effect[17].

A link between CSF volume and the extent of SSB while using iso- or hyperbaric bupivacaine at the lumbar level and its volume has also been inversely correlated with motor block initiation and regression at L1 and L2[17]. Various studies have indicated that in the elderly, SSB increases compared with younger ones, possibly due to age-related physiological changes leading to a decrease in CSF volume[18]. Herewith, our study revealed that the diabetic group had a higher average age, which may have contributed to these differences. The association between the age and the number of blocked dermatomes demonstrated a direct relationship in the diabetic one. In contrast, it was only observed for the maximum dermatomal block duration in SSBwDM.

Long-term complications of diabetes, both microvascular and macrovascular, are a significant cause of morbidity and mortality, and glycated hemoglobin (HbA1c) plays a crucial role in developing and progressing microvascular complications like diabetic neuropathy[19-25]. In this study, higher HbA1c levels had longer block durations, suggesting that poor glycemic control may contribute to extended durations. Of note, the exact mechanisms behind this phenomenon remain unclear. Still, pharmacodynamic (increased sensitivity of sodium currents) and pharmacokinetic (decreased nerve blood flow leading to prolonged local anesthetic residence) factors have been proposed. Endocrine disorders might experience various changes in the autonomic system. Herein, we have reported a significantly higher heart rate in the diabetic group. At the same time, blood pressure values did not differ between them, possibly due to changes in the autonomic nervous system.

Limitations

Limitations of the study include a relatively small sample size, single-center nature, heterogeneity among diabetic cases, age differences between groups, and the absence of long-term follow-up data.

CONCLUSION

Our study provides evidence in support of the idea that diabetic patients tend to experience prolonged block durations after subarachnoid intervention. Of note, several factors could contribute to this phenomenon, including the heightened sensitivity of diabetic nerves to local agents, age-related changes, and potentially the impact of glycemic control on duration. In addition, we suggest the need for lower doses of local agents in diabetic neuropathy might enhance early patient mobilization, attenuate thromboembolic events, and expedite the recovery of gastrointestinal function. This issue merits further investigation.

ACKNOWLEDGMENTS

The authors thank all the study participants.

AUTHORS' CONTRIBUTIONS

TA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft. **MC:** Investigation, Methodology, Project administration, Validation, Visualization. **IS:** Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. **ATG:** Investigation, Validation, Visualization. **DS:** Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. MA: Investigation, Validation, Visualization. **TK:** Investigation, Validation, Visualization. **EC:** Investigation, Validation, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240068

Relationship between sacroiliitis and inflammatory markers in familial Mediterranean fever

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SUMMARY

OBJECTIVE: Familial Mediterranean fever is the most common monogenic autoinflammatory disease. This study aimed to evaluate the relationship between sacroiliitis observed in familial Mediterranean fever and hematological inflammatory markers.

METHODS: In this study, 168 familial Mediterranean fever patients were examined. A total of 61 familial Mediterranean fever patients who had sacroiliac magnetic resonance imaging due to waist and hip pain were included in the study. According to the magnetic resonance imaging findings, patients were divided into two groups: with and without sacroiliitis. The relationship between hematological inflammatory markers and sacroiliitis was investigated.

RESULTS: The frequency of sacroiliitis was found to be 13.6% in all familial Mediterranean fever patients and 37.8% in patients with low back pain who underwent sacroiliac magnetic resonance imaging. Neutrophil count, neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and systemic immune-inflammatory index were significantly higher in the sacroiliitis group than in the other group, and this difference was found to be statistically significant ($p<0.05$). As a result of the receiver operating characteristic analysis, it was observed that neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and systemic immune-inflammatory index were very sensitive parameters in determining sacroiliitis in patients with familial Mediterranean fever.

CONCLUSION: It was observed that the frequency of sacroiliitis was increased in familial Mediterranean fever patients. It is predicted that hematological inflammatory markers such as neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and systemic immune-inflammatory index can be used in the diagnosis of sacroiliitis.

KEYWORDS: Familial Mediterranean fever. Inflammation. Sacroiliitis.

INTRODUCTION

Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease. It is characterized by peritonitis, pleuritis, and acute synovitis attacks and is the prototype of relapsing fever syndromes[1]. It is more common in populations of Mediterranean origin such as Arabs, Turks, Jews, and Armenians. Acute relapsing arthritis is the most common form of musculoskeletal involvement in FMF. However, chronic arthritis, including sacroiliitis, may develop in ≤5% of FMF patients[2]. Studies conducted on Turkish FMF patients have reported a high frequency of sacroiliitis[3-5].

The MEFV gene, which is mapped on the short arm of chromosome 16, is associated only with FMF. The most common MEFV mutations among Arabs, Turks, Jews, and Iranians are M694V, E148Q, M680I, and V726A. These genes differ in terms of penetrance and correlation with the severity of clinical symptoms[6].

Inflammation: In many diseases, especially rheumatological diseases, it was found to be related to the severity, clinical presentation, and prognosis of the disease. Various ratios are employed to determine the level of inflammation, which are considered to be superior to the quantitative count of white blood cells. These ratios, which are obtained by dividing cell counts, include the neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and monocyte/lymphocyte ratio (MLR), as well as the systemic immune-inflammatory index (SII)[7-9]. NLR is a diagnostic inflammatory marker studied in gastrointestinal[10-12], endocrine[13], cardiac[14], and infectious[15] diseases. Similarly, MLR is another inflammatory marker studied in malignancies[16], gastrointestinal diseases[12], and endocrine pathologies such as diabetes mellitus[17], showing its significance. SII, which is another inflammation marker, has been investigated for its diagnostic value in various diseases, including sacroiliitis, and is a valuable indicator[18,19]. FMF is an autoinflammatory rheumatic disease, and sacroiliitis can be observed in FMF patients as it is associated with inflammation[20].

This study aimed to investigate the presence of sacroiliitis in FMF patients, the relationship between sacroiliitis and gene mutation, and the diagnostic value of inflammatory markers in FMF patients with sacroiliitis.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 14, 2024. Accepted on February 04, 2024.

METHODS

This is a single-center, retrospective, cross-sectional study. Approval for the study was received from the local university ethics committee. Patients who were previously diagnosed with FMF by Tel-Hashomer criteria had low back and hip pain and underwent sacroiliac magnetic resonance imaging (MRI) in the Department of Radiology between January 2018 and October 2023 were included in the study. All MRIs were prospectively reinterpreted by a musculoskeletal radiologist and rheumatologist blinded to the patient's clinical status. Evaluations were made according to the Assessment of SpondyloArthritis International Society (ASAS) MRI working group definition of active sacroiliitis, and erosions, subchondral bone edema, and synovitis were findings that suggested the diagnosis of sacroiliitis[21]. According to the Declaration of Helsinki, the rights of all participants were protected. Coexistence of FMF and spondyloarthritis, psoriasis, recurrent oral-genital aphtha, inflammatory bowel disease, and amyloidosis were determined as exclusion criteria.

The patient's age, gender, genetic test results, and laboratory test results such as neutrophils, lymphocytes, monocytes, platelets, sedimentation, and C-reactive protein were recorded. NLR, MLR, and SII were used to evaluate the inflammation level. NLR and MLR levels were obtained by dividing the values measured in the complete blood count. SII value was calculated according to the formula platelet ´ neutrophil/lymphocyte ratio.

Statistical analysis

The data from the study were analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0. The normality of the variables was assessed using the Kolmogorov-Smirnov test. For continuous variables that followed a normal distribution, the mean±standard deviation (SD) was reported, while for non-normally distributed variables, the median and minimum-maximum values were provided. Categorical data were presented in terms of frequency and percentage. To compare normally distributed continuous variables, the independent-sample t-test was used, and for non-normally distributed continuous variables, the Mann-Whitney U test was employed. Qualitative data analysis was performed using the chi-square (χ^2) test. In evaluating some quantitative data, diagnostic performance (DP) was determined through receiver operating characteristic (ROC) analysis. The significance level for statistical tests was set at 0.05.

RESULTS

Between January 2018 and October 2023, 168 FMF patients were scanned from medical records. A total of 61 FMF patients who had sacroiliac MRI and met the inclusion and exclusion criteria were evaluated. These patients were divided into two groups, with and without sacroiliitis, according to the available MRI findings. The frequency of sacroiliitis was found to be 13.6% in the screened FMF cohort and 37.8% in patients with low back pain who underwent sacroiliac MRI. The demographic characteristics and laboratory findings of the patients are presented in comparative detail in Table 1.

A significant difference was detected in the NLR, MLR, and SII values in the group with sacroiliitis compared with the other group. Based on this result, the ROC curve was used to

Table 1. Comparison of demographic and laboratory findings of the study group and neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and systemic immune-inflammatory index values.

Gender (n, %)	Sacroiliitis (+)	Sacroiliitis (-)	p
Female	14 (23%)	27 (44.2%)	
Male	9 (14.8%)	11 (18%)	0.41[a]
Age, years (mean±SD)	36.60±12.14	37.73±11.36	0.71[b]
CRP, mg/L median (min–max)	3.28 (0.66–6.90)	2.56 (0.21–7.58)	0.30[c]
ESR, mm/h median (min–max)	11 (2–34)	11.5 (2–39)	0.74[c]
MPV, fl (mean±SD)	10.04±0.97	10.20±0.84	0.50[b]
Neutrophil count (10^9/L) median (min–max)	4.52 (2.95–12.70)	3.41 (1.27–6.49)	**0.001***[c]
Lymphocyte count (10^9/L) median (min–max)	2.19 (1.32–3.88)	2.51 (1.15–5.73)	0.23[c]
Monocyte count (10^9/L) median (min–max)	0.5 (0.27–1.30)	0.42 (0.22–1.21)	0.11[c]
Platelet count (10^9/L) median (min–max)	293 (208–645)	273 (175–526)	0.12[c]
NLR [median (min–max)]	2.01 (1.40–4.35)	1.38 (0.73–3.61)	**0.0001***[c]
MLR [median (min–max)]	0.24 (0.11–0.47)	0.18 (0.09–0.57)	**0.009***[c]
SII [median (min–max)]	738.63 (409.15–1239.55)	387.72 (190.0–987.92)	**0.0001***[c]

Statistically significant values are indicated in bold. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MPV: mean platelet volume; NLR: neutrophil/lymphocyte ratio; MLR: monocyte/lymphocyte ratio; SII: systemic immune-inflammatory index. *p<0.05: statistically significant. [a]Chi-square analysis was used. [b]Independent-sample t-test was used. [c]Mann-Whitney U test was used.

evaluate the cutoff values, sensitivity, and specificity in terms of the importance of NLR, MLR, and SII in the development of sacroiliitis in patients with FMF (Table 2 and Figure 1).

Genetic analysis results of the patients included in the study were as follows: homozygous M694V (M694V/MV94V) in 13 patients (21.3%), heterozygous M694V (M694V/-) in 10 patients (16.3%), heterozygous R202Q (R202Q/-) in 7 patients (11.4%), heterozygous E148Q (E148Q/-) in 7 patients (11.4%), homozygous V726A (V726A/V726A) in 6 patients (9.8%), combined heterozygous M694V/M680I in 5 patients (8.2%), heterozygous M680I (M680I/-) in 4 patients (6.5%), homozygous M680I (M680I/M680I) in 3 patients (5.1%), heterozygous A744S (A744S/-) in 2 patients (3.2%), heterozygous P369S (P369S/-) in 1 patient (1.7%), heterozygous PGLN1678 (PGLN1678/-) in 1 patient (1.7%), homozygous R202Q (R202Q/R202Q) (1.7%) in 1 patient, and heterozygous V722M (V722M/-) in 1 patient (1.7%).

As the majority of patients had the M694V mutation, the relationship between this genotype and sacroiliitis was evaluated. The result was found to be statistically significant ($p<0.05$).

DISCUSSION

Although sacroiliitis is generally known as a distinguishing feature of spondyloarthropathies, it is also observed with increasing

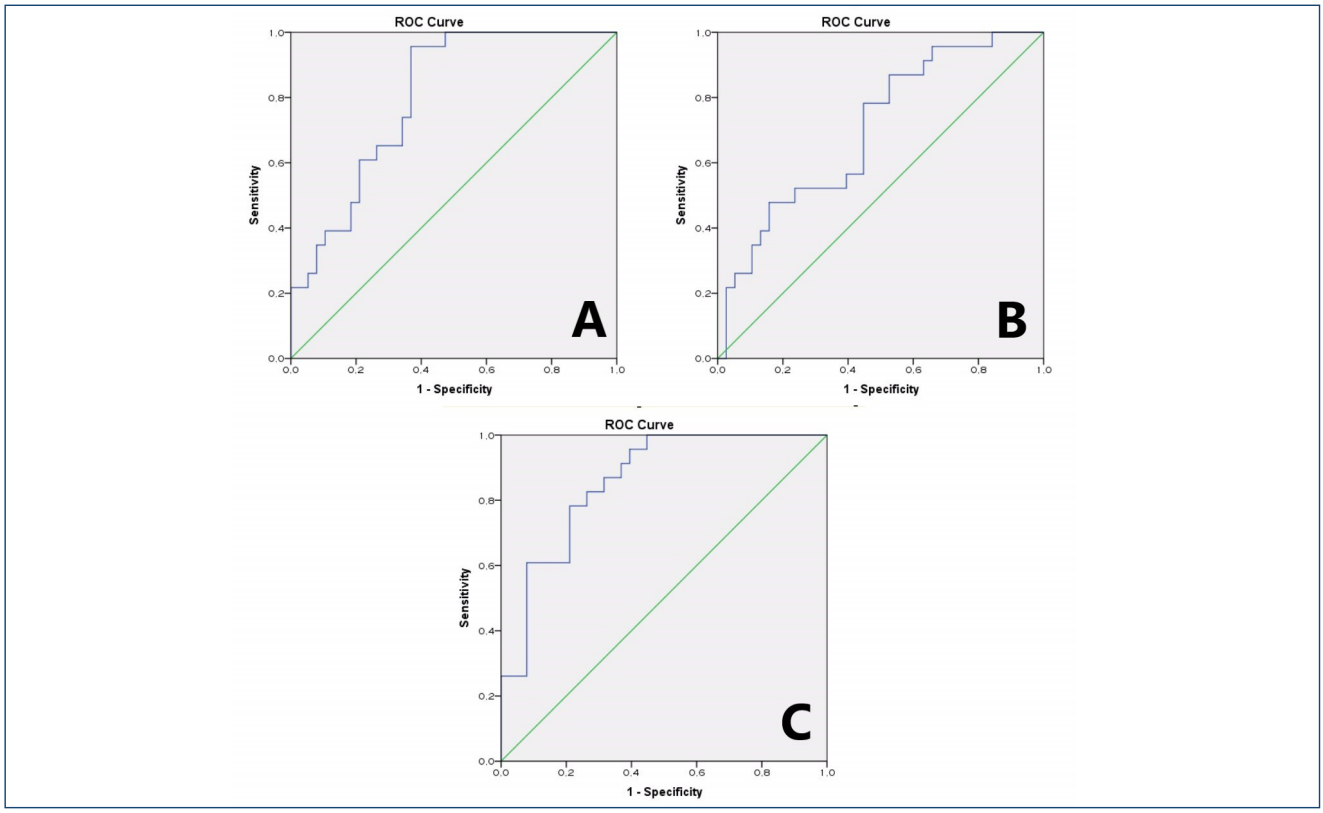

Figure 1. (A) Receiver operating characteristic curve for neutrophil/lymphocyte ratio measurement according to the presence of sacroiliitis in patients with familial Mediterranean fever (AUC: 0.801). (B) Receiver operating characteristic curve for monocyte/lymphocyte ratio measurement according to the presence of sacroiliitis in patients with familial Mediterranean fever (AUC: 0.700). (C) Receiver operating characteristic curve for systemic immune-inflammatory index measurement according to the presence of sacroiliitis in patients with familial Mediterranean fever (AUC: 0.858).

Table 2. Diagnostic screening tests for neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and systemic immune-inflammatory index based on the presence of sacroiliitis and receiver operating characteristic curve results.

	Diagnostic scan			ROC curve		p
	Cutoff	Sensitivity	Specificity	Area	95% confidence interval	
NLR	***≥1.70***	73.90	65.80	**0.801**	0.693–0.908	***0.0001****
MLR	***≥0.193***	78.30	55.30	**0.700**	0.567–0.833	***0.009****
SII	***≥524.07***	78.30	78.90	**0.858**	0.767–0.949	***0.0001****

Statistically significant values are indicated in bold or italic. NLR: neutrophil/lymphocyte ratio; MLR: monocyte/lymphocyte ratio; SII: systemic immune-inflammatory index. *p<0.05: statistically significant.

frequency in Turkish and Jewish FMF patients. In the study conducted by Cefle et al. in Turkish FMF patients, the frequency of sacroiliitis was found to be 10.5%, and in the study by Kaşifoğlu et al. it was found to be 7% in all FMF patients and 32.7% in those with complaints[3,5]. Similarly, in this study, the frequency of sacroiliitis was 13.6% in all FMF patients and 37.8% in those with low back pain. Yildiz et al. found that the rate was found to be 50% in cases in which sacroiliitis was evaluated with Tc99m-MDP bone scintigraphy[4]. It is thought that the differences in the results of many studies may be due to changes such as differences in the patient population and radiological screening methods.

Many studies have been conducted showing that FMF involvement may be associated with different genotypes. M694V mutation is the most common mutation known to be associated with severe disease in Turkish patients[22]. Many studies have shown that the M694V mutation is associated with many different severe phenotypes, such as earlier disease onset, more frequent attacks, higher prevalence of arthritis, pleuritis, erysipelas-like erythema, requirement for high doses of colchicine, and increased risk of amyloidosis. In this study, the risk of developing sacroiliitis in patients with the M694V mutation was found to be statistically significantly higher than in those without it. Gene mutation results of FMF patients who were not included in the study are unknown. This result may not be generalizable to all FMF patients. This is among the limitations of this study.

Another aim of this study was to evaluate subclinical inflammation in the attack-free period in FMF patients who develop sacroiliitis and to determine its contribution to the development of sacroiliitis. To determine subclinical inflammation, NLR, MLR, and SII were used, which have been used in many studies before and are considered to guide in the progression, diagnosis, and treatment of diseases. We found these values to be significantly higher in FMF patients with sacroiliitis compared with those without. Kelesoglu et al. evaluated subclinical inflammation in pediatric FMF patients according to mutation type and attack status and found the CRP level in patients with M694V mutation to be significantly different compared with other mutations[23].

Neutrophil/lymphocyte ratio, PLR, mean platelet volume (MPV), and red cell width distribution (RDW) were used in the study by Özer et al. to determine a new inflammatory marker to indicate subclinical inflammation in FMF patients. While it was concluded that all of them could be used to determine subclinical inflammation, the most powerful marker was NLR[24]. In this study, markers such as NLR, MLR, and SII were used, whose relationship with sacroiliitis in FMF patients has not been investigated before. Additionally, we did not find any other study in the literature that investigated the role of SII and its relationship with the disease in FMF patients. We concluded that NLR, PLR, and SII are very valuable markers in predicting sacroiliitis in patients with FMF. In addition, the cutoff values are found with ROC analysis and are intended to be easily used daily.

Magnetic resonance imaging is the most sensitive method recommended for detecting sacroiliitis[25]. However, MRI is an imaging method that is not available in every center and is costly. Considering these factors, we believe that inflammatory markers may contribute to the diagnosis and at least guide first-line treatment in patients with symptoms.

The positive aspects of this study are the evaluation of the usability of NLR, MLR, and SII, whose relationship with sacroiliitis in FMF patients has not been studied before, in the diagnosis of sacroiliitis and the simple evaluation and conclusion of the results with hemogram examination, which can be used frequently in many patients. There are some limitations in this study. This study was designed retrospectively, which may cause selection bias. Another limitation is that the relationship between mutations and sacroiliitis could not be evaluated due to the lack of sufficient patients in all genotype groups. Studies with larger numbers of patients are needed. We believe that the long-term results of the association of FMF and sacroiliitis can be evaluated by long-term follow-up of these patients.

CONCLUSION

The frequency of sacroiliitis is increased in FMF patients. Inflammatory markers such as NLR, MLR, and SII can be used in clinical practice to predict sacroiliitis in patients with symptoms.

ETHICS APPROVAL

Local ethics committee approval was obtained before undertaking the study (19.10.2023, decision no: 2023-10/37). The participants were provided with written and verbal information, and all provided their consent form. This study was conducted in accordance with the Declaration of Helsinki.

AUTHORS' CONTRIBUTIONS

IA: Conceptualization, Data curation, Formal Analysis, Methodology, Supervision, Validation, Visualization, Writing – original draft. **SA:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – review & editing.

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ORIGINAL ARTICLE

https://doi.org/10.1590/1806-9282.20240076

Relationship between platelet-related parameters and new-onset atrial fibrillation after coronary bypass surgery

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SUMMARY

OBJECTIVE: Inflammation plays a key role in the pathogenesis of postoperative atrial fibrillation after coronary artery bypass graft surgery. In this study, we aimed to investigate the changes in mean platelet volume and platelet values during the spring and autumn seasons in patients who underwent isolated coronary artery bypass graft surgery and the possible effect of these occurrences on postoperative atrial fibrillation.

METHODS: Consecutive patients who underwent elective isolated coronary bypass surgery at our clinic in the spring and autumn months, between August 2020 and July 2022, were retrospectively included in this study. Variables were evaluated according to the spring and autumn seasons. Patients who did not develop in-hospital postoperative atrial fibrillation were identified as Group 1, and those who did constituted Group 2.

RESULTS: A total of 622 patients were included in the study. The patients were divided into two groups: those who were operated on in the spring (n=277, median age=62 years, male gender ratio=77.3%) and those who were operated on in the autumn (n=345, median age=61 years, male gender ratio=81.4%). There was no statistically significant difference between the patients operated on in both seasons in terms of age, gender, hypertension rates, and the frequency of chronic obstructive pulmonary disease. In multivariate analysis, being over 70 years old (OR: 1.934, 95% confidence interval (CI) 1.489–2.995, p<0.001), having a left ventricular ejection fraction below 30% (OR: 1.550, 95%CI 1.190–2.236, p=0.012), and having chronic obstructive pulmonary disease (OR: 1.663, 95%CI 1.339–2.191, p<0.001) were found to be independent predictors in predicting the development of postoperative atrial fibrillation.

CONCLUSION: In this study, we first demonstrated that mean platelet volume and platelet mass index values were higher in patients in the autumn months. Additionally, for the first time in the literature, we showed that there is a significant relationship between platelet mass index value and the development of postoperative atrial fibrillation in patients who underwent isolated coronary artery bypass graft.

KEYWORDS: Platelets. Inflammation. Coronary artery bypass surgery. Atrial fibrillation.

INTRODUCTION

Coronary artery disease (CAD) is a significant atherosclerotic cardiovascular disease and its appropriate treatment is of vital importance. Although endovascular interventions are increasing today, coronary artery bypass graft (CABG) surgery remains foremost. Although these operations are performed with high success rates, some postoperative complications may occur[1]. One of the most critical complications is postoperative atrial fibrillation (PoAF), which is detected at a rate of 20–40% after CABG operations and is significant in that it causes heart failure, cerebrovascular events, and prolonged hospitalization[2].

Inflammation plays a key role in the pathogenesis of PoAF. Platelets have an important place in the pathogenesis of inflammation, and mean platelet volume (MPV) is an important marker of platelet functions[3]. Various studies have demonstrated the effects of platelet-related parameters on PoAF after CABG operations[4]. It has also been shown that there may be changes in MPV values depending on the seasons[5].

In this study, we aimed to investigate the changes in MPV and platelet values, during the spring and autumn seasons, in patients who underwent isolated CABG surgery and the possible effect of these occurrences on PoAF.

METHODS

Consecutive patients who underwent elective isolated coronary bypass surgery at our clinic in the spring and autumn months, between August 2020 and July 2022, were retrospectively included in this study. Prior approval was obtained from the clinical research ethics committee of our hospital (2011-KAEK-25 2022/10-10). Demographic data (age, gender, chronic disease, etc.), preoperative blood values (hemoglobin, neutrophil, lymphocyte, platelet, MPV, urea, creatinine, etc.), operative characteristics (total perfusion time, cross-clamp time, etc.), and postoperative characteristics (PoAF development, etc.) of all patients during the perioperative period were recorded.

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 17, 2024. Accepted on January 20, 2024.

After the exclusion criteria, a total of 622 consecutive patients were included in the study. Variables of the patients were evaluated according to spring and autumn seasons. Also, patients who did not develop in-hospital PoAF were identified as Group 1, and those who did constituted Group 2.

Statistical analysis

The SPSS 21.0 (IBM Statistical Package for the Social Sciences Statistic Inc., version 21.0, Chicago, IL, USA) computer program was used to analyze the data. Means and standard deviations (SDs) were calculated using descriptive methods for continuous and ordinal data. Kolmogorov-Smirnov test and Shapiro-Wilk test were used for normality distribution. For normally distributed data, Student's t-test was used, and the data were shown as mean±SD. For data that did not comply with normal distribution, the Mann-Whitney U test was used and the data were expressed as mean (minimum–maximum). Frequency and percentage analysis was performed for nominal data, and the chi-square test was used to compare these data. Multivariate logistic regression analysis was used to analyze the predictors of PoAF. For correlation analysis, Spearman correlation analysis was used. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 622 patients were included in the study. The patients were divided into two groups: those who were operated on in the spring (n=277, median age=62 years, male gender ratio=77.3%) and those who were operated on in the autumn (n=345, median age=61 years, male gender ratio=81.4%) (Table 1). There was no

Table 1. Characteristics of patients according to spring and autumn seasons.

Variables	Spring (n=277)	Autumn (n=345)	p-value
Age (years)	62 (39–89)	61 (38–85)	0.342‡
Male gender, n (%)	214 (77.3%)	281 (81.4%)	0.197*
Hypertension, n (%)	189 (68.2%)	239 (69.3%)	0.780*
Diabetes mellitus, n (%)	87 (31.4%)	103 (29.9%)	0.676*
Hyperlipidemia, n (%)	109 (39.4%)	146 (42.3%)	0.454*
Smoking, n (%)	108 (39%)	124 (35.9%)	0.435*
COPD, n (%)	29 (10.5%)	41 (11.9%)	0.579*
Cerebrovascular event, n (%)	17 (6.1%)	32 (9.3%)	0.149*
BMI, (kg/m^2)	27.3 (23–39.7)	26.6 (24–41.1)	0.491
LVEF (%)	50 (20–72)	50 (25–67)	0.114‡
SYNTAX score I	15 (9–41)	17 (7–39)	0.362‡
PoAF, n (%)	93 (33.6%)	110 (31.9%)	0.655
White blood cell (10^3/μL)	8.5 (3.8–10.6)	8.4 (4.3–9.6)	0.635
Hematocrit (%)	40.8 (29–52.2)	39.6 (23–55)	0.466
Platelet (10^3/μL)	248 (91–487)	239 (110–556)	0.763
Neutrophil (10^3/μL)	5.6 (0.8–9.6)	5.3 (2.2–7.1)	0.717
Lymphocyte (10^3/μL)	2 (0.3–7.8)	2.1 (0.5–6.3)	0.325
MPV (fL)	9.8 (6–14.4)	10.2 (5.9–16)	<0.001
Creatinine (mg/dL)	0.92 (0.47–1.9)	0.92 (0.34–1.9)	0.643
Urea (mg/dL)	16 (14–44)	16 (12–46)	0.526
C-reactive protein (mg/dL)	5.3 (3–27)	5 (3–35)	0.334
NLR	2.7 (0.35–4.8)	2.4 (0.19–5.9)	0.360
PMI	2,332.6 (968–4,378)	2,437.8 (1,104–5,112)	0.005
Number of grafts (n)	3 (1–7)	3 (1–6)	0.258
Total perfusion time (min)	85 (44–189)	88 (39–185)	0.407
Cross clamp time (min)	58 (12–130)	62 (11–133)	0.255

*Chi-square test, ‡Mann-Whitney U test [data are expressed as median (minimum–maximum)]. ACE-I: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; COPD: chronic obstructive pulmonary disease; SYNTAX: synergy between percutaneous coronary intervention with taxus and coronary artery bypass surgery; LVEF: left ventricular ejection fraction; PoAF: postoperative atrial fibrillation; PMI: platelet mass index; NLR: neutrophil-to-lymphocyte ratio.

statistically significant difference between the patients operated on in both seasons in terms of age, gender, hypertension rates, diabetes mellitus, hyperlipidemia rates, and the frequency of chronic obstructive pulmonary disease (COPD). In addition, the ejection fractions, body mass indexes, white blood cell values, hematocrit values, and platelet counts of the patients operated on in both seasons were statistically similar. Only MPV and PMI values were statistically significantly higher in patients operated in the autumn (p<0.001 and p=0.005, respectively).

The patients included in the study were divided into two groups according to the development of PoAF (Group 1: those who did not develop PoAF and Group 2: those who developed PoAF) (Table 2). There were 419 patients in Group 1 and their median age was 62 (38–89) years, and there were 203 patients in Group 2 and their median age was 69 (41–85) years (p<0.001).

There was no difference between the groups in terms of gender, smoking, diabetes mellitus, hyperlipidemia rate, history of cerebrovascular accident, body mass index, white blood cell, hematocrit values, lymphocyte, neutrophil, MPV, urea, creatinine, and NLR values. In Group 2, the frequency of hypertension, COPD frequency, SYNTAX score I value, and PMI value were significantly higher, while the left ventricular ejection fraction (LVEF) value was significantly lower (p=0.023, p<0.001, p<0.001, p=0.026, and p=0.032, respectively) (Table 2).

Univariate and multivariate logistic regression analyses were performed to reveal the predictive factors for the development of PoAF (Table 3). In univariate analysis results, being over 70 years of age [odds ratio (OR): 2.845, 95% confidence interval (CI) 1.750–5.388, p<0.001], having hypertension (OR: 0.654 95%CI 0.358–0.845, p=0.028), having LVEF below

Table 2. Demographic data and perioperative characteristics of the patients according to the development of postoperative atrial fibrillation.

Variables	Group 1 PoAF (-) (n=419)	Group 2 PoAF (+) (n=203)	p-value
Age (years)	62 (38–89)	69 (41–85)	<0.001‡
Male gender, n (%)	338 (80.7%)	157 (77.3%)	0.334*
Hypertension, n (%)	276 (65.9%)	152 (74.9%)	0.023*
Diabetes mellitus, n (%)	127 (30.3%)	63 (31%)	0.854*
Hyperlipidemia, n (%)	166 (39.6%)	89 (43.8%)	0.315*
Smoking n (%)	149 (35.6%)	83 (40.9%)	0.198*
COPD, n (%)	33 (%7.9)	37 (18.2%)	<0.001*
Cerebrovascular event, n (%)	34 (8.1%)	15 (7.4%)	0.753*
BMI (kg/m^2)	26.9 (23–39.6)	27.1 (23–41.1)	0.298
LVEF (%)	50 (30–72)	50 (20–65)	0.032‡
SYNTAX score I	12 (8–35)	17 (7–41)	<0.001‡
White blood cell (10^3/μL)	8.8 (3.8–10.6)	8.6 (4.4–9.6)	0.410
Hematocrit (%)	40.6 (25.5–55)	39.9 (23–52)	0.213
Platelet (10^3/μL)	236 (91–550)	241 (99–556)	0.091
Neutrophil (10^3/μL)	5.5 (1.7–9)	5.2 (0.8–9.6)	0.210
Lymphocyte (10^3/μL)	2.1 (0.5–7.8)	1.9 (0.3–6.9)	0.091
MPV (fL)	10 (6–14.2)	10.3 (5.9–16)	0.165
Creatinine (mg/dL)	0.9 (0.34–1.9)	0.98 (0.45–1.9)	0.294
Urea (mg/dL)	18 (16–38)	16 (12–46)	0.391
C-reactive protein (mg/dL)	5 (3–235)	5.3 (3–115)	0.569
NLR	2.5 (0.19–52.41)	2.7 (0.36–34.8)	0.092
PMI	2,397 (991.9–4,680)	2,450.1 (968–5,112)	0.026
Number of grafts (n)	3 (1–7)	3 (1–6)	0.259
Total perfusion time (min)	88 (39–189)	90 (40–180)	0.416
Cross clamp time (min)	62 (11–130)	59 (20–133)	0.234

*Chi-square test, ‡Mann Whitney U test [data are expressed as median (minimum–maximum)] ACE–I, angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; BMI: body mass index; COPD: chronic obstructive pulmonary disease; LVEF: left ventricular ejection fraction; SYNTAX: synergy between percutaneous coronary intervention with taxus and coronary artery bypass surgery; MPV: mean platelet volume; NLR: neutrophil-to-lymphocyte ratio; PMI: platelet mass index.

Table 3. Logistic regression analysis of risk factors for postoperative atrial fibrillation.

Variables	Univariate analyses			Multivariate analyses		
	p	Exp(B) Odds ratio	95% CI Lower–Upper	p	Exp(B) Odds ratio	95% CI Lower–Upper
Age> 70 years	<0.001	2.845	1.750–5.388	<0.001	1.934	1.489–2.995
Hypertension	0.028	0.654	0.358–0.845	0.321	0.794	0.471–1.183
LVEF< 30%	<0.001	1.959	1.360–3.070	0.012	1.550	1.190–2.236
COPD	<0.001	2.154	1.660–4.227	<0.001	1.663	1.339–2.191
SYNTAX Score I	<0.001	1.290	1.070–1.889	0.088	1.115	0.897–1.440
MPV	0.217	0.896	0.654–1.190	–	–	–
PMI	0.029	0.816	0.604–0.965	0.275	1.090	0.769–1.337
NLR	0.114	1.197	0.789–1.698	–	–	–

COPD: chronic obstructive pulmonary disease; LVEF: left ventricular ejection fraction; SYNTAX: synergy between percutaneous coronary intervention with taxus and coronary artery bypass surgery; MPV: mean platelet volume; PMI: platelet mass index; NLR: neutrophil-to-lymphocyte ratio.

30% (OR: 1.959, 95%CI 1.360–3.070, p<0.001), having COPD (OR: 2.154, 95%CI 1.660–4.227, p<0.001), and having high SYNTAX score I (OR: 1.290, 95%CI 1.070–1.889, p<0.001) and PMI values (OR: 0.816, 95%CI 0.604–0.965, p=0.029) were found to be significantly correlated with PoAF. According to the results of multivariate analysis, being over 70 years old (OR: 1.934, 95%CI 1.489–2.995, p<0.001), having an LVEF below 30% (OR: 1.550, 95%CI 1.190–2.236, p=0.012), and having COPD (OR: 1.663, 95%CI 1.339–2.191, p<0.001) were found to be independent predictors in predicting the development of PoAF.

Correlation analysis was performed between platelet mass index (PMI) and SYNTAX score I value. As a result of the analysis, a weakly significant correlation was detected between the two values (r=0.185, p<0.001).

DISCUSSION

Coronary artery bypass surgery is performed with high success rates with the developing techniques today, and PoAF is an important problem that may occur after these operations. In this study, we investigated the risk factors for PoAF by dividing patients who underwent CABG according to the spring and autumn seasons. We did not detect any differences in PoAF rates between seasons in our study group. However, we found preoperative MPV and PMI values to be significantly higher in patients who operated in the autumn months. In our analysis to reveal PoAF risk factors, we revealed a significant relationship between PMI and PoAF. As a result of multivariate analysis, we determined that being over 70 years old, having an EF value below 30, and having COPD were independent predictors of PoAF.

It is known that there may be changes in hematological parameters depending on the seasons. For example, it has been shown that there are decreases in hematocrit values during the summer months. It has also been demonstrated that there are increases in platelet numbers and activation due to low air temperatures. Accordingly, it has been determined that coronary thromboembolism, cerebrovascular thrombosis, and peripheral thromboembolism increase in the winter months[6]. Another study showed that platelet counts were higher in the autumn months in the northern hemisphere than in the spring months[5]. We also found MPV and PMI values to be significantly higher in the autumn months in our study group. In a study that investigated PoAF status according to seasons, the most common PoAF rate was observed in the winter months. In this study, PoAF rates were found to be similar in spring and autumn, which is consistent with our study[7].

Platelets have an important place in the pathogenesis of inflammation and thrombosis. Average platelet volume value has an important place in platelet activation. Large platelets secrete important mediators for coagulation, inflammation, thrombosis, and atherosclerosis. In this regard, it has been revealed that there may be a relationship between MPV and cardiovascular diseases[8]. The effects of platelet-related factors on PoAF have been investigated in various studies in the literature.

In a retrospective study conducted by Erdem et al., 208 patients who underwent elective isolated CABG surgery were included. PoAF developed in 38 (22%) of the patients, and as a result of multivariate analysis, MPV (OR: 2.564, p=0.005) and CRP (OR:1.055, p=0.05) values were shown to be independent predictors for PoAF[9]. In another study conducted by Şaşkın et al., 1,138 patients who underwent CABG surgery

were included retrospectively. PoAF occurred in 294 patients (25.8%). In this study, it was concluded that there was a significant correlation between MPV values and PoAF[5]. In another study conducted by Özsin et al., 93 patients who underwent off-pump CABG surgery were included. In this study, MPV values were found to be higher in patients who developed PoAF, and no statistically significant difference was detected. In this study, only the age variable was shown to be an independent predictor for PoAF[10]. In our study, we found that MPV values were higher in patients who developed PoAF. However, there was no statistically significant difference between the groups.

Platelet mass index is a parameter obtained by multiplying the average platelet volume and platelet count and is an important indicator of platelet activity[11]. A study revealed a significant relationship between high MPV values and the presence of atherosclerosis in psoriasis patients[12]. In another study conducted by Korkmaz et al., the importance of PMI value in the treatment of premature retinopathy was investigated. At the end of the study, it was revealed that there was a significant decrease in PMI values in parallel with the improvement of retinopathy[13]. In a recent study by Guzelburc et al., the relationship between PMI and PoAF was investigated. A total of 848 consecutive patients who underwent CABG or valve surgery combined with CABG were included in the study. As a result of the analysis, the preoperatively calculated PMI value was shown to be an independent predictor for PoAF (OR: 1.01, p<0.01)[14]. In our study conducted on isolated CABG patients, although we did not find the PMI value as an independent predictor for the development of PoAF, we concluded that there was a significant positive correlation between the development of PoAF and the PMI value (OR: 0.816, p=0.029).

Hypertension is a factor that increases stress on the vascular wall. This situation triggers inflammation. Thus, the risk of PoAF increases in hypertensive patients[15]. In our study, we found that there was a significant correlation (OR: 0.654, p=0.028) between hypertension and PoAF. With advancing age, increasing collagen deposits in cardiac tissues cause fibrosis, which leads to conduction problems. In addition, mobilization problems and noncompliance with breathing exercises in the postoperative period in elderly patient groups increase the risk of atrial fibrillation[16]. Accordingly, in this study, we revealed that being over 70 years of age is a strong independent predictor of the development of PoAF (OR: 1.934, p<0.001).

Histopathological changes occur in the heart chambers due to reduced ejection fraction. Due to this situation, the risk of atrial fibrillation increases in patients with low ejection fraction[17]. In a meta-analysis conducted by Yamashita et al., low EF was shown to be an important predictor for PoAF[18]. In our study, we found that the EF value below 30% was an independent predictor for PoAF (OR: 1.550, p=0.012). Pulmonary arterial pressures and right ventricular functions are affected due to COPD[19]. For this reason, the risk of PoAF increases in COPD patients[20]. In our study, we revealed that there is a significant relationship between the presence of COPD and PoAF, which is consistent with the literature.

Limitations

The most important limitation of this study is that it is a retrospective study. Additionally, this is a single-center study and the number of patients was limited.

CONCLUSION

In this study, we first demonstrated that MPV and PMI values were higher in patients in the autumn months. However, we concluded that there was no difference between the two seasons in terms of PoAF rates. Additionally, for the first time in the literature, we showed that there is a significant relationship between PMI value and the development of PoAF in patients who underwent isolated CABG.

ETHICS APPROVAL

The study was approved by the Clinical Research Ethics Committee of Bursa Yuksek Ihtisas Training and Research Hospital (date: 05/10/2022, protocol number: 2011-KAEK-25 2022/10-10.)

AUTHORS' CONTRIBUTIONS

AD: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ME:** Conceptualization, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **FT:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **KKÖ:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **AKA:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **UA:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **YA:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ŞY:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

https://doi.org/10.1590/1806-9282.20231211

Long COVID-19 and mnemonic effects: an integrative literature review

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INTRODUCTION

The COVID-19 epidemic emerged in Wuhan in December 2019, soon becoming a pandemic with millions of global deaths. Due to the rapid spread of the disease, collapse of health systems, and lack of treatment, radical measures were adopted, such as quarantines, in order to minimize impacts, especially on risk groups[1].

From this pandemic, the post-COVID syndrome or long COVID-19 emerged, clinically defined by the Delphi method and announced in Geneva[2]. This syndrome generates sequelae in the systems of recovered patients, persisting for more than 3 months after the acute infection, with frequent symptoms such as fatigue and dyspnea. It also causes psychological and neurobiological sequelae linked to the trauma experienced by the patient[3].

Recent research suggests a relationship between the immune response to the virus and these effects, showing that COVID-19 damages the central nervous system, affecting cognitive functions such as memory. It was noted that 67% of patients affected by long COVID-19 had "brain fog,"[4] impairing concentration and verbal expression, affecting declarative memory, described as a network of synapses that allows retrieving specific information[5]. Therefore, it is important to research the subject to understand whether, in fact, there is a relationship between the disease and cognitive impairment. Thus, the gap of uncertainty about this cause-consequence will be closed.

METHODS

This is an integrative literature review, whose guiding theme was the pathophysiological effects of long COVID-19 on memory. This research model is presented as a descriptive study, which consists of the precise detailing of certain facts and phenomena of reality[6]. We opted for an integrative review to obtain a holistic and critical view of the topic[7]. In addition, as only public data were used, there was no need to submit the work to ethical appreciation.

Thus, research was carried out in the bases: BVS, SciELO, MEDLINE, Periódicos CAPES, and Scopus, using a strategy with descriptors: (COVID OR SARS-CoV-2) AND long-term effects AND memory AND (neurology OR psychiatry OR psychology OR cognition OR cognitive functions OR mental health OR brain). This strategy was based on the search for DeCS/MesH descriptors in the Portuguese, English, and Spanish versions. Moreover, the literature review on the mnemonic effects of post-COVID syndrome was enhanced by incorporating the clinical definition established through the Delphi Consensus, along with a thorough exploration of credible scientific sources.

Inclusion criteria for papers were as follows: (a) studies addressing the persistent effects of COVID-19 or SARS-CoV-2; (b) studies published and indexed in peer-reviewed scientific journals; (c) studies that examined memory. Furthermore, as exclusion criteria, the following were applied: (a) observational studies that do not examine memory through recognized methods; (b) studies that were conducted in animals or cells in culture; (c) studies whose sample has diseases that serve as confounding factors for changes in memory; and (d) studies in which socio-environmental aspects (such as social isolation) altered by COVID-19 were related to memory impairment.

The screening of articles was carried out by all authors, independently, using pre-defined criteria. To avoid discrepancies in choices, all screening results were evaluated by the two supervisors, one of whom is a specialist in neurology, regarding study

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Conflicts of interest: the authors declare there is no conflicts of interest. Funding: none.
Received on January 20, 2024. Accepted on January 24, 2024.

designs, evaluation tools, results, and discussion, in addition to the conclusion, in order to filter the most suitable materials for the research in question.

After analyzing the complete articles, a PRISMA flowchart of the selection process and a table with a summary of the information of the 13 chosen articles were created, using the following parameters: year of publication; authors; title; database; study design; place of study; results; and considerations.

Finally, the data and information in the table were structured in order to guide the analysis and discussion of the results obtained. Such structuring aimed at composing a core of integrated knowledge about the effects of long COVID-19 on memory.

RESULTS

In total, 267 articles were identified in MEDLINE and underwent a screening stage, as illustrated in Figure 1. From this group, the following were excluded: 107 studies involving animals or cell cultures; 89 articles focused mainly on the socio-environmental impacts of the COVID-19 pandemic on memory; 51 articles that presented confounding factors in the analysis of the relationship between COVID-19 and memory, such as neurodegenerative diseases; and seven observational studies that did not investigate memory by recognized clinical or laboratory methods. Finally, 13 articles that were published between 2021 and 2023 met the criteria and are summarized in Table 1.

DISCUSSION

The National Institute for Health and Care Excellence (NICE) guidelines define post-COVID-19 syndrome as "signs and symptoms that develop during or after an infection consistent with COVID-19, that persist for more than 12 weeks and are not explained by an alternative diagnosis."

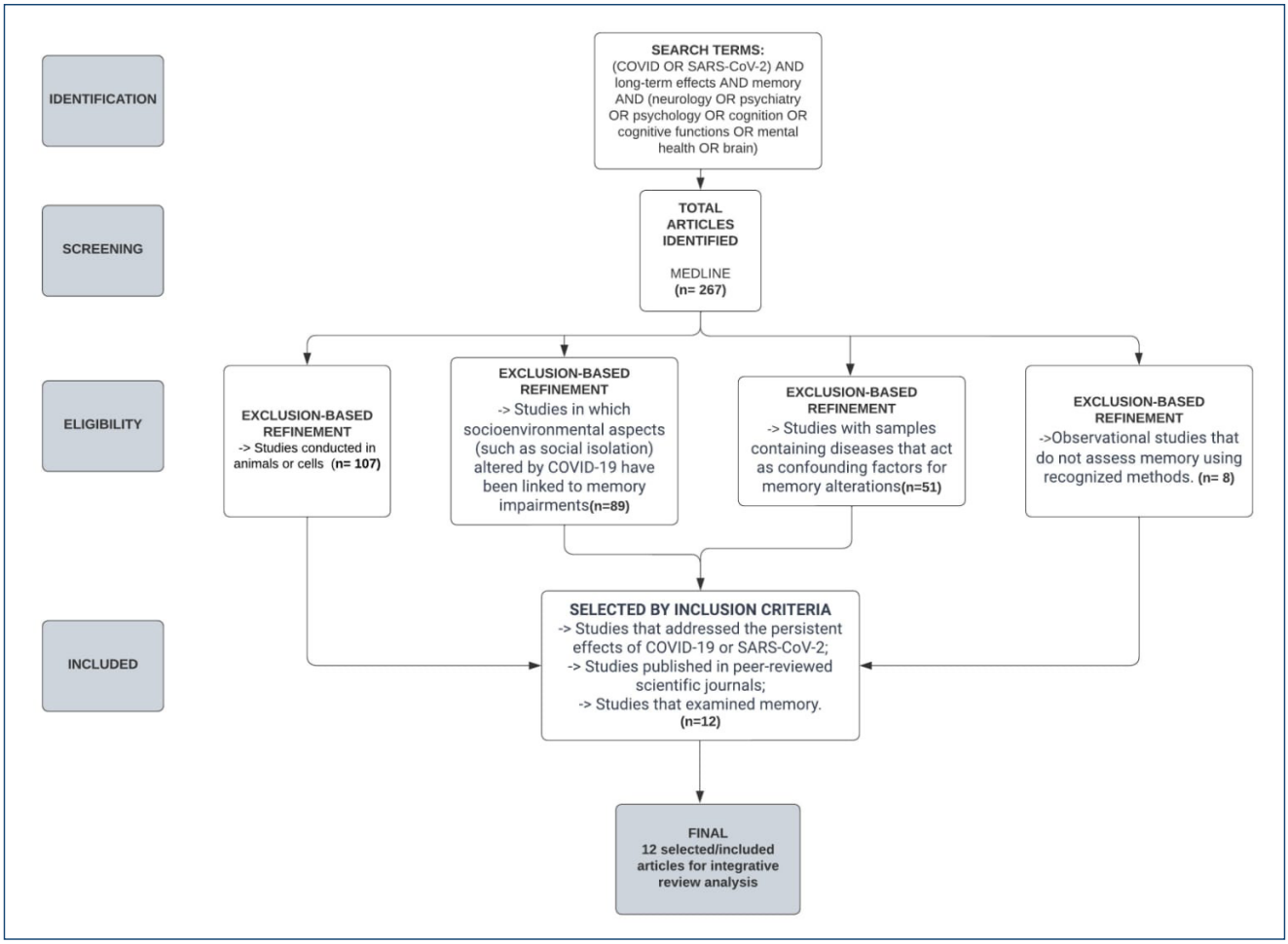

Figure 1. Article selection flowchart. Source: realization by the authors themselves, 2023.

Table 1. Summary of review articles.

Year	Authors	Title	Kind of study	Place of study	Results	Considerations
2021	Hellmuth J. et al.[9]	Persistent COVID-19-associated neurocognitive symptoms in non-hospitalized patients.	Case series.	Ambulatory.	Of the first 100 participants evaluated, 20 had cognitive problems at one or more study visits, 12 had cognitive symptoms at the baseline visit, and 8 had no baseline symptoms but noticed during follow-up.	Neurocognitive symptoms associated with COVID-19 have been observed in young and middle-aged adults who have not undergone hospitalization.
2021	Graham EL. et al.[8]	Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 "long haulers".	Prospective cohort.	Neuro-Covid-19 Clinic of Northwestern Memorial Hospital (EUA).	Non-hospitalized SARS-CoV-2 infected had working memory deficit at follow-up, relative to the US median (T-score 43 vs 50, p = 0.007) in the NIH Toolbox test.	About 80% had neurological impairment, 50% with changes in short-term memory and attention.
2022	Bungenberg J. et al.[10]	Long COVID-19: Objectifying most self-reported neurological symptoms	Transversal.	RWTH Aachen University Hospital, Germany.	Patients reported difficulties with attention (56%), memory (38%), and word search (18%). Hospitalized had worse performance in MoCA, logical reasoning, and verbal memory.	The authors point to mild deficits in attention, processing speed, and memory in cognitive performance. Some had severe impairment in attention and executive functions.
2022	Premraj L. et al.[11]	Mid and long-term neurological and neuropsychiatric manifestations of post-COVID-19 syndrome: A meta-analysis.	Systematic review.	–	The overall prevalence of memory problems was 28% (22–35%) in a total sample of 10,530 properly screened patients.	Memory impairment stood out even 3 months after the onset of the acute illness.
2022	Crivelli L. et al.[12]	Changes in cognitive functioning after COVID-19 A systematic review and meta-analysis	Systematic review.	–	In the study, memory was one of the most affected domains, in which delay and impairment of immediate verbal memory were noted. In the longer follow-up study, deficits in memory coding were found.	The meta-analysis provided preliminary evidence suggesting that individuals may exhibit reduced cognitive performance in some domains after recovery from COVID-19.
2022	Nakamura A. et al.[13]	Long-Term Sequelae in Young Convalescent COVID-19 Patients.	Prospective cohort.	Duke University Memory Disorders Clinic.	The three patients scored above 26 on the MoCA. All were diagnosed with subjective cognitive impairment. Neuropsychological tests a month later showed improvement, except in verbal memory.	The authors conclude that young patients with cognitive sequelae of SARS-CoV-2 infection symptoms tend to improve with time.
2022	Keijsers K. et al.[19]	Memory impairment and concentration problems in COVID-19 survivors 8 weeks after non-ICU hospitalization: A retrospective cohort study.	Retrospective cohort.	Jeroen Bosch Hospital.	Among symptoms 8 weeks after hospitalization, complaints of memory impairment were 33%.	Memory issues are common post-COVID, so there should be extensive care for such affected patients.
2022	Zawilska JB, Kuczyńska K.[16]	Psychiatric and neurological complications of long COVID.	Integrative review.	–	Memory impairment was observed in different age groups. In adults, deficits in short-term memory and general memory loss have been observed.	Neurological symptoms seem to be characteristic of long-term COVID, with cognitive deficits being the most recurrent.

Continue...

Table 1. Continuation.

Year	Authors	Title	Kind of study	Place of study	Results	Considerations
2022	Soh HS, Cho B.[15]	Long COVID-19 and Health-Related Quality of Life of Mild Cases in Korea: 3-Months Follow-up of a Single Community Treatment Center.	Prospective cohort.	Seongnam Community Treatment Center, in South Korea.	Factors linked to persistent symptoms and reduced quality of life are: female gender, metabolic disease, and anxiety in the acute phase of COVID-19.	At baseline, 89.1% of patients had one or more symptoms. The most common persistent symptoms were memory impairment, fatigue, and loss of quality of life.
2022	Perrottelli A. et al.[14]	Cognitive Impairment after Post-Acute COVID-19 Infection: A Systematic Review of the Literature.	Systematic review.	—	Memory is among the most affected cognitive domains.	A relationship was noted between SARS-CoV-2 infection and the emergence of memory deficits.
2023	Cavaco S. et al.[18]	Predictors of Cognitive Dysfunction One-Year Post COVID-19.	Retrospective cohort.	Porto University Hospital Center.	After 1 year, more than 50% of patients had abnormal performance on at least one cognitive test and more than one-third had significant cognitive complaints.	Part of patients with long-term COVID have persistent cognitive changes
2023	Taruffi L. et al.[17]	Neurological Manifestations of Long COVID: A Single-Center One-Year Experience.	Retrospective observational	University of Bologna, Italy.	Out of 130 patients, 46 had cognitive disorders. Among the 30 assessed, 37% had changes in memory; 86.4% had acute neurological symptoms.	Of the cognitive abilities, memory was the most impaired, with a prevalence of 37% of patients.
2023	Gamberini J. et al.[20]	Previously independent patients with mild-symptomatic COVID-19 are at high risk of developing cognitive impairment but not depression or anxiety.	Prospective observational.	Unit of the "Mons. L. Novarese" Rehabilitation Center (Moncrivello, Italy).	In the MoCA test, 81.1% of the patients had impaired memory, specifically in 76.7% of the youngest and 83.3% of the elderly (>65 years).	In the MoCA test, the elderly had a higher prevalence of mild cognitive impairment in memory.

Source: realization by the authors themselves, 2023.

Memory, which is the central focus of this study, is defined as a facet of cognition, similar to learning, attention, language, and executive function. Thus, a cognitive deficit indicates a reduction in a previously normal domain, negatively affecting the individual's daily life.

In a prospective cohort, developed in 2020, 100 patients were analyzed, without previous hospitalization for pneumonia or hypoxemia and with clinical manifestations of COVID-19 compatible with the guidelines of the Infectious Diseases Society of America (IDSA) for at least 6 weeks[8]. Upon confirmation of diagnosis using reverse transcription polymerase chain reaction (RT-PCR), 50 patients tested positive for SARS-CoV-2, and the other 50 tested negative for SARS-CoV-2. A complete neurological examination was performed on the 52 patients who attended the neurology clinic and a limited neurological examination on the 48 patients who were followed up virtually. Overall, 53% had an abnormal examination, with the most frequent neurological signs of short-term memory deficit (32%) and attention deficit (27%).

On another occasion, a case series was conducted, presenting two reports of female patients recovering from COVID-19, without hospitalization, with persistent neurocognitive symptoms[9]. The first patient, who was evaluated in a memory clinic, mentioned that cognitive symptoms appeared in the first week, including difficulty concentrating, and memory problems improved by cues and information processing. Despite showing deficits in working memory, she scored 30/30 on the Montreal Cognitive Assessment (MoCA). The second patient, who was assessed by telemedicine 37 days after the onset of COVID-19 symptoms, scored perfect (30/30) on the Mini-Mental State Exam (MMSE). However, she reported difficulty expressing herself and reduced organization, leading to missed deadlines. In conclusion, the need for further research to determine the prevalence of neurocognitive symptoms associated with COVID-19 was highlighted.

A cross-sectional study was carried out with 50 patients after infection with SARS-CoV-2 having persistent symptoms for at least 4 weeks[10]. These patients were divided into hospitalized

(n=21) and non-hospitalized (n=29). A neurologist conducted complete neurological examinations and structured interviews. Verbal episodic memory was assessed using the verbal auditory memory test (VLMT) or Word List from the CERAD-Plus Battery. Visual memory was analyzed with the Rey-Osterrieth complex figure test (ROCFT) or CERAD-Plus figure subtest. Patients mentioned memory complaints (38%) and word search problems (18%). Neuropsychological performance, in general, remained within the reference standards. Although hospitalized patients had lower verbal performance, cognitive performance was comparable between groups and, for the most part, without impairment. However, the study has limitations due to the small sample size and the absence of a control group.

In a future meta-analysis, 18 studies were included in the systematic review, which included 10,530 patients for the final analysis[11]. We determined the prevalence of neurological and neuropsychiatric symptoms reported from 3 months after acute onset of COVID-19 in adults. A mean prevalence of memory problems was obtained around 28% of the patients, with a variation between 22 and 35%. The authors came to the conclusion that cognitive dysfunction (brain fog, memory problems, and attention disorder) was the main neurological feature present in patients with long-term COVID-19.

A pooled analysis of 27 studies involving 2,049 participants and an additional study with 16 moderate to severe SARS-CoV-2-positive patients revealed that one of the most affected cognitive domains is immediate verbal memory (38% moderate and 11.2% severe)[12]. The larger study, which followed patients over 7 months and included 749 participants, also found memory coding deficits in 24% (n=178) and memory recall deficits in 23% (n=170). However, the authors of the systematic review highlight the scarcity of articles to conclude how the severity of COVID-19 or the types of symptoms directly influence cognition and, consequently, memory.

During the conduct of a case series, which included three reports of female patients under the age of 60 years, who sought the Duke University Memory Disorders Clinic due to complaints of cognitive dysfunction after recovering from SARS-CoV-2 infection[13], the Gold Standard MoCA test or a follow-up neuropsychological test was administered to all three patients. The first patient scored 28/30 on her initial assessment and 4 months later achieved a score of 30/30. At the baseline visit, the second patient achieved a MoCA score of 29/30, and after 1 month, during follow-up, she demonstrated normal general cognitive function. The third patient received a MoCA score of 26/30 during the initial assessment and underwent neuropsychological tests a month later, which revealed difficulties in verbal memory. In summary, neuropsychological findings suggest that patients who experience cognitive sequelae after SARS-CoV-2 infection tend to experience improvement in their symptoms over time.

Some studies broadly address cognitive impairments resulting from COVID-19. Focusing on memory for this integrative review, in studies such as the one mentioned above, an attempt was made to assess the impacts on memory in comparison with other cognitive areas. In this context, memory proved to be one of the main affected aspects, which was individually considered in 37 studies for the preparation of a systematic review[14], of which 30 indicated some degree of impairment. The conclusion highlighted by the authors is that all subdivisions of memory showed some level of impairment.

As can be observed, there are several implications of the post-COVID syndrome on the quality of life of patients, caused by neurological depression and neuropsychiatric sequelae.

Another cut proposed in the analyzed studies is that of age groups, which describe the occurrence of systemic dysfunctions in different age groups[15]. When considering children as less affected by COVID-19 infection, therefore, they are the least affected by long-term COVID-19. In young and elderly adults, the picture is different. The study points out, within memory impairment, that the highest percentage of adults is affected by "short-term" memory loss. In this population, the problems are accumulated, and when considering the multifactorial nature of the post-COVID syndrome, the consequences are greater. The author mentions that cognitive impairment even hinders the return to work.

We analyzed 103 patients who attended an outpatient clinic for their first consultation, on average 243 days after the initial diagnosis of COVID-19[16]. Among the evaluated patients, 30 were submitted to the application of neurological scales, such as the MoCA. The results of the analysis indicated that the most impaired cognitive function was memory, affecting approximately 37% of patients. These findings may be related to later findings, which also observed neurological impairments in COVID-19-infected patients, however, in a period of at least 1 year after infection[17]. In this context, it is possible to identify similarities in the results obtained, and the main discrepancy is the average time interval for the onset of symptoms.

The studies in this literature review help clarify the connection between coronavirus infection and later-stage memory dysfunction. However, due to the recency of events, it is likely that the evidence confirming the causal link between post-COVID syndrome and memory problems will strengthen over time.

By establishing inclusion and exclusion criteria that prioritized objective and quantitative research, the researchers limited the scope of the study, neglecting investigations that

integrated social determinants into the analysis of memory. It is important to highlight that, even given the scarcity of articles on the topic, post-COVID syndrome has emerged as a triggering factor for complex complications in the memory of individuals affected by this specific condition. Therefore, we suggest a reflection on the need for more comprehensive and holistic approaches to fully understand the impacts of the syndrome on the mnemonic function, considering both objective aspects and underlying social elements.

CONCLUSION

The study aimed to research the connection between SARS-CoV-2 infection and cognitive dysfunctions, using MEDLINE sources. However, reaching a broad conclusion about the pathological impact on memory through the selected articles is complex, due to different participant variables, such as age and severity of cases. There was also variation in location, with frequent mentions of the American, European, and Asian regions, and a slight majority of female participants, with emphasis on adults and the elderly.

Despite the differences, there are some similarities between the articles: mild or asymptomatic cases of the disease can cause cognitive problems, affecting memory and concentration, affecting quality of life[18], which highlighted the negative influence of neuropsychiatric symptoms on quality of life assessed by the EuroQol-5 dimensions-5 levels questionnaire (EQ-5D-5L). Most patients demonstrated improvement in symptoms over time. Children and adolescents represent a minority in cases of COVID-19 and long-term COVID-19.

In addition, due to the novelty of the topic, the post-COVID consequences are still poorly understood. Most studies[9,12-17] mention possible pathological mechanisms contributing to cognitive symptoms, including direct damage by virus invasion in the central nervous system[9,12,14], decreased activity of angiotensin-converting enzyme 2 (ACE-2) – linked to memory changes in the hippocampus[12,14] – and hyper-inflammatory state, increasing levels of inflammatory markers such as cytokines, TNF-a and G-CSF, in addition to high levels of antinuclear antibodies.

Cases showed evidence of changes in the cortex, ranging from atrophy in the orbitofrontal cortex and occipital regions[13] to white matter abnormalities, impairing the frontal and parietal lobes[14]. Conditions such as decreased lung function, delirium, sepsis, hypoxia, vascular dysfunctions, ongoing endothelial activation, residual immune activation, and residual lesions accumulated during acute illness can all worsen cognitive performance.

Regarding the drug approach, the studies were not carried out in-depth due to the complexity of the possible mechanisms in this clinical condition, which highlights the need for more research.

AUTHORS' CONTRIBUTIONS

ALO: Data curation, Investigation, Resources, Software, Writing – original draft. **GSF:** Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **IFLF:** Data curation, Investigation, Resources, Software. **JEM:** Conceptualization, Data curation, Investigation, Resources, Software, Writing – original draft, Writing – review & editing. **JGD:** Data curation, Investigation, Resources, Software. **JSM:** Data curation, Investigation, Resources, Software. **LNA:** Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. **TGL:** Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Writing – original draft. **TVC:** Project administration, Supervision, Validation. **WRMM:** Project administration, Supervision, Validation.

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