

RAMMB

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SARS-CoV-2-associated Guillain-Barre syndrome is not infrequent

Josef Finsterer^{1*} , Fulvio Alexandre Scorza² ,
Carla Alexandra Scorza² , Ana Claudia Fiorini^{3,4} 

Dear Editor,

We read with interest the review article by Medeiros et al.¹ about patients with SARS-CoV-2-associated Guillain-Barre syndrome (SAG) collected via a search of various databases. It was found that the main manifestations of SAG were fever, coughing, dyspnea, sore throat, ageusia, anosmia, and respiratory failure, in addition to paresthesias of the upper and lower limbs, quadraparesis, facial diplegia, areflexia, asthenia, mastoid pain, acute ataxia, fatigue, numbness, dysphagia, and moderate low back pain¹. It was concluded that SARS-CoV-2 can trigger GBS¹. This study is appealing but raises concerns that require discussion.

We do not agree with the notion that only few studies on the topic SAG are available as mentioned in the abstract and the discussion¹. In a recent review about SAG, collecting available data until the end of July 2021, 120 articles reporting 300 patients with SAG were retrieved (Finsterer, submitted). The age of these patients ranged between 7 and 94 years. There was male preponderance. Latency between the onset of COVID-19 and SAG ranged between -10 and 90 days. Acute inflammatory demyelinating polyneuropathy (AIDP) was diagnosed in 171 patients, acute, motor axonal neuropathy (AMAN) in 24, and acute, motor, and sensory axonal neuropathy (AMSAN) in 16 patients. Intravenous immunoglobulins (IVIGs) were given to 241 patients, plasmapheresis to 28 patients, and steroids to 7 patients. A total of 59 patients were in need of artificial ventilation. Complete recovery was achieved only in 42 cases and partial recovery in 163 cases. Notably, 17 patients died.

The classification of GBS subtypes has been missed out in the review. GBS manifests with a variety of clinical presentations, such as AIDP, AMAN, AMSAN, Miller Fisher syndrome (MFS), polyneuritis cranialis (PNC), the pharyngo-cervico-brachial (PCB) subtype, and Bickerstaff encephalitis (BFE)². There are also patients with SAG who present with pure dysautonomia³.

The results of cerebrospinal fluid (CSF) findings have also been missed out. GBS may be characterized not only by the “dissociation cyto-albuminque” but also by the absence of virus mRNA in the CSF and elevated cytokines, particularly IL6, IL8, TNF- α , and IL1b⁴. Only few patients have been reported in whom the CSF was positive for SARS-CoV-2^{4,5}, suggesting that SAG is truly immunogenic.

A discussion about GBS triggered by SARS-CoV-2 vaccinations is missing in the review. GBS may occur not only during COVID-19 infections but also after SARS-CoV-2 vaccinations. In a recent review about 19 gender-balanced patients with SARS-CoV-2 vaccination-associated GBS (SVAG), aged 20–86 years, all patients developed SVAG after the first dose of the vaccine. The AstraZeneca vaccine was used in 14 cases, the Pfizer vaccine in 4 cases, and Johnson & Johnson vaccine in 1 case. Latency between vaccination and the onset of SVAG ranged between 3 h and 39 days. SVAG patients responded favorably to IVIGs (n=13) or steroids (n=3). Six patients were in need of mechanical ventilation. Only one patient achieved complete recovery. Partial recovery was achieved in nine patients (Finsterer, submitted).

There is a discrepancy between the search dates given under “Information sources” (until April 2020) and the search date

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given under “Search” (until June 26) without mentioning the year¹. This ambiguity should be removed.

Overall, the review has several limitations that challenge the results and their interpretations. These limitations should be met to further strengthen the conclusions.

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JF: Conceptualization, Data curation, Validation, Writing – original draft. **FS:** Formal analysis, Validation, Writing – review & editing. **CS:** Formal analysis, Validation, Writing – review & editing. **AF:** Formal analysis, Validation, Writing – review & editing.

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A closer look at the size cutoff of 10 mm, below 10 mm in particular, in thyroidology: debate is still ongoing

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To date, the size cutoff of 10, 15, and 20 mm has been one of the most challenging issues in endocrine pathology, endocrine surgery, endocrinology, head and neck surgery, head and neck radiology, and thyroidology. Of note, the size cutoff of 10 mm particularly remains crucial in the evaluation and management of thyroid nodules with suspicious clinical findings, sonographic features, and/or cytology¹⁻⁴.

More recently, Borges et al.⁵ reported a valued research article, entitled “Thyroid nodules 1 cm or less are related to Bethesda System nondiagnostic and suspicious for malignancy categories.” In terms of the size cutoff of 10 mm, they sought to investigate the fine-needle aspiration (FNA) cytology of the nodules below and above 10 mm. They had analyzed 3,703 nodules, had undergone FNA during January 2016 to December 2019, and declared the size cutoff ≤ 10 mm was associated with cytology of nondiagnostic/unsatisfactory (prevalence ratio [PR]: 3.0, 95%CI 2.2–4.2) and suspicious of malignancy (PR: 1.6, 95%CI 1.1–2.4) for Categories I and V, *secunda edition*, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), respectively⁵. It is important to note that the size cutoff of 10 mm, *per se*, has been set as not being underestimated gauge by some recommendations on the size selection criteria for the thyroid nodule, that is, FNA is recommended for the nodules:

1. Above 10 mm, solid and hypoechoic on ultrasound, the American Association of Clinical Endocrinologists (AACE)/Associazione Medici Endocrinologi (Italian Association of Clinical Endocrinologists or AME) (Grade B; best evidence level [BEL] 3);
2. >10 mm, high-risk category, the novel European Thyroid Imaging and Reporting Data System (EU-TIRADS 5);

3. ≥ 10 mm, possessing microcalcifications, the Society of Radiologists in Ultrasound (SRU);
4. ≥ 10 mm with microcalcifications and hypoechoic solid nodules, Revised American Thyroid Association (ATA) Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer (2009); and
5. ≥ 10 mm with high-to-intermediate suspicion sonographic pattern, 2015 ATA Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer, last ATA guidelines (Recommendation 8, IID)^{1,3}. While the recommendations have emphasized the size cutoff of 10 mm^{1,3}, the study by Borges et al.⁵ provides data demonstrating that the nodules ≤ 10 mm were associated with Categories I and V, TBSRTC, 2nd ed. We have currently focused on thyroid nodules in suspense, 10–15 mm with repeat cytology of Category III, TBSRTC, whether or not necessitating a needful upgrade in thyroidology, has published in issue 2, volume 67, *Revista da Associação Médica Brasileira*⁶. In addition, we recently reported the size cutoff of 10 mm whether being prepotential for three diagnostic tools; strain elastography, ultrasound (US)-guided FNA cytology, and histopathology and our 3-year preliminary results revealed no significance of the thyroid nodules above 10 mm for indetermined and malignant cytology, Categories III, IV, V, and VI, of TBSRTC, with an additional calculation of area under the curve (AUC) as 0.517¹. Interestingly, our surveillance could not demonstrate the superior effect of the nodules above 10 mm on the prediction of Category V, TBSRTC, among the indetermined and malignant cytology¹, congruently

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the study by Borges et al.⁵, proclaiming the size cutoff ≤ 10 mm was associated with Category V, TBSRTC, 2nd ed. They suggested FNA as a feasible diagnostic tool even for nodules ≤ 10 mm, even utilizing more than one procedure⁵. In addition, we recently emphasized that even strain elastography might not be a beneficial tool to discriminate benign and malignant thyroid nodules with a size above 10 mm as the largest diameter³. Herewith, might it be propounded that a *strong* debate is still ongoing for the nodules below 10 mm of diameter?

Finally, we have an opinion that in case of waiting for such clarification, *grammatici certant*, we encourage appropriate discussion and incorporation of the thyroid nodules below 10 mm whether or not requiring the application of image-guided interventional diagnostic procedures^{7,8}. *Bene diagnosticur, bene*

curratur. As a matter of fact, this issue merits further investigation. We thank Borges et al.⁵ for their valuable study.

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

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Mobile learning in teaching radiology in times of social isolation

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INTRODUCTION

On March 11, 2020, the World Health Organization declared COVID-19, a highly transmissible disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, as a pandemic and recommended social isolation as an emergency measure to control the disease. On March 17, 2020, onsite academic activities were suspended indefinitely in the city of Juiz de Fora (MG), Brazil. In view of this, alternative learning strategies were employed in several institutions so that students would not be left unattended.

This present work describes the experience that medical students from a Teaching Institution in Juiz de Fora (MG), members of an Extension Project (EP) in radiology, had with remote teaching — a resource used to keep the project going.

The students expressed their desire to continue with the activities of the EP remotely, but there were some difficulties, such as:

1. Instability of the Internet connection that would not make it possible to attend classes synchronously;
2. Students who had to return to their home cities and did not have adequate equipment to participate in simultaneous classes on educational platforms; and
3. Incompatibility of schedules due to extracurricular tasks.

To remedy these difficulties, it was proposed that the students themselves record content to be watched by their colleagues and by the group advisor according to the availability of each one, asynchronously, with a deadline for the delivery of the responses of the clinical cases presented in the recordings.

The use of technologies as auxiliary tools for education promotes environments of learning opportunities, favors

cooperative and collaborative construction through media sharing, and encourages discussions among students¹. Over the past decade, there has been a progressive increase in the use of smartphones in different personal and professional contexts, with its use among medical students highly prevalent. These devices can assist in the teaching-learning process, configuring an alternative capable of uniting technology and education: Mobile Learning, M-Learning, or Mobile Learning is an alternative of learning in various contexts, through social interactions, using personal electronic devices².

Social media is an important tool for collaboration, information, and knowledge sharing between colleagues, as well as between students and facilitators, being useful for improving personal skills and professional communication through continuous posting of photos, videos, audios, and texts. Mobile Learning provides a motivating environment that promotes the development of critical thinking, engagement, motivation, collaboration, and carrying out learning activities through the digital environment².

The EP students were already included in a group in the WhatsApp[®] application, which did not function as the focus of scientific discussions, since these were held in person every 15 days. During the period of social isolation, the application was chosen by students as a vehicle for discussions and remote learning, considering that it is a free application that allows the transfer of text, images, videos, and audios for groups of people. All of this makes it possible for users to share information in real time in a common interface and with selected members³.

The use of the WhatsApp[®] application in the educational context allows for greater dynamism, ease of interaction, fluency

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in dialogue, and the exchange of information and adherence by students. In addition, it enables an immediate interaction with other platforms to search in real time for articles and evidence that support the discussion and effective learning of students through didactic–pedagogical involvement through a tool used by them routinely^{4,5}.

Negative aspects and barriers in the use of social media include:

1. Addiction to social media, which acts as a distractor and distracts students' attention;
2. Cyberbullying;
3. Workload concerns and time constraints;
4. Doubt about the maintenance of privacy and integrity;
5. Lack of interest in feedback from colleagues;
6. Lack of adequate training; and
7. Students who use social media excessively find it difficult to communicate face-to-face². Such effects were minimized due to the emergency and temporary context of the proposed follow-up of the project's activities during the pandemic.

In this way, the Mobile Learning proposal in the context of the pandemic aimed to continue the academic activities in a remote and safe way, guaranteeing the learning of the contents of the radiology discipline through the interaction between students and the teacher responsible for the EP in a group on WhatsApp[®].

METHODS

For 4 months, the methodology was applied to a WhatsApp[®] group with 11 members, medical students members of the EP in radiology, and an EP supervising teacher. Initially, it was proposed that each student be responsible every 2 weeks for recording an expository class with topics of relevance in clinical practice combined with image examinations and, at the end of each presentation, clinical cases were exposed so that the other students should solve in a period of up to 1 week.

Synchronous discussions were promoted in the WhatsApp[®] group through radiography and computed tomography examinations, based on real clinical cases, sent by the teacher-advisor in the video format. After sharing the content, the advisor invited the group to discuss the radiological patterns identified and elaborate diagnostic hypotheses, actively participating in the debate as a facilitator and contributing to the quality of the discussion. As they were made live, times were scheduled to be held with as many members as possible.

In addition, the teacher and the students promoted online events in the group, such as congresses, courses, and webinars related to the radiology area, seeking to foster continuous learning through various sources.

RESULTS

From the students' and supervising professor's point of view, this study gave satisfactory results. For the students, the main positive aspects were the maintenance of the link between the participants of the project, the knowledge acquired in the elaboration and exhibition of the proposed works, as well as the resolution of the questions related to the various themes presented, in addition to stimulating the maintenance of the search for information and knowledge.

Motivational aspects were also mentioned as relevant factors for the continuity of studies and for the search for a more in-depth knowledge on subjects proposed by group participants. In the context of the pandemic, the teaching favored by the new media is innovative, since it changes the conventional learning paradigms, which generally keep teachers and students apart⁴. In addition, physicians must know how to handle theoretical information from books and scientific articles, as well as to build knowledge in a critical and shared way, using various technologies at their fingertips⁵.

The use of the WhatsApp group for educational activities, in the context of the EP in radiology, enabled a quick and dynamic dialogue between the members of the group, the sharing of theoretically based information, and the construction of critical and collective knowledge on the part of the group. In addition, the continuity of academic activities, maintained remotely, allowed for the maintenance of the link between students and professor, becoming a stimulus for the search for information.

Another factor of great importance considered by most students was the possibility of using the virtual space to disseminate and stimulate participation in congresses and courses, all carried out online. Some students considered this to be one of the first opportunities for entry and participation in recognized academic means of discussion.

It is important to emphasize that the feasibility of sharing information that could cover different types and levels of difficulty of content in Medicine was also considered a relevant and positive aspect of the project, which were also used by students from different grades (from the third to the eighth grades).

For some group participants, the experience of the EP in radiology exceeded the expectations of the possibilities offered by a usual EP and encouraged the creation of a diary with records of all the activities carried out by the group during the pandemic period (Figure 1A).

The main difficulty presented in relation to the results was the variability of the Internet standard used, which sometimes prevented the questions from being answered immediately when exposed and the insecurity in relation to the possibility of concluding the EP due to the uncertainty of when the return to face-to-face activities would be carried out and how activities carried out during the remote period would be validated.

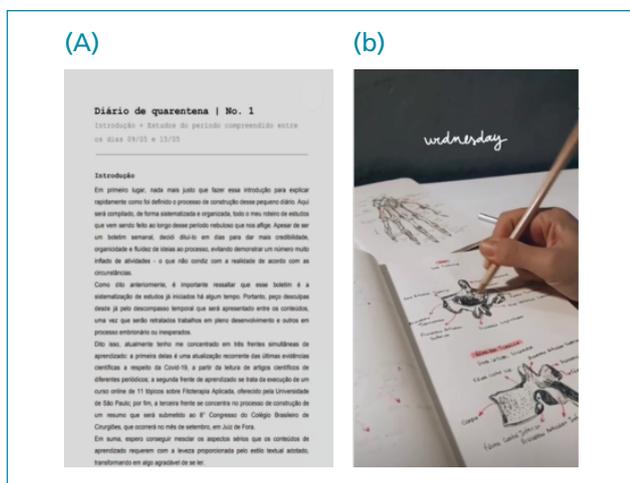


Figure 1. (A) Diary with records of activities carried out by the group during the pandemic period. (B) Drawing of a student from the group who was inspired and motivated to express his study on this form.

For the advisor, the main positive aspect was the confirmation of the students' interest and commitment in the proposed activities. On the other hand, the complicating factor was the impossibility of carrying out the training of students in the practice scenarios. It also highlights the motivational aspect during this tragic period. In a moment of extreme discouragement and uncertainty, the continuity with teaching projects and activities was both a possibility for theoretical and curricular development and a positive psychological stimulus. It is essential to highlight the participation in courses at congresses as well as the possibility for some students to dedicate more time to recreational activities in line with radiology since some even expressed the moment in the form of artistic drawings (Figure 1B).

Since learning is an active process, it is necessary to emphasize that the final result is individual and depends on the interest and commitment of each student.

DISCUSSION

In the pre-pandemic period, digital learning resources were underemployed in higher education institutions in Brazil, being restricted to only a few sources complementary to the tasks developed in person in the classroom^{5,6}.

With the advent of the public health emergency brought about by the new SARS-CoV-2, the demand for the use of Mobile Learning resources increased due to the social isolation measures required and the closure of teaching and learning institutions followed by the suspension of face-to-face activities.

In this way, teaching platforms such as Microsoft Teams[®], Google Classroom[®], and Zoom[®] and communication platforms such as Instagram[®] and WhatsApp[®], which previously had other

priority purposes, have now been converted into true centers of access and dissemination of knowledge — previously restricted to the physical space of educational institutions.

In this scenario, the platform chosen to continue the EP in radiology, WhatsApp[®], allows the sending of images, videos, voice messages, and other features. This whole framework of applicability helped in the experience and enriched the implemented ones, being essential for learning about radiology. In addition, it is evident that the WhatsApp[®] communication platform is widely disseminated and used because it allows the creation of groups within the application, making simple and simultaneous contact between EP members at any distance and at any time. In addition, it allows accessibility independent of the mobile device and can be operated on Android[®], iOS[®], or Windows[®].

Nevertheless, WhatsApp[®] fosters student-student and student-teacher interaction, in addition to providing dynamism to the process, without any additional cost to those involved. This makes it possible to have a questioning reflection on the content and the appearance of doubts — such as which are quickly resolved thanks to the practicality that the application offers.

However, it should be noted that the existence of some obstacles permeates the use of this means of communication in practice. For example, one can mention problems with an Internet connection, the need to have a smartphone, and even the high consumption of cell phone memory by files that need a large storage space can be obstacles to the full enjoyment of the EP.

CONCLUSIONS

The alternative methodology of Mobile Learning used to continue teaching and extension in the area of radiology remotely by a group of students and teacher in a context of social isolation is easily reproducible and of low cost. In addition, the possibility of using new media and communication vehicles as resources for health education. The activities carried out with the help of the WhatsApp[®] application were useful in learning, sharing ideas, case studies, stimulating learning, and publicizing events. However, it does not replace the knowledge acquired in the student-teacher relationship that occurs in person.

AUTHORS' CONTRIBUTIONS

ALS: Conceptualization, Writing – original draft. **ALBT:** Writing – review & editing. **BGM:** Writing – original draft, Writing – review & editing. **CMC:** Writing – original draft, Writing – review & editing. **LFV:** Conceptualization, Writing – original draft. **PHAPB:** Conceptualization, Writing – original draft. **YHFD:** Conceptualization, Writing – review & editing. **GBC:** Conceptualization, Writing – review & editing.

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Itraconazole versus potassium iodide for cutaneous sporotrichosis: weighing up the pros and cons

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Sporotrichosis is a versatile disease caused by a ubiquitous genus of soil-dwelling fungus called *Sporothrix* spp. and is frequently seen in tropical global areas. Several clinical manifestations have been reported in the literature ranging from localized cutaneous lesions to disseminated presentations.

Both “fixed” lesions and the lymphocutaneous pattern are expected to respond well to oral treatment, either with itraconazole or with potassium iodide; nonetheless, the question “which one to choose?” remains a matter of debate. Even though neither treatment has shown indubitable superiority, this discussion on the pros and cons of each therapy should not be taken for granted, since the appropriate care for the patient requires further practical observations in addition to the medication effectiveness alone, such as affordability and local drug availability.

Over the past decades, many outbreaks of *Sporothrix* spp. have been registered in different areas of Brazil. From 2010–2020, 374 cases of cutaneous sporotrichosis were notified in the city of São Paulo, shedding light on the topic and calling the attention to a possibly emerging public health issue¹. Similarly, in 2019, more than 240 cases were notified in the state of Rio de Janeiro alone, picturing yet another scenario of great concern². In fact, cases of localized sporotrichosis keep appearing throughout Brazil requiring from the attending physician smarter therapeutic decisions, since social conditions vary a lot from region to region and so does the prevalence of comorbidities, such as heart failure and renal insufficiency, which, in turn, imposes some limitations to drug usage.

Itraconazole is used to treat sporotrichosis. According to the Mycoses Study Group of the Infectious Diseases Society of America, itraconazole is considered the drug of choice for lymphocutaneous or fixed cutaneous sporotrichosis due to its good tolerability and low relapse rate³. It is suggested that most patients will benefit from a dose of 100–200 mg/day and that a period of 3–6 months is quite enough to cure most cases.

Especially for patients with limited budget and for those with contraindications to itraconazole (Table 1), successful treatment of sporotrichosis may be obtained with oral potassium iodide, which is less expensive and well tolerated⁴. For instance, approximately R\$120–240 (30–60 tablets) is required per month to treat a patient with itraconazole, while the same treatment with potassium iodide will cost nearly R\$100.

The patient is usually given a saturated solution of potassium iodide (SSPI). The mechanism by which SSPI works against *Sporothrix* spp. is not clear; however, its level of evidence in the current literature is the same of itraconazole³. Furthermore, SSPI is safe for children and common adverse effects are usually mild³.

SSPI must be administered three times a day, starting with five drops (each drop containing 67 mg of potassium iodide) ideally admixed in milk or juice^{1,4}. The dose is increased by 3–5 drops per day, and some patients may require up to 20 drops three times daily to show a satisfactory response^{1,4}. Lesions usually remit within 2–4 weeks. Perhaps, posology pictures the most challenging part of the treatment with SSPI since it may

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Table 1. Comparison between saturated potassium iodide and oral itraconazole for localized presentations of cutaneous sporotrichosis.

Level of evidence	Itraconazole	Saturated potassium iodide
	A2	A2
Common adverse effects	Usually seen at doses around 400 mg/day. Nausea and vomiting, abdominal pain, diarrhea.	Metallic taste and nausea.
Contraindications	Hypersensitivity to itraconazole (absolute). Heart failure, liver disease, pregnancy (category C) and lactation.	Hypersensitivity to iodide (absolute). Patients with thyroid dysfunction, renal and cardiac failure (relative).
Average time for clinical response	2–3 months	2–3 months
Length of therapy	Clinical cure – absence of disease activity in the lesion (pus, oozing, crust, mild erythema)	Clinical cure – absence of disease activity in the lesion (pus, oozing, crust, mild erythema)
Infancy	6–10 mg/kg, maximum 400 mg/day	1–2 g/day
Posology	100–200 mg/day	4–6 g/day
Fungus resistance	Rare	Rare

be difficult for some patients to copy with. Nonetheless, a thorough explanation at the time of prescription usually suffices.

Regarding the patients' response to therapy, a thorough research in the literature has shown that even though refractory cases of sporotrichosis are uncommon, they do exist with both itraconazole and SSPI⁵. Recently, Lyra et al. has published an interesting observation reporting on a case of a 25-year-old female patient who was treated with itraconazole for 11 months and then with a combined itraconazole and terbinafine for more than seven months with poor clinical response. As a result, complete remission was achieved only after two months of SSPI inclusion⁵.

Finally, despite the apparently inexistent practical difference between SSPI and itraconazole in the treatment of localized cutaneous sporotrichosis, the authors would like to encourage attending physicians to do more than merely flipping a coin. Instead, ploughing more time into listening to the patients' limitations and explaining in detail the whole treatment process

may be the key for reaching better outcomes. In the authors' experience, SSPI is considered a cost-effective drug for the treatment of localized cutaneous sporotrichosis.

AUTHORS' CONTRIBUTIONS

HDR: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **ADM:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **EVMBF:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **ADM:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **SLMD:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **ACTM:** Data curation, Writing – original draft, Writing – review & editing. **JVCP:** Data curation, Writing – original draft, Writing – review & editing. **PACARC:** Data curation, Writing – original draft, Writing – review & editing

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Chest computed tomography findings of 1271 patients with COVID-19 pneumonia and classifications with different age groups: a descriptive study from Istanbul, Turkey

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SUMMARY

OBJECTIVE: The objectives of this study were to describe lung computed tomography findings of patients with COVID-19 diagnosed by real-time reverse transcription polymerase chain reaction test, investigate whether the findings differ regarding age and gender, and evaluate the diagnostic performance of chest computed tomography based on the duration of symptoms at the time of presentation to the hospital.

METHODS: From March 11 to May 11, 2020, 1271 consecutive patients (733 males and 538 females) were included in this retrospective, cross-sectional study. Based on age, patients were divided into five separate subgroups. Then based on the duration of symptoms, patients were divided into five separate phases. The presence of lung lesion(s) and their characteristics, distribution patterns, and the presence of concomitant pleural thickening/effusion and other findings (malignancy, metastasis, chronic obstructive pulmonary disease, interstitial lung disease, bronchiectasis, cardiomegaly, pericardial effusion) were evaluated by five radiologists independently.

RESULTS: The “normal lung computed tomography finding” was the most common chest CT finding (37%), followed by ground-glass opacity (31%). Regardless of the shape of the lesion, the distribution features were significant (peripheral, subpleural, and lower lobe distribution) ($p < 0.05$). The presence of pleural thickening posteriorly and adjacent to the lesion was statistically different in groups 1–3 ($p < 0.05$). Other concomitant pathologies, except pulmonary congestion, did not suppress the typical findings of COVID-19.

CONCLUSION: Chest computed tomography findings were mostly normal in the early phase (P1). Therefore, it may be appropriate to perform the first computed tomography screening of COVID-19 after 6 days to decrease the radiation exposure.

KEYWORDS: COVID-19. X-ray computed tomography. Lung. Pleura.

INTRODUCTION

A novel coronavirus (SARS-CoV-2) infection, which emerged as “unknown viral pneumonia” in Wuhan, China, in December 2019, spread worldwide in about two months^{1,2}. The first case was detected in Turkey on March 11, 2020. After this date, comprehensive diagnostic workup algorithms of possible cases

were implemented to ensure the rapid recognition and isolation of patients³. In addition to the reverse transcription polymerase chain reaction (RT-PCR) test, chest computed tomography (CT) was commonly used as a complementary examination⁴.

As reported in the literature, the sensitivity of the RT-PCR test ranges between 60 and 89% (5,6), which varies based on

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the location of the sample, as well as the collection of samples, transport, and kit performance^{5,6}.

There are many articles in the literature which emphasize the importance of radiological imaging with CT in the diagnosis of the disease^{4,7,9}. According to these studies, CT scan shows typical ground-glass opacity (GGO) in the lung, suggesting viral pneumonia. CT findings change rapidly based on the course of the disease. When the RT-PCR test was accepted as a reference, the sensitivity of chest CT was 97% for the diagnosis of COVID-19 disease^{8,9}. For these reasons, knowing the COVID-19 CT radiological findings well is extremely important.

This study aimed to document lung CT findings in order of frequency in patients who had a positive RT-PCR test and determine whether lung CT findings vary based on age and gender. In addition, this study also aimed to evaluate the diagnostic performance of chest CT based on the duration of symptoms at the time of presentation to the hospital.

METHODS

This is a single-center, cross-sectional, retrospective study. It was approved by the ethical committee (54132726-000-8605), and informed consent was received from all the patients. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

All RT-PCR tests were performed at repurposed genomic laboratory with the SARS-CoV-2 detection kit (Coyote Bioscience Co., Ltd.). The primers of the commercial kit were designed to target *ORF1ab* and *N* gene. The results of RT-PCR were extracted from the patients' electronic medical records in the Hospital Information System.

Patients

From March 11 to May 11, 2020, 1500 consecutive patients who underwent chest CT scan and whose RT-PCR test results positive were included in the study. Patients who had more than 5 days interval between RT-PCR test and CT examination (n=51), patients with positive RT-PCR test results but no chest CT examination (n=152), and children below 18 years (n=26) were excluded from the study.

The mean age of all patient population was 44 years.

Based on age, patients below 44 years and those above 44 years were divided into two groups. Patients above 65 years were considered a separate group due to the high comorbidity (Group 5). Thus, a total of five separate groups were formed.

Patients were divided into five groups as follows: group 1: 18–30 years; group 2: 31–45 years; group 3: 46–55 years; group 4: 56–65 years; and group 5: >65 years old.

Finally, all the patients included in the study were analyzed based on their complaints with the duration of symptoms. Based on this, chest CT findings were classified radiologically (0–2 days=early phase, 3–5 days=advanced-early phase, 6–10 days=peak phase, and >11 days=resolution or recovery phase)^{4,7}.

Image analysis

Image data sets were transferred to a Picture Archiving and Communication System (PACS) workstation for analysis (Centricity® PACS; GE Healthcare, Milwaukee, WI, USA). The images were evaluated by five radiologists independently. All patients were randomly divided into five groups. All observers had over five years of experience in thoracic radiology. Observers evaluated the chest CT findings as follows: type of lesion (GGO and/or consolidation), lung involvement (upper, middle, or lower zone), distribution of the lesion (central, peripheral, or peribronchovascular), and shape of lesion (nodular/round shape, ill-defined borders). In addition, the presence of pleural findings was evaluated (pleural thickening and/or effusion). All the patients were equally shared to five radiologists. Suspected cases were classified in consultation with the most senior radiologist.

Interpretation of the findings

Based on the previously identified COVID-19 CT findings in the literature^{10,11}, the CT impression was collected under four categories:

1. negative for pneumonia,
2. typical appearance for COVID-19,
3. indeterminate appearance for COVID-19, and
4. atypical appearance for COVID-19.

If none of the lesions mentioned above were present, the chest CT was interpreted as normal.

The presence of other existing lung diseases of patients was also recorded as follows: malignancy, metastasis, chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), bronchiectasis, cardiomegaly (CMG), and pulmonary congestion, and such patients were included in the category of atypical findings.

Computed tomography protocol

CT scans were performed in two multidetector scanners (128-slice GE Optima CT660 CT scanner, and 16-slice GE Optima CT 520 CT Scanner; GE Healthcare). To minimize motion

artifacts, the patients were instructed to hold their breath. The CT protocol consisted of tube voltage=120 kVp (for those below 40 years, tube voltage was 100 kVp), automatic tube current modulation (30–70 mAs), pitch=0.99–1.22 mm, matrix=512×512, slice thickness=10 mm, field of view=350 mm×350 mm. All images were then reconstructed with a slice thickness of 0.625–1.250 mm.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 22.0; SPSS Inc., Chicago, IL, USA).

The distribution of outcome categories was assessed using the Shapiro–Wilk test. Data are presented as mean±standard deviation, based on the normality of data. And categorical variables were reported as counts and percentages.

When the data distributed normally, a one-way analysis of variance test was performed to check the differences between the groups. If the test showed a significant difference, then the data were further analyzed with a *post hoc* Tukey's test.

A $p < 0.05$ was considered statistically significant.

RESULTS

Patients and demographic findings

A total of 1271 patients (733 males and 538 females) who met the study criteria were included.

Group 2 was the majority of our patient population with a ratio of 34%. This was followed by groups 1, 3, 5, and 4, in order of frequency (24, 20, 11 and 0.9%, respectively).

The mean interval between the RT-PCR test and the first CT scan was 0.97 ± 1.83 days.

The most common symptom at the time of presentation to the hospital was cough in all groups. The second most common symptom was fever. It was also noteworthy that the frequency of hyposmia was 10% in Group 1 and <5% in other groups.

Most of the patients in all groups had applied to the hospital in 6–12 days after the onset of symptoms (63% on average for all groups).

Chest computed tomography findings based on age

The “normal lung CT findings” was the most common chest CT finding at the time of presentation to the hospital, except Group 2. This was followed by GGOs and GGOs with consolidation with the percentage of 31 and 22%, respectively (Figure 1).

In all groups, GGOs tended to be in nodular/round shape, and it was statistically different in group 2 ($p < 0.05$; Table 1).

The distribution of CT findings at the time of admission by groups is summarized in Table 2.

GGOs with an ill-defined shape was encountered as often as the nodular form in Group 2 ($p < 0.05$). However, whether GGO was nodular or ill-defined in shape, it was statistically significant to have a peripheral distribution in groups 1–3 ($p < 0.05$; Table 1).

The lesions were predominantly peripheral and subpleural in 659 (49%) patients, and there was tendency to be in lower zone (53%). In terms of peribronchovascular distribution of GGOs, there was no statistically difference between the groups ($p > 0.05$; Table 1).

The presence of consolidation without GGO in chest CT was extremely rare (0.01%). The coexistence of GGO and consolidation with peripheral distribution in the lower lobes were common in all groups ($p < 0.05$). If accompanying halo finding, the consolidation with GGO was statistically different in Group 2 ($p < 0.05$) compared to other groups ($p > 0.05$) (Table 1).

In all groups, 134 (10%) patients had focal pleural thickening. Of these, 115 were in the posterior pleura and 75 were adjacent to the lung lesion (Figure 2). This finding was statistically different for Groups 1–3 compared to other groups ($p < 0.05$).

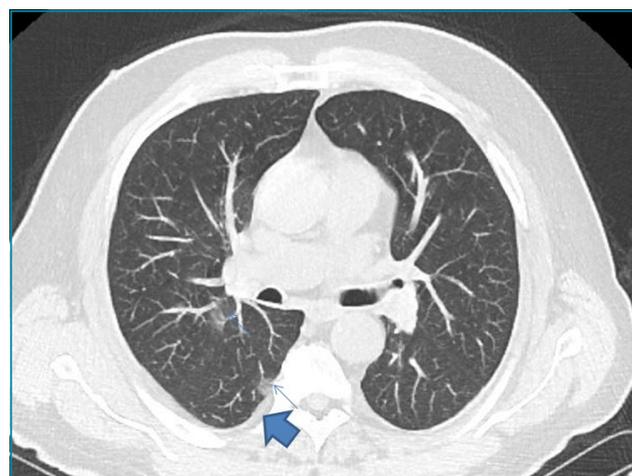


Figure 1. Graph showing the chest computed tomography findings of all patients at the time of presentation to the hospital. The most common chest computed tomography findings were normal. The presence of ground-glass opacity was the second most common lesion group, especially if it was bilateral. The cases with ground-glass opacity and consolidation were the third most common finding. The presence of consolidation alone was a very rare finding that the vast majority were due to atelectasis.

Table 1. Chest computed tomography findings of ground-glass opacity in patients' groups.

Groups		Group 1	Group 2	Group 3	Group 4	Group 5	
Range of age (year)		18–30	31–45	46–55	56–65	>65	
Number of patients		(n=309)	(n=434)	(n=260)	(n=119)	(n=149)	
ground-glass opacity	How the lung is involved?						
	Upper lobes (n)	81	28	103*	54	73	
	Middle lobes (n)	71	29	107*	57	69	
	Lower lobes (n)	141*	72	140*	70	103*	
	Unilateral (n)	61	64	30	12	15	
	Bilateral (n)	101	181*	120	66	96	
	Unifocal (n)	38	48	19	5	5	
	Multifocal (n)	118	197*	129	73	106	
	Number of diseased segments (average±SD)	7±5.2	8.1±0.7	8.5±2.1	8.38±5.65	9.16±1.4	
	Distribution feature of the lesion/lesions						
	Central (n)	31	27	17	4	2	
	Peripheral (n)	135*	205*	122*	68	94	
	Peribronchovascular distribution (n)	45	81	68	53	47	
	Shape of lesion/lesions						
	Nodular/round shape (n)	84	113*	59	36	45	
	Lesions with irregular border (n)	63	113*	75	41	66	
	Coalescent lesions (n)	19	37	28	19	27	
	Accompanying CT findings						
	Consolidation	62	99*	47	34	41	
	Halo sign	35	86*	27	13	9	
Ters halo sign	4	6	5	1	3		
Septal thickening	50	63	52	34	64		

In our patient groups, the dominant lung lesion in chest computed tomography was ground-glass opacity. It tended to be bilateral in all groups. Groups 1–3 also had the feature of peripheral distribution in the lower lobes. In the presence of accompanying consolidation, the lower lobes and peripheral distribution were statistically significant, especially in Group 2. The presence of ground-glass density and halo finding to consolidation was statistically significant in Group 2. *Statistically significant at $p < 0.05$. Since the findings described above can be seen together, the ratio has not been given. Statistical significance was specified.

Table 2. Distribution of the frequency of COVID-19 lung computed tomography findings by groups.

Groups	Negative for pneumonia	Typical appearance for COVID-19	Indeterminate appearance for COVID-19	Atypical appearance for COVID-19
Group 1 n=309, n (%)	196 (63)	80 (26)	24 (8)	9 (3)
Group 2 n=434, n (%)	168 (39)	182 (42)	63 (14)	21 (5)
Group 3 n=260, n (%)	82 (32)	74 (28)	66 (25)	38 (15)
Group 4 n=119, n (%)	14 (12)	65 (54)	28 (23)	12 (11)
Group 5 n=149, n (%)	12 (8)	42 (28)	46 (31)	49 (33)

In the Group 1, the frequency of normal lung findings (negative for pneumonia) is remarkable. The frequency of normal Chest computed findings in Groups 2 and 3 is remarkable as typical appearance for COVID-19. When all groups are examined, it is observed that the most common lung Chest computed finding is "negative for pneumonia" at the time of first admission to the hospital.

Chest computed tomography findings based on the duration of symptoms

There were 786 chest CTs assessed in accordance with the early phase (P1). Normal chest CT findings were found in 404 patients in P1.

There were 310 patients evaluated as advanced-early phase (P2). Of these patients, 248 had chest CT findings typical of COVID-19.

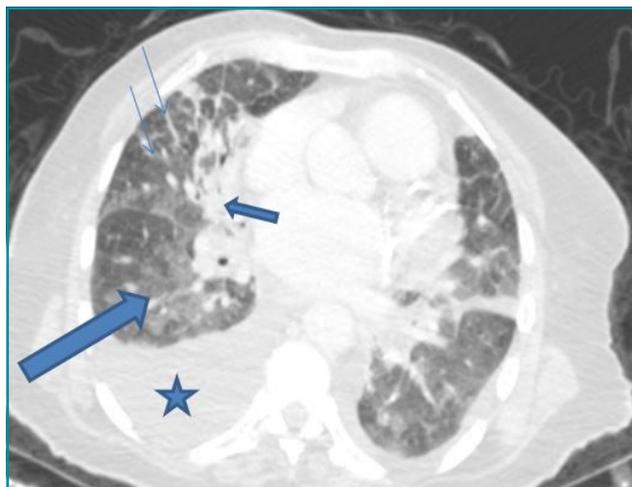


Figure 2. A 52-year-old male has been suffering from weakness and cough for about five days. There are ground-glass opacity adjacent to the fissure and subpleural area (thin blue arrows). Focal pleural thickening is noticed in the right lower lobe adjacent to the lesion (thick blue arrow).

Typical chest CT findings for COVID-19 in the peak phase (P3) were 83%.

There were 19 patients in the resolution phase (P4) in the study population. Typical chest CT findings for COVID-19 were the most common findings in P4.

There were no patients in recovery phase (P5) in our study population.

Of the 630 patients with typical findings, 343 were males and 287 were females. In patients with typical CT findings, the most common symptom was cough (52%), followed by fever (26%) and dyspnea (23%).

Twenty-seven patients with pulmonary congestion findings were also clinically confirmed (Figure 3).

Chest computed tomography findings based on the gender

There was no statistically significant difference between men and women for any finding ($p > 0.05$).

DISCUSSION

COVID-19 is the disease caused by a new subtype whose genetic structure is 82% similar to SARS-CoV¹. The source of infection is wild animals and it can be transmitted through droplets or contact with a rapid infection, thus posing a huge threat to the public health^{1,3,12}. Therefore, detecting the disease

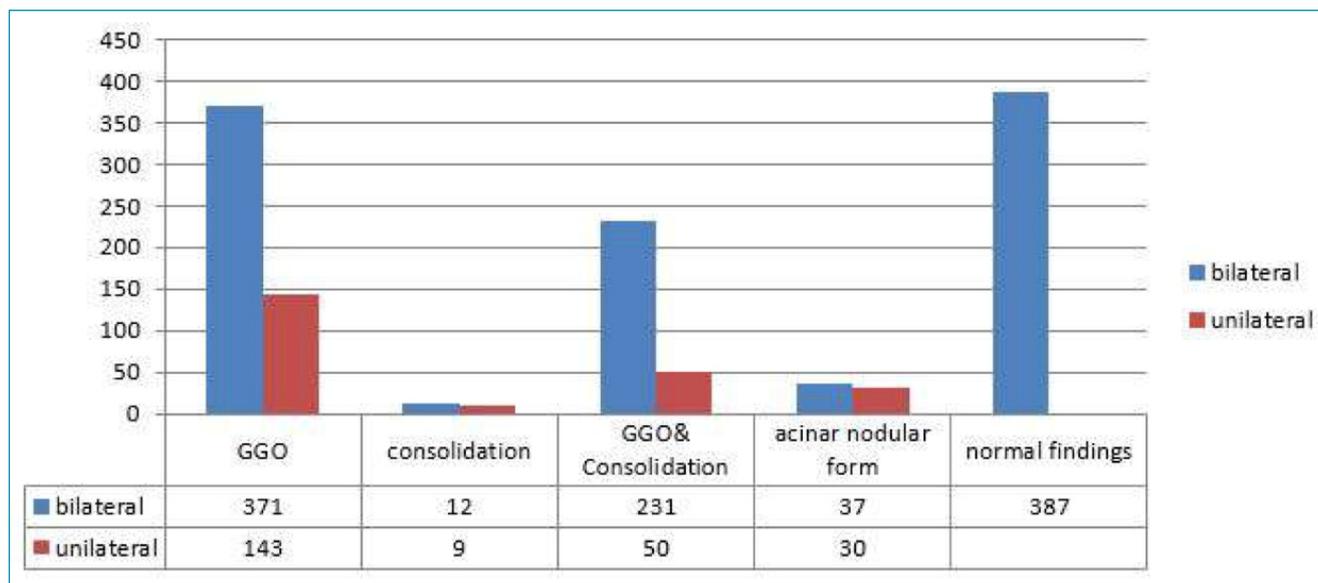


Figure 3. GGO: ground-glass opacity. A 76-year-old female patient has wheezing for the past 15 days and severe abdominal pain for one week. There are bilateral pleural effusion and cardiomegaly (blue stars). Bilaterally moderate interlobular septal thickening is observed (thin blue arrows). There is a focal consolidation in the right middle lobe (small thick blue arrow). Perihilar ground-glass opacity are present (long bold blue arrow). Chest computed tomography findings were classified as atypical findings for COVID-19. Pulmonary congestion findings in chest computed tomography suppress typical radiological findings for COVID-19.

accurately and quickly should be the target¹². As mentioned in the literature, chest CT has an important role in early diagnosis and follow-up of the disease^{8,9}.

In our study, we evaluated chest CT findings of patients diagnosed with COVID-19 by RT-PCR test. We also evaluated the findings at the time of presentation to the hospital and whether they change with age, as well as how the duration of the findings affected chest CT findings.

The imaging manifestations of COVID-19 are similar to common viral pneumonia, especially SARS pneumonia, but there are some specific imaging and distribution characteristics of lesions^{1,3,4}.

Peripheral distribution of GGOs and multisegment involvement of GGOs in early phase were typical^{11,12}. This situation varied depending on the age^{11,12}. In our younger age groups, typical findings were not observed in the early phase.

Therefore, it is important that the first CT scan is not performed in the first and second days of symptoms.

For the lung CT findings to be seen for COVID-19 as positive, the time interval required is found to be 3–5 days after the onset of complaints in our study. Before this period, the diagnostic sensitivity of lung CT for COVID-19 is low¹³.

MERS pneumonia appears on CT images as subpleural and basilar airspace lesions, with extensive GGO and consolidation¹.

In our patient groups, this was found to be quite significant, especially in group 2 ($p < 0.05$). The reason for this may be the late presentation to the hospital in this age group, which was most commonly between 6 and 12 days¹³⁻¹⁵.

In our study, we also evaluated the pleura in terms of pleural thickening. In the first three Groups (Groups 1–3), pleural thickening posteriorly or adjacent to the lesions was significantly more common than other locations ($p < 0.05$).

In the literature, vascular engorgement, pleural thickening, and subpleural lines were reported in patients with COVID-19⁷⁻⁹. The presence of pleural thickening may be a guiding radiological finding in patients whose parenchymal lesions have not yet developed or in those with suspicious findings in our study.

In other studies in the literature, it was stated that the presence of pleural effusion is a finding in the advanced stage of COVID-19 pneumonia¹³⁻¹⁵. In our study, we observed pleural effusion mostly in the older age group with CMG and clinically pulmonary congestion (Groups 4 and 5). In our study, early chest CT findings were evaluated at the time of presentation to the hospital. There were no follow-up chest CTs in the study. Multicenter studies will be needed to confirm this^{14,15}.

It was, however, not clear whether it was due to COVID-19 or pulmonary congestion, both clinically and radiologically.

The clinician was advised to repeat the control chest CT after the treatment to relieve pulmonary congestion. In patients with other known malignancies, the typical findings of COVID-19 were easily recognized.

In the elderly patient group, the first CT findings had more severe parenchymal involvement compared to the other age group. The reason for this was interpreted as the prevalence of comorbidity in the patient group and the fact that first admission to the hospital was delayed compared to other groups.

It is also interesting that the older patient group had more indeterminate and atypical CT findings. This could be due to the severity or comorbidities of the older patients.

This study has some limitations. First, patients with negative RT-PCR tests were not included. As a result, the sensitivity of the chest CT could not be evaluated. Second, there were no children in our population. Third, the patient evolution or outcome was not evaluated. The frequency of thromboembolism has been reported as high in COVID-19¹³⁻¹⁶. However, in our study protocol, we performed chest CT scan without administration of intravenous (IV) contrast agent. Therefore, we could not evaluate our patients in this regard. If the patient's clinic is suspicious in terms of pulmonary thromboembolism, we performed chest CT by adding IV contrast. And, we detected only for the presence of additional lung diseases (COPD, ILD, etc.) and hence there was lack of sufficient data on hypertension, diabetes mellitus, and other metabolic diseases at the time of diagnosis. Finally, sudden onset of pandemic caused inadequate documentation in the early stages of the disease.

CONCLUSIONS

It is noteworthy that the typical lung CT findings for COVID-19 occur on days 6 to 10 (P3) of the illness period. This applies to all age groups. In addition, the most common symptoms in these patients with typical CT findings were cough, fever, and dyspnea. Therefore, it may be appropriate to perform the first CT screening of COVID-19 after 6 days based on the start time of the complaints for all age groups. In this way, the frequency and number of unnecessary radiation exposure can be reduced.

AUTHORS' CONTRIBUTIONS

SSDB: Conceptualization, Data curation, Formal analysis. **ZS:** Data curation. **ASO:** Data curation. **FK:** Data curation. **MTA:** Data curation. **LD:** Data curation. **AI:** Data curation. collected data. **LD:** Writing – original draft, Writing – review & editing. **YB:** Writing – original draft, Writing – review & editing.

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Relationship of ERCC5 genetic polymorphisms with metastasis and recurrence of gastric cancer

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SUMMARY

OBJECTIVE: This study aims to explore the role of *ERCC5* genetic polymorphisms in gastric cancer and their relationship with metastasis and recurrence of gastric cancer.

METHODS: A total of 200 patients with gastric cancer and 133 healthy subjects were enrolled. MassARRAY iPLEX® technology was used to genotype *ERCC5* rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 between the control group and the gastric cancer group. The relationship of *ERCC5* genetic polymorphisms with metastasis and recurrence of gastric cancer was explored. The differences in sociodemographic characteristics between patients with gastric cancer and control subjects were compared using the chi-square test. The genetic loci between the control group and the gastric cancer group were analyzed by the chi-square test.

RESULTS: There was no significant difference in the metastasis of gastric cancer between males and females ($p=0.628$), but there was a significant difference in the metastasis of gastric cancer ($p=0.005$). Patients aged ≤ 60 years and >60 years showed no significant difference in the metastasis of gastric cancer ($p=0.420$), but there was a significant difference in the recurrence of gastric cancer ($p<0.001$). The loci rs2016073, rs751402, and rs2094258 in the gastric cancer group showed no significant differences compared with the control group ($p=0.194$), and the loci rs2296147 and rs2296148 showed significant differences.

CONCLUSIONS: The results suggested that *ERCC5* polymorphisms (e.g., rs201607, rs751402, rs2094258, rs2296147, and rs2296148) may be associated with metastasis and recurrence of gastric cancer.

KEYWORDS: ERCC5. Genetic polymorphism. Metastasis. Recurrence. Gastric cancer.

INTRODUCTION

Gastric cancer is the second most common cause of cancer-related deaths in the world, the epidemiology of which has changed within the past decades¹. Gastric cancer remains a considerable health burden throughout the world². Metastasis is a crucial impediment to the successful treatment of gastric cancer³. Recurrence of gastric cancer after 10 years of surgical resection is highly rare, and local recurrence is common after radical surgery⁴. Many studies have explored biomarkers

to monitor metastasis and recurrence of gastric cancer⁵⁻⁷, but further study is still needed.

The common polymorphic variant in the 5'-untranslated region of the excision repair cross-complementation group 5 (*ERCC5*) gene was described to generate an upstream open reading frame that regulates both the basal *ERCC5* expression and its ability to be synthesized following DNA damage⁸. Genetic variations in *ERCC5* might influence individual vulnerability to gastric cancer⁹.

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A previous study indicated that *ERCC5* rs2094258 polymorphism may contribute to the risk of breast cancer¹⁰. Our previous study found that *ERCC5* rs2094258 and rs751402 polymorphisms were not associated with the development of gastric cancer in co-dominant, dominant, and recessive models¹¹. But in a meta-analysis, it was demonstrated that rs751402 C>T, rs2296147 T>C, and rs873601 G>A polymorphisms of *ERCC5* gene were associated with the susceptibility of gastric cancer¹². In another study, the *ERCC5* rs751402 gene polymorphism may influence the susceptibility to gastric cancer in the Chinese population¹³.

In this study, the roles of *ERCC5* genes (e.g., rs2016073, rs751402, rs2094258, rs2296147, and rs2296148) in gastric cancer were explored, and their relationship with metastasis and recurrence of gastric cancer was studied by MassARRAY iPLEX[®] technology.

METHODS

Study population

In this study, 333 subjects were enrolled from our hospital between October 2012 and December 2014, including 200 patients with gastric cancer who were newly diagnosed as case group and 133 subjects as control group. The case group included 170 men and 30 women, with age ranging from 39 to 78 years (56.9±10.1). The control group included 80 men and 53 women, with age ranging from 20 to 87 years (49.4±14.2). The percentage of metastasis and recurrence in the case group was 9.0% and 18.0%, respectively. Exclusion criteria for patients with gastric cancer were primary tumors outside the esophagus, other malignant neoplasms, recurrent tumors, and serious liver and kidney diseases. Exclusion criteria for the control group were a history of malignant cancers, serious liver and kidney diseases, and digestive system disease. The basic medical data of patients with gastric cancer were obtained from medical records. A standardized questionnaire including sociodemographic characteristics was used to interview both patients and controls. Before the study, all subjects signed the informed consent, and this study was approved by our hospital.

DNA extraction and single-nucleotide polymorphisms genotyping

DNA from peripheral blood samples that were collected from patients was extracted using TIANamp Blood DNA Kit (Tiangen, Beijing, China), according to the instructions

of the manufacturer. The *ERCC5* rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 were genotyped by MassARRAY iPLEX[®] technology (Shanghai Genechem Co., Ltd.). The polymerase chain reaction (PCR) fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme. The PCR began with an initial denaturation at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 60 s, and a final extension at 72°C for 5 min. After desalted with resin, the Typer software automatically interpreted the molecular weight peaks detected by the mass spectrometry, and the transformation showed the molecular mass spectrum peak map corresponding to the single-nucleotide polymorphism (SNP) site. The PCR primers were as follows:

rs2016073	ACGTTGGATGCTCCTTTGGAAAGGCTTATC (2nd-PCR) ACGTTGGATGAAGCAGGAAGGGCTCTAGG (1st-PCR)
rs751402	ACGTTGGATGGTATTAGACGGAACCGAGC (2nd-PCR) ACGTTGGATGAAACAGCCAGAAGATGTCCC (1st-PCR)
rs2094258	ACGTTGGATGCAATTTCCCGTACTACTCTG (2nd-PCR) ACGTTGGATGAACTCAGTGAAGGCTGAC (1st-PCR)
rs2296147	ACGTTGGATGCAGACGTTTGGGCTAAGC (2nd-PCR) ACGTTGGATGAACACGTCTCAGCAGCTGTC (1st-PCR)
rs2296148	ACGTTGGATGATTCTTCTACGACGGACTGC (2nd-PCR) ACGTTGGATGCTTTGTTGTGTAGGAGCAGG (1st-PCR)

Statistical analysis

Statistical analyses were performed using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). The differences in sociodemographic characteristics between patients with metastasis and recurrence of gastric cancer were compared using the chi-square test. Genetic loci between the control group and the gastric cancer group were analyzed using the chi-square test. All tests were two-sided. A p-value <0.05 was considered statistically significant.

Ethical issue and informed consent

The authors declare that they have no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study. The study protocol and the comprehensive written informed consent used in this study protocol were reviewed.

RESULTS

Relationship of gender and age with metastasis and recurrence of gastric cancer

In patients with metastasis, there were 16 males and 2 females. In patients without metastasis, there were 154 males and 28 females. There was no significant difference in the metastasis of gastric cancer between males and females ($p=0.628$). In patients with recurrence, there were 36 males and 0 female. In patients without recurrence, there were 134 males and 30 females. Results showed that the recurrence rate of males was higher than that of females ($p=0.005$). Among patients aged ≤ 60 years, patients with metastasis were 13, and patients without metastasis were 114. Among patients aged >60 years, patients with metastasis were 5, and patients without metastasis were 68. There was no significant difference in age for the metastasis of gastric cancer. Among patients aged ≤ 60 years, patients with recurrence were 0, and patients without recurrence were 126. Among patients >60 years, patients with recurrence were 36, and patients without recurrence were 60. There was a significant difference in age for the recurrence of gastric cancer ($p<0.001$) (Table 1).

Comparison of genetic loci between the control group and the gastric cancer group

As for loci rs2016073, rs751402, and rs2094258, there were no significant differences between the control group and the gastric cancer group ($p=0.194$, $p=0.257$, and $p=0.686$, respectively). As for loci rs2296147 and rs2296148, there were significant differences between the control group and the gastric cancer group ($p<0.001$ and $p=0.005$, respectively) (Table 2).

Relationship of gene loci with metastasis and recurrence of gastric cancer

As for locus rs2016073, there was a significant difference between different genotypes and gastric cancer metastasis

Table 1. Relationship of gender and age with metastasis and recurrence of gastric cancer.

	Metastasis			Recurrence		
	Yes	No	p-value	Yes	No	p-value
Gender						
Male	16	154	0.628	36	134	0.005
Female	2	28		0	30	
Age, year						
≤ 60	13	114	0.420	0	126	<0.001
>60	5	68		36	60	

($p=0.021$). As for locus rs751402, there was a significant difference between different genotypes and gastric cancer metastasis ($p=0.021$). As for locus rs2094258, there was a significant difference between different genotypes and gastric cancer metastasis ($p<0.001$). As for locus rs2296147, there was a significant difference between different genotypes and gastric cancer metastasis ($p<0.001$). There was no significant difference between different genotypes of rs2296148 locus and gastric cancer metastasis ($p=0.197$) (Table 3).

As for locus rs2016073, there was no significant difference between different genotypes and gastric cancer recurrence. As for locus rs751402, there was no significant difference between different genotypes and gastric cancer recurrence ($p=0.094$). As for locus rs2094258, there was a significant difference between different genotypes and gastric cancer recurrence ($p=0.021$). As for locus rs2296147, there was no significant difference between different genotypes and gastric cancer recurrence ($p=0.434$). As for locus rs2296148, there was a significant difference between different genotypes and gastric cancer recurrence ($p<0.001$) (Table 3).

DISCUSSION

Functions pertaining to DNA repair and synthesis are believed to play a critical role in cancer development and seem to be affected by genetic polymorphisms¹⁴. Several SNPs in *ERCC5* gene have been identified and have been studied for their association with different cancer risk, such as rs751402 C>T, rs2296147 T>C, rs873601 G>A, rs2094258 C>T, rs1047768 T>C, rs17655G>C, rs2018836G>A, and rs3818356G>A^{12,15-17}. In this study, a case-control study was performed to investigate the association of *ERCC5* genetic polymorphisms with metastasis and recurrence of gastric cancer.

In a previous study, *ERCC5*-763 A>G (rs2016073) may be associated with chemosensitivity of oxaliplatin-based chemotherapy in Chinese patients with advanced colorectal cancer¹⁸. In our study, locus rs2016073 showed no significant differences between the control group and the gastric cancer group and between different genotypes and gastric cancer recurrence, but a significant difference between different genotypes and gastric cancer metastasis was observed. So, results indicated that locus rs2016073 may play a role in gastric cancer, especially in metastasis.

Our results found that locus rs751402 in the gastric cancer group showed no significant difference in the control group and in the gastric cancer recurrence group, which was consistent with our previous study¹¹. Another study using unconditional multiple logistic regression analysis revealed that the AA genotype of rs751402 significantly increased risk of gastric cancer compared with the GG genotype¹⁶. Stratification by cancer type

Table 2. Comparison of genetic loci between the control group and the gastric cancer group.

Loci	Single-nucleotide polymorphisms	Control group	Gastric cancer group	χ^2	p-value
rs2016073	A	67	90	3.281	0.194
	G	20	22		
	AG	46	88		
rs751402	A	20	22	2.72	0.257
	G	67	90		
	GA	48	88		
rs2094258	C	47	44	0.753	0.686
	T	16	21		
	CT	72	76		
rs2296147	C	4	7	17.11	<0.001
	T	76	153		
	CT	55	40		
rs2296148	C	128	170	7.902	0.005
	TC	7	30		

Table 3. Relationship of gene loci with metastasis and recurrence of gastric cancer.

Loci	Single-nucleotide polymorphisms	Metastasis		χ^2	p-value	Recurrence		χ^2	p-value
		Yes	No			Yes	No		
rs2016073	G	22	0	7.758	0.021	1	21	4.721	0.094
	A	89	1			15	75		
	GA	80	8			21	67		
rs751402	AG	80	8	7.758	0.021	21	67	4.721	0.094
	G	89	1			15	75		
	A	22	0			1	21		
rs2094258	C	52	9	21.475	<0.001	9	52	7.758	0.021
	CT	109	0			28	81		
	T	30	0			0	30		
rs2296147	C	6	1	31.131	<0.001	0	7	1.67	0.434
	CT	32	8			8	32		
	T	153	0			29	124		
rs2296148	C	161	9	1.663	0.197	22	148	23.227	<0.001
	TC	30	0			15	15		

indicated that rs751402 polymorphism may increase the risk of gastric cancer and hepatocellular carcinoma, which was further confirmed by a false-positive report probability analysis¹⁹. We found that locus rs751402 was related to gastric cancer metastasis. So, all these results indicated that locus rs751402 may play a role in gastric cancer, especially in metastasis.

The locus rs2094258 as an important polymorphism plays an important role in many cancers^{15,20}. Positive *Helicobacter pylori* individuals with rs2094258 TT genotypes demonstrated an increased risk of gastric cancer²¹. The rs2094258 polymorphism may be associated with an increased risk of gastric cancer in Southern China²². The rs2094258 interacted

with rs2094258 and metabolic gene *GSTP1* rs1695 and formed the basis for various inter-individual susceptibilities to atrophic gastritis²³. Our results indicated that locus rs2094258 was significantly related to metastasis and recurrence of gastric cancer. The above results demonstrated that locus rs2094258 may be a critical DNA repair gene for gastric cancer.

The locus rs2296147 showed a significant difference between the control group and the gastric cancer group and was significantly related to metastasis of gastric cancer, but not to the recurrence of gastric cancer. A case-control study showed that the polymorphic locus on *ERCC5* rs2296147 could reduce the risk of esophageal cancer, which will help further understand the pathogenesis of esophageal cancer²⁴. *ERCC5* rs2296147 C variant genotypes were associated with a significantly lower esophageal squamous cell carcinoma risk²⁵. The rs2296147 polymorphism might alter the risk of developing gastric cancer, especially the diffuse subtype²⁵. Therefore, the rs2296147 polymorphism could be used as surrogate markers for gastric cancer.

The locus rs2296148 showed no significant difference between the control group and the gastric cancer group and was not significantly related to metastasis of gastric cancer, but showed a significant difference in the recurrence of gastric cancer. Until now, there is no relevant literature report about locus rs2296148 for gastric cancer. Our results indicated that locus rs2296148 may be related to the recurrence of gastric cancer.

CONCLUSIONS

In this study, five *ERCC5* gene polymorphisms were explored. The results found that two loci (rs2296147 and rs2296148) in gastric cancer were significantly different from the control group, four loci (rs201607, rs751402, rs2094258, and rs2296147) were related to metastasis of gastric cancer, and two loci (rs2094258 and rs2296148) were related to the recurrence of gastric cancer. *ERCC5* gene polymorphisms may perform important functions

in gastric cancer and its metastasis and recurrence. Due to the small sample size, further validation by larger population-based case-control studies is needed.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All participants signed the informed consent, and this study was approved by the Gansu Provincial Hospital.

IMPACT STATEMENT

This study aims to explore the role of *ERCC5* genetic polymorphisms in gastric cancer. Genotype *ERCC5* rs2016073, rs751402, rs2094258, rs2296147, and rs2296148 were detected. The loci rs2016073, rs751402, and rs2094258 in the gastric cancer group showed no significant differences compared with the control group, and loci rs2296147 and rs2296148 showed significant differences. *ERCC5* polymorphisms (e.g., rs201607, rs751402, rs2094258, rs2296147, and rs2296148) may be associated with metastasis and recurrence of gastric cancer. *ERCC5* gene polymorphisms may perform important functions in gastric cancer and its metastasis and recurrence.

AUTHORS' CONTRIBUTIONS

SW: Conceptualization, Data curation, Resources, Writing-original draft, Writing-review & editing. **XC:** Conceptualization, Data curation, Investigation, Resources. **YF:** Conceptualization, Data curation, Formal analysis, Investigation, Resources. **HZ:** Conceptualization, Data curation, Formal analysis, Investigation, Resources. **WL:** Data curation, Formal analysis, Investigation, Methodology, Resources. **XS:** Data curation, Formal analysis, Investigation, Methodology, Resources. **XM:** Data curation, Formal analysis, Investigation, Methodology, Resources. **SC:** Data curation, Formal analysis, Investigation, Methodology, Resources. **JL:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Supervision, Writing-original draft, Writing-review & editing.

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Anthropometric clinical indicators of visceral adiposity as predictors of nonalcoholic fatty liver disease

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SUMMARY

OBJECTIVE: This study aims to evaluate the role of anthropometric clinical indicators of visceral adiposity as predictors of NAFLD, identifying the cutoff points based on gender.

METHODS: This was a cross-sectional study conducted in patients with or without NAFLD. Waist circumference (WC), body mass index (BMI), waist-to-height ratio (WHtR), Conicity Index (C Index), and lipid accumulation product (LAP) were evaluated.

RESULTS: A total of 107 individuals were evaluated, of which 46.7% were diagnosed with NAFLD. Individuals with NAFLD presented higher values of WC, BMI, C Index, LAP, and WHtR when compared with those without NAFLD ($p < 0.05$). For the total sample, the indicators WC, BMI, WHtR, LAP, and C Index had an area under the receiver operator characteristic curve (AUC) above 0.87, with no difference in the prediction of NAFLD in both sexes. WHtR (AUC=0.934) was the indicator of visceral adiposity with the best discriminatory power for NAFLD, followed by LAP (0.919), WC (0.912), C Index (0.907), and BMI (0.877).

CONCLUSIONS: The anthropometric clinical indicators of visceral adiposity showed high performance, especially the WHtR indicator, as NAFLD predictors.

KEYWORDS: Anthropometry. Non-alcoholic fatty liver disease. Adiposity. Health status indicators. Abdominal fat. Adult.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological condition characterized by histopathological changes, involving abnormal accumulation of lipid in the cytoplasm of hepatocytes, especially triglycerides, in individuals with no history of excessive ethanol consumption¹.

The occurrence of NAFLD is strongly related to obesity, insulin resistance (IR), and other components of the metabolic syndrome (MS)². Studies indicate that abdominal obesity, especially the accumulation of visceral fat, is closely related to the severity of the disease and hepatic steatosis³⁻⁶.

Currently, anthropometric clinical indicators such as waist-to-height ratio (WHtR), lipid accumulation product (LAP), and Conicity Index (C Index) are described in the literature as more sensitive and specific to discriminate visceral fat, when compared to classical parameters such as waist circumference (WC) and body mass index (BMI)⁷.

However, few studies have evaluated the predictive capacity as well as the cutoff points of these new indicators in NAFLD⁸⁻¹¹. The objective of this study is to evaluate the role of anthropometric clinical indicators of visceral adiposity in the prediction of NAFLD, identifying the cutoff points based on gender.

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METHODS

Study design and population

A cross-sectional study conducted at an outpatient clinic specialized in NAFLD, Nonalcoholic Steatohepatitis (NASH) Outpatient Clinic at the University Hospital – Bahia Federal University, Brazil, between April 2015 and August 2017. Inclusion criteria were individuals of both sexes, aged >18 years and <60 years, and the presence or absence of NAFLD confirmed by abdominal ultrasonography (US),

Patients with a previous diagnosis of steatosis who followed up at the clinic were taken as study group and voluntary individuals without steatosis as comparison group.

In both the groups, individuals with ingestion above 140 g ethanol/week, diagnosis of hypothyroidism, other chronic liver diseases (e.g., hepatitis A, B, and C; autoimmune diseases; Wilson's disease; and hemochromatosis); pregnant or lactating women; those with hepatomegaly or splenomegaly, ascites, abdominal tumors, and recent abdominal surgeries; or any physical limitation that compromised the anthropometric evaluation were not included in the study.

Hepatic ultrasonography

Abdominal US was performed by a single evaluator to measure intrahepatic fat.

Laboratory evaluation

No biochemical tests were performed, but we evaluated serum triglyceride (TG) values from test results within 3 months of the patient's assessment date.

Anthropometric clinical indicators

The anthropometric measures were calculated in duplicate by a well-trained and standardized team. Body weight (kg), height (cm), and WC (cm) were measured^{12,13}. These variables measurements were carried out according to the techniques proposed by Lohman et al.¹² and the World Health Organization¹³.

The WHtR was calculated as WC (cm) divided by height (cm), and the BMI was calculated by weight (kg) divided by height (m²).

The C Index was given using the equation proposed by Valdez¹⁴:

$$C \text{ Index} = WC / 0.109 \times \sqrt{(\text{weight}/\text{height})}$$

The LAP was obtained using formula proposed by Kahn¹⁵:

For males,

$$LAP = (WC [\text{cm}] - 65) \times (\text{triglyceride concentration} [\text{mmol/L}])$$

For females,

$$LAP = (WC [\text{cm}] - 58) \times (\text{triglyceride concentration} [\text{mmol/L}])$$

Statistical analysis

Data were expressed as absolute and relative frequency for the categorical variables and as median and interquartile range for non-normally distributed variables. For comparison of continuous variables between the two groups, the Mann-Whitney test and Pearson's chi-square test were used.

To evaluate the performance of anthropometric clinical indicators in the prediction of NAFLD, the receiver operator characteristic (ROC) curve was used. The areas under ROC curves (AUC) and confidence intervals (95%CI) were used to determine the positive predictive value (PPV), negative predictive value (NPV), and accuracy. The values of specificity, sensitivity, and Youden's Index were used to determine the optimal cutoff point.

For statistical analysis, the SPSS version 20.0 software program was used. A p-value <0.05 was considered statistically significant. The power of the test was based on sample size calculation method for nonparametric Wilcoxon-Mann-Whitney test, resulting in a higher power test of 80%, prevalence of control group with BMI above normal by 48%, and prevalence of case group with BMI above normal by 90% ($t=0.53$; $\alpha=0.05$).

RESULTS

A total of 107 individuals were evaluated, of which 46.7% were diagnosed with NAFLD. There was a predominance of the female gender among the individuals with NAFLD (68.0%), while in the comparison group, there was a predominance of the male gender (54.4%) ($p<0.05$). There was no difference in the median age of the individuals with NAFLD and the comparison group ($p<0.05$). Based on the WC, BMI, C Index, LAP, and WHtR scores, it was observed that individuals with NAFLD presented higher values than those without the disease ($p<0.05$) (Table 1).

It was found that the anthropometric indicators showed AUC above 0.87, with no difference in the prediction of NAFLD in both sexes (Figure 1).

The WC, WHtR, LAP, and C Index presented higher values of the Youden's Index relative to BMI. When analyzed by gender, it was observed that only WC, WHtR, and LAP showed the highest values of the Youden's Index in both sexes (Table 2).

The PPV was above 0.8 for all indicators of visceral adiposity, meaning more than 80% of the patients are diagnosed with NAFLD, with BMI of 0.68. The NPV was above 0.9 for WC, WHtR, and BMI and above 0.8 for LAP and C Index, meaning that more than 80% of the patients are without NAFLD (Table 2).

DISCUSSION

In this study, the anthropometric clinical indicators of visceral adiposity such as WC, C Index, WHtR, BMI, and LAP showed a high predictive capacity for NAFLD. Few studies have evidenced the indicators (WC, WHtR, BMI, and LAP) as more specific and sensitive in the prediction of NAFLD^{8,9,16,17}.

Overweight and obesity are closely related to the increase in the prevalence of NAFLD, and visceral obesity is considered the most important predictor of the occurrence of NAFLD¹⁸. Visceral adipose tissue has a greater influence on metabolic disorders due to the production of adipokines that induce IR, type 2 diabetes mellitus, and hypertriglyceridemia^{19,20}, which predispose the occurrence of NAFLD. Anthropometric indicators such as BMI and WC are widely used in the evaluation of patients with NAFLD, and several studies have shown an association of these indicators with steatosis and NASH^{3,6,8}. However, it is important to emphasize on the limited use of BMI in the evaluation of the distribution and body composition, not allowing to distinguish between central obesity²¹, and this type of obesity seems to be an important risk factor for NAFLD, even in those individuals with normal BMI²².

Despite our optimal cutoff point for WC similar to those suggested by the International Diabetes Federation²³ for the diagnosis of MS, Ju et al.¹⁰ identified other cutoff points, showing that there is still controversy over the best anthropometric parameter to predict NAFLD.

Table 1. Demographic characteristics and anthropometric clinical indicators of visceral adiposity of individuals with and without nonalcoholic fatty liver disease.

Variable	NAFLD (n=50)	Comparison group (n=57)	p
Sex, n (%)			
Female	34.0 (68.0)	26.0 (45.6)	0.02*
Male	16.0 (32.0)	31.0 (54.4)	
Age (years) [†]	48.5 (39.0–55.0)	32.0 (28.0–36.5)	<0.01 [‡]
WC (cm) [†]	97.7 (91.0–107.1)	78.0 (71.5–85.0)	<0.01 [‡]
BMI (kg/m ²) [†]	30.1 (27.1–33.8)	24.0 (22.4–26.2)	<0.01 [‡]
C Index [†]	1.2 (1.2–1.3)	1.1 (1.0–1.1)	<0.01 [‡]
LAP [†]	61.1 (40.6–85.7)	14.1 (9.2–24.2)	<0.01 [‡]
WHtR [†]	0.6 (0.5–0.6)	0.4 (0.4–0.5)	<0.01 [‡]

NAFLD: nonalcoholic fatty liver disease; WC: waist circumference; BMI: Body Mass Index; C Index: conicity index; LAP: lipid accumulation product; WHtR: waist-to-height ratio. [‡]Pearson's chi-square test; [†]Results presented in Median and Interquartile range; [‡]Mann-Whitney test.

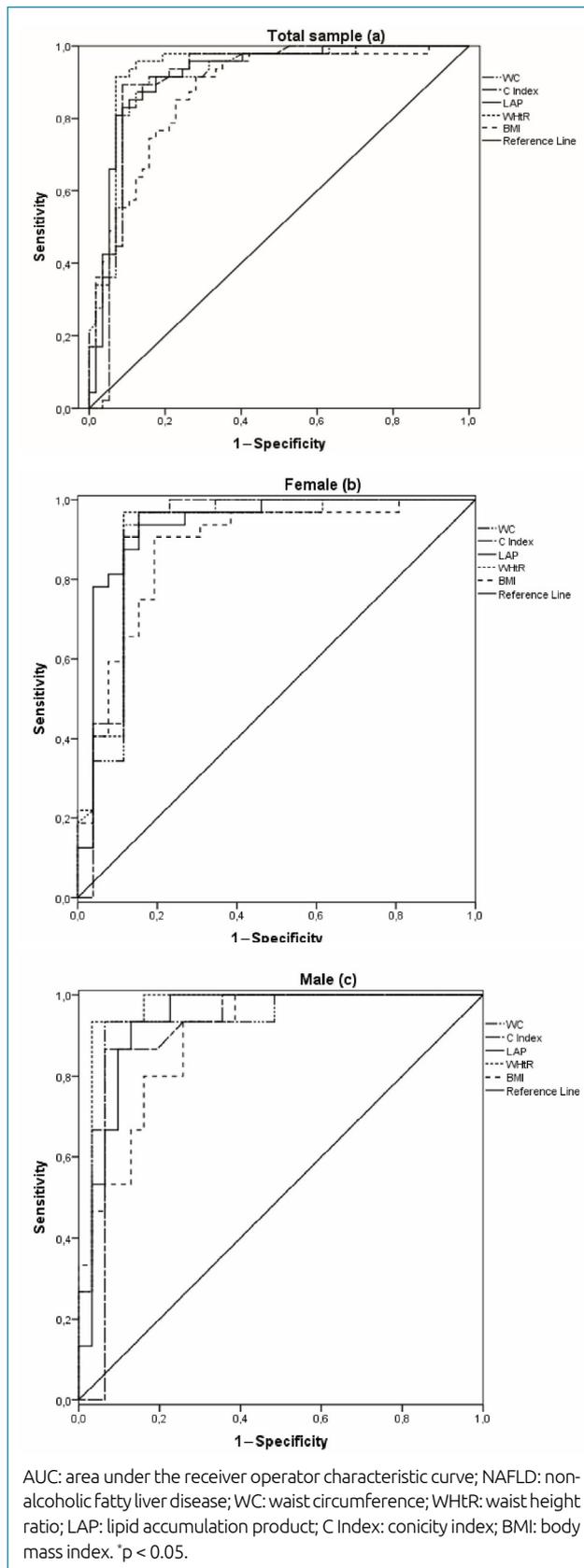


Figure 1. Receiver operator characteristic analysis with areas under the curve of the anthropometric clinical indicators in the prediction of nonalcoholic fatty liver disease by sex.

Zheng et al.⁹ found that WHtR presented greater discriminatory power for NAFLD in relation to BMI. However, if both WC and WHtR have similar discriminatory power, NAFLD was better discriminated by waist-to-hip ratio.

The divergences between the cutoff points of the WHtR for the prediction of NAFLD could be related to the age and ethnicity of the individuals¹⁷. In the Iranian study¹¹, the sample composed of individuals over 60 years of age, different from our study that did not include the elderly. Thus, it should be noted that there are changes in body composition with the aging process, which could generate different WHtR cutoffs by age group.

LAP was identified to have a good discriminatory capacity for NAFLD^{8,16}. The LAP is an indicator that evaluates if the concentration of lipids exerts an effect in the evaluation of the cardiovascular risks and is considered better than the BMI¹⁵.

In the literature, studies conducted with the C Index specifically in NAFLD have not been found, which makes this study the first to evaluate the predictive capacity. C Index has been considered as one of the most accurate indicators in the

discrimination of visceral obesity, in relation to other anthropometric clinical indicators of visceral fat in the adult population⁷, and also as a good predictor of high coronary risk²⁴ and cardiovascular risk^{7,25} factors that are related to the occurrence of NAFLD. The C Index incorporates three easy-to-measure anthropometric measures, including WC, which is common to the other indicators of visceral adiposity.

It should be emphasized that although LAP and C Index presented good performance, the clinical use of these indicators may be limited, since serum levels of TG and use of specific formula are required. Anthropometric indicators (BMI, WC, and WHtR) are easy to perform, indicating a positive factor for clinical applicability.

The main benefits of this study include the evaluation of several anthropometric indicators in the prediction of NAFLD in a single sample; the evaluation of the C Index; and a pioneering study in the literature in the population with NAFLD. However, our study had some limitations. First, the number of individuals evaluated in this work. Thus, studies with larger sample groups would be important to better analyze the

Table 2. Cutoff points, Youden's Index, sensitivity, specificity, positive predictive value and negative predictive value, and accuracy of anthropometric clinical indicators in the prediction of nonalcoholic fatty liver disease.

Indicator	Cutoff	Youden's Index	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy
WC							
Sample	86.7	0.74	87	87	0.87	0.94	0.90
Male	90.1	0.86	93	93	0.87	0.93	0.91
Female	82.5	0.81	93	88	0.86	0.95	0.90
WHtR							
Sample	0.5	0.82	95	87	0.82	0.95	0.88
Male	0.5	0.80	93	87	0.75	0.96	0.87
Female	0.5	0.84	96	88	0.86	0.95	0.90
LAP							
Sample	26.7	0.83	91	82	0.89	0.81	0.84
Male	32.7	0.80	93	87	0.81	0.82	0.82
Female	23.3	0.77	93	84	0.92	0.80	0.86
C Index							
Sample	1.2	0.79	89	91	0.88	0.83	0.85
Male	1.1	0.66	86	80	0.81	0.82	0.82
Female	1.1	0.78	90	88	0.90	0.85	0.88
BMI							
Sample	26.7	0.56	78	78	0.68	0.94	0.77
Male	27.3	0.63	80	83	0.53	1.00	0.70
Female	25.9	0.67	87	80	0.80	0.90	0.83

PPV: positive predictive value; NPV: negative predictive value; WC: waist circumference; WHtR: waist-to-height ratio; LAP: lipid accumulation product; C Index: conicity index; BMI: body mass index.

prediction and cutoff points of visceral adiposity indicators by sex in individuals with NAFLD. Second, the median age of the two groups is different. Third, the diagnosis of hepatic steatosis was confirmed by US, a method that has significant limitations, since individuals with normal US may still have NAFLD.

CONCLUSIONS

The anthropometric clinical indicators of visceral adiposity showed high performance, especially WHtR, as NAFLD predictor. Based on the results of this study, we can conclude that indicators of visceral adiposity, also BMI, play a role to the diagnosis of NAFLD and can be used in the clinical evaluation of individuals who are at risk of NAFLD, allowing clinical intervention and consequently early nutritional intervention, as well as the monitoring of these individuals.

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ETHICAL STATEMENT

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

The study was approved by the research ethics committee of the School of Nutrition of the Federal University of Bahia (Opinion n° 774.353/2014). Informed consent was obtained from all participants. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>).

AUTHORS' CONTRIBUTIONS

NSA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **RR:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CD:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CAS:** Data curation, Project administration, Resources, Supervision, Validation. **RLPDS:** Data curation, Investigation, Methodology, Project administration. **MACS:** Data curation, Project administration. **HPC:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Late pregnancy: impact on prematurity and newborn's weight

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SUMMARY

OBJECTIVE: This study aimed to evaluate the frequency of late pregnancies in Brazil, the age-specific fertility rate (ASFR) in the regions, the rate of prematurity, and the rate of low birth weight (LBW) and their association with advanced maternal age compared with 20–34-year-old women.

METHODS: This was a cross-sectional study conducted by searching the Information System on Live Births (*Sistema de Informações Sobre Nascidos Vivos* [SINASC]). Data from 1995 to 2018 were collected, and pregnant women were divided into three categories based on their age range: 35–39, 40–44, and ≥45 years. The study calculated the frequency of deliveries of mothers of advanced age in Brazil, the ASFR, and the rates of prematurity and LBW in each group.

RESULTS: The frequency of newborns (NB) of mothers aged ≥35 years increased by 64%. The 35–39-year-old ASFR increased in all regions, except in the northeast. At maternal age ≥35 years, NB increased by 58% between 28 and 36 weeks during the study period. LBW increased between 500 and 1,499 g in 68.7% and between 1,500 and 2499 g in 57% of cases. In 2018, regarding the age range of 20–34 years, the chance of premature delivery was 29% at 35–39 years (OR=1.29), 54% higher at 40–44 years (OR=1.54), and 114% higher at ≥45 years (OR=2.14); while the chance of LBW increased by 28% at 35–39 years (OR=1.28), 56% at 40–44 years (OR=1.56), and 139% at 45 years or older (OR=2.39).

CONCLUSIONS: The frequency of deliveries and ASFR ≥35 years increased between 1995 and 2018. The chances of prematurity and LBW were higher with increased maternal age.

KEYWORDS: Prematurity. Maternal age. Fertility rate. Pregnancy complications. Low birth weight.

INTRODUCTION

In recent decades, there has been an increased presence of women in the labor market along with the advent of debates on female empowerment, gender roles, and reproductive rights. While taking on other roles and considering the political, economic, and cultural contexts, many women choose to postpone motherhood¹. With this, many mothers aged 35 years or older will

be considered as “late pregnancy”². Data from the Information System on Live Births (*Sistema de Informações Sobre Nascidos Vivos* [SINASC])² in Brazil show that the number of live births in mothers aged ≥35 years was 275,277 in 2000 and 457,109 in 2018, with an increase of 66%, which justifies the study of this population^{2,3}.

Pregnancy with advanced maternal age is a risk factor for obstetric and neonatal complications such as preeclampsia,

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hypertension, and gestational diabetes, resulting in higher rates of cesarean delivery and low birth weight (LBW)⁴. Therefore, it is important to study women who choose to postpone pregnancy to decrease the rates of peripartum complications due to higher maternal-fetal risk^{2,4,5}.

This study aims to evaluate the frequency of late pregnancies in Brazil, the age-specific fertility rate (ASFR) per region, the rates of prematurity and LBW, and their association with advanced maternal age as compared with 20–34-year-old women.

METHODS

This cross-sectional study obtained information from the SINASC database of the Department of Informatics of the Unified Health System (*Departamento de Informática do Sistema Único de Saúde [DATASUS]*) of the Ministry of Health in Brazil from 1995 to 2018³. The estimated total female population was obtained from the SINASC database until 2000 and from the Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística [IBGE]*) after 2001⁶.

The 24-year period was grouped into four groups for data analysis: 1995–2000, 2001–2006, 2007–2012, and 2013–2018.

The maternal ages that were considered advanced for pregnancy were divided into the following: 35–39 years, 40–44 years, and 45 years or more. The control group included pregnant women between 20 and 34 years of age.

Gestational age was subdivided into 22–27 weeks (limit of fetal viability), 28–36 weeks (premature newborns, NB), 37–41 weeks (full-term pregnancies), and 42 weeks or more (postdatism situations).

Birth weight was subdivided into four subgroups: <500 g, 500–1,499 g; 1,500–2,499 g, 2,500–3,999 g, and ≥4,000 g.

The exclusion criteria were gestational age of <22 weeks (for miscarriage) and ignored maternal age. Since cases with the studied variable marked as “ignored” were not computed, the distribution of some variables did not reach 100%.

The frequency of late pregnancies was calculated by dividing the number of live births (LB) with maternal age ≥35 years by the total number of LB in the same period and region multiplied by 100. The fertility rate was obtained as the ratio between the number of LB and the total number of women living in the same period and region multiplied by 1,000. Fertility rate growth was calculated as the rate difference between 2013–2018 and 1995–2000 on the initial period value. The relationship between maternal age and birth weight used the number of LB with a given birth weight at maternal age ≥35 years divided by the total number of LB in the same period of a given birth weight multiplied by 100.

The graphs and data analysis were performed using Excel and R-Project software.

RESULTS

After applying the exclusion criteria, the total number of LB in Brazil was 18,408,043 with 1,498,193 mothers aged ≥35 years in 1995–2000, which is equivalent to 8.1% of births in the period. Between 2001 and 2006, there were 18,219,699 LB and 1,655,517 mothers of advanced age (9.1%). Between 2007 and 2012, the total number of LB decreased to 17,388,554, but mothers aged ≥35 years increased to 10.4% (1,808,578). Between 2013 and 2018, the total number of LB (17,627,221) was stable, with a significant increase in the number of LB of mothers aged ≥35 years to 2,354,305 (13.4% of the total) (Figure 1).

The frequency of LB between 1995 and 2018 increased in mothers in the following age groups: ≥35 years (64% increase), 35–39 years (69.8% increase), and 40–44 years (51.7% increase).

The comparison between 1995–2000 and 2013–2018 showed a 13.7% increase in ASFR for mothers aged 35–39 years in Brazil.

In the north of the country, ASFR increased by 4.9% for mothers aged between 35 and 39 years. For mothers aged between 40–44 and 45–49 years, it reduced by 19.6% and 53.2%, respectively.

In the northeast region, ASFR decreased in the three age groups by 3.5, 28.7%, and 33.6%, respectively.

In the central-west region, ASFR increased by 48.9% between 35 and 39 years and by 32.1% between 40 and 44 years. It reduced by 26.6% in the age range of 45–49 years.

In the southeast region, ASFR increased by 26.7% between 35 and 39 years and by 20.1% between 40 and 44 years. For mothers aged between 45 and 49 years, it was reduced by 16.7%.

In the southern region of the country, ASFR/1,000 increased by 6.4% between 35 and 39 years. The other two age groups reduced by 12.2% and 50.9%, respectively (Table 1).

As for the duration of early pregnancy, the number of LB at 22–27 weeks increased by 70.2% between 1995 and 2018. Between 28 and 36 weeks and between 37 and 41 weeks, it increased by 58% and 66.3%, respectively (Table 2). Therefore, there was a higher frequency of pregnancies ≥35 years in all subgroups, which was more pronounced in extremely premature and full-term infants.

As for LBW, there was an increase in all subgroups between 1995 and 2018 in mothers aged ≥35 years. The frequency of LB increased by 68.7% between 500 and 1,499 g and by 57% between 1,500 and 2,499 g.

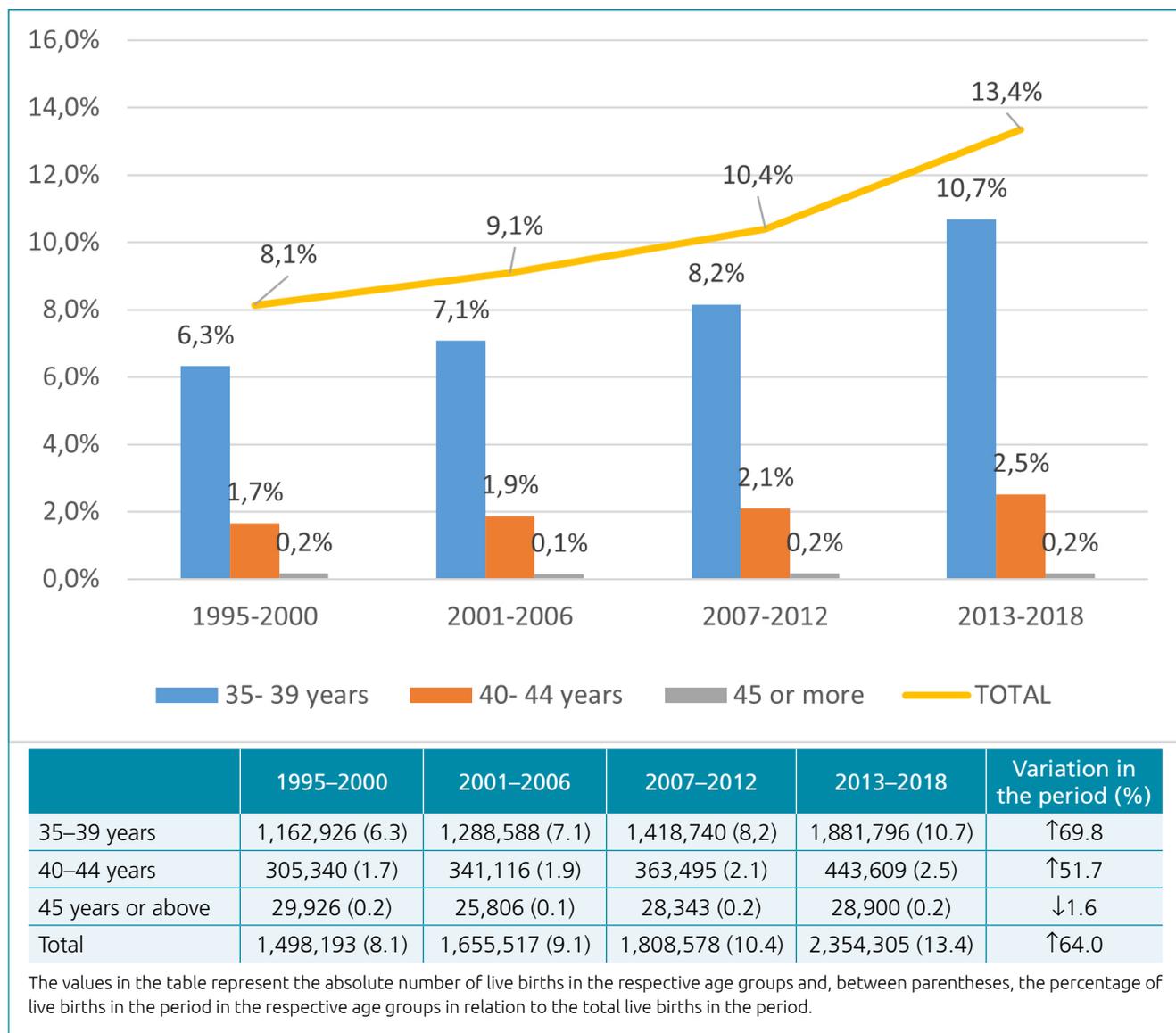


Figure 1. Frequency of pregnancy in women aged ≥35 years or more in Brazil (1995-2018).

The chances of premature delivery and LBW were analyzed based on data from 2018. The prevalence of premature delivery in the control group (20-34 years) was 10.3%. These rates increase proportionally to maternal age, being 12.9% between 35 and 39 years, 15.0% between 40 and 44 years, and 19.7% in ≥45 years. Pregnant women aged 35-39 years have a 29% higher chance of premature delivery than those aged 20-34 years [p<0.001, OR=1.29 (95%CI 1.27-1.30)]. At 40-44 years, the chance increases to 54% [p<0.001, OR=1.54 (95%CI 1.51-1.57)], and at ≥45 years, the chance of preterm delivery doubles in relation to 20-34 years [p<0.001, OR=2.14 (95%CI 1.99-2.30)].

The prevalence of LBW was 7.9% in the control group. Between 35 and 39 years, it increased to 9.8, 11.7% at 40-44

years, and 18.3% at ≥45 years. The chance of LBW between 35 and 39 years was 28% higher than that between 20 and 34 years [p<0.001, OR=1.28 (95%CI 1.26-1.30)]. At 40-44 years, the chance of NB with LBW increases to 56% [p<0.001, OR=1.56 (95%CI 1.53-1.59)] and 139% at ≥45 years [p<0.001, OR=2.39 (95%CI 2.23-2.57)] (Table 2).

DISCUSSION

The last decades of the 20th century showed increased participation of women in the labor market. This includes a process of continuous expansion, political and economic changes, and movement to seek equity between men and women⁷. This movement followed a decreased birth rate that reflected

Table 1. Age-specific fertility rate/1,000 women aged ≥35 years in the regions of Brazil (1995–2018).

North region													
Maternal age (years)	1995–2000			2001–2006			2007–2012			2013–2018			Variation 2013–2018 to 1995–2000
	Fem. pop.	N° LB	ASFR/1,000										
35–39	2,019,196	64,013	31.7	2,512,602	79,561	31.7	3,119,241	97,624	31.3	3,984,599	132,498	33.3	4.9%
40–44	1,590,054	1,9250	12.1	2,040,078	22,423	11.0	2,626,489	25,694	9.8	3,312,505	32,224	9.7	-19.6%
45–49	1,212,189	2,293	1.9	1,742,507	2,608	1.5	2,194,995	2,471	1.1	2,680,054	2,375	0.9	-53.2%
Total	8,612,299	85,556	9.9	10,555,205	104,592	9.9	14,082,620	125,789	8.9	9,977,158	167,097	16.7	68.6%
Northeast region													
Maternal age (years)	1995–2000			2001–2006			2007–2012			2013–2018			Variation 2013–2018 to 1995–2000
	Fem. pop.	N° LB	ASFR/1,000										
35–39	8,407,568	290,313	34.5	9,863,931	329,563	33.4	11,048,258	345,828	31.3	13,234,508	441,184	33.3	-3.5%
40–44	7,043,303	92,026	13.1	8,162,943	98,462	12.1	10,206,393	95,591	9.4	11,529,569	107,354	9.3	-28.7%
45–49	26,515,402	11,463	0.4	30,959,251	9,120	0.3	39,417,304	7,820	0.2	24,764,077	7,106	0.3	-33.6%
Total	42,247,527	394,329	9.3	48,986,125	437,374	8.9	60,671,955	449,822	7.4	49,528,154	556,289	11.2	20.3%
Central-west region													
Maternal age (years)	1995–2000			2001–2006			2007–2012			2013–2018			Variation 2013–2018 to 1995–2000
	Fem. pop.	N° LB	ASFR/1,000										
35–39	2,333,033	57,883	24.8	2,880,175	72,237	25.1	3,335,263	95,671	28.7	3,934,303	145,312	36.9	48.9%
40–44	1,880,647	12,956	6.9	2,344,215	15,807	6.7	3,007,460	21,139	7.0	3,486,192	31,722	9.1	32.1%
45–49	1,444,370	1,167	0.8	2,047,978	990	0.5	2,611,653	1,304	0.5	3,075,702	1,823	0.6	-26.6%
Total	9,589,369	72,006	7.5	12,061,893	89,034	7.4	16,313,916	118,114	7.2	10,496,197	178,857	17.0	126.9%

Continue...

Table 1. Continuation.

North region																
Southeast region																
Maternal age (years)	1995-2000			2001-2006			2007-2012			2013-2018			Variation 2013-2018 to 1995-2000			
	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	
35-39	16,060,684	527,001	32.8	18,278,641	590,144	32.3	18,539,827	660,988	35.7	21,046,815	875,035	41.6	21,046,815	875,035	41.6	26.7%
40-44	13,754,946	123,286	9.0	16,264,584	146,153	9.0	17,807,649	164,219	9.2	19,052,960	205,111	10.8	19,052,960	205,111	10.8	20.1%
45-49	10,986,161	8,886	0.8	14,665,077	8,632	0.6	16,744,024	9,623	0.6	17,542,724	11,814	0.7	17,542,724	11,814	0.7	-16.7%
Total	76,884,903	659,173	8.6	91,095,225	744,929	8.2	111,158,805	834,830	7.5	57,642,499	1,091,960	18.9	57,642,499	1,091,960	18.9	121.0%
South region																
Maternal age (years)	1995-2000			2001-2006			2007-2012			2013-2018			Variation 2013-2018 to 1995-2000			
	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	
35-39	5,519,462	223,716	40.5	6,379,145	217,083	34.0	6,121,427	218,629	35.7	6,674,118	287,767	43.1	6,674,118	287,767	43.1	6.4%
40-44	4,701,741	57,822	12.3	5,526,089	58,271	10.5	6,157,495	56,852	9.2	6,220,413	67,198	10.8	6,220,413	67,198	10.8	-12.2%
45-49	3,806,339	4,697	1.2	5,005,921	3,735	0.7	5,867,858	3,426	0.6	6,027,612	3,652	0.6	6,027,612	3,652	0.6	-50.9%
Total	26,305,429	286,235	10.9	31,199,313	279,089	8.9	37,957,265	278,907	7.3	44,120,618	358,617	8.1	44,120,618	358,617	8.1	-25.3%
Brazil																
Maternal age (years)	1995-2000			2001-2006			2007-2012			2013-2018			Variation 2013-2018 to 1995-2000			
	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	Fem. pop.	N° LB	ASFR/1,000	
35-39	34,339,943	1,162,926	33.9	39,914,494	1,288,588	32.3	42,164,016	1,418,740	33.6	48,874,343	1,881,796	38.5	48,874,343	1,881,796	38.5	13.7%
40-44	28,970,691	305,340	10.5	34,337,909	341,116	9.9	39,805,486	363,495	9.1	43,601,639	443,609	10.2	43,601,639	443,609	10.2	-3.5%
45-49	24,492,362	109,069	4.5	31,624,426	114,427	3.6	37,624,923	112,415	3.0	40,855,661	127,018	3.1	40,855,661	127,018	3.1	-30.2%
Total	87,802,996	1,577,335	18.0	105,876,829	1,744,131	16.5	119,594,425	1,894,650	15.8	133,331,643	2,452,423	18.4	133,331,643	2,452,423	18.4	2.4%

Source: SINASC/IBGE; Fem. pop.: female population; ASFR: age-specific fertility rate; N°: number; LB: live born.

Table 2. Total number of live births of mothers ≥ 35 years by gestational age and birth weight, and association with prematurity and low birth weight.

	1995–2000		2001–2006		2007–2012		2013–2018		Variation in the period 1995–2018 (%)
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	
Gestational age (weeks)									
22–27	7,178	8.4	6,170	9.9	8,291	11.4	12,362	14.3	70.2
28–36	32,828	10.0	127,307	11.5	176,266	13.1	294,299	15.8	58.0
37–41	1,209,895	8.0	1,483,394	8.9	1,567,948	10.2	1,955,544	13.3	66.3
≥ 42	40,873	7.7	17,807	8.3	21,882	7.7	43,633	8.4	9.1
Total	1,290,774	8.1	1,634,678	9.1	1,774,387	10.4	2,305,838	13.4	65.4
NB weight (g)									
Less than 500	247	1.0	547	12.1	1,525	11.1	2,445	13.5	1,200.2
500–1,499	8,945	10.5	22,906	12.3	27,865	14.2	36,628	17.7	68.7
1,500–2,499	90,200	10.2	142,155	11.4	154,775	12.8	196,437	16.1	57.0
2,500–3,999	1,069,815	7.7	1,349,170	8.7	1,633,763	11.1	1,931,823	13.0	69.3
4,000 or more	106,274	10.9	108,288	11.5	108,812	12.5	137,914	15.7	44.3
Total	1,275,481	8.1	1,623,066	9.1	1,926,740	11.3	2,305,247	13.4	67.2
Association between maternal age and prematurity (Brazil, 2018)									
Maternal age (years)	Freq.	%	p-value	OR (95%CI)					
20–34	205,831	10.3							
35–39	46,451	12.9	<0.001	1.29 (1.27–1.30)					
40–44	12,707	15.0	<0.001	1.54 (1.51–1.57)					
>45	954	19.7	<0.001	2.14 (1.99–2.30)					
Association between maternal age and low birth weight (Brazil, 2018)									
Maternal age (years)	Freq.	%	p-value	OR (95%CI)					
20–34	159,564	7.9		c					
35–39	36,004	9.8	<0.001	1.28 (1.26–1.30)					
40–44	10,076	11.7	<0.001	1.56 (1.53–1.59)					
≥ 45	900	18.3	<0.001	2.39 (2.23–2.57)					

Freq.: frequency; c: denotes comparative group.

the behavior of a significant part of the Brazilian population, with the demographic profile changing to a lower number of children per family unit due to pregnancy postponement, among other factors¹.

This study showed an increase of 64% in the frequency of advanced-age pregnancies between 1995 and 2018 in Brazil. Those with ages of 35–39 years represented the largest portion of pregnancies in all periods, but the frequency increased by 51% between 40 and 44 years. This information corroborates the data presented in 2005³, showing an increased proportion of births in mothers aged ≥ 40 years, from 1.75% to 1.95%, between 1996 and 2002^{3,8}.

The contribution of women to the household income increased relatively regularly from 15.7% in 1981 to 23.8% in

2002, which is a leap that reflects economic, social, and political changes in this period⁷. More specifically, in the last decade, late pregnancy increased by 84% in the United States due to behavioral changes in the family. Some studies also show that the proportion of deliveries after 40 years of age varies from 2% to 5%^{8,9}.

The number and quality of oocytes decrease at advanced ages, with a higher decline after 35 years¹⁰. Nevertheless, the increased frequency of pregnancies of $>50\%$ in the age group ≥ 40 years indicates the influence of other factors, such as the search for assisted reproduction techniques (ART).

The risk of placenta previa, cesarean section, preterm delivery, and LBW is higher in the ART group, but advanced age also poses a risk for these outcomes regardless of the form of

conception¹¹. After 40 years of age, with 18.2% of births by ART, a significant increase in preeclampsia and prematurity was observed in the ART group. At ≥ 45 years, the risk was significantly higher among women who conceived naturally⁵.

The ASFR increased between 35 and 44 years in 1995–2018 in Brazil. This increase was related to a higher frequency of late pregnancies between 2013 and 2018. The south, southeast, and center-west regions comprised approximately 65% of the Brazilian female population between 35 and 39 years in 2018. Therefore, the national standard reflects these three regions⁶. They are economically more developed and populous regions, have reference centers in assisted reproduction, higher levels of education, and access to contraception, which is probably related to the increasing participation of women in the workforce leading to the postponement of pregnancy^{1,7}.

This study showed that prematurity increases proportionally with maternal age, doubling the chance at ≥ 45 years of age. This result corroborates the results of several previous studies. A meta-analysis of pregnant women aged ≥ 45 years showed twice the chance (OR=1.96) of premature birth and four times the chance of cesarean delivery¹². Another meta-analysis evaluated pregnant women aged 35–40 and >40 years with a greater propensity to overweight, hypertension, and gestational diabetes, as well as adverse perinatal results such as premature delivery, LBW, higher perinatal mortality, and stillbirth¹³. Bouzaglou et al. also found a significant increase in gestational hypertension (3.1×1.1%, $p<0.001$), premature delivery (10.4×6.5%, $p<0.001$), and intrauterine fetal death (2.1×0.5%, $p<0.001$) at ≥ 40 years⁴. Ogawa et al. reported a higher risk of severe preeclampsia, placenta previa, and premature delivery in pregnant women ≥ 45 years⁵.

LBW is directly associated with premature birth. Gravena et al.¹⁴ reported a prevalence of 12.5% and a 24% higher chance of LBW at >35 years, similar to the 12.8% in NB between 1,500 and 2,499 g between 2007 and 2012 obtained in this study.

The proportion of NB with weight <500 and $>4,000$ g in women of advanced age increased between 1995 and 2018. Although LBW is the focus of this study, due to its close relationship with unfavorable perinatal outcomes, it is worth

reflecting on maternal factors involved in the increased number of macrosomic complications at advanced maternal age, such as gestational diabetes¹⁵.

One of the limitations of an observational study is that the formulated hypotheses cannot be tested and proven as an association of cause and effect. Women who became pregnant later are not necessarily the same women who postponed pregnancy due to greater participation in the labor market, among other reasons. However, this phenomenon is observed worldwide, leading to a reflection on the historical and economic contexts in which the period studied is inserted. In addition, it is worth emphasizing the insufficient filling of official data and the obtention of the real number of pregnancies since fetal deaths and abortions were not included.

CONCLUSIONS

There was an increased frequency of LB and ASFR for ages ≥ 34 years between 1995 and 2018. The chances of prematurity and LBW were higher with increased maternal age. Advanced maternal age is a factor of gestational risk; therefore, adequate access and quality of care are important in the pre-, peri-, and postnatal periods for these women who, by chance or by choice, become pregnant in the late end of their reproductive life.

AUTHORS' CONTRIBUTIONS

LKSS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **DLMM:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CRS:** Investigation, Project administration, Visualization. **FEFM:** Formal analysis, Project administration, Resources, Software. **FMS:** Formal analysis, Methodology, Supervision, Validation, Visualization. **NCPR:** Conceptualization, Formal analysis, Resources, Software.

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Efficacy of shear wave elastography in predicting preeclampsia in the first trimester

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SUMMARY

OBJECTIVE: This study aimed to investigate the predictive value of shear wave elastography (SWE) for preeclampsia (PE) in first-trimester pregnancies.

METHODS: Singleton pregnant women aged 18–45 years, who underwent routine first-trimester prenatal examinations (11–13 weeks+6 days) were enrolled. Pregnancies with anterior placenta and normal first-trimester screening test results were included in the study group. The SWE measurements of six areas of the placenta were performed, and the mean value was estimated. The perinatal outcomes and the demographic data were also collected. The receiver operating characteristic curve analysis was used for the accuracy of predicting PE.

RESULTS: This study consisted of 84 patients, of which 9 were diagnosed with PE during the follow-up. The mean SWE value of the PE patients was higher than that of patients with normal pregnancies ($p=0.002$). The analysis showed that the optimal cutoff value was 7.43 kPa to predict PE in the placentas of first-trimester pregnancies, with 88% sensitivity and 78% specificity.

CONCLUSIONS: The SWE values of the placenta in the first trimester were different between normal patients and those who are subsequently developing PE. SWE may be a suitable tool for predicting PE in pregnant women.

KEYWORDS: Shear wave elastography. Preeclampsia. High-risk pregnancy. First-trimester screening.

INTRODUCTION

Shear wave elastography (SWE) is a type of sonoelastography method that measures the stiffness and elasticity of soft tissues¹. This new technique has already been used in the differential diagnosis of diseases such as hepatic fibrosis, tumors, and inflammation in musculoskeletal tissues^{1,2}. Recently, it has become popular in screening the elasticity of the placenta in high-risk pregnancies such as those with preeclampsia (PE), intrauterine growth restriction, and placental dysfunction³⁻⁵. The application of SWE is considered safe during pregnancy, and performing this technique has already been documented in obstetrics^{5,6}.

PE, a known multisystem disease, generally affects 2–5% of pregnant women, and it is associated with a perinatal and neonatal mortality rate of 10%^{7,8}. Many studies have been conducted to predict and prevent PE⁹⁻¹¹. They investigated maternal factors including maternal pulse wave analysis, mean arterial blood pressure, uterine artery pulsatility index (UtA-PI), serum placental protein 13, serum pregnancy-associated plasma protein A (PAPP-A), and placental growth factor^{7-9,12}. While the cause of PE remains an enigma, clinical and pathological studies suggest that the placenta is central in the pathogenesis of this syndrome. Instead of combining the laboratory markers, the use of a noninvasive ultrasound method for screening

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the placenta seemed appealing. Therefore, we aimed to investigate the predictive value of SWE for PE in first-trimester single-euploid pregnancies.

METHODS

This prospective study included singleton pregnant women aged 18–45 years, who underwent routine first-trimester prenatal examination (11–13 weeks+6 days) between May 2019 and December 2019 at a tertiary hospital. Ethical documents, including the protocols as per the Declaration of Helsinki, were approved by the local Institutional Review Board (Approval number: 2019/50), and written informed consent was obtained from all patients.

Patients with normal first-trimester screening test results (single-euploid pregnancies) were enrolled in the study group. Exclusion criteria were multiple gestations, pregnancies with fetal anomalies, abnormal placental location, and uterine malformations. Patients with systemic autoimmune diseases (chronic hypertension, diabetes, hepatitis, etc.), history of PE, high blood pressure, and smoking habits were also excluded.

First-trimester prenatal examinations including nuchal translucency (NT) and UtA-PI of both maternal sides were performed as previously described^{13,14}. Routine laboratory markers, including PAPP-A and beta-human chorionic gonadotropin (β -hCG) for the first-trimester screening test, were also obtained. Measurements were expressed as multiples of the median (MoM) values according to the percentiles adjusted for fetal crown rump length. Additionally, SWE was performed during the ultrasonographic evaluation of the first trimester. All ultrasonographic measurements were conducted by a single perinatologist. Patients with anterior placenta were included in the study to avoid reducing the effectiveness of the method. Considering the disc-like structure of the placenta, it was divided into the following six regions for measurement: maternal right, maternal central, maternal left, fetal right, fetal central, and fetal left. The mean arithmetic value was calculated. The SWE measurements of the six areas of the placenta were performed for every patient to record the measurements from different parts of the placenta. Since the placental structure is small in the first trimester and the measurement areas were close to each other, an average value was calculated. The demographic data and the detailed medical and obstetrical history were examined and recorded during patient admission.

Perinatal outcomes, including the delivery week and birth weight, were also recorded. All patients were followed up at the perinatology clinic, and the delivery timings were organized

individually based on obstetrical indications. PE was diagnosed according to the following guidelines: two measurements of diastolic blood pressure ≥ 90 mmHg of at least 4 h apart in a previously normotensive woman and proteinuria ≥ 300 mg in 24 h; or two readings of at least “++” on the dipstick analysis of midstream urine or catheter specimen urine (if no 24-h urine collection is available) after 20 weeks of gestation¹⁵. The primary measurement outcome was to predict PE by using SWE in the normally detected first-trimester screening.

Shear wave elastography

SWE is a safe, noninvasive technique with the advantage of being performed in the same session as the routine antenatal scanning^{2,6}. There is no need to wait for the results like other screening markers; SWE measurement can alleviate patient anxiety. The stiffer the tissue, the higher is the shear wave velocity (SWV). Samsung HS70A ultrasound system with a Samsung CA1-7A convex transducer was used. SWE measures alterations in tissue deformation, reflective of its elastic properties when an internal or external force is applied. Once force is applied, tissue displacement can then be displayed directly as an image property, as SWE. During the examination, patients were made to lie in the supine position, breathing levels were kept steady to prevent noise and artifacts, and a minimum pressure was applied on the transducer to eliminate misleading tissue compression. A region of interest box of 10×10 mm was positioned on the placenta, and six measurements were obtained from the central and peripheral zones of the placenta while avoiding vascular structures (Figure 1). All measurements were obtained in kPa formats.

Statistical analysis

Statistical Package for the Social Sciences version 20 program (SPSS Inc., Chicago, Illinois, USA) was used for the statistical evaluation of the data obtained in this study. The continuous data were summarized as mean and standard deviation, while the categorical data were summarized as numbers and percentages. The receiver operating characteristic (ROC) curve was used to investigate the accuracy of predicting the presence of PE. According to this method, for the best test definition, the sensitivity was 100%, the area under the curve (AUC) was 1, and the diagnostic value of AUC was $p < 0.05$.

The sampling size was calculated based on the ability of the SWE measurements to predict PE in pregnancies by the ROC analysis. In this case, considering the structure of the ROC curve, the value of the AUC was assumed to be approximately 0.85 and the minimum sample size at 80% power at a 10% error level was determined in 76 patients.

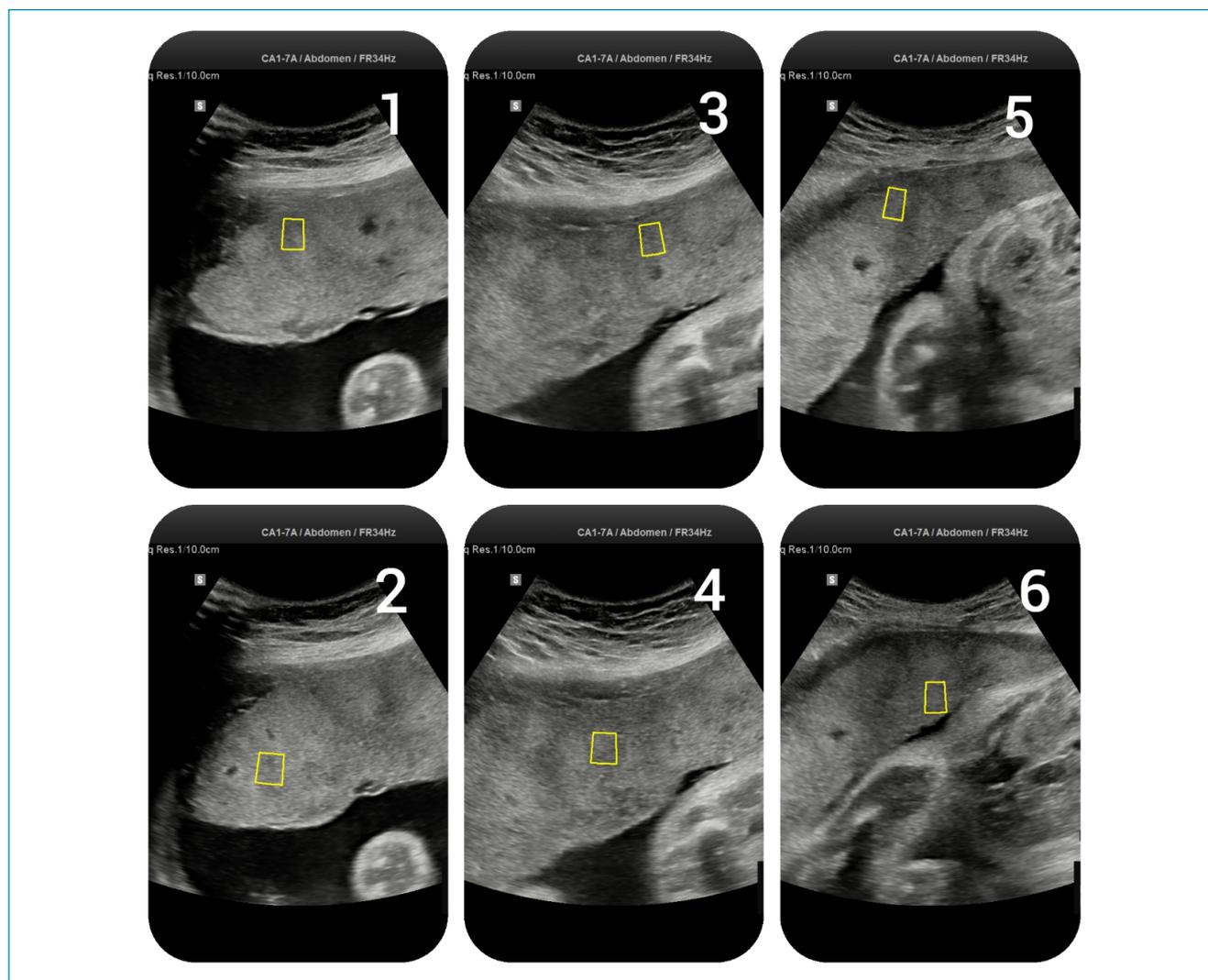


Figure 1. Placental measurements were obtained from central and peripheral zones of placenta while avoiding vascular structures. Numbers 1, 3, and 5 were maternal side and 2, 4, and 6 were fetal side; respectively.

RESULTS

This study included 113 pregnancies, of which 11 patients were lost to follow-up and 1 to abortion. All pregnancies were followed up until delivery. Patients who subsequently developed gestational diabetes mellitus, gestational hypertension, and fetal growth restriction were excluded. Since it is not clear which patient will be diagnosed with PE according to the follow-ups in the first trimester, a long time was required in terms of the follow-up period and results. The final study population consisted of 84 patients, of which 9 were diagnosed with PE later during the follow-up. The characteristics of the patients, including pregnancy outcomes and the results of screening tests, are shown in Table 1. Pregnancies with PE had earlier deliveries and lower birth weights. The mean age of the women in this study was 29 ± 5.13 years,

and the mean gestation period was 11 weeks and 6 days. All mothers and babies were healthy, and only two babies were taken to the neonatal intensive care unit. There was no statistically significant difference between the patients with PE and those with normal pregnancies regarding age, gravidity, parity, body mass index, and the results of first-trimester screening tests, except for the mean SWE value. The mean SWE value in the PE patients was significantly higher than that of the patients with normal pregnancies ($p=0.002$). The ROC analysis for the mean values of SWE showed that the optimal cutoff value was 7.43 kPa to predict PE in the placentas of first-trimester pregnancies, with 88% sensitivity and 78% specificity (Figure 2). Regarding the detection rates including NT, PAPP-A, β -hCG, and UtA-PI for PE, there was no better prediction parameter than the mean value of SWE.

Table 1. Summary of characteristics of the patients, pregnancy outcomes, and the results of screening tests between groups (preeclampsia and normal pregnancies).

	Normal pregnancies (n=75)	Preeclampsia (n=9)	p-value
Age (year)	29.93±4.29	26.77±7.13	0.081
Gravidity (min–max)	2 (1–7)	2 (1–7)	0.828
Parity (min–max)	1 (0–3)	1 (0–3)	0.847
BMI	25.47±2.93	25.61±4.22	0.926
Birth (weeks)	38.66±1.09	36.22±2.63	0.024
Birth weight (g)	3390±372	2782±646	0.023
PAPP-A (MoM)	1.22±0.81	1.05±1.01	0.564
β-hCG (MoM)	1.16±0.91	1.20±0.99	0.905
NT (MoM)	0.94±0.23	0.97±0.26	0.736
UtA-PI			
Right	1.15±0.55	1.28±0.52	0.933
Left	1.08±0.50	1.28±0.61	0.279
SWE (mean)	6.03±2.23	9.04±2.57	0.04
SWE (min–max)	2.37–13.78	3.43–11.77	

BMI: body mass index; PAPP-A: pregnancy-associated plasma protein A; MoM: multiples of the median; β-hCG: human chorionic gonadotropin; NT: nuchal translucency; UtA-PI: uterine artery pulsatility index; SWE: shear wave elastography; p<0.05 indicates the statistical significance.

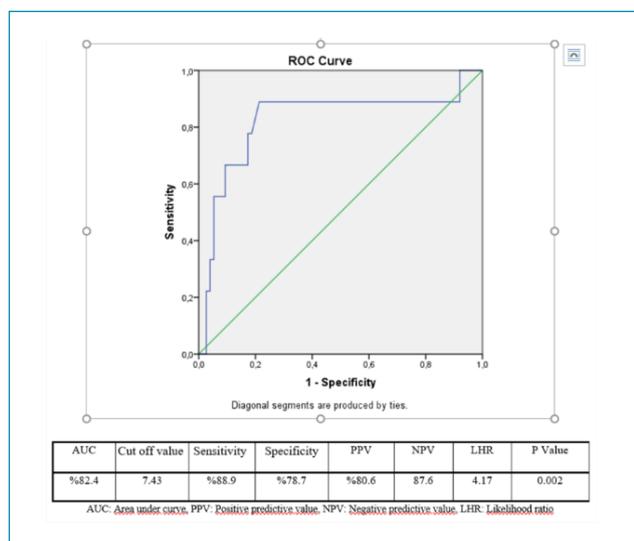


Figure 2. The receiver operating characteristic curve analysis for the positive and negative likelihood ratio of the value of 7.43 kPa.

DISCUSSION

In this study, we found that a mean SWE value above 7.43 kPa predicted PE with very high sensitivity and specificity in low-risk pregnancy patients with normal first-trimester screening test results.

Fujita et al.¹⁶ measured point SWV to evaluate the placental elasticity for predicting the onset of PE in the second trimester. They examined 16–32 weeks of singleton pregnancies with anterior placenta and compared values between 185 low-risk and 36 high-risk patients for PE¹⁶. A higher SWV was found in the high-risk group¹⁸. Therefore, it has been suggested that SWV can be used as a parameter to predict the onset of PE. Similarly, higher placental SWV values were observed in the second- and third trimester of pregnancies with mild-severe PE or gestational hypertension as compared with the controls^{3,17}. The UtA-PI value was found to be significantly higher in the PE group than in the controls of these studies^{3,17}.

Cimsit et al.⁵ investigated SWE in the placentas of normal pregnancies (n=101) and those complicated with PE (n=28) at 20–23 weeks of gestation. A higher overall mean value of SWE was found in pregnancies complicated with PE as compared with the controls (7.01 kPa vs. 2.53 kPa). There was no statistically significant difference in the SWE values between the center and edges of the placenta in both groups. UtA notching was detected in the PE group (60.7% bilateral and 39.2% unilateral). The SWE findings were in line with the results of our study, even in the second trimester. The efficacy of UtA-PI for the prediction of PE was found to be low and variable¹⁸. Although the first-trimester screening tests to detect aneuploidy have become an essential part of perinatology with recent evidence of serum or ultrasound markers, their results for the prediction of PE are not impressive¹⁹.

In our study, there was no difference between the UtA-PI values and the results of the other first-trimester screening tests. Since patients with normal first-trimester screening test results were included in this study, it could explain the nonsignificant difference between the two groups. The mean SWE value in patients with PE was significantly higher than in patients with normal pregnancies (p=0.04). The reason could be inflammation and resistance in the fetal–maternal unit (atherosis, infarction, and hyperplasia)²⁰. PE should not be considered as a single form with different underlying pathophysiological conditions.

In a recent study²¹, compared with healthy pregnancies, placentas of preeclamptic pregnancies were found stiffer and more heterogeneous. Placental stiffness was not affected by the gestational age or the severity of PE. In another point of view, the process of PE is known to be started in the first trimester, and pharmacological intervention with medications such as low-dose aspirin is recommended to reduce the prevalence of complications. But, there is still a lack of knowledge that plays

a major role in pathology, inflammation, or factors leading to inadequate invasion of the placenta. Both the insufficient invasion to the maternal unit and the tissue inflammation or changes were reported as stiffness in SWE in PE or fetal growth restriction. Therefore, it should be the answer to the high SWE values in our study results in the first trimester.

The major strength of our study was the SWE measurement in the first-trimester placenta and the follow-up for maternal and fetal outcomes. We believe that SWE can effectively predict PE in the first trimester.

Our study has some limitations. First, the posterior placenta was not evaluated due to the technical limitations of the SWE. Second, the distribution of PE cases was appeared to be higher than that of normal cases because the study was conducted in a tertiary hospital. Moreover, the study may be improved with larger patient numbers.

CONCLUSIONS

The SWE values differed between normal pregnancies and those that are subsequently developing PE in the first trimester. SWE may be a suitable tool, and we suggest its use clinically to predict PE.

AUTHORS' CONTRIBUTIONS

HAS: Conceptualization, Funding acquisition, Methodology, Resources, Software, Supervision, Writing – original draft. **GU:** Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing. **HN:** Data curation, Investigation, Visualization, Writing – review & editing. **BC:** Data curation, Investigation, Validation. **SG:** Formal analysis, Project administration, Validation. **OP:** Formal analysis, Funding acquisition, Visualization.

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Effect of Tirofiban on new cerebral microbleeds after mechanical thrombectomy in patients with acute ischemic stroke

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Yongying Ren¹ , Xin Huang¹ , Lihua Wang¹ 

SUMMARY

OBJECTIVE: The aim of this study was to analyze the effect of tirofiban on new cerebral microhemorrhage after mechanical thrombectomy in patients with acute ischemic stroke.

METHODS: In total, 203 patients with acute ischemic stroke treated by mechanical thrombectomy in our department of neurology were enrolled as the research objects. The patients were divided into two groups: the patients who used tirofiban within 24 h after surgery were assigned to the study group (78 subjects), while patients who did not use tirofiban were assigned to the conventional group (125 subjects). Magnetic resonance imaging was used to detect new-onset cerebral microbleeds in patients with stroke after surgery. The National Institute of Health Stroke Scale, modified ranking scale, and activity of daily living scale were used to assess the prognosis of patients, and the general data and the occurrence of adverse effects between two groups were compared to comprehensively evaluate the efficacy and safety of tirofiban.

RESULTS: The proportion of atrial fibrillation in the research group was significantly lower than that in the conventional group. The research group had a much lower rate of new-onset cerebral microbleeds than the conventional group ($p < 0.001$). There was no significant difference in the proportion of adverse reactions between the two groups ($p > 0.05$).

CONCLUSION: The application of tirofiban in mechanical thrombectomy of patients with acute ischemic stroke has high safety, effectively reduces the occurrence of new cerebral microhemorrhage, and provides a guarantee for patient safety.

KEYWORDS: Tirofiban. Acute ischemic stroke. Neurologic deficits.

INTRODUCTION

Acute cerebral infarction often leads to neurological impairment in patients. It is one of the common critical illnesses in the emergency department of hospitals and has a high incidence^{1,2}. Intravascular mechanical thrombectomy is widely used in clinical practice due to the gradual extension of the “time window” and higher opening rate. However, due to mechanical damage to the vascular endothelium, etc., platelet activation

will be formed after thrombosis occurs. One of the important links of mechanical thrombectomy treatment is how to quickly and effectively inhibit excessive platelet activation for such patients³. To reduce the risk of occlusion, tirofiban can be injected intravenously during and after the operation. It is reported that tirofiban, a highly selective, fast-acting non-peptide glycoprotein IIb/IIIa (Gp IIb/IIIa) platelet receptor antagonist, could reversibly block the specific binding of platelets

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to fibrinogen ligands and directly inhibit platelet aggregation. Tirofiban is not immunogenic, so it also does not activate complement to damage platelets. The antiplatelet effects would readily be apparent after five min of intravenous infusion, and the bleeding time would normalize 4 h after discontinuation^{4,5}.

A tiny intracerebral hemorrhage that cannot be detected by conventional computed tomography (CT) is called cerebral microbleeds (CMBs). It will occur in the acute phase of patients with acute ischemic stroke, and the incidence rate is higher than symptomatic intracranial hemorrhage. Studies have confirmed that patients with ischemic stroke with new-onset CMBs are more susceptible to symptomatic intracranial hemorrhage, which indicate that CMBs may be one of the risk factors for symptomatic intracranial hemorrhage⁶. Researches⁷⁻⁹ have shown that the use of tirofiban in patients with acute ischemic stroke does not increase the risk index of symptomatic intracranial hemorrhage in the acute phase. However, a few studies have been carried out to clarify the specific application safety of tirofiban on CMBs in patients with acute ischemic stroke. Therefore, we conducted a prospective case-control study to analyze the impact of tirofiban on new CMBs after mechanical thrombectomy in patients with acute ischemic stroke and related risk factors.

METHODS

General information

In total, 203 patients with acute ischemic stroke treated in the Department of Neurology in our hospital from January 2017 to December 2020 were enrolled in this study as the research objects. The patients were grouped according to the use of tirofiban within 24 h after operation. The patients who used tirofiban and those who did not use tirofiban were divided into the research group and the conventional group, respectively. The number of patients in the above two groups was 78 and 125, respectively.

The inclusion criteria were as follows:

- (1) age older than 16 years;
- (2) patients with acute ischemic stroke were treated with mechanical thrombectomy;
- (3) occlusion of the intracranial segment of the internal carotid artery, the M1 or M2 segment of the middle cerebral artery, and the front A1 segment of the brain to cause infarction;
- (4) the National Institute of Health Stroke Scale (NIHSS) score was 6 points or above;
- (5) the Diffusion-Weighted Imaging-Alberta Stroke Program Early Computed Tomography Score (DWI-ASPECT) ≥ 6 points; and

- (6) informed consent signed by patients or their agents.

The exclusion criteria were as follows:

- (1) the modified rankin scale (mRS) ≥ 3 points before the onset of the disease and
- (2) head CT detection showed changes in cerebral hemorrhage or early cerebral infarction with low density or higher than one-third of one hemisphere and other obvious early cerebral infarction changes.

Mechanical thrombectomy method

The specific operation of the mechanical thrombectomy method is as follows. The whole cerebral angiography is performed on the patient. If the result of the angiography shows that there is no recanalization of the responsible artery, the micro-guide wire and micro-catheter are passed through the guide catheter to the distal end of the thrombus. The Solitaire™ FR blood flow reconstruction device produced by Medtronic, Minneapolis, MN, USA is used to repeatedly deliver to the distal end of the thrombus to open and retract it to the outside of the body to remove the whole or divided thrombus, and the above whole process can be repeated. After the operation is completed, the opening of the occluded artery is confirmed. The above operation is repeated until the occluded artery is opened, but the number of repetitions should be controlled within five times. During the treatment activities, the patient's vital signs, consciousness, limb activities, etc., should be closely monitored. If bleeding occurs, the operation must be stopped immediately. Attention should be paid to the patients in order to reduce the adverse reactions after the operation. The enrolled cases are admitted to the stroke unit, and those with the severe illness must be admitted to the intensive care unit, and all patients will undergo rehabilitation training after their illness is stable^{7,8}.

Use of medication

If there is a high risk of reocclusion such as permanent stent implantation and large vessel stenosis during the treatment, the treatment will start during the operation. The specification of tirofiban (National Medicine Standard: H20041165) manufactured by the Chinese pharmaceutical company Yuanda Pharmaceutical is 100 ml. Later, it is changed to a maintenance dose of $0.12 \text{ kg}^{-1} \cdot \text{h}^{-1}$ for 24 h (if there is no bleeding symptom during the 18-h reexamination, then give aspirin 300 mg and clopidogrel 75 mg, and bridging with tirofiban for 6 h). For patients in the conventional group who did not use tirofiban during the treatment process, they were treated with oral antiplatelets (aspirin/clopidogrel) when there was no bleeding on the head CT after 24 h (aspirin 100 mg and clopidogrel 75 mg once a day)^{9,10}.

Magnetic resonance imaging examination of cerebral microbleeds

Notably, 24 h after completing the above operations, the patients are reexamined with CT or MRI. All patients in the group are examined on the head susceptibility-weighted imaging (SWI) within 48 h after the onset of the disease and 8 days after admission. SWI usually can detect minor bleeding after 48 h. Therefore, SWI examination within 48 h of onset can confirm old CMBs, but SWI examination at about eight days after onset can observe the condition of new CMBs. Four imaging specialists read the SWI images and analyze whether they are new images of microbleeds¹¹.

Observation indicators

General information

General information, such as age, sex, history of smoking, history of drinking, history of atrial fibrillation routine blood, urine routine, blood lipids, fasting blood glucose, and other laboratory test indicators of the patients after admission, were collected and retrospectively analyzed.

Clinical indicators

- (1) Use NIHSS to assess the patient's stroke status before and 7, 14, and 28 days after treatment. The lower the score indicated the less severe the condition.
- (2) Use mRS to evaluate the prognosis of the enrolled patients on 7, 14, and 28 days after treatment. The lower the score indicated the better the prognosis.
- (3) The activity of daily living (ADL) scale (Barthel Index) was assessed on 7, 14, and 28 days after treatment and divided into two categories: satisfactory (Barthel Index ≥ 95 points) and dissatisfaction (Barthel Index < 95 points) index.

Safety indicators

To study the occurrence of adverse effects, the number of bleeding events three months after discharge should be followed up, according to the bleeding grading standard of the reported infarction-related artery-opening strategy:

- (1) severe life-threatening bleeding;
- (2) if there is moderate bleeding, blood transfusion is needed; and
- (3) slight bleeding such as bleeding gums, ecchymoses under the skin, etc., and whether there are any allergic reactions.

Statistical methods

In this study, SPSS 20.0 statistical software was used to perform the statistical analysis of the data. The count data

were expressed as %. The data between groups are compared and analyzed with the χ^2 test or the Fisher's exact test. The measurement data were expressed as mean \pm standard deviation (SD). The two groups of measurement data were compared using the *t*-test. $p < 0.05$ was considered statistically significant.

RESULTS

The general data between the two groups

The general data between the two groups were shown in Table 1. After detailed comparison, the proportion of atrial fibrillation in the research group was lower than that in the conventional group, the difference was statistically significant ($p < 0.05$). However, the remaining clinical indicators were not a significant difference between the two groups ($p > 0.05$).

Comparison of new-onset cerebral microbleeds in each group

The new-onset CMB rates of the two groups are shown in Table 1. After surgery, the number of new-onset CMBs in the research group and the conventional group was 12 and 56, accounting for 15.38 and 44.80% in each group, respectively. The research group had a much lower rate of new-onset CMBs than the conventional group ($\chi^2 = 18.656$, $p < 0.001$), and there was a significant difference in the data between the groups, and the difference was statistically significance.

Comparison of National Institute of Health Stroke Scale, activity of daily living, and modified ranking scale scores between the two groups

The NIHSS, ADL, and mRS scores of the two groups are shown in Figure 1. After surgery, the NIHSS of the research group and conventional group were significantly decreased with the increase of follow-up time. However, at each follow-up time, the NIHSS of the research group was much lower than that of the conventional group ($p < 0.05$). The mRS scores of the two groups had a similar trend, while the ADL scores of the two groups had an opposite trend.

Adverse reactions

After comparison, the two groups of patients did not experience gum bleeding, massive bleeding, and blood transfusion. The difference between the groups was not significant ($p > 0.05$).

Table 1. General data and new-onset CMB rate of the two groups (n=203).

	Research Group (n=78)	Conventional Group (n=125)	χ^2/t	p-value
Age (years)	70.45±8.13	68.42±7.03	1.883	0.061
Male/female (n)	49/29	85/40	0.574	0.449
Smoking [n (%)]	35 (44.87)	60 (48.00)	0.189	0.664
Drinking [n (%)]	38 (48.72)	63 (50.40)	0.054	0.816
Atrial fibrillation [n (%)]	8 (10.26)	30 (24.00)	5.963	0.015*
Fasting blood glucose (mmol/L)	5.73±1.26	5.85±1.88	0.498	0.619
Systolic blood pressure (mmHg)	142.17±16.35	146.48±18.52	1.686	0.093
Diastolic blood pressure (mmHg)	86.77±13.41	88.45±10.10	1.014	0.312
Total cholesterol (mmol/L)	4.75±1.12	4.96±1.04	1.358	0.176
Low-density lipoprotein (mmol/L)	3.03±0.94	3.12±0.82	0.719	0.473
Cause of stroke [n (%)]			3.197	0.362
Atherosclerotic stroke	52 (66.67)	76 (60.80)		
Cardiogenic cerebral embolism stroke	12 (15.38)	32 (25.60)		
Arteriolar occlusive stroke	7 (8.97)	9 (7.20)		
Other strokes with definite etiology	7 (8.97)	8 (6.40)		
New-onset CMB rate [n (%)]	12 (15.38)	56 (44.80)	18.656	<0.001**

CMBs: cerebral microbleeds. *p<0.05 versus conventional group; **p<0.001 versus conventional group.

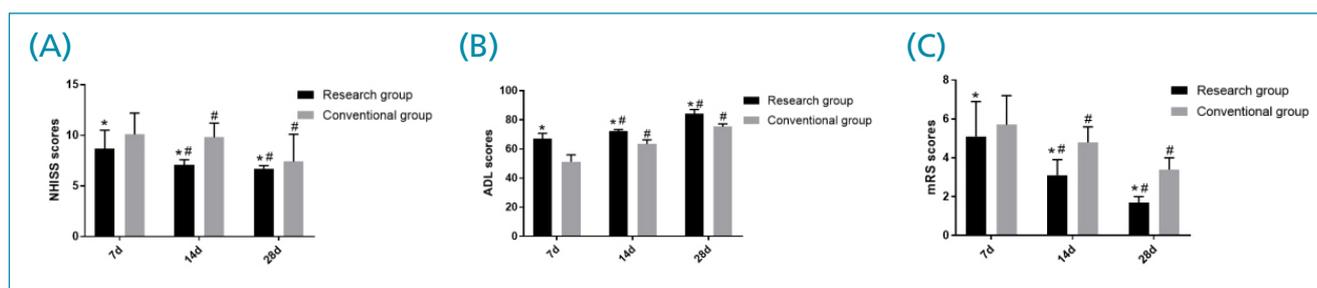


Figure 1. NIHSS: the National Institute of Health Stroke Scale; mRS: the modified ranking scale; ADL: activity of daily living. The NIHSS (A); ADL (B); and mRS (C) scores of the research group and conventional group after treatment. *p<0.05 versus conventional group; #p<0.05 versus the score measured on day seven.

DISCUSSION

The emergence of new CMBs in the acute phase is a risk factor for symptomatic intracranial hemorrhage in patients with acute ischemic stroke treated with mechanical thrombectomy¹²⁻¹⁴. In the analysis of this study, the risk factors for CMBs are finally obtained, such as cardiogenic cerebral embolism, the use of antithrombotic drugs (especially anticoagulant drugs), etc. The relevant literature at the present stage has partially confirmed that there is no statistically significant difference between the data in the two cases of no hemorrhagic transformation and asymptomatic hemorrhage transformation in patients with mechanical thrombectomy for acute ischemic stroke. In addition, the research on the treatment of patients with asymptomatic

hemorrhage transformation has been slow, and a more reliable improvement plan has not yet been obtained¹⁵. The proportion of patients with hemorrhagic transformation in the two groups participating in the study is basically the same as the relevant data in the previous literature. Finally, the conclusion on the effect of the application of tirofiban on the transformation of intracranial hemorrhage is more inclined to produce a favorable effect. However, it was found that the group of patients with acute ischemic stroke treated with tirofiban had significantly lower data of new-onset CMBs compared with the conventional group of patients treated with mechanical thrombectomy, indicating that the use of tirofiban could decrease the occurrence of CMBs after mechanical thrombectomy.

Studies¹⁶ have shown that the risk factor for new CMBs is DWI-ASPECTS, ASPECTS is related to infarct size and NIH. The experimental data can only confirm that tirofiban has no significant effect on the increase of new-onset CMBs, but it cannot prove the clinical effect of the application of the drug in reducing new-onset CMBs. This result may be caused by the combined action of other influencing factors. The safety and feasibility of using tirofiban for the treatment of patients with acute ischemic stroke by mechanical thrombectomy are supported by various research data⁷⁻⁹. However, the clinical judgment of whether to use tirofiban is based on the patient's condition. The levels of antiplatelet strength, anticoagulation ratio, cardiogenic embolism, NIHSS, and other indicators in the two groups of patients with different treatment plans were compared and analyzed, and we found that the research group had a better patient report prognosis.

Antiplatelet therapy is one of the most commonly used regimens for mechanical thrombectomy in patients with acute ischemic stroke, and the combination of clopidogrel and aspirin has a very high clinical effect on patients with mild stroke with an early NIHSS of less than three, for example, it can significantly reduce the late recurrence rate of patients. In this study, the drug was administered according to the antiplatelet therapy guidelines. Compared with the conventional group, the proportion of patients in the study group who are treated with clopidogrel and aspirin is significantly higher, which suggests that the intensity of antiplatelet therapy in the study group is higher. In patients with platelet therapy, when the disease control effect is not good or even further development occurs, the clinical risk of using clopidogrel and aspirin combination therapy is lower. Tirofiban should be added to patients who have not been able to effectively control the development of the disease after the application of dual antiplatelet, so the antiplatelet strength is significantly higher in the study group compared to the conventional group. The application of anticoagulation therapy can significantly reduce the recurrence rate of patients with cardiogenic embolism, so most patients will use anticoagulation therapy. As a result, there is a certain difference in the data of anticoagulation and cardiogenic embolism between the two groups. The main reason is that the vast majority of patients using tirofiban had mild clinical symptoms at admission, so the NIHSS of the study group was significantly lower than that of the conventional group.

Antiplatelet drug treatment regimen occupies an important position in the clinical treatment of patients with clinical

acute ischemic stroke, and the specific efficacy of intravenous antiplatelet drugs in patients with clinical acute ischemic stroke treated by mechanical thrombectomy is still in the stage of clinical exploration¹⁷. Research¹⁷ has confirmed that after mechanical thrombolytic blood flow is recanalized, maintenance for tirofiban is given to observe the efficacy of tirofiban. A total of 32 patients were enrolled this time, with (16.1 ± 4.4) as the average baseline NIHSS. After treatment, 75.15% of the patients showed recanalization, of which 43.75% was completely recanalized and 31.28% was partially recanalized. At the two-month follow-up, 56.26% of patients had an mRS score lower than three, indicating a good prognosis, only two patients had symptoms of symptomatic intracranial hemorrhage. The data of this study show that the effective treatment for patients with acute ischemic stroke is the use of tirofiban in the local arteries after the formation of mechanical thrombolytic antegrade blood flow in acute ischemic stroke. Arterial tirofiban can reduce the risk of reocclusion after intra-arterial thrombolysis.

Several limitations should be taken into consideration when interpreting the results of this analysis. The dosing regimens between the two groups were not completely consistent, especially the intra-arterial administration of tirofiban in the research group, which made this study not a well-controlled trial. The sample size of this study is still insufficient, and there is a lack of relatively rich literature reviews. Besides, the long-term efficacy and safety studies of tirofiban on stroke remain to be conducted.

CONCLUSION

In summary, clinical drug treatment for patients with ischemic stroke in the acute phase has certain safety and feasibility. Tirofiban treatment has the clinical effect of increasing the rate of vascular recanalization and reducing the risk of in-stent thrombosis for patients with acute ischemic stroke, safeguarding the safety of patients.

AUTHORS' CONTRIBUTIONS

YDS: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **HJZ:** Data curation. **HL:** Formal analysis. **ZFJ:** Formal analysis. **LHW:** Formal analysis. **YYR:** Formal analysis. **XH:** Formal analysis.

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Virtual consent and the use of electronic informed consent form in clinical research in Brazil

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SUMMARY

OBJECTIVE: In view of the need to apply term free and informed consent (IC) in clinical research involving humans, in accordance with the Brazilian ethical standards (CNS Resolution No. 466/2012), it is necessary to assess whether this practice is being effective and can be improved. The aim of this study was to evaluate the use of the IC in electronic format (e-IC), regarding its feasibility and suitability, as a complement to the written/physical consent form. **METHODS:** Quantitative-qualitative research with a questionnaire instrument. **RESULTS:** Greater retention of information and fewer wrong answers were observed after the application of the e-IC. **CONCLUSIONS:** The use of e-IC is of great value to research participants in Brazil.

KEYWORDS: Clinical trials as topic. Informed consent. Ethics committees. Research.

INTRODUCTION

For the first time in world history, the need for research participants to voluntarily authorize their participation in clinical trials arose in the Nuremberg code of ethics¹. In this document, as a first principle, the consent of the volunteer was presented as being essential.

However, the nomenclature term *free and informed consent* (IC) had not yet been mentioned as a formal consent document. This term appears in 1964, with the Declaration of Helsinki. In this statement, it is stated that in any research with human subjects, each potential participant must be adequately informed about the objectives, methods, anticipated benefits, potential risks of the study, and the inconvenience that the study may entail. Participants must be informed that they are free to withdraw their consent at any time during the study².

The regulation in Brazil, which dealt for the first time on the mandatory nature of the IC, was in Resolution No. 196/1996. This resolution brought the competences of the Institutional Review Board (IRB), the National Research Ethics Commission — Conep, and the need for the IC in research in

Brazil³. However, it cannot be said that this term did not exist in Brazil before that, considering that international research, which took place in centers in several countries, such as Brazil, already had this obligation.

Currently, in Brazil, the legislation that deals with the details regarding this issue is Resolution No. 466/2012. This resolution also brings all the information requirements that need to be clearly present in the IC. In addition, it also informs the situations in which it is not possible to apply the IC in the standard format for some populations, including children, adolescents, patients with psychological disabilities, and brain death. Considering these cases, another document needs to be applied, the Informed Consent Term⁴.

The IC has ceased to be just a paper document for many years. The practice of free and informed consent is a process of continuous and effective communication⁵. Thus, it is necessary that the signing of the IC is not just an isolated fact, but it is the result of an awareness process so that the participant can be provided with all the information, clearly, and that they can make the decision accordingly, as autonomous and enlightened as possible.

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All research involving human beings needs the application of the IC; this research can be direct or indirect. Clinical research is considered direct, since the participant undergoes an intervention during the study, while the indirect occurs when the participant participates in the research through data collection, questionnaires, or forms, but does not receive any type of intervention that somehow alters its current state⁶.

Even after all these precautions, it is debated whether this term is really understood by the research participants in a satisfactory way. Therefore, Souza et al.⁷ carried out a study to assess the readability of ICs in Brazil with the aim of correlating the acceptance of the research participant with demographic status, social factors, risk-to-benefit ratio, and the level of education. It was observed that the ICs presented high degrees of difficulty in reading.

It is noteworthy that the research participant's consent needs to be performed in the best possible way and the patient needs to understand perfectly about the research or procedure in which they will be submitted. If the participant's autonomy is not respected and there is any assessment of medical misconduct, the responsible physician will respond civilly in the subjective and objective modalities⁸⁻¹⁰.

Thus, even with all this concern expressed in Brazilian regulations, it is possible to observe that there are still problems with the application of these terms in the clinical practice.

In addition, the form of application of the IC needs to be improved and this became clear with the advent of the worldwide pandemic COVID-19, a highly contagious disease caused by the new coronavirus (SARS-CoV-2), which appeared for the first time in Wuhan, China, spreading rapidly around the world in just 2 months¹¹. Along with the protective measures of quarantine and social distancing, the use of electronic IC (e-IC) form in Brazil can represent a gain for Brazilian clinical research.

For these reasons, this work aims to assess the applicability of an electronic consent form in Brazil, evaluating the viability and preference of volunteers, considering this new format. It is important to mention that this is not an exclusive study, the e-IC is not an instrument to replace the written informed consent, but rather, it will be used, as in other countries, in a complementary way. In this sense, the study aims to assess whether the use of the e-IC will be effective for greater understanding and retention of information, after the application of the written consent, in Brazil, and the feasibility of the completely virtual consent procedure.

METHODS

This study is a qualitative research, approved by the Ethics Committee of the University of Brasília (CAAE 26314719,1,0000,0030), with information collection through forms applied to volunteers who agreed to participate in this

analysis. These volunteers were informed that they would be part of a simulation of participation in hypothetical clinical research and that they would help the research team with relevant information to improve this practice in Brazil.

The sample for this research was invited to participate by electronic means, such as cell phone messages or email. Recruitment was through email and telephone contacts, mainly through contacts via the University of Brasília. A total of 60 volunteers were included for this research. The profile chosen for the hypothetical research was phase 1, a study with a small number of participants, focusing on the safety of a new product, usually with healthy participants, since the volunteers for the hypothetical study would also be healthy. This number of participants was defined based on the median accepted for phase 1 clinical trials (20–100), that is, 60 research participants¹².

The inclusion criterion was >18 years old. The exclusion criteria were not having Brazilian Portuguese as a native language, presenting some type of cognitive deficiency that compromises the understanding of the material, and/or presenting complete or functional illiteracy.

All volunteers were informed that they would be participating in a survey to evaluate the e-IC instrument in a clinical research simulation; this IC was presented and should be signed by those interested in participating. In addition, these volunteers received a consent form simulating participation in the clinical research for a hypothetical new pain medication.

Regarding the hypothetical phase 1 study, all participants received the written consent form for reading this document. After reading, a questionnaire on their understanding of the study was applied and, after completing this form, an electronic consent form was also presented, in the form of a video lasting less than 5 minutes, containing all the information available in the consent form. After the end of the video, the same questionnaire was applied again to the group. This format was chosen to assess whether there would be greater retention of information about the study after the application of the e-IC. After the two questionnaires were answered, a third one was applied to assess preferences, suitability, and feasibility of applying this new tool in Brazil.

The video was recorded using an actor, for better performance in front of the cameras, who addressed all the points present in the written consent form, verbally, in the video.

The qualitative-quantitative interview guide, applied after reading the informed consent and after presenting the video, was developed based on a similar study carried out in the United States¹³ and the knowledge of the research team on ethics, regulatory matters, and patient safety. Tables 1 and 2 list the questions that were asked during the interviews. The research and interviews in this study were purposely designed in brief to lessen the research burden on individuals.

Table 1. Questionnaire after reading the informed consent and post-e-informed consent.

Interview		
Questions	Answer option	Type of analysis and generated data
Sample identification		
Initials of your name	Open response	Sample identification
Date of birth	Date	Sample identification
Sociodemographic profile		
Sex	F; M; Not declared	Profile of research participants
Education	Education; Literacy; Elementary School; High school; University education; Postgraduate lato sensu; Master's degree; Doctorate degree; PhD	Profile of research participants
Do you have health insurance?	Yes; No	Profile of research participants
Do you have social networks?	Yes; No	Profile of research participants
Have you participated in previous clinical research?	Yes; No	Profile of research participants
Questionnaire about the IC after reading and after video		
What do you remember as relevant in relation to the research procedures presented in the Electronic Informed Consent Form?	Open response	Assess the amount of information retained for each participant
Can you say what the purpose of the survey was?	Open response	Assess the amount of information retained for each participant
Can you explain to me who should not participate in the study?	Open response	Assess the amount of information retained for each participant
Can you tell me what the risks are for this study?	Open response	Assess the amount of information retained for each participant
Can you tell me what the benefits of this study are?	Open response	Assess the amount of information retained for each participant
Can you tell me what assistance is offered at the end of the study?	Open response	Assess the amount of information retained for each participant

IC: Informed consent form.

Table 2. Objective questionnaire on feedback about the survey.

Interview		
Questions	Answer option	Type of analysis and generated data
Sample identification		
Was Electronic Informed Consent Form relevant for a better understanding of the research?	Yes; No	Objective
Do you think Informed Consent Form should be implemented in all surveys?	Yes; No	Objective
Do you think the Informed Consent Form is enough for the understanding of the research or the face-to-face care with the doctor is essential?	e-IC is sufficient; I still need face-to-face medical service	Objective
Do you think Informed Consent Form should be implemented in all surveys?	Yes; No	Objective
On a scale of 1 to 5, how important did you think the Informed Consent Form was for understanding the research? Being 1, not relevant, and 5, fundamental for understanding	1; 2; 3; 4 e 5	Objective

IC: Informed consent form.

RESULTS

The predicted number for this study was 60 volunteers and all were included. The profile of the population included in this study was evaluated, showing that the majority were females (55%) and had postgraduation *lato sensu* (43.31%). Notably, 73.3% of the participants had a health insurance plan, 96.7% had access to a social network, and 83.3% had never participated in a previous clinical research.

The results of this qualitative research were evaluated as follows. The answers after just reading the IC and the answers after reading and applying the video were, for all participants, read and classified as “correct,” when they were in agreement with what was described in the text or in the video of the IC of the drug curadorzil, and as “wrong,” when they did not correspond to what was presented. Correct answers for the same question were also compared in the postwritten consent and postelectronic consent questionnaires.

Considering the classification presented above, it was observed that the wrong answers decreased by 36% after the application of the e-TCLE and the more complete answers increased by 835% after the application of the term.

In addition to retaining and understanding the information presented in the IC and e-IC, the preference of the volunteers who participated in this study was quantitatively evaluated.

The first question asked was about the relevance of the presentation of the e-TCLE for the understanding of the research. Of the 60 volunteers who answered the questionnaire, 50 (83.3%) judged the e-IC as relevant for a better understanding of the research. Another question asked was in relation to the opinion of the volunteers if they thought that the e-IC should be implemented in all clinical trials. Notably, 49 (81.7%) thought that the e-IC should be part of all research in Brazil. As for the ease of understanding the research, 33 (55%) thought that the written consent form was easier to understand the content of the research. Regarding the question, “Do you think that the e-IC is sufficient for the understanding of the research or the face-to-face care with the doctor is essential?” 50 (83.3%) answered that the e-IC would be enough, while 10 participants answered that they would still need face-to-face medical care.

The volunteers were also asked to rate, on a scale of 1–5 (1=not relevant, 5=fundamental for understanding), how much they thought the e-IC was important for understanding the research. More than half of the volunteers, 32 (53.3%), marked the item referring to the maximum relevance of this instrument for the understanding of the research (item 5). The result is shown in Figure 1.

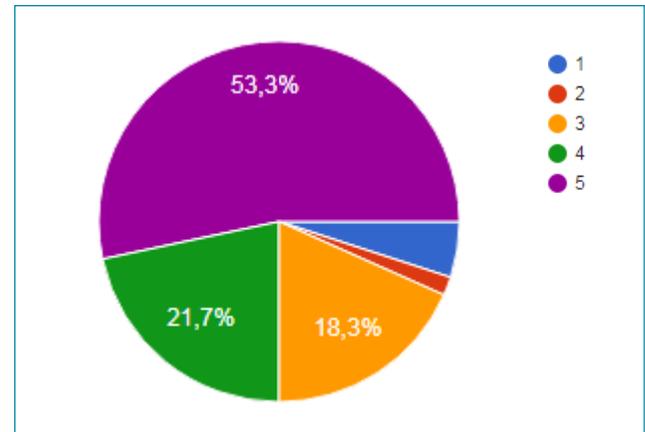


Figure 1. Ranking the relevance of adding e- informed consent in research in Brazil, with 1 indicating not relevant and 5 fundamental for understanding.

DISCUSSION

According to the results, it is possible to note that after the presentation of the e-IC, there was a more expressive number of correct and more complete answers than just the presentation of the IC in isolation. In addition, the number of responses considered “wrong” was lower after the volunteers watched the e-IC. Thus, it is possible to observe that, indeed, after the presentation of the e-IC, there was an increase in the understanding and retention of information about the study.

Considering the results previously presented, it was possible to verify, qualitatively and quantitatively, that the e-IC had a positive impact on the understanding of the hypothetical clinical research study.

Presenting a video, after having access to the written consent form, allowed a greater understanding of the research and greater retention of information, and, according to the opinion of the volunteers, the e-IC should be implemented in all trials. This format was intentionally chosen, as the presentation of the e-IC after the written consent is the methodology used internationally for the application of this instrument.

One result that stands out is related to the understanding that e-IC could replace face-to-face service. This is important considering that the implementation of this tool can help in critical moments of social distancing, such as the COVID-19 pandemic.

The constant updating of the IC is necessary, and recent efforts are being made in Brazil to cover patients with visual impairment in Brazil¹⁴. In this sense, this work has the benefit of extrapolating the understanding of the IC to different population groups.

A limitation of this study is that there was the small number of the sample and the recruitment hampered by the COVID-19 pandemic situation; thus, the population studied was the one with access to the Internet and with the highest level of education.

CONCLUSIONS

Given the above, the implementation of the e-IC in Brazilian research is characterized as a valuable procedure to increase the understanding and retention of information by volunteers, and, in cases of need for social distance, this resource can be used as a strategy in clinical research in Brazil.

Furthermore, it is important to present experience with virtual consent, mainly as a strategy for clinical research in need of social distancing.

It is noteworthy that further studies are needed, including the analysis of the applicability of this tool in other phases of clinical research.

AUTHORS' CONTRIBUTIONS

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

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Prognostic value of inflammatory markers determined during diagnosis in patients with sarcoidosis: chronic versus remission

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SUMMARY

OBJECTIVE: This study aimed to evaluate the prognostic value of inflammatory markers determined during admission among patients with sarcoidosis with chronic and remission groups.

METHODS: This study was designed as retrospective single-center study. Patients with sarcoidosis without treatment and who had at least two years of follow-up were included in this study. Patients were divided into two groups as chronic and remission. The primary outcome is to evaluate hematological parameters in remission and chronic sarcoidosis groups.

RESULTS: Out of 348 patients with sarcoidosis, 142 patients without treatment and followed up for at least two years were included in this study. Groups had similar demographic features with the predominance of females (80.4 and 77.9%, respectively) and stage I disease (78.6 and 68.6%, respectively). Lymphocyte count [median (IQR) 1.7 (1.3–2.3) 10⁹/L *versus* 2.1 (1.6–2.4) 10⁹/L, $p=0.034$] was significantly lower, whereas neutrophil to lymphocyte ratio (NLR) was significantly higher [median (IQR) 2.6 (2.0–3.1) *versus* 2.0 (1.6–2.8), $p=0.006$] at admission in the chronic group. No significant difference was determined in inflammatory parameters at admission between groups.

CONCLUSION: Lower lymphocyte count and higher neutrophil to lymphocyte ratio were determined in patients with chronic sarcoidosis compared with the remission group, based on monitoring of radiological staging up to five-year after the initial diagnosis. Accordingly, the identification of neutrophil to lymphocyte ratio at diagnosis seems to be a potential prognostic marker in patients with sarcoidosis beside its low cost and easy determination in routine clinical practice.

KEYWORDS: Sarcoidosis. Chronic granulomatous disease. Prognosis.

INTRODUCTION

Sarcoidosis is a systemic inflammatory granulomatous disease of unknown etiology, with pulmonary involvement in most cases, and associated with heterogeneous clinical presentation and disease course¹⁻⁴. Although a favorable outcome with

self-limiting clinics occurs in most of the patients, progression to fibrosis and thus permanent organ impairment occurs in one-third of the patients^{5,6}.

Several clinical, physiological, and radiographic parameters were under investigation for objective disease-specific biomarkers

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that can predict the severity and prognosis of sarcoidosis^{3,4,7,8}. Diagnostic and prognostic roles of inflammatory markers have been addressed by several studies in inflammatory and chronic respiratory diseases^{4,5,9-14}. Besides, limited data about serum inflammatory markers were available in the monitoring of patients with sarcoidosis^{4,5,9,13,14}.

Neutrophil to lymphocyte ratio (NLR) has been recently emerged as a cost-effective, potential inflammatory marker and found to be associated with the severity and prognosis of systemic inflammatory diseases, cancer, and respiratory and cardiac diseases. Although there are a limited number of studies, NLR was shown to be increased in patients with sarcoidosis as compared with the control group^{4,5,13,14}.

Therefore, this retrospective study was designed to evaluate the prognostic value of serum inflammatory markers [i.e., NLR, mean platelet volume (MPV), platelet/MPV ratio, angiotensin-converting enzyme (ACE), and C-reactive protein (CRP)] determined at initial diagnosis in the clinical course of disease among patients with sarcoidosis.

METHODS

Study population

Patients with sarcoidosis without treatment and who had at least 2 years of follow-up were included in this retrospective study. Patients were divided into two groups, namely, chronic and remission. Figure 1 shows the flowchart of patient enrollment.

Diagnosis of Sarcoidosis

Sarcoidosis was diagnosed as if clinical and radiological data are supported by the presence of non-caseating granulomas in a biopsy specimen, and alternative causes of granulomatous inflammation are ruled out. For patients who did not allow biopsy and/or had Löfgren syndrome, sarcoidosis was diagnosed by clinical, radiological, and laboratory compatibilities.

There are five roentgenographic stages as follows: Stage 0: normal chest X-ray; Stage 1: bilateral hilar lymphadenopathy; Stage 2: bilateral hilar lymphadenopathy together with parenchymal involvement; Stage 3: parenchymal involvement without bilateral hilar lymphadenopathy; and Stage 4: pulmonary fibrosis¹.

- *Remission*: Spontaneous clinical and radiological improvement¹⁵.
- *Chronic sarcoidosis*: Persistence of sarcoidosis more than 2 years¹⁵.
- *Extrapulmonary involvement*: The criteria specified in A Case–Control Etiologic Study of Sarcoidosis (ACCESS) trial were used for the definition of organ involvement¹⁶.
- *Hematological analysis*

Complete blood count analysis was performed *via* flow cytometry (Beckman Coulter LH 780 Analyzer; Beckman Coulter Inc., Miami, FL, USA). Serum CRP levels were determined by using the turbidimetric method using a BN II System (Siemens, Munich, Germany). ACE (U/L) was measured by using the spectrophotometric methods (EN Biochemical Enterprise, Italy). NLR was calculated by the ratio of neutrophil to lymphocyte counts. The platelet to MPV ratio was also calculated by the ratio of the platelet count to MPV.

Outcome measures

Data on patient demographics, stage of the disease, duration of follow up, and total blood count (i.e., neutrophil, lymphocyte, and thrombocyte) and inflammatory markers (i.e., ACE, CRP, MPV, NLR, and platelet/MPV ratio) determined at diagnosis were obtained from medical records in both chronic and remission groups. The percentage of survivors and non-survivors was also recorded in both groups.

Ethics

The study was approved by the Ethical Committee of the institution (05.11.2020/116.2007.197) and was in accordance with the Declaration of Helsinki. As informed consent from patients to review their medical records was not obtained, patient data were de-identified and strictly protected.

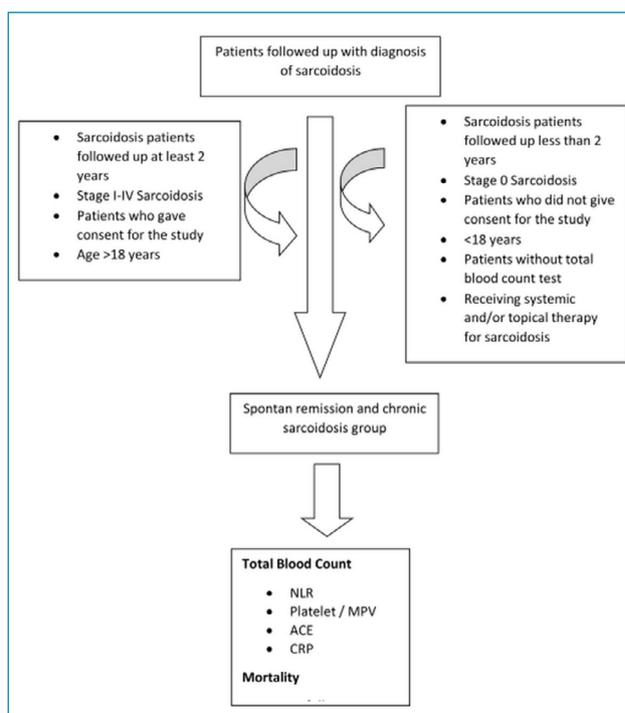


Figure 1. Flowchart of this study.

Statistical analysis

Statistical analysis was made using the computer software Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 21.0, released 2012, IBM Corp., Armonk, New York, USA). Fisher's exact test and Pearson's chi-square analysis were performed for categorical variables. Mann–Whitney U test was used for comparison of numerical variables. Data were expressed as “mean (standard deviation; SD)”, “n (%)” and “median (minimum and maximum)” values, where appropriate. $p < 0.05$ was considered statistically significant.

RESULTS

Among 348 patients with sarcoidosis, 142 patients with stage I–III sarcoidosis without treatment and had at least two years of follow-up were included in this retrospective study.

Remission and chronic groups had similar demographic and sarcoidosis features with the predominance of females (80.4 and 77.9%, respectively) and Stage I disease (78.6 and 68.6%, respectively). Stage IV sarcoidosis did not exist in this study population. Extrapulmonary involvement was determined in one-third of patients in both groups. Remission occurred in 39.4% of patients (Table 1).

Patients in the remission and chronic groups had a similar duration of follow-up (median 62.5 and 60.0 months, respectively) and low mortality (0.0 and 4.7%, respectively; Table 1).

Lymphocyte count [median (IQR) 1.7 (1.3–2.3) $10^9/L$ versus 2.1 (1.6–2.4) $10^9/L$, $p = 0.034$] was significantly lower, whereas

NLR was significantly higher [median (IQR) 2.6 (2.0–3.1) versus 2.0 (1.6–2.8), $p = 0.006$] at admission in the chronic group when compared with remission group. No significant difference was noted in other inflammatory parameters between groups including ACE, CRP, MPV, and PLT/MPV ratio (Table 2).

DISCUSSION

In this study, despite the similar baseline demographic and disease characteristics, the chronic sarcoidosis group had decreased lymphocyte count and increased NLR, compared with the remission group. Lymphocyte count and NLR levels were found to indicate the prognosis in patients with sarcoidosis. Thus, patients with low lymphocyte count and high NLR values had a higher likelihood of developing chronic disease than the clinical remission.

Sarcoidosis is observed most frequently in women in different studies^{17,18}. In this study, the ratios of female patients were similar in both groups. In recent studies, the ratios of patients with stages I and II were higher in all patients with sarcoidosis, which is compatible with the literature¹⁷⁻¹⁹. In this study, patients with stage IV sarcoidosis did not exist, besides the ratio of stage II disease in the chronic group was almost two-fold greater than the remission group without statistical significance.

In recent studies, remission in sarcoidosis was reported to occur in more than half of patients within 3 years^{6,7}. In a study conducted by Scadding et al., complete radiological remission after >5-year follow-up was reported to occur in 84%, 58%,

Table 1. Demographic and disease-related characteristics in study groups.

		Remission (n=56)	Chronic course (n=86)	p-value
Age (year)	Mean (SD)	41.5 (11.8)	44.1 (12.3)	0.174 ^a
	Median (IQR)	42.0 (31.3–51.8)	47.0 (34.0–52.3)	
Gender, n (%)				
Female		45 (80.4)	67 (77.9)	0.448 ^b
Male		11 (19.6)	19 (22.1)	
Disease stage				
Stage I		44 (78.6)	59 (68.6)	0.425 ^b
Stage II		10 (17.9)	23 (26.7)	
Stage III		2 (3.6)	4 (4.7)	
Extrapulmonary involvement		19 (33.9)	25 (29.1)	0.334 ^b
Duration of follow up (month), median (IQR)		62.5 (32.5–90.8)	60.0 (41.3–120.0)	0.393 ^a
Survival, n (%)				
Died		0 (0.0)	4 (4.7)	0.131 ^b
Survived		56 (100.0)	82 (95.3)	

^aMann-Whitney U test; ^b χ^2 test.

Table 2. Hematological parameters in study groups.

	Remission (n=56)	Chronic course (n=86)	p-value ^a
ACE	53.0 (35.5–82.8)	56.0 (32.8–96.0)	0.666
Neutrophil (10 ⁹ /L)	4.1 (3.2–5.07)	4.3 (3.5–5.4)	0.277
Lymphocyte (10 ⁹ /L)	2.1 (1.6–2.4)	1.7 (1.3–2.3)	0.034
NLR	2.0 (1.6–2.8)	2.6 (2.0–3.1)	0.006
Platelet (10 ⁹ /L)	271.0 (220.3–308.6)	256.3 (216.8–317.0)	0.757
MPV (fL)	8.6 (7.7–9.2)	8.5 (7.9–9.0)	0.784
Platelet/MPV	31.2 (24.3–38.1)	30.6 (25.9–38.4)	0.948
CRP ^b	3.5 (3.0–16.5)	12.5 (4.5–26.5)	0.228

Data are shown as median (IQR). ^aMann-Whitney U test; ^bbased on data from 12 patients in the remission and 10 patients in the chronic groups. ACE: angiotensin-converting enzyme; CRP: C-reactive protein; MPV: mean platelet volume; NLR: neutrophil to lymphocyte ratio. Numbers in bold are statistically significant.

and 10–20% of patients with initial radiological stages I, II, and III, respectively²⁰. Besides, in this study, the ratio of the patients with chronic sarcoidosis was nearly 25%, which was mentioned 10–30% in different studies^{20,21}.

Hematological changes in sarcoidosis include mild anemia, leukocytosis, leukopenia, lymphopenia, eosinophilia, and thrombocytopenia, which were frequently observed in recent studies^{4,22}. Notably, in this study compared with the remission group, the chronic group had a lower lymphocyte count, while a similar neutrophil count was determined during the diagnosis. This seems to indicate the prominent role of lymphocytopenia, as a marker of physiological oxidative stress.

The NLR is a simple composite marker providing the objective measurement of changes in both neutrophils and lymphocytes, while calculated easily from a routine complete blood count without needing extra effort or cost^{4,5,13}. Hence, in this study, the presence of high NLR during diagnosis as a poor prognostic marker seems notable with at least 5-year follow-up.

The NLR is novel prognostic, diagnostic marker for inflammatory diseases^{11,13,14,23}. In the study by İliaz et al., the NLR was found to be supportive in differentiation between tuberculosis and sarcoidosis with the cutoff value of 2.55¹³. In a study by Dirican et al., NLR values were reported to be higher in patients with sarcoidosis than in healthy controls and in extrapulmonary involvement⁵. In a large-scale study by Gungor et al. among patients with sarcoidosis, high NLR (≥ 2.0) values have demonstrated the correlation with well-known inflammatory markers such as ACE, erythrocyte sedimentation rate (ESR), and CRP¹⁴. In this study, since there are limited data about the role of NLR in the chronic sarcoidosis and remission group, the NLR was significantly higher at admission in the chronic sarcoidosis.

Patients with sarcoidosis with stage II and III disease and extrapulmonary involvement were reported to be more likely to have a high NLR⁵. Despite significantly higher NLR in the chronic group in this study, two groups had a similar NLR in terms of initial disease stage and extrapulmonary involvement. Thus, in this study, baseline similarity of disease characteristics between two clinical outcome groups further emphasizes the need for potential biomarkers in sarcoidosis that could predict the disease-specific prognosis following the initial diagnosis.

In recent studies, MPV values were found to be higher in patients with sarcoidosis than in the control group^{5,24}. While the stage increases, no changes in MPV values were detected in the study by Dirican et al.⁵. In contrast, in this study, no significant difference was found between the two groups according to MPV and PLT/MPV values. While serum ACE is the most widely used biomarker of diagnostic and prognostic values in patients with sarcoidosis, it has been associated with relatively low specificity, as elevated serum ACE activity was also shown in multiple granulomatous and non-granulomatous diseases. ACE was also shown to be insignificant in determining the severity of the disease and inappropriate to be used during follow-up⁹. In this study, there was no significant change in serum ACE values measured at the time of admission, between chronic and remission groups.

In recent studies, CRP levels were found to be useful to predict the effectiveness of treatment in chronic pulmonary sarcoidosis. Besides, studies have also analyzed the usefulness of CRP levels reporting higher significant levels with respect to healthy controls²⁵. In this study, according to CRP values, no significant difference was determined between the two groups.

Both stage III sarcoidosis and extrapulmonary involvement were reported to be associated with chronic and progressive diseases and increased the likelihood of relapse²⁰. In this study,

the mortality rate in the chronic group (4.7%) was similar to the meta-analysis designed by Reich et al. (4.8%). The ratio of stage III disease (4.7%) in this study is related to the mortality rate (4.7%).

The major limitation of this study is its retrospective single-center design and relatively low sample size due to the rarity of the disease, which makes our findings not generalizable to the general patient population with sarcoidosis. As patients were asymptomatic in both groups, a measurable outcome in terms of clinical implications of NLR might add strength to the study. Since the data on the prognostic role of inflammatory markers in sarcoidosis are not well defined, our findings make a valuable contribution to the literature.

CONCLUSION

This study demonstrated that the chronic sarcoidosis group had a higher NLR value and lower lymphocyte count at diagnosis compared with the remission group in long-term follow-up. Therefore, NLR values obtained during diagnosis seem to have prognostic value in the long-term monitoring of patients with sarcoidosis in routine clinical practice. The similarity between

chronic and remission groups in terms of initial disease stage and prevalence of extrapulmonary involvement, this study emphasizes the importance of developing novel serum biomarkers to offer a reliable and objective measure to predict disease-specific prognosis among patients with sarcoidosis. Future studies in larger series are needed to justify the diagnostic and prognostic significance of inflammatory markers in patients with sarcoidosis.

AUTHORS' CONTRIBUTIONS

SAB: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **MY:** Conceptualization, Data curation, Writing – original draft, writing – review & editing. **SG:** Conceptualization, Data curation, Writing – original draft, writing – review & editing. **ET:** Investigation, Methodology, Resources, Formal analysis, Writing – original draft, Writing – review & editing. **FTA:** Data curation, Formal analysis, Methodology, Resources, Writing – original draft. **PS:** Conceptualization, Data curation, Resources, Writing – review & editing. **DY:** Data curation, Investigation, Resources, Visualization, Writing – original draft. **SB:** Formal analysis, Investigation, Resources, Writing – review & editing.

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Effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *caspase 3* in the testes of gamma-irradiated rats

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OBJECTIVE: Ionizing radiation has various applications, including uses in medicine, industry, agriculture, and research. However, ionizing radiation is accompanied by side effects in normal radiosensitive tissues. Probiotics as natural radioprotective agents can protect normal tissues from ionizing radiation. In this regard, this study aimed to investigate the effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *caspase 3* (*CASP3*) in the testes of gamma-irradiated rats.

METHODS: A total of 30 male Wistar rats were involved in this study. The animals received the whole-body radiation with the dose rate of 2 Gy gamma-ray and were orally gavaged with 0.2 mL of 1×10^{10} *Lactobacillus* species in phosphate-buffered saline (PBS) for 4 weeks. Then, the relative gene expression levels of *BCL2*, *BAX*, and *CASP3* in the testis were assessed by using the quantitative real-time polymerase chain reaction (qRT-PCR).

RESULTS: Compared with the control group, radiation significantly downregulated the *BCL2* and upregulated the *BAX* and *CASP3* genes ($p < 0.0001$). However, *Lactobacillus* species significantly reversed these effects.

CONCLUSION: All in all, according to our results, employing *Lactobacilli* probiotics as a natural radioprotector may protect radiosensitive tissue from damage.

KEYWORDS: Gamma radiation. Apoptosis. Probiotics. *Lactobacillus* species.

INTRODUCTION

It has been estimated that about 50–70% of all oncology patients undergo radiation therapy or a combination of chemotherapy and radiation therapy¹. Although radiotherapy has turned into one of the most common treatments for cancer, the detrimental effects on the radiosensitive normal tissues limit the radiation exposure amount that can be applied². The radiation damages radiosensitive normal tissues through various mechanisms³. Reactive oxygen species (ROS) are the major

cause of cell apoptosis and DNA damage through increasing *BAX* and *caspase 3* (*CASP 3*) levels and decreasing *BCL2* levels in radiation-exposed normal tissues⁴. Therefore, the administration of certain antioxidants as radioprotective agents is a critical procedure to attenuate the radiation-related harmful effects on normal tissues.

Recently, probiotics, as a natural radioprotector, have attracted scientific interest. Probiotics, especially lactic acid bacteria (LAB), are live nonpathogenic microorganisms that

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have health benefits when consumed in adequate quantity⁵. They can increase antioxidant capacity through different ways such as scavenging the excess free radicals and producing various metabolites such as glutathione (GSH), butyrate, and folate⁶. Some studies have also shown that probiotics, especially *Lactobacillus* species, exert their radioprotective effect through the regulation of the nuclear factor kappa B (NF- κ B) pathway^{7,8}. Additionally, the anti-apoptosis and anti-inflammation effects of *Lactobacillus* species on the irradiated normal tissues were reported by other studies^{9,10}.

Since the testis is one of the most radiosensitive tissues, this study aimed to investigate the effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *CASP 3* in the testes of gamma-irradiated rats.

METHODS

Irradiation

The animals received the whole-body radiation with the dose rate of 2 Gy gamma-ray (⁶⁰Co) and 100 cGY/min and a source-to-skin distance (SSD) of 100 cm. At the same time, the field of view (FOV) was set at 36×36, and SSD was 80 cm. The single 2 Gy dose of X-ray irradiation was conducted in Rad Source Model RS2000 Irradiator with a 0.3-mm copper filter and X-ray tube settings of 160 kVp and 24 mA (Rad Source Technologies, USA).

Preparation of probiotic strains

Lactobacillus casei (LC) and *Lactobacillus acidophilus* (LA) isolates were provided from the Iranian Biological Resource Center (IBRC). The bacteria were aerobically grown in de Man, Rogosa, and Sharpe (MRS) medium (Sigma-Aldrich, UK) at 37°C for 16 h. The live bacteria were harvested by centrifugation (10 min, 4000×g, room temperature). Thus, plate bacteria were mixed with phosphate-buffered saline (PBS) at the desired concentration. During the experiment, the rats received 0.2 ml of PBS containing 1×10¹⁰ colony-forming unit (CFU) of the probiotics daily.

Animals and treatment

The male Wistar rats (6–8 weeks old) weighing 220±20 g were purchased from the Animal House of Tehran University of Medical Sciences. They were housed under standard laboratory conditions (12-h light/dark cycle at 22±1°C temperature and 55±10% humidity).

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Tehran University of Medical Sciences (the ethical code: 34613).

The rats were divided into six groups (five rats in each group) and treated as it follows:

- Group 1 (healthy control): the animals only received PBS.
- Group 2: the animals only received radiation using the dose rate of 2 Gy gamma-rays.
- Group 3: the animals were orally gavaged with 0.2 mL of 1×10¹⁰ LC in PBS.
- Group 4: the animals were orally gavaged with 0.2 mL of a suspension of 1×10¹⁰ CFU LC in PBS, and their whole body was exposed to radiation.
- Group 5: the animals were orally gavaged with 0.2 mL of a suspension of 1×10¹⁰ CFU LA in PBS.
- Group 6: the animals were orally gavaged with 0.2 mL of a suspension of 1×10¹⁰ CFU LA in PBS, and their whole body was exposed to radiation.

After 4 weeks, the rats were anesthetized intraperitoneally (i.p.) with 250 mg/kg 2,2,2-tribromoethanol (TBE; Avertin®, Sigma-Aldrich) and sacrificed by cervical dislocation. Then, the rats' testes were isolated and immediately frozen in liquid nitrogen and then stored at -80°C for the subsequent analyses.

Quantitative analysis of real-time PCR (qRT-PCR)

The total RNA was extracted from the rat's testis using TRIzol[®] LS (Invitrogen Corp., USA) reagent according to the manufacturer's directions. Then, the single-stranded complementary DNA (cDNA) was synthesized from equal amounts of RNA using the Prime Script cDNA Synthesis Kit (Takara Bio, Japan), following the manufacturer's directions. The relative expression levels of the target gene were measured by qRT-PCR using SYBR Green with the following primer sets: *BCL2* (forward, 5'-GGTGAAGTGGGGGAGGATTG-3'; reverse, 5'-GCATGCTGGGGCCATATAGT-3') (product size: 197 bp), *BAX* (forward, 5'-GGCGATGAACTGGACAACAA-3'; reverse, 5'-CAAAGTAGAAAAGGGCAACC-3') (product size: 151 bp), *CASP3* (forward, 5'-AGCTGGACTGCGGTATTGAG-3'; reverse, 5'-ATGGCGCAAAGTGAAGTGGAT-3') (product size: 189 bp), and *Hypoxanthine-guanine phosphoribosyl transferase* (HPRT) (forward, 5'-TCAGTCAACGGGGGACATAAA-3'; reverse, 5'-GGGGCTGTACTGCTTAACCAG-3') (product size: 142 bp). The relative amounts of *BCL2*, *BAX*, and *CASP3* mRNA were normalized against the endogenous control, HPRT, and calculated with the 2^{- $\Delta\Delta C_t$} formula.

Statistical analysis

The graphs and the statistical analysis of the data were performed using SPSS 16. The results were represented as the mean±SD. One-way analysis of variance (ANOVA) was followed by the Tukey's *post hoc* test for multiple comparisons. p≤0.05 was considered statistically significant.

RESULTS

Effect of *Lactobacillus* species on the *BCL2* and *BAX* gene expression in the testes of gamma-irradiated rats

As shown in Figure 1, radiation significantly downregulated the *BCL2* gene expression in the testis tissues in comparison with the control group ($p < 0.0001$). Conversely, *Lactobacillus* spp. administration significantly reversed this effect ($p < 0.01$). Moreover, the mRNA level of *BCL2* slightly decreased in the LC and LA groups compared with the control group.

The qRT-PCR results also showed that radiation significantly upregulated the *BAX* gene expression in the testis tissues in comparison with the control group ($p < 0.0001$) (Figure 2). However, in the ionization radiation-treated rats and LC (IR+LC) and (ionization radiation-treated rats and LA (IR+LA) groups compared with the irradiated group, *Lactobacillus* spp. significantly decreased the expression of the *BAX* gene ($p < 0.05$) (Figure 2). Additionally, our results showed that the mRNA level of *BAX* did not change between *Lactobacillus* spp. (LC and LA) treated groups and control groups.

Effect of *Lactobacillus* species on the *CASP3* gene expression in the testes of gamma-irradiated rats

Similar to the *BAX* gene, the mRNA level of *CASP3* significantly increased in the irradiated group compared with the

control group ($p < 0.0001$) (Figure 3). However, this effect was significantly reversed by treatment with *Lactobacillus* spp. in the IR+LC and IR+LA groups compared with the irradiated group ($p < 0.01$) (Figure 3). We also found that the mRNA level of *CASP3* did not change in the LC and LA groups compared with the control group.

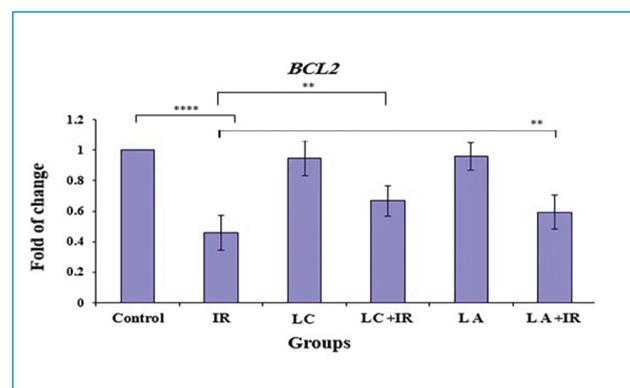


Figure 2. Effect of *Lactobacillus* species on the *BAX* gene expression in the testes of gamma-irradiated rats. Control: PBS-treated rats as control group; IR: radiation-treated rats; LC: *Lactobacillus casei*-treated rats; LC+IR: *L. casei* and ionization radiation-treated rats; LA: *Lactobacillus acidophilus*-treated rats; LC+IR: *L. acidophilus* and ionization radiation-treated rats. Results were expressed as mean; error bars (SD); $n=6$. Statistical analysis was performed using one-way ANOVA test. ** $p < 0.05$; **** $p < 0.0001$.

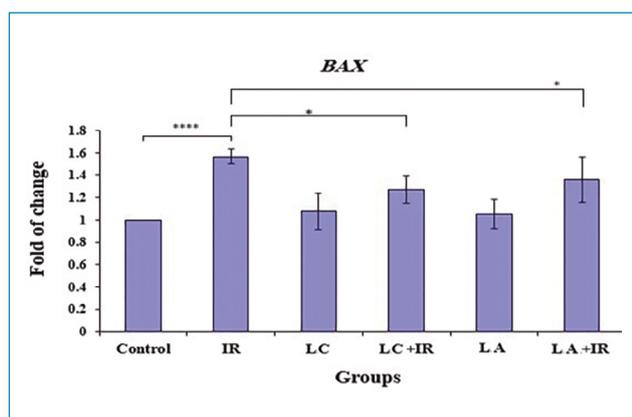


Figure 1. Effect of *Lactobacillus* species on the *BCL2* gene expression in the testes of gamma-irradiated rats. Control: PBS-treated rats as control group; IR: radiation-treated rats; LC: *Lactobacillus casei*-treated rats; LC+IR: *L. casei* and ionization radiation-treated rats; LA: *Lactobacillus acidophilus*-treated rats; LA+IR: *L. acidophilus* and ionization radiation-treated rats. Results were expressed as mean; error bars (SD); $n=6$. Statistical analysis was performed using one-way ANOVA test. * $p < 0.01$; **** $p < 0.0001$

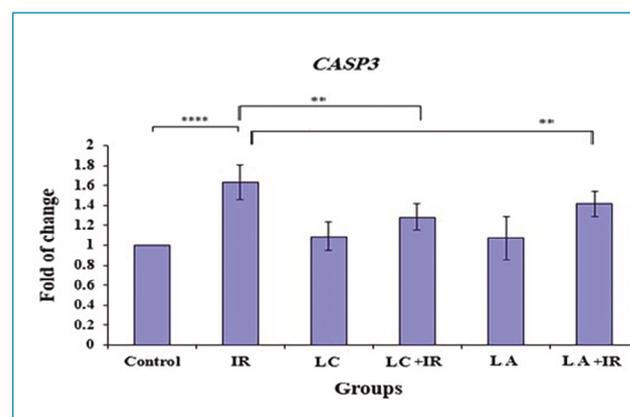


Figure 3. Effect of *Lactobacillus* species on the *CASP3* gene expression in the testes of gamma-irradiated rats. Control: PBS-treated rats as control group; IR: radiation-treated rats; LC: *Lactobacillus casei*-treated rats; LC+IR: *L. casei* and ionization radiation-treated rats; LA: *Lactobacillus acidophilus*-treated rats; LC+IR: *L. acidophilus* and ionization radiation-treated rats. Results were expressed as mean; error bars (SD); $n=6$. Statistical analysis was performed using one-way ANOVA test. ** $p < 0.01$; **** $p < 0.0001$.

DISCUSSION

Ionizing radiation plays a critical role in medical diagnosis and cancer-related therapy. It has been shown that the testis is one of the most radiosensitive organs because very low doses of radiation lead to abnormalities in spermatogenesis by mutagenesis, apoptosis, and necrosis¹¹. Exposure of the testes to radiation leads to the induction of apoptosis in the radiosensitive normal cells, which may result in temporary or permanent infertility¹². Additionally, Mingote et al. reported that changes in the brain lipid's intensities are early tissue responses to radiation exposure¹³{Mingote, 2020 #1}. The development of natural radioprotective agents with less toxicity and high effectiveness is attractive and interesting. Therefore, this study pursued the goal to determine the probiotic treatment effects on modulating apoptosis-related genes *BCL2*, *BAX*, and *CASP3* in the testes of gamma-irradiated rats. The probiotics such as LAB are the beneficial bacteria employed as an adjunct to reduce the adverse effects of ionizing radiation through several mechanisms¹⁴. Some studies indicated that certain probiotics modulate the activation of signaling pathways in radiation therapy⁸. The cell survival and death are regulated by the equilibrium of pro-apoptotic and the anti-apoptotic *BCL2* family proteins and *BAX/BCL2* ratio determines the cell susceptibility to apoptosis¹⁵. Our qRT-PCR results revealed that radiation downregulated the *BCL2* and increased the expression of the *BAX* gene in the normal testicular cells. The reaction of ionizing radiation with the cellular contents such as the small molecules of water in the normal testicular cells leads to the generation of ROS¹⁶. The high ROS level induces apoptosis *via* controlling the phosphorylation and ubiquitination of *BCL2* family proteins, which results in the upregulation of pro-apoptotic genes (e.g., *BAX*) and the downregulation of anti-apoptotic (e.g., *BCL2*)⁴.

Some studies indicated that probiotics modulate the cellular signaling pathway in mammals by direct attachment to the cell surface¹⁷. For instance, Lutfi et al. showed that probiotic *Lactobacillus rhamnosus* negatively regulates appetite markers possibly through melatonin receptors¹⁸. In this study, we showed that *Lactobacillus* spp. significantly upregulates the *BCL2* gene and downregulates the *BAX* and *CASP3* genes in the testes of irradiated rats. In the human body, LAB such as LA and LC are part of the normal microbiota or microflora. The protective effects of probiotics against radiation were reported by many studies. Liu et al. demonstrated that the probiotic *Lactobacillus Plantarum* 299v reduced gastrointestinal injury and inflammation in the rats that were locally irradiated with 10 Gy¹⁹. In addition, it was shown that *L. rhamnosus* GG ATCC 53103 reduced intestinal epithelial apoptosis and improved crypt survival following whole-body gamma

radiation at a dose of 12 Gy⁸. Intestinal bacteria also lowered the negative effect of radiation on intestinal barrier integrity by regulating the expression of tight junction-related proteins and restoring intestinal permeability²⁰. A recently published study reported that probiotics improved the testes' function by neutralizing the toxins, improving sperm quality and testosterone levels, and modulating the immune system²¹. Researchers suggested that probiotics reduced the ROS activation evoked by radiation *via* the production of antioxidant enzymes such as superoxide dismutase, GSH peroxidase, GSH reductase, and catalase^{22,23}. Shokri et al. demonstrated that melatonin with its antioxidant property can decrease oxidative damage induced by radiofrequency electromagnetic radiation (RF-EMR) of mobile phones on testis tissue²⁴. Moreover, melatonin has been reported to have an important anti-apoptotic action by attenuating the production of ROS and pro-apoptotic proteins, such as *BAX*²⁵. As above mentioned probiotics affect the melatonin pathway, therefore, it seems that melatonin might also be involved in the radioprotective effects of probiotics on testis tissue.

Certain limitations should be noted in this study. Primarily, immunohistochemical studies or tunnel analysis were not used to exactly evaluate the apoptosis induction in the testis tissue. However, in view of these findings, it is probable, therefore, that *Lactobacillus* species protect the testes of gamma-irradiated rats by modulating apoptosis-related genes *BCL2*, *BAX*, and *CASP3*. This study is the first report, to the best of our knowledge, indicating the modulatory effect of *Lactobacillus* species on apoptosis-related genes *BCL2*, *BAX*, and *CASP3* in the testes of gamma-irradiated rats.

CONCLUSION

In summary, we concluded that *Lactobacillus* spp., particularly LC protects the testicular tissue against high-dose radiation (2 Gy) through modulating the *BAX* and *BCL2* genes expression, which play a significant role in the activation of apoptosis.

AUTHORS' CONTRIBUTIONS

VC: Conceptualization, Data curation, Formal analysis, Supervision, Validation. **OA:** Investigation, Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **RG:** Data curation, Formal analysis, Writing – original draft. **HB:** Validation, Writing – original draft, Writing – review & editing. **EM:** Conceptualization, Data curation, Validation, Methodology. **PK:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft.

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Older people's knowledge of the purpose of drugs prescribed at primary care appointments

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SUMMARY

OBJECTIVE: This study aimed to assess older people's knowledge of the purpose of drugs prescribed at medical appointments in primary care units and the possible factors related to their level of knowledge about their medications.

METHODS: This was a cross-sectional study conducted in 22 basic health units in Brazil. Patients aged ≥ 60 years were included in this study ($n=674$). Knowledge of prescribed medications was assessed by comparing the responses to the questionnaire and the medication and prescription information. Multivariate analyses were conducted using the Poisson regression with robust variance.

RESULTS: The mean age of the sample was 70.1 (standard deviation: ± 7.1) years. Among 674 patients, 272 (40.4%) did not know the indication of at least 1 of their prescribed drugs; among them, 78 (11.6%) did not know the indication of any of their prescribed drugs. In the final multivariate analysis, polypharmacy, illiteracy, and cognitive impairment were found to be associated with misunderstanding the purpose of at least one prescribed drug. Moreover, illiteracy and cognitive impairment were associated with a greater misunderstanding of the purpose of all prescribed drugs.

CONCLUSIONS: In the studied sample, patients demonstrated a high rate of misunderstanding of the purpose of prescribed drugs. Therefore, it is necessary for health services and professionals to implement strategies that increase the quality of the guidance and instructions given to older people in order to promote adherence to treatment.

KEYWORDS: Aged. Primary health care. Older adults. Medication adherence. Patient education. Polypharmacy.

INTRODUCTION

A prescription for medication typically results from a consultation with a physician¹. Prescribing the correct therapy for older people is difficult compared with that for younger adults, due to differences in pharmacodynamics and pharmacokinetic profiles, potential drug side-effects, and the chronic use of drugs². The majority of this population lives with multimorbidity as

a result of physiologically declining functional organ reserve caused by the natural process of aging. Therefore, they are frequently prescribed more than one drug².

Older people are more likely to have visual, hearing, and memory impairments³. Each type of impairment impacts the quality of life in a particular way, and when combined, they can cause extensive incapacities in an individual³. These impairments

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are associated with other factors, such as environmental, social, and financial support, and are usually the causes of medication non-adherence among older people³. Consequently, they are more likely to have problems in reading, listening to, and understanding medical instructions; taking the correct drugs at the correct time; and following the treatment plan³.

Patient adherence to treatment is related to successful pharmacotherapy. Many patients do not take their medications as prescribed because they lack knowledge about them⁴. Non-adherence, prescription adjustments, adverse drug reactions, and pharmacotherapy complications have been associated with this lack of knowledge, as well as poor perceptions about drugs in general, which may result in the increased use of health services⁵.

Therefore, this study aimed to assess older people's knowledge of the purpose of drugs prescribed at medical appointments in primary health units in Brazil and to identify the possible factors related to their levels of knowledge.

METHODS

This cross-sectional study used the baseline data from a randomized clinical trial titled "Development and evaluation of a mobile application for supporting the prescription of appropriate medications to the elderly." This study was conducted in 22 public primary healthcare units in Brazil.

Data were collected from September 2016 to March 2019 using a multidimensional questionnaire adapted from an instrument used in a previous project named "Health, Wellbeing, and Aging in Latin America and the Caribbean⁶." A digital data collection platform (Kobotoolbox® [Harvard Humanitarian Initiative, Cambridge, MA, USA]) was used to administer the questionnaire. Eligible patients consisted of people aged 60 years or above, who were waiting for medical consultations in the study facilities. Individuals who left the medical consultations without receiving a medical prescription and those who had hearing impairments and/or severe cognitive impairments and were not accompanied by a person who could answer questions related to the participant's functional status in the interview process were excluded. The participant interviews were conducted before and after their medical consultations at the primary care facilities previously mentioned.

Measurement tools

Dependent variables

The level of knowledge of prescribed medications was assessed using two questions: "Do you know the purpose of this medication?" (yes/no). If the patient answered "yes," then they were asked, "What is the purpose?" The patient's understanding of

the drug's purpose was assessed after the consultation and was determined by comparing their responses with the information in the medication prescription. Popular terms such as "lowering blood sugar" or "improving diabetes" were classified as correct responses. Patients were classified into two groups as follows: lower insight of drug's purpose (not knowing at least one purpose of their medications) and absent insight of drug's purpose (not knowing the purposes of any of their medications).

Independent variables

The questionnaire contained variables related to sociodemographic characteristics, clinical and functional characteristics, and medical characteristics. The sociodemographic data included information on sex, skin color, age, marital status, work situation, personal income, and literacy. The clinical and functional data included information on self-rated health, self-rated memory, cognitive impairment (assessed using the Mini-Mental State Examination (MMSE) with different cutoff points according to the education level)⁷, functional status (assessed using the Katz Index of Independence in Activities of Daily Living)⁸, sensory deficits (assessed using self-perceived visual and hearing impairments), insomnia (defined as difficulty in falling or staying asleep), clinical and self-reported diseases, chronic use of medications (continuous use of drugs), and hospitalization (any hospital admission within the past 12 months). The medical data included information on polypharmacy (prescriptions for ≥ 5 medications)⁹, medical consultation time (the length of time was recorded for each appointment), and prescribed drugs and pharmacotherapeutic complexity (assessed using the medication regimen complexity index [MRCI]). The MRCI cutoff points used to distinguish complexity were as follows: values of < 2.7 were considered as very low; 2.7–5.0 as low; 5.0–12.0 as average; 12.0–24.5 as high; and > 24.5 as very high¹⁰.

Statistical analyses

The descriptive analyses of the variables were performed. Two analytical models were created, and the dependent variables were determined based on the patient's knowledge of the drug's purpose. The associations between categorical variables were assessed using the chi-squared test, and the prevalence ratio (PR) was measured to estimate the strength of the association. A multivariate analysis (Poisson regression) was used to adjust for potential confounders. All variables included in the bivariate analysis were associated with the dependent variables at a significance level of $< 20\%$. A significance level of 5% was used for all tests and to identify variables for the final model. R statistical software (R Foundation for Statistical Computing, Vienna, Austria) was used to calculate the PR while all other analyses were performed with the Statistical Package for the Social Sciences version 24 (serial number 10101161149; IBM; Armonk, NY, USA).

Ethics approval

This study was carried out according to the guidelines laid down in the Declaration of Helsinki and was approved by the appropriate institutional review board (number 38.198). Written informed consent was obtained from all participants included in this study.

RESULTS

Of the 854 older patients interviewed, 180 were excluded due to the absence of a prescription after the medical consultation. Ultimately, 674 interviewed individuals were included in this study. Their mean age was 70 years (± 7.1 years). Overall, 11.6% of patients did not know the purpose of any of their prescribed drugs, and 40.4% did not know the purpose of at least one prescribed drug. The sociodemographic characteristics of the study population are described in Table 1.

Table 1. Sociodemographic characteristics of the study population (n=674).

Characteristics	Mean (SD)
Age	70 years (± 7.1 years)
	% (n)
Sex	
Male	30.9 (208)
Female	69.1 (466)
Literacy	
Illiterate	42.3 (279)
Literate	57.7 (381)
Marital status	
Single, widowed, divorced	53.7 (362)
Married	46.3 (312)
Skin color	
White	23.9 (159)
Other (such as black and brown)	76.1 (507)
Personal income	
\leq Minimum wage	91.6 (610)
$>$ Minimum wage	8.4 (56)
Working currently	
Yes	27 (182)
No	73 (492)
Cognitive impairment	
Yes	58.5 (393)
No	41.5 (279)
Visual impairment	
Yes	62.6 (418)
No	37.4 (250)

Table 1. Continuation.

Characteristics	Mean (SD)
Hearing impairment	
Yes	34.2 (230)
No	65.8 (443)
Insomnia	
Yes	54.8 (368)
No	45.2 (304)
Poor self-rated memory	
Yes	53.5 (359)
No	46.5 (312)
Poor self-rated health	
Yes	70.1 (471)
No	29.9 (201)
ADL impairment	
Yes	29.1 (196)
No	70.9 (478)
Hospitalization in last year	
Yes	12.9 (87)
No	87.1 (587)
Polypharmacy	
Yes	18.8 (127)
No	81.2 (547)
Diagnose of hypertension and/or diabetes	
Yes	84.3 (568)
No	15.7 (106)
Length of consultation < 10 min	
Yes	47.6 (321)
No	52.4 (353)
MRCI	
High to very high	27.9 (188)
Very low to average	72.1 (486)
Not knowing purpose of at least 1 prescribed drug	
Yes	40.4 (272)
No	59.6 (402)
Not knowing purpose of all prescribed drugs	
Yes	11.6 (78)
No	88.4 (596)
Not knowing purpose of at least 1 drug already used	
Yes	18.7 (126)
No	81.3 (548)
Not knowing purpose of at least 1 drug for diabetes or cardiovascular disease	
Yes	20.2 (136)
No	79.8 (538)

ADL: activities of daily living; MRCI: medication regimen complexity index.

Regarding the prescriptions, the mean number of prescribed drugs was 2.93 (± 1.8), and 18.8% of the patients had prescriptions for ≥ 5 drugs. The median value of the MRCI was 8, and 27.9% of patients had a high or very high MRCI score. Of the 1991 prescribed drugs, patients did not know the purpose of 537. The top 10 most prescribed drugs for which patients did not know the purpose are shown in Table 2.

Table 2. Top-10 most prescribed drugs for which patients did not know the purpose.

Ranking	Drug	% (n)
1	Hydrochlorothiazide	7.3 (39)
2	Salicylic acid	6.7 (36)
3	Losartan	5.6 (30)
4	Metformin	4.5 (24)
5	Simvastatin	4.3 (23)
6	Loratadine	3.4 (18)
7	Amlodipine	2.8 (15)
	Omeprazole	
8	Glyburide	2.0 (11)
	Enalapril	
9	Atenolol	1.9 (10)
	Ibuprofen	
10	Azithromycin	1.7 (9)
	Multivitamin	

In multivariate analysis, polypharmacy, illiteracy, and cognitive impairment were associated with not knowing the purpose of at least one drug (Table 3), and illiteracy and insomnia were associated with the misunderstanding of the purpose of all prescribed drugs (Table 4).

DISCUSSION

In this study, patients' knowledge of the purpose of drugs prescribed in primary care settings in Brazil was evaluated. The data showed that almost 60% of patients were able to report the purposes of all their drugs, while about 40% did not know the indication of at least one of their prescribed drugs, and more than 11% did not know the indication of any of their prescribed drugs. In the final multivariate analysis, polypharmacy, illiteracy, and cognitive impairment were identified as factors associated with a misunderstanding of the purpose of at least one prescribed drug. Moreover, illiteracy and cognitive impairment were associated with a greater misunderstanding of the purpose of all prescribed drugs.

Other studies have reported that 51% of older adults before geriatric consultation¹¹ and 20% of older adults admitted at geriatric unit¹² knew the purposes of their prescribed drugs. Those studies did not accept generalized terms, such as "lowering blood sugars," as correct answers, which could explain the differences in the results of this study and the previous studies. Another study that did accept generalized terms as correct

Table 3. Patient factors associated with not knowing the purpose of at least one prescribed drug.

	Yes % (n/N)	No % (n/N)	Univariate		Multivariate	
			PR (95%CI)	p-value	PR (95%CI)	p-value
Sex						
Female	39.3 (183/466)	60.7 (283/466)	0.92 (0.76–1.11)	0.39		
Male	42.8 (89/208)	57.2 (119/208)	1.0			
Literacy						
Illiterate	46.6 (130/279)	53.4 (149/279)	1.30 (1.08–1.56)	0.01	1.34 (1.12–1.60)	0.002
Literate	36 (137/381)	64 (244/381)	1.0		1.0	
Marital status						
Single, widowed, divorced	40.1 (145/362)	59.9 (217/362)	0.98 (0.82–1.18)	0.86		
Married	40.7 (127/312)	59.3 (185/312)	1.0			
Skin color						
Other (such as black and brown)	40.4 (205/507)	59.6 (302/507)	1.02 (0.82–1.27)	0.86		
White	39.6 (63/159)	60.4 (96/159)	1.0			
Personal income						
Yes	40.5 (247/610)	59.5 (363/610)	0.94 (0.69–1.30)	0.73		
No	42.9 (24/56)	57.1 (32/56)	1.0			

Table 3. Continuation.

	Yes % (n/N)	No % (n/N)	Univariate		Multivariate	
			PR (95%CI)	p-value	PR (95%CI)	p-value
Working currently						
No	40 (197/492)	60 (295/492)	0.97 (0.79–1.19)	0.78		
Yes	41.2 (75/182)	58.8 (107/182)	1.0			
Cognitive impairment						
Yes	44.8 (176/393)	55.2 (217/393)	1.32 (1.08–1.60)	0.01	1.36 (1.12–1.65)	0.002
No	34.1 (95/279)	65.9 (184/279)	1.0		1.0	
Visual impairment						
Yes	43.1 (180/418)	56.9 (238/418)	1.25 (1.02–1.53)	0.03	1.19 (0.98–1.44)	0.087
No	34.4 (86/250)	65.6 (164/250)	1.0		1.0	
Hearing impairment						
Yes	43.5 (100/230)	56.5 (130/230)	1.12 (0.93–1.35)	0.24		
No	38.8 (172/443)	61.2 (271/443)	1.0			
Insomnia						
Yes	41.3 (152/368)	58.7 (216/368)	1.05 (0.87–1.26)	0.63		
No	39.5 (120/304)	60.5 (184/304)	1.0			
Poor self-rated memory						
Yes	43.5 (156/359)	56.5 (203/359)	1.19 (0.99–1.44)	0.07		
No	36.5 (114/312)	63.5 (198/312)	1.0			
Poor self-rated health						
Yes	41 (193/471)	59 (278/471)	1.06 (0.86–1.30)	0.60		
No	38.8 (78/201)	61.2 (123/201)	1.0			
ADL impairment						
Yes	43.4 (85/196)	56.6 (111/196)	1.11 (0.91–1.35)	0.31		
No	39.1 (187/478)	60.9 (291/478)	1.0			
Hospitalization in last year						
Yes	39.1 (34/87)	60.9 (53/87)	0.96 (0.73–1.28)	0.79		
No	40.5 (238/587)	59.5 (349/587)	1.0			
Polypharmacy						
Yes	70.1 (89/127)	29.9 (38/127)	2.09 (1.78–2.47)	<0.001	1.93 (1.60–2.33)	<0.001
No	33.5 (183/547)	66.5 (364/547)	1.0		1.0	
Diagnosis of cardiovascular disease and/or diabetes						
Yes	41 (233/568)	59 (335/568)	1.11 (0.85–1.46)	0.42		
No	36.8 (39/106)	63.2 (67/106)	1.0			
Length of consultation <10 min						
Yes	33.6 (108/321)	66.4 (213/321)	0.72 (0.60–0.88)	<0.001	0.83 (0.69–1.01)	0.054
No	46.5 (164/353)	53.5 (189/353)	1.0		1.0	
MRCI						
High to very high	51.6 (97/188)	48.4 (91/188)	1.43 (1.19–1.72)	<0.001	1.12 (0.92–1.36)	0.284
Very low to average	36 (175/486)	64 (311/486)	1.0		1.0	

PR: prevalence ratio; CI: confidence interval; ADL: activities of daily living; MRCI: medication regimen complexity index.

Table 4. Patient factors associated with not knowing the purpose of any prescribed drug.

	Yes % (n/N)	No % (n/N)	Univariate		Multivariate	
			PR (95%CI)	p-value	PR (95%CI)	p-value
Sex						
Female	10.3 (48/466)	89.7 (418/466)	0.71 (0.47–1.09)	0.12		
Male	14.4 (30/208)	85.6 (178/208)	1.0			
Literacy						
Illiterate	14.3 (40/279)	85.7 (239/279)	1.56 (1.02–2.39)	0.04	1.51 (0.99–2.32)	0.058
Literate	9.2 (35/381)	90.8 (346/381)	1.0		1.0	
Marital status						
Single, widowed, divorced	11.3 (41/362)	88.7 (321/362)	0.96 (0.63–1.45)	0.83		
Married	11.9 (37/312)	88.1 (275/312)	1.0			
Skin color						
Other (such as black and brown)	11.2 (57/507)	88.2 (450/507)	0.94 (0.58–1.53)	0.81		
White	11.9 (19/159)	88.1 (140/159)	1.0			
Personal income						
Yes	12 (73/610)	88 (537/610)	1.34 (0.57–3.18)	0.50		
No	8.9 (5/56)	91.1 (51/56)	1.0			
Working currently						
No	11.4 (56/492)	88.6 (436/492)	0.94 (0.59–1.50)	0.80		
Yes	12.1 (22/182)	87.9 (160/182)	1.0			
Cognitive impairment						
Yes	13.5 (53/393)	86.5 (340/393)	1.57 (0.99–2.48)	0.05		
No	8.6 (24/279)	91.4 (255/279)	1.0			
Visual impairment						
Yes	12 (50/418)	88 (368/418)	1.20 (0.76–1.88)	0.44		
No	10 (25/250)	90 (225/250)	1.0			
Hearing impairment						
Yes	12.6 (29/230)	87.4 (201/230)	1.14 (0.74–1.75)	0.55		
No	11.1 (49/443)	88.9 (394/443)	1.0			
Insomnia						
Yes	14.4 (53/368)	85.6 (315/368)	1.75 (1.12–2.75)	0.01	1.63 (1.04–2.57)	0.035
No	8.2 (25/304)	91.8 (279/304)	1.0		1.0	
Poor self-rated memory						
Yes	13.1 (47/359)	86.9 (312/359)	1.36 (0.88–2.10)	0.16		
No	9.6 (30/312)	90.4 (282/312)	1.0			
Poor self-rated health						
Yes	11.9 (56/471)	88.1 (415/471)	1.09 (0.68–1.73)	0.73		
No	10.9 (22/201)	89.1 (179/201)	1.0			
ADL impairment						
Yes	13.8 (27/196)	86.2 (169/196)	1.29 (0.83–2.00)	0.25		
No	10.7 (51/478)	89.3 (427/478)	1.0			

Table 4. Continuation.

	Yes % (n/N)	No % (n/N)	Univariate		Multivariate	
			PR (95%CI)	p-value	PR (95%CI)	p-value
Hospitalization in last year						
Yes	11.5 (10/87)	88.5 (77/87)	0.99 (0.53–1.85)	0.98		
No	11.6 (68/587)	88.4 (519/587)	1.0			
Polypharmacy						
Yes	7.9 (10/127)	92.1 (117/127)	0.63 (0.34–1.20)	0.15		
No	12.4 (68/547)	87.6 (479/547)	1.0			
Diagnosis of hypertension and/or diabetes						
Yes	10.4 (59/568)	89.6 (509/568)	0.58 (0.36–0.93)	0.03	0.57 (0.35–0.93)	0.024
No	17.9 (19/106)	82.1 (87/106)	1.0		1.0	
Length of consultation <10 min						
Yes	11.5 (37/321)	88.5 (284/321)	0.99 (0.65–1.51)	0.97		
No	11.6 (41/353)	88.4 (312/353)	1.0			
MRCI						
High to very high	10.1 (19/188)	89.9 (169/188)	0.83 (0.51–1.36)	0.46		
Very low to average	12.1 (59/486)	87.9 (427/486)	1.0			

PR: prevalence ratio; CI: confidence interval; ADL: activities of daily living; MRCI: medication regimen complexity index.

answers found that 69.4% of their participants in home interview knew all drugs' purposes¹³.

Polypharmacy was found in 18.8% of patient's prescriptions, similar to the results of a prior study¹⁴. Patients with polypharmacy were 93% less likely to report a drug's purpose correctly. Negative health outcomes, particularly in older adults, have been associated with polypharmacy¹⁴, and knowledge of the purpose of all medications was inversely associated with this phenomenon¹⁵. Polypharmacy was also linked to non-adherence, and this association may be explained by the fact that patients who are unaware of a drug's purpose may be less likely to use it.

The understanding of pharmacotherapy was associated with literacy in previous studies of adults¹⁶ and older adults¹⁷. Patients with lower levels of education have difficulties with reading, memorizing, and understanding instructions, as well as poor understanding of the information provided by healthcare workers¹⁷. The proportions of older people who did not recognize the purpose of at least one prescribed drug or of any prescribed drug were 34% and 56%, respectively, and were greater among illiterate patients. This finding can be explained by the fact that a successful therapeutic medication regimen depends on patient participation in the healthcare setting. Moreover, basic skills in reading, writing, and numeracy are crucial for this process¹⁸.

Patients with insomnia are less likely to recognize a drug's purpose, which could be explained by the fact that insomnia

affects the ability to accomplish complex and simple tasks, as well as working and episodic memory and problem-solving¹⁹. Additionally, patients with insomnia are more likely to perform poorly in complex tasks measuring reaction time, information processing, and selective attention¹⁹. Cognitive deficits and problems with medication management are relevant and underdiagnosed problems in older adults. Cognitive dysfunction and the lack of basic knowledge of the medication regimen coexist in a large number of patients²⁰. The established cutoff points of the MMSE are higher than those of other proposed methodologies²¹, which increases the sensitivity of the test, allowing early detection of cognitive impairment and decreases specificity²¹.

Most patients (84.3%) had a diagnosis of cardiovascular disease and/or diabetes. Around 20% of them did not know the purpose of at least one drug for diabetes or cardiovascular disease. Additionally, the most frequently prescribed drugs for which patients did not know the purpose were typically used to treat these types of diseases. Similar to the findings of this study, the lack of knowledge of cardiovascular drugs was more common than with diabetic drugs²². In contrast, another study found less knowledge regarding those prescribed drugs for acute conditions¹⁷. However, having a diagnosis of diabetes or cardiovascular disease was a protective factor against not knowing all prescribed drugs' purposes. This could be explained by the

fact that patients with chronic diseases are more likely to use the same drugs for a long time; therefore, they are likely to be more familiar with their medications^{4,17}.

This study has several potential limitations. This study was a secondary analysis of the baseline data from a previous study, which was not designed for the specific objectives of this study. Furthermore, a non-probability sampling procedure was used, and some data were obtained through self-reporting. Therefore, some data were not optimally collected, making it impossible to answer all aspects of the study question fully, such as evaluating the patient's perception of instructions given by family physicians for prescribed drugs. Furthermore, health literacy was not evaluated and is directly associated with a lack of knowledge about medications and low educational levels.

CONCLUSIONS

This study demonstrated that there is a considerable lack of knowledge about prescribed medications among older Brazilian adults after a medical appointment. These results suggest that pharmacotherapy in older adults is complex. There was a high prevalence of older people who did not understand the purpose of or the instructions to use their medications, which may be associated with multiple factors. Health professionals need to be aware and assess patient's understanding of medication prescriptions. Misunderstanding of how to use medications leads to safety and efficacy issues.

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DATA AVAILABILITY STATEMENT

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

AUTHORS' CONTRIBUTIONS

RSG: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **LCSP:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **WWA:** Conceptualization, Formal analysis, Investigation, Methodology. **RMS:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **HMQ:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **JCM:** Conceptualization, Formal analysis, Investigation, Methodology. **LGON:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MGO:** Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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An evaluation of people's knowledge of adult vaccination information level and attitudes during the pandemic Era

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SUMMARY

OBJECTIVE: This research was carried out to evaluate people's knowledge of adult vaccination and their attitude and to observe the effect of the pandemic era on this situation.

METHODS: A total of 1,425 people (18–80 years old) were included in this study. The types of questions like the province where they live, age, gender, occupation, education status, and the presence of chronic diseases, as well as knowing which vaccines are used in adult vaccination, which of these vaccines they had in the last 10 years, which ones they plan to have this year, and whether COVID-19 pandemic changed their perspective on adult vaccinations or not were asked to people.

RESULTS: In the last 10 years, while participants stated that they had the highest rate of tetanus vaccine with 29.8%, hepatitis B vaccine with 23.1%, influenza vaccine with 22.7%, human papillomavirus vaccine with 1.3%, and zoster vaccine with 0.3% were the lowest levels of vaccines.

CONCLUSIONS: As a result, it seems that we are far from the goals set by the health authorities for adult vaccination. We observed that the COVID-19 pandemic raised awareness toward pneumococcus and influenza vaccines and interest toward adult vaccinations and at the same time changed the thoughts against adult vaccinations.

KEYWORDS: Vaccination. Pandemic. Pneumococcal vaccines. Influenza vaccines.

INTRODUCTION

The incidence of vaccine-preventable diseases has been reduced by more than 99% after vaccination, and some of them (smallpox) have been completely eradicated¹. With an effective vaccination, it is possible to reduce the number of referrals to healthcare institutions and hospitalizations, as well as by using less antibiotics and reducing infections with resistant microorganisms. Thus, a serious reduction in mortality and morbidity can be achieved. The success achieved in childhood vaccination in our country and in the world is not yet at the desired level for adult vaccination; it is still reported as a neglected issue

in many countries; and solutions are being sought to increase application rates^{2,3}.

We observed that the interest and demand for adult vaccination have increased after the effects of the COVID-19-induced pandemic on the world. The increasing number of cases and deaths, especially with the pandemic, causes fear and anxiety as well as confusion about adult vaccination. In the literature review, it is seen that there are limited studies on this subject.

This research was carried out to evaluate people's knowledge of adult vaccination and their attitude and to observe the effect of the pandemic era on this situation.

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METHODS

During the pandemic period, it was planned to conduct a descriptive study to evaluate the adult vaccination knowledge and attitudes; approval was obtained from the Ethics Committee; and patient information were collected. This research was conducted in the form of an online questionnaire, and 1,425 people aged 18 years and older were included in this study.

Participants who voluntarily accepted to participate in this research were informed about the research and their rights, and their “informed consent” was obtained before the research.

The online survey for the participants consisted of 21 questions; in addition to their provincial, age, gender, occupation, education status, presence of chronic disease, marital status, presence of children, which vaccines were used in adult vaccination that they know (e.g., pneumococcus, influenza, tetanus, meningococcus, hepatitis A, hepatitis B, zoster, human papillomavirus (HPV) vaccine, etc.) or which of these vaccines they had in the last 10 years, which ones they plan to have this year, whether COVID-19 pandemic has changed their view on adult vaccines, the protectiveness of the pneumococcal and influenza vaccine, its usage periods, risk groups, and its effects on COVID-19, and their perspectives on coronavirus vaccines in production were asked.

IBM SPSS statistics version 22.0 program was used for statistical analysis in this study. While evaluating the data of this study, besides descriptive statistical methods (i.e., mean, standard deviation, and frequency), the chi-square test was used to evaluate the relationship between variables, the Student's *t*-test for the comparison of normally distributed data, and the Mann-Whitney *U* test for comparing non-normally distributed data. Results were evaluated at 95% confidence interval and significance level of $p < 0.05$.

RESULTS

A total of 1,425 people were included in this study, including 850 (59.6%) women and 575 (40.4%) men. The mean age of the participants was 37.4 ± 10.78 years, while the men were 37.73 ± 10.64 years and the women were 37.19 ± 10.88 years, and there was no statistically significant difference in age between women and men ($p > 0.05$).

Among 286 out of 1,425 people participating in this study, they had one or more chronic diseases. While the mean age of this group was 44.40 ± 11.58 years, the mean age of 1,139 people without chronic disease was 36.65 ± 9.82 years. There was a statistically significant difference in age between the groups ($p < 0.05$).

In adult vaccination, the highest awareness rate was the tetanus vaccine with 96.6%, while the lowest awareness rate was the zoster vaccine with 27.6% (Table 1). While there was no difference in awareness between the genders in the zoster vaccine ($p > 0.05$), the awareness of all other vaccines was statistically

significantly higher in women. No significant difference was found in comparing the individuals' awareness of vaccine with and without chronic diseases.

When we compared the patients above the age of 35 years with those below the age of 35 years, the HPV vaccine was significantly higher in those who aged above 35 years, but there was no statistically significant difference in terms of other vaccines.

Vaccines received by the participants in the last 10 years are shown in Table 2. The vaccines that the participants plan to have this year are shown in Table 3.

Table 1. Awareness levels of vaccines used in adult vaccination.

Adult vaccines	(%)
Tetanus vaccine	96.3
Hepatitis B vaccine	91.9
Influenza vaccine	91.6
Pneumococcal vaccine	86.7
Hepatitis A vaccine	74.7
Meningococcus vaccine	73
Human papilloma virus vaccine	50.4
Zoster vaccine	27.6

Table 2. Levels of adult vaccines that people have had in the last 10 years.

Adult vaccines	(%)
Tetanus vaccine	29.8
Hepatitis B vaccine	23.1
Influenza vaccine	22.7
Pneumococcal vaccine	8.6
Hepatitis A vaccine	6.5
Meningococcus vaccine	2
Human papilloma virus vaccine	1.3
Zoster vaccine	0.3

Table 3. Levels of adult vaccines people plan to have this year.

Adult vaccines	(%)
Influenza vaccine	30.3
Pneumococcal vaccine	17.8
Hepatitis B vaccine	5.5
Tetanus vaccine	5.1
Human papilloma virus vaccine	3.4
Hepatitis A vaccine	2.2
Meningococcus vaccine	0.6
Zoster vaccine	0

To the question we asked the participants “Have coronavirus pandemic changed your perspective on adult vaccinations?” 886 (62.2%) participants answered as “No, it hasn’t,” while 539 (37.8%) participants answered as “I’m thinking of having one or more than one of these vaccines that I have not thought about having before the coronavirus pandemic for myself or one of my family members.”

When we asked the question about the effects of pneumococcal vaccine on pneumonia and COVID-19 infection, 930 (65.2%) participants said, “This vaccine does not protect against either coronavirus or pneumonia, it only protects from pneumococcal pneumonia,” 380 (26.6%) people said, “This vaccine is not protective against coronavirus, but I think it protects from pneumonia caused by coronavirus,” and 115 (8.1%) people responded as “I think this vaccine protects against coronavirus.”

To the question we asked about the effects of influenza vaccine on influenza and COVID-19 infection, 1,294 (90.8%) participants answered as “I do not think this vaccine protects against coronavirus, I think it only prevents influenza infection caused by influenza virus,” while 131 (9.2%) people answered as “I think this vaccine protects against coronavirus” in the form.

Participants answered the question “To whom should the pneumococcal and influenza vaccine be given first?” The most common answer was “people over 65, those with chronic diseases and healthcare workers.”

To the question we asked about coronavirus vaccines in production, 552 (38.7%) people answered as “I do not think that a fully effective vaccine can be produced against coronavirus,” 672 (47.2%) people answered as “I think the vaccine to be produced against coronavirus will be partially or short-term effective,” and 201 (14.1%) people answered as “I think the vaccine to be produced against coronavirus will be completely effective.”

Finally, to the question “If the coronavirus vaccine is produced, do you plan to have it?” 562 (39.4%) people answered as “If I see that there is no serious side effect in those who had the vaccine for a while after the vaccine is produced, I will have it done later,” 270 (18.9%) people answered as “I consult my doctor and decide according to their suggestions,” 159 (11.2%) people said, “Yes, I’ll immediately have it.” In addition, 278 (19.5%) people answered “I’m undecided,” and 156 (10.9%) people answered as “I will definitely not have it.”

DISCUSSION

Despite the increasing efforts in recent years, due to reasons such as people have doubts about the efficacy and side effects of vaccines, misleading news, anti-vaccination movements,

insufficiency of national health policies on adult immunization, and the economic burden the vaccination brings, the literature reveals that only 10–20% of the targeted adult vaccination groups can be vaccinated⁴. Due to the COVID-19 pandemic, we observed that the interest and demand for adult vaccines, especially pneumococcal and influenza vaccines, have increased in 2020. When we considered the findings of our study, in the last 10 years, the highest rates of vaccines received by the participants were tetanus vaccine with 29.8%, hepatitis B vaccine with 23.1%, and influenza vaccine with 22.7%. In the study by Kanitz et al., they found that the most commonly used vaccine in European countries is the influenza vaccine, followed by the tetanus vaccine⁵. In the study performed by Özişik et al. in our country with 522 patients, while it was found that the most commonly used vaccine was the tetanus vaccine, the most commonly used vaccine in patients with chronic lung disease, cardiovascular diseases, and diabetes was found to be influenza vaccine⁶. The results found in the study by Özişik et al.⁶ are in line with the results of our study. The reason for this is that the tetanus vaccine is routinely administered to prevent neonatal tetanus during pregnancy before compulsory military service in our country, and it is thought that tetanus vaccine is the most known and administered vaccine due to the administration of tetanus vaccine after trauma and accidents. Unlike the study conducted by Özişik et al.⁶, in our study, the tetanus vaccine was the most commonly used vaccine in patients with chronic diseases within 10 years, followed by the influenza vaccine⁶. The reason for this difference is thought to be due to the fact that chronic diseases were wider in our study, the number of patients was higher, the 10-year process was questioned, and the patient profiles were different.

In our study, there was no significant difference between men and women in terms of adult vaccines they had in the last 10 years, except for HPV (more common in women) and zoster (more common in men) vaccines. However, the awareness rate in all adult vaccines, except for the zoster vaccine, was statistically significantly higher in women. This reveals that awareness is higher about adult vaccination in women, although it is not yet fully implemented in practice.

In our study, HPV and zoster vaccines were the least vaccines that individuals had in the last 10 years. The study in which Yuruyen et al. evaluated the awareness of physicians in adult vaccination performed on 200 physicians from different branches in our country, the vaccines recommended by the physicians to patients were the lowest with 1% while the rate of recommending the HPV vaccine was found to be 4.5%⁷. Since it is not recommended a lot by physicians and its payment is not covered by social security institutions, it is expected that these vaccines will have the lowest rate in our country.

Again, in the same study, the three vaccines frequently recommended by physicians with a rate of 22% are influenza, hepatitis B, and pneumococcal vaccines⁷. The low rates of these recommendations can be considered as one of the main reasons for not achieving the goals in adult vaccination. In the study performed by Özişik et al.⁶ on 155 patients, it was found that 93.5% of the patients had the indication for influenza and pneumococcal vaccines, but only 17.2% of these patients had the pneumococcal vaccine and 29.7% had the influenza vaccine. When we asked the patients who were vaccinated, they answered that they had the highest rate of vaccinations because their physicians recommended it⁸. Considering the studies conducted, it is seen that the rate of vaccination is affected by the doctor's recommendation. Similarly, there is evidence that the majority of patients will be vaccinated if recommended by healthcare providers⁸⁻¹⁰.

When we asked the participants in our study "Have coronavirus pandemic changed your perspective to vaccines?" 539 (37.8%) people replied as "I'm thinking of having one or more than one of these vaccines that I have not thought about having before the coronavirus pandemic for myself or one of my family members," and again when we asked these individuals which vaccines they plan to have in this year, it was stated by them that they will have influenza vaccine, especially with the highest rates of 30.3 and 17.8% pneumococcal vaccine later. These rates were 1.5 times for influenza vaccination and two times for pneumococcal vaccine more than the rates that patients stated that they had received these vaccines in the last 10 years. These results show that the pandemic has led to an increased interest in adult vaccines, especially toward influenza and pneumococcal vaccines.

In our study, when we asked the patients about the effects of pneumococcal and influenza vaccines on coronavirus infection, pneumonia, and influenza, 380 (26.6%) people said, "This vaccine is not protective against coronavirus, but I think it protects from pneumonia caused by coronavirus" for the pneumococcal vaccine and 115 (8.1%) people answered as "I think this vaccine protects against coronavirus," while 131 (9.2%) people answered as "I think this vaccine protects against coronavirus"

for the influenza vaccine. These responses can be considered as the main reason for the serious increase in interest in these vaccines, as there is a perception forming in the society that these vaccines are directly effective against coronavirus.

In our study, to the question we asked about the coronavirus vaccines that are in the production phase, 672 (47.2%) people with the highest rate answered as "I think the vaccine to be produced against coronavirus will be partially or short-term effective." This shows that although the participants are hopeful against the coronavirus vaccine, the expectation that a fully effective vaccine will be produced is less.

Finally, to the question "If a coronavirus vaccine is produced do you plan to have it?" only 159 (11.2%) people have answered as "Yes, I definitely do." This situation shows that the participants harbor doubts about the COVID-19 vaccines in the production phase.

When we evaluated the limitations of our study, although there was participation from all around Turkey, the majority of the contributions from urban areas, rural areas may have led to an inadequate reflection of the data.

CONCLUSIONS

As a result, it seems that we are far from the goals set by the health authorities for adult vaccination. We observed that the COVID-19 pandemic raised awareness toward pneumococcus and influenza vaccines and interest toward adult vaccinations, and at the same time it changed the thoughts against adult vaccinations. To achieve the desired goals of adult vaccination, we think that national and international organizations should work in cooperation with individuals, society, and authority-based regulations.

AUTHORS' CONTRIBUTIONS

MZ: Conceptualization, Data curation, Writing – original draft. **MK:** Conceptualization, Data curation, Supervision, Writing – review & editing. **NK:** Writing – original draft. **AS:** Investigation, Methodology, Data curation.

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Characterization of clinical, laboratory, IL-6 serum levels, and IL-6-174 G/C genetic polymorphisms in patients with rheumatoid arthritis and Sjögren's syndrome

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SUMMARY

OBJECTIVE: This study aimed to characterize the clinical (disease activity and exocrine gland function), laboratory, interleukin 6 (IL-6) serum levels, and IL-6-174G/C (rs1800795) genetic polymorphisms among rheumatoid arthritis (RA), RA plus Sjögren's syndrome (RA+SS), and control subjects.

METHODS: A case-control study enrolling 137 women (52±11 years old) were divided into three groups as follows: RA (n=70), RA+SS (n=29), and healthy control (C, n=38). Individuals underwent clinical evaluation composed of Schirmer's test, unstimulated salivary flow rate, and evaluation of disease activity and functional capacity (Disease Activity Score [DAS28] and Health Assessment Questionnaire [HAQ]). IL-6 serum levels and IL-6-174G/C polymorphisms were assessed.

RESULTS: RA and RA+SS presented higher serum levels of IL-6 than controls ($p<0.001$). Also, higher IL-6 levels were related to swollen joints ($p=0.038$), limited functional capacity ($p=0.004$), and disease activity ($p\leq 0.001$). However, neither IL-6-174G/C genetic polymorphism nor its allele frequency was associated with RA or RA+SS.

CONCLUSION: IL-6 serum is an important marker of RA activity and functional incapacity, but IL-6-174G/C genetic polymorphism did not differ among healthy controls and cases.

KEYWORDS: Sjögren's syndrome. Rheumatoid arthritis. Genetic polymorphism. IL-6.

INTRODUCTION

Interleukin 6 (IL-6) is a pro-inflammatory cytokine related to the pathogenesis and perpetuation of both rheumatoid arthritis (RA) and Sjögren's syndrome (SS)¹, as well as of other autoimmune diseases.

Although high IL-6 rates have been observed in patients with RA and considered to be largely genetically determined^{2,3}. Studies indicate that single nucleotide polymorphism, such as -174G/C (rs1800795) of the IL-6 gene promoter region, plays a role in influencing IL-6 protein transcription and serum levels⁴.

In tandem with it, there is a positive correlation between IL-6 serum levels and RA activity⁵.

Although studies suggest the involvement of IL-6 in disease progression, the predominant genetic profile in RA needs to be better understood, thus providing insights for the development of future patient management strategies. Therefore, this study aimed to assess the serological levels of IL-6 and the genetic polymorphism of IL-6-174G/C in patients with RA and RA+SS, as well as to correlate them with clinical, ophthalmological, and oral evaluation.

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METHODS

Study design and sample characteristics

Cross-sectional control-matched study enrolling women who are 18 years or older, in a convenience sample. RA, RA+SS, and SS volunteers were invited in a tertiary center (Rheumatology Unit of Hospital das Clínicas, Universidade Federal de Pernambuco). Controls were invited to the Stomatology Unit of the same university and matched for age with individuals with RA.

All individuals were assessed for sicca syndrome and classified as SS according to the criteria of the American-European College of Consensus⁶. The diagnosis of RA was based on the criteria determined by the American College of Rheumatology⁷.

Exclusion criteria were the history of radiotherapy in the head and neck, HIV or HCV infection, sarcoidosis, amyloidosis, graft versus host disease, and using anticholinergic drugs. The approval by the ethical review board was obtained, and all individuals agreed voluntarily to participate.

Assessment of disease activity

The Disease Activity Score 28 (DAS28) was applied. It assesses 28 joints for pain and swell, rate of erythrocyte sedimentation or C-reactive protein as an inflammatory marker, and a scale of self-perception of disease activity from 0 to 100.

Clinical evaluations

Functional capacity was determined using the Portuguese version of the Health Assessment Questionnaire (HAQ). This instrument assesses the functional status of patients with RA. For each of the eight categories, the patient indicated the degree of difficulty to perform daily activities in four possible responses ranging from “no difficulty=0” to “unable to do it=3.”

Visual analog scales (VAS), ranging from 0 to 100, were also used to assess the self-perception of general condition (VAS of general condition affliction) and fatigue in the previous week reported by the patient.

Evaluation of xerostomia

We applied the Portuguese version of the Xerostomia Inventory. This inventory consists of 11 questions with a 5-item Likert scale for each item. The sum ranges from 11 to 55, and higher values correspond to a more pronounced perception of xerostomia.

Resting salivary flow (RSF) collection

Saliva to evaluate resting salivary flow (RSF) was collected by obtaining whole non-stimulated saliva. Collection occurred by the spitting method, lasting 15 min and administered in the morning.

Evaluation of IL-6 concentration in serum

IL-6 was measured from serum samples. The human Cytometric Bead Array (CBA) Kit Th1/Th2/Th17 (BD™ Cytometric Bead Array (CBA), Catalog #560484, BD Bioscience, San Jose, CA, USA) was used according to the manufacturer's recommendations. The IL-6 detection limit is 2.4 pg/mL. Data acquisition was performed using the FACSCalibur cytometer (BD Bioscience), and the analyzes were performed using FCAP Array v3.0 software (Soft Flow Inc.).

DNA extraction and detection of the polymorphic variant

The DNA of the samples was isolated from whole blood (EDTA) with the QIAamp Mini Spin Columns DNA Kit (QIAGEN) following the manufacturer's instructions. The samples were genotyped for IL-6-174G/C (rs1800795) using a TaqMan assay (Applied Biosystems, Foster City, CA, USA). Allele-specific probes were labeled with the fluorescent dyes VIC and FAM, respectively.

Statistical analysis

The data are presented according to the following three groups based on the clinical evaluation: RA, RA+SS, and control. Data are expressed in mean±standard deviation. Quantitative variables were assessed in groups with Kruskal-Wallis or ANOVA, according to the normality distribution by the D'Agostino and Pearson's test. Binary categorical variables were analyzed using the Fisher's test; the chi-square test was used for categorical non-binary variables. Spearman's correlation was applied for the analysis of two quantitative variables.

The data were analyzed using the GraphPad Prism software version 6.0 for Windows. An alpha coefficient of 5% was adopted.

RESULTS

Sample characteristics

Our sample is composed of 70 women with RA, 29 with RA+SS, and 38 controls. Table 1 describes sample characteristics.

Oral assessment

Xerostomia was reported for 28/29 (97%) in individuals with RA+SS and for 27/70 (39%) in individuals with RA ($p<0.001$) and controls 5/38 (13%). Reduced resting salivary flow rate was more frequent in the SS+RA group (26/29, 90%) compared with RA (5/70, 7%) and controls (6/38, 16%) ($p<0.001$). The mean RSF was significantly lower in the RA+SS group (Table 1).

Table 1. Sample characterization.

Characteristics	Group			p
	Control (n=38)	RA+SS (n=29)	RA (n=70)	
Age	52.3±12.2	53.6±9.1	50.9±11.4	0.736
RSF positive	6 (16)	26 (90)	5 (7)	<0.001*
RSF	0.3±0.3	0.06±0.08	0.4±0.3	<0.001*
ESR	19.9±14.9	37.4±25.5	35.0±25.3	0.004*
Schirmer	12.5±11.1	2.1±1.8	10.4±9.8	<0.001*
Schirmer positive	7 (19)	29 (100)	21 (27)	<0.001*
Xerophthalmia	4 (11)	21 (72)	29 (41)	<0.001*
Xerostomia	5 (13)	28 (97)	27 (39)	<0.001*
Xerostomia inventory	17.4±7.1	31.6±11.1	21.3±9.3	<0.001*
DAS28	–	4.6±1.8	4.4±1.6	0.539
DAS28 [†]	–	22 (79)	51 (74)	0.796
HAQ	–	1.3±0.9	1.3±0.9	0.814
HAQ	–	16 (57)	38 (56)	1.000
EVA	–	56.1±28.5	53.9±32.9	0.921
Fatigue	–	55.0±36.6	53.8±35.4	0.802
Disease time	–	10.9±9.3	9.1±6.8	0.540
Joint pain [‡]	–	7.0±8.9	7.1±9.9	0.876
Swollen joint [‡]	–	3.4±4.0	2.3±4.1	0.048*

Data expressed as mean±SD and n (%). RA: rheumatoid arthritis; SS: Sjögren's syndrome; RSF: resting salivary flow; ESR: erythrocyte sedimentation rate; DAS28 = Disease Activity Score 28; HAQ: Health Assessment Questionnaire. *Statistical significance; age and time of illness in years; [†]Moderate and severe strata; [‡]Number of joints.

Ophthalmological evaluation

Xerophthalmia was reported by 4/38 (11%) controls, 29/70 (41%) patients with RA, and 21/29 (72%) individuals with RA+SS ($p<0.001$). All individuals in the RA+SS group showed an aqueous deficiency (29/29) on the Schirmer's test, while it was observed on 21/70 (27%) in the RA group and 7/38 (19%) among controls ($p<0.001$) (Table 1).

Disease activity and functional capacity assessment

The mean DAS28 score was similar in the RA+SS and RA groups ($4.6±1.8$ vs. $4.4±1.6$, $p=0.539$). For functional capacity, there was also no significant difference between them.

Joint edema

The number of swollen joints was significantly higher in the RA+SS group ($3.4±4.0$) than in the RA group ($2.3±4.1$, $p=0.048$). There was no difference in the number of painful joints between them (Table 1).

Correlation of IL-6 serum levels and clinical and laboratory variables in RA and RA+SS groups

In RA and RA+SS groups, IL-6 levels positively correlate with erythrocyte sedimentation rate (ESR), DAS28, HAQ, and joint pain ($r=0.421$, $p<0.001$; $r=0.334$, $p<0.001$; $r=0.292$, $p=0.004$; $r=0.209$, $p=0.038$, respectively).

Association of IL-6 with polymorphism and allele frequencies

Allele frequencies did not differ significantly between the RA+SS, RA, and C groups (Table 2). The genotype distributions are shown in Table 3. Patients in the RA group (GG 53.5%, GC 62.5%, and CC 66.6%) and the RA+SS group (GG 21.1%, CG 15.6%, and CC 0%) had no significant difference from controls (GG 25.3%, CG 21.8%, and CC 33.3%) ($p=0.691$).

IL-6 levels by clinical groups

IL-6 serum levels were significantly higher in the RA and RA+SS groups when compared with the C individuals (RA= $10.8±33.4$, RA+SS= $6.5±8.2$, and C= $0.41±0.9$; $p<0.001$). Nevertheless, no difference was observed between RA and RA+SS groups ($p=0.391$).

Table 2. Allele frequency of interleukin-6 174G/C in all clinical groups.

Group	Allele				p	95%CI
	C		G			
	n	%	n	%		
Case	33	75	82	66	0.276	0.72–3.42
Control	11	25	43	44		
RA+SS	5	31	35	44	0.410	0.18–1.80
Control	11	69	43	46		
RA	27	71	96	69	1.000	0.50–2.42
Control	11	19	43	31		
RA+SS	5	15	35	27	0.254	0.18–1.42
RA	27	85	96	73		

Case: rheumatoid arthritis and rheumatoid arthritis+secondary Sjögren's syndrome; RA: rheumatoid arthritis; SS: secondary Sjögren's syndrome.

Table 3. Interleukin-6 174G/C genotype per groups.

Genotype	Clinical profile						p
	Control		RA+SS		RA		
	n	%	n	%	n	%	
CC	2	7.4	0	0.0	4	6.4	0.691
GC	7	25.9	5	25.0	20	32.3	
GG	18	66.7	15	75.0	38	61.3	
Total	27	100	20	100	62	100	-

RA: rheumatoid arthritis; SS: secondary Sjögren's syndrome.

DISCUSSION

We found that IL-6 serum levels correlated with RA activity and ERS, but we found no association between allelic frequency of IL-6-174G/C polymorphism and clinical phenotypes.

IL-6 is a cytokine produced by lymphocytes, monocytes, and fibroblasts, which plays a key role in the maturation of B cells and the production of autoantibodies⁸. IL-6 acts as a pro-inflammatory mediator in the face of inflammatory stimuli^{9,10}. During the acute inflammation phase of RA, serum IL-6 levels can be used as a biomarker of inflammation or disease activity⁹, in line with our finding that the higher levels of IL-6 in patients with higher DAS28 scores. Several lines of studies have pointed that individuals with elevated IL-6 serum levels are likely to present higher levels of those in the synovial fluid, and these findings are

associated with disease activity and joint destruction^{11,12}. HAQ is a measure that evaluates the functional disability reported by the patient, it also had a significant association with the serum level of IL-6.

In individuals with RA, the coexistence of SS has been associated with more severe diseases and worse prognoses^{13,14}. We observed a higher number of swollen joints in the RA+SS group. SS is a chronic systemic inflammatory disorder that mainly affects exocrine glands, leading to xerostomia and xerophthalmia. It is characterized by the infiltration of mononuclear cells in the exocrine glands as well as acinar and ductal destruction, with consequent glandular hypofunction¹⁵. Salivary and lacrimal glands are the target organs for autoimmune attack mediated mainly by T cells. CD4 cells in SS¹⁶ and IL-6 are capable of stimulating numerous biological processes including T cell activation, B cell differentiation, and autoantibody production.

The GG genotype has already been associated with an increase in the production of this cytokine that is possibly related to the lymphocytic infiltration in the glandular stroma REF. This infiltration can lead to acinar and ductal destruction of epithelial cells and loss of the glandular parenchyma resulting in clinical signs and classic symptoms of dry mouth and eyes¹⁷. Although the presence of xerostomia and xerophthalmia has been associated with the diagnosis of SS, these clinical findings correlated neither with the serum levels nor with the genetic polymorphism of IL-6 in this study.

Regarding the allelic and genotypic frequencies of the IL-6-174G/C genetic polymorphism, they were not associated with the presence of RA or SS. Report from the British population comparing patients with RA and controls also did not observe differences among IL-6 levels and the same polymorphism¹⁸. In fact, a systematic review showed heterogeneity in the association of IL-6 genotypes and between individuals with RA from Europe, Asia, and eastern China and suggests that this divergence can probably be explained by different antecedent genetic disorders or linkage imbalance¹⁹. In fact, studies have already reported that one gene variant plays different roles in the risk of RA in different populations and regions²⁰⁻²². These findings suggest complex interactions with environmental and probably other genetic factors leading to clinical phenotypes^{23,24}, as well as the importance of establishing specific databases of populations and regions.

This study has limitations. This was an unicenter study, and we have a relatively small sample size; then, maybe our results could not be representative, and the possibility of type II error should be kept in mind.

CONCLUSIONS

IL-6 polymorphisms and allele frequency were not associated with the diagnosis of RA or RA+SS, but IL-6 levels were correlated with clinical findings, such as ESR, DAS28, and HAQ. In this context, the use of IL-6 as a marker of clinical activity may be useful. More studies are needed to evaluate its impact on disease progression.

AUTHORS' CONTRIBUTIONS

TSM: Conceptualization, Data curation, Investigation, Methodology. **MLS:** Conceptualization, Data Curation, Investigation. **MLMSJ:** Conceptualization, Data Curation, Formal analysis. **APD:** Project administration, Validation, Visualization. **LAG:** Project administration, Supervision, Validation, Visualization.

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Factors associated with bleeding complications in hernia repair of warfarin users

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SUMMARY

OBJECTIVE: In this retrospective study, we aimed to determine factors associated with bleeding complications in patients on long-term warfarin, undergoing inguinal hernia repair using low-molecular-weight heparin (LMWH) bridging.

METHODS: Two-year hospital records yielded 44 inguinal hernia repair patients on long-term warfarin (26 men, 4 women, aged 57.4 [38–72] years). All patients were managed with LMWH bridging. Patient and operative characteristics, LMWH bridging characteristics, and international normalized ratio (INR) values were compared between patients with and without postoperative bleeding complications.

RESULTS: Indication for warfarin use was heart valve disease (n=15), atrial fibrillation (n=7), deep venous thrombosis (n=3), cerebrovascular event (n=3), and pulmonary embolism (n=2). Four of the operations were urgent, while the remaining were elective. There were four ecchymosis cases and three hematoma cases in a total of seven patients. Baseline (2.94±0.26 versus 2.16±0.38, p<0.001) and preoperative INR values (1.69±0.67 versus 1.31±0.35, p=0.027) were significantly higher, while postoperative INR values (1.04±0.09 versus 1.2±0.13, p=0.004) were significantly lower in patients having bleeding complications.

CONCLUSIONS: Baseline, preoperative INR, and postoperative INR were the only variables associated with postoperative bleeding complications in patients undergoing LMWH-bridged inguinal hernia repair. We suggest close monitoring of INR levels in long-term warfarin users, even for relatively low-bleeding risk operations such as inguinal hernia repair.

KEYWORDS: Inguinal hernia. Anticoagulants. Warfarin. Low-molecular-weight heparin.

INTRODUCTION

Inguinal hernia repair is the most common operation in general surgery, with more than 20 million hernia repair operations conducted worldwide, every year¹. The lifetime risk of having inguinal hernia repair is 27% for men and 3% for women². Patients at increased risk of arterial thromboembolism due to conditions, such as atrial fibrillation or prosthetic heart valve, and patients with a history of venous thromboembolism require long-term anticoagulation treatment³.

Vitamin K antagonist warfarin is a commonly used oral anticoagulation agent; however, it may pose a bleeding risk in

patients requiring surgery. Warfarin therapy is interrupted in most types of major surgery in order to minimize blood loss. Patients at high risk of a thromboembolic event are recommended to discontinue warfarin and bridge the perioperative period by switching to a short-acting anticoagulant such as subcutaneous low-molecular-weight heparin (LMWH) or intravenous unfractionated heparin⁴. However, guidelines are less clear in patients at moderate risk of bleeding and/or moderate risk of a thromboembolic event. As common as hernia repair may be, the number of studies investigating postoperative complications following LMWH-bridged hernia repair surgery is few.

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In this study, we aimed to determine the incidence of bleeding complications and investigate factors related to bleeding complications in long-term warfarin users undergoing inguinal hernia repair via LMWH bridging protocol.

METHODS

This was a retrospective study of LMWH bridging of patients on long-term warfarin treatment, undergoing inguinal hernia repair. We reviewed files of patients having inguinal hernia repair within the past 2 years and identified 30 patients who had been on long-term warfarin therapy prior to the operation. All patients were managed with LMWH bridging. Patient characteristics, reason for warfarin use, operative characteristics, timing of warfarin discontinuation and reinitiation, timing of LMWH bridging, preoperative and postoperative international normalized ratio (INR) values, and postoperative complications were recorded.

Factors related to complications were investigated. The design of this study has been approved by local Ethics Committees. Due to the retrospective nature of this study, the Ethics Committee did not require written consent from the participants.

Statistical analysis

SPSS version 17.0 was used in the statistical analysis. Descriptive statistics were used to summarize data: continuous variables were expressed as mean and standard deviation, while categorical variables were expressed as number and percentage. Dependent group comparisons were performed using repeated-measures analysis of variance and paired-sample *t* test. Independent group comparisons were performed using Mann-Whitney *U* test. Comparison of categorical variables was performed using chi-square or Fisher's exact test. The limit of statistical significance was set at $p < 0.05$.

RESULTS

A review of hospital records from the past 2 years revealed 30 inguinal hernia repair patients (4 women and 26 men) who had been on long-term warfarin treatment. The mean age of the patients was 57.4 years, ranging between 38 and 72 years. Indication for warfarin use was heart valve disease ($n=15$), atrial fibrillation ($n=7$), deep venous thrombosis (DVT) ($n=3$), cerebrovascular event ($n=3$), and pulmonary embolism ($n=2$) (Table 1). The majority of patients had a primary hernia (90%), and most patients were diagnosed with indirect inguinal hernia (70%) (Table 1).

Surgical characteristics are summarized in Table 2. Elective surgery was performed on 26 patients, while emergency surgery

was performed on 4 patients. In total, 7 (23.3%) patients had postoperative bleeding complications, with 4 (13.3%) patients having ecchymosis and 3 (10%) developing hematoma. All cases with hematoma resolved naturally without surgical intervention. No venous thromboembolic events were recorded in the postoperative follow-up of patients. There was no significant

Table 1. Patient characteristics.

	n (%)
Male/female	26/4 (86.7/13.3)
Indication for warfarin use	
Heart valve disease	15 (50)
Arterial fibrillation	7 (23.3)
Deep venous thrombosis	3 (10)
Cerebrovascular event	3 (10)
Pulmonary embolism	2 (6.7)
Hernia type	
Primary/secondary	27/3 (90/10)
Direct	6 (20)
Indirect	21 (70)
Femoral	2 (6.7)
Pantaloon	1 (3.3)
Hernia location	
Right	19 (63.4)
Left	9 (30)
Bilateral	2 (6.6)

Table 2. Surgical characteristics.

	n (%)
Elective surgery	26 (86.7)
Emergency surgery	4 (13.3)
Type of anesthesia	
Local	1 (3.3)
Regional	22 (73.3)
General	7 (23.4)
Surgical drain present	
<24 h	5 (71.4)
24–48 h	1 (14.2)
48–72 h	1 (14.2)
Bleeding complications	
Hematoma	4 (13.3)
Ecchymosis	3 (10)

difference between patients with and without complications in terms of patient or surgical characteristics (Table 3). In addition, the timing of the LMWH bridging protocol was not significantly different between the groups (Table 4). INR readings were taken on the day of warfarin discontinuation (baseline INR), preoperatively on the morning of surgery (pre-op INR), and on the day of warfarin reinitiation (post-op INR).

Baseline (2.94 ± 0.26 versus 2.16 ± 0.38 , $p < 0.001$) and pre-op INR values (1.69 ± 0.67 versus 1.31 ± 0.35 , $p = 0.027$) were significantly higher, while post-op INR values (1.04 ± 0.09 versus 1.2 ± 0.13 , $p = 0.004$) were significantly lower in patients having bleeding complications (Figure 1).

DISCUSSION

In this retrospective study, we found the incidence of all bleeding complications was 23.3%, while the incidence of hematoma development was 10%. Baseline, pre-op INR, and post-op INR were the only variables associated with postoperative bleeding complications following LMWH-bridged inguinal hernia repair surgery. Mean INR on the day of surgery was 1.69 in patients developing complications and 1.31 in patients without complications.

Perioperative management of patients on long-term anticoagulation therapy depends on the patient's risk of having a thromboembolic event and the risk of bleeding. However, there are no clear-cut recommendations on perioperative

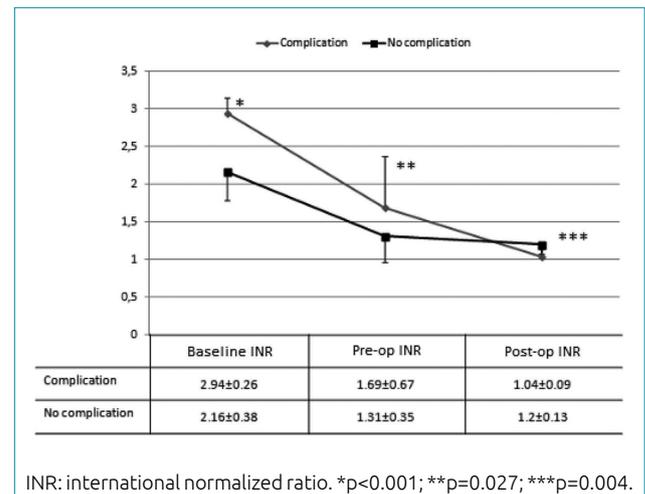


Figure 1. Comparison of international normalized ratio on the day of warfarin discontinuation (baseline INR), on the day of surgery (pre-op INR), and on the day of warfarin reinitiation (post-op INR) in patients with and without bleeding complications.

Table 3. Comparison of patient and surgical characteristics according to complication.

	Complication, n=7	No complication, n=23	p
Gender			
Male/female	6/1 (85/15)	20/3 (87/13)	1.000
Hernia location			
Right/left/bilateral	5/2/0 (70/30/0)	14/7/2 (60/30/10)	0.667
Hernia type			
Primary/secondary	6/1 (85/15)	21/2 (91/9)	1.000
Surgery			
Emergency/elective	0/7 (0/100)	4/19 (18/82)	0.559
Anesthesia			
Local/regional/general	0/4/3 (0/50/50)	2/16/5 (8/70/22)	0.057
Surgical drain			
Yes/no	3/4 (43/57)	4/19 (18/82)	0.971

Table 4. Comparison of low-molecular-weight heparin bridging periods according to complication.

	Complication, n=7	No complication, n=23	p
Warfarin discontinuation and LMWH initiation*	6.00 ± 2.61	6.29 ± 1.71	0.926
Warfarin reinitiation†	2.67 ± 0.82	3.14 ± 0.67	0.138
LMWH discontinuation†	5.34 ± 0.52	5.45 ± 0.61	0.562

LMWH: low-molecular-weight heparin. *Number of days before surgery; †Number of days after surgery.

anticoagulation management for the majority of patients with a mechanical heart valve, atrial fibrillation, or DVT, who have a moderate risk of thromboembolism and a low/moderate risk of bleeding⁴.

Our standard perioperative anticoagulation management protocol in hernia repair surgery involves warfarin interruption and LMWH bridging. LMWH bridging was shown to be a safe and effective method to manage anticoagulation during the perioperative period of a variety of surgeries, in patients with mechanical heart valves, atrial fibrillation, or DVT in retrospective observational studies and prospective registries⁵⁻⁷. LMWH is preferred to unfractionated heparin as it can be safely delivered at fixed doses in an outpatient setting, minimizing hospital stay and its related costs⁸.

The incidence of bleeding complications found in this study was similar to the rates reported by Bombuy et al.⁹, who employed the same standard LMWH bridging protocol for patients on long-term warfarin therapy undergoing elective hernia repair. In their study, among 47 patients bridged with LMWH in the perioperative period, 6 (13%) had surgical-site hematoma and 1 patient had major bleeding, necessitating surgical intervention⁹. No thromboembolic events or deaths were seen in either study.

As hernia repair is considered a low-bleeding risk surgery, some groups investigated the possibility of continuing therapeutic dose warfarin in the perioperative period. In a retrospective study, Sanders et al.¹⁰ investigated the outcome of inguinal repair surgery in 49 patients while using full-dose warfarin. The rate of small hematomas requiring no intervention was 14.2%, while the rate of large hematomas requiring surgical or medical intervention was 8.2%. They found a significantly higher risk of hematoma in patients with INR >3 and suggested that patients may undergo inguinal repair while on warfarin therapy, as long as INR is <3 . In another study, McLemore et al.¹¹ reported a similar rate of postsurgical hematoma in inguinal hernia repair patients who continued warfarin (2/19) and those who had heparin bridging (2/15), even though most patients continuing warfarin had an INR >3 and those with heparin bridge had INR <1.5 . However, they indicated that due to a limited number of patients, their study may lack statistical power to detect a significant difference between surgical-site hematomas. Although these studies appear to undermine the importance of having INR <1.5 preoperatively, there could be inherent biases in the selection of patients, as warfarin continuation was not their standard protocol.

The American Academy of Neurology published a recent guideline on periprocedural management of antithrombotic medications in patients with ischemic cerebrovascular

disease¹², where inguinal herniorrhaphy was listed among procedures that possibly do not increase bleeding risk. Thus, they had a Level C recommendation to continue warfarin in patients undergoing hernia repair. In contrast, we preferred to exercise caution against the continuation of warfarin during hernia repair, since INR was the only significant factor associated with bleeding in our study. As far as we know there is no increased risk of thromboembolism associated with the use of LMWH bridging, warfarin continuation would only serve to simplify the perioperative management protocol. Warfarin continuation during hernia repair would necessarily mean having INR >2 or even >3 and may yet show an increased risk of bleeding when practiced in larger numbers.

Smoot et al.¹³ recommended meticulous management of patients requiring warfarin, as chronic warfarin may be a risk factor for postoperative hematoma development in inguinal hernia repair. The recommended current procedure is for warfarin interruption that is omitted for 3 days before elective surgery and then used bridging LMWH for patients with the low/moderate bleeding risk category concomitant high-risk thromboembolism¹⁴. The biological half-life of warfarin is 36–42 h, which is based on this timing of warfarin interruption. In this process, the INR falls below 2¹⁵. Postprocedure INR >3 significantly increases the risk of major bleeding (associated decrease in hemoglobin ≥ 20 g/L) in patients on long-term warfarin¹⁶. Although the value of INR is more clearly defined for major bleeding, it is difficult to estimate the value for minor bleeding. Although the surgeon feels safe to prevent postoperative bleeding with an INR of <1.5 preoperatively, INR <2 is acceptable in patients at high risk of thromboembolism. In this study, the difference between the values in this INR range seems to be significant for minor bleeding.

CONCLUSIONS

This study has limitations. Due to the insufficient number of patients included in this study, we could not obtain a significant INR cutoff value for minor bleeding. Since warfarin continuation during inguinal hernia repair is being discussed as a possibility, studies focused on perioperative management of hernia repair are needed. On the other hand, the strength of this study is the investigation of LMWH bridging in a homogeneous group of hernia repair patients, while the less number of such patients is a limitation. Future studies with large-scale patient series may be useful to determine the appropriate INR range to prevent minor bleeding in low/moderate-risk groups on long-term warfarin.

INR was the only factor associated with bleeding complications in patients receiving long-term warfarin therapy, undergoing LMWH-bridged inguinal hernia repair. We suggest close monitoring of INR levels in long-term warfarin users, even for relatively low bleeding risk operations such as inguinal hernia repair.

AUTHORS' CONTRIBUTIONS

EGD: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **IK:** Data curation. Software. **ÖP:** Formal analysis. **MÖ:** Project administration. **MK:** Investigation, Supervision.

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Readmission rates of patients with COVID-19 after hospital discharge

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SUMMARY

OBJECTIVE: The purpose of this study was to inspect return and readmission reasons and rates of discharged patients with coronavirus disease 2019 (COVID-19).

METHODS: This is an observational descriptive retrospective study that was conducted with patients who had confirmed COVID-19 diagnosed with severe respiratory syndrome coronavirus-2 (SARS-CoV-2) polymerase chain reaction (PCR) and hospitalized between April 2020 and June 2021 in a tertiary care university hospital. Patients returning to the hospital after treatment for COVID-19, with symptoms related to COVID-19 within 30 days, were included. Patients under 18 years of age and who were hospitalized in the intensive care unit were excluded.

RESULTS: It was determined that of 369 discharged patients, 87 (23.5%) returned to the hospital, 9 (2.4%) were readmitted, and 1 (0.02%) was deceased within 30 days. The most frequent reasons for returning to the hospital were dyspnea and cough complaints. Existence of pneumonia at first admission, levels of aspartate aminotransferase, lactate dehydrogenase, C-reactive protein, D-dimer, neutrophil counts, lymphocyte counts, and neutrophil-to-lymphocyte count ratios were found to be higher in patients who returned to hospital, compared with the patients who did not return.

CONCLUSIONS: Return rate of patients to hospital after discharge with COVID-19 was comparatively high, but readmissions to hospital and mortality rate were low. Comparatively, the higher rate of return to hospital within 30 days of discharge was thought to be resulting from prolonged signs and symptoms related to COVID-19. Since COVID-19 is a new and enigmatic disease and its long-term effects still need to be elucidated, long-term follow-ups of discharged patients will be adequate.

KEYWORDS: COVID-19. Patient readmission. 30-day readmission.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) is a viral infection caused by subacute severe respiratory syndrome coronavirus-2 (SARS-CoV-2), which spreads by air as respiratory droplets and may result in severe pneumonia and respiratory failure. After the first case in Wuhan, China, in December 2019, the disease has become pandemic in a very short time. It has been first reported in March 2020 in Turkey, and up to now, more than 5 million and 400 thousand patients and 50 thousand deaths have been reported¹.

Symptoms and signs related to COVID-19 may persist for a long time. Information about the readmission rate of patients with COVID-19 is insufficient². In two previous studies, readmission rates to the hospital for symptoms related to COVID-19 after discharge were 1.9 and 29.4%, respectively^{3,4}. There are very few studies inspecting readmissions of patients with COVID-19 in Turkey. One study reports a readmission rate of 7.1% within 30 days of discharge⁵. Detailed information about the clinical course and follow-up in the convalescence period after discharge may be

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beneficial for the management of the course. In addition, if characteristics and clinical findings of patients whose clinical course has worsened in the convalescence period can be determined better; estimating the best timing for hospital discharge and issues that must be considered in the follow-up period may be more reliable and convenient for those patients⁶.

In the inspection of readmissions to the hospital after treatment for COVID-19, periods of 14–60 days were considered in different reports^{7,8}. A follow-up period of 30 days after discharge is a reliable time to evaluate the development of unwanted outcomes related to COVID-19³. Rate of readmission is strongly related to the existence of concomitant diseases, course, severity, and recovery rate of COVID-19 disease. Higher rates of readmission are related to bad prognosis for these patients².

The aim of this study was to inspect reasons for returning or readmissions to the hospital and return, readmission rates, and mortality rates of patients with COVID-19 within 30 days of discharge.

METHODS

Study design and population

This is an observational descriptive retrospective study that was conducted with patients who had confirmed COVID-19 diagnosed and hospitalized between April 2020 and June 2021 in the internal medicine department of a tertiary care university hospital. Diagnosis, hospitalization criteria, treatment, and discharge decisions were made according to the guidelines of the Scientific Advisory Committee of the National Ministry of Health⁹.

This study was approved by the Local Ethics Committee (approval no: 2021/9). This study was conducted in compliance with Helsinki Declaration and Good Clinical Practice Directives.

Inclusion criteria

Patients who were hospitalized and discharged after treatment for COVID-19 disease and who had positive SARS-CoV-2 polymerase chain reaction (PCR) test results were included.

Exclusion criteria

Patients below 18 years of age, patients who were hospitalized in the intensive care unit, and patients who had negative PCR test results were excluded. Patients who returned to the hospital for previously planned admissions or symptoms unrelated to COVID-19 were excluded.

COVID-19 diagnostic criteria

COVID-19 diagnosis was confirmed with the detection of specific viral RNA sequences in rRT-PCR by nucleic acid amplification test.

Study setting

This study was conducted in a tertiary university hospital with 250 beds located in the capital city of Turkey.

Data collection

Patients were inspected for returning to a health care facility (either the hospital in which treatments for COVID-19 were given or admissions to other hospitals) for COVID-19-related signs and symptoms within 30 days of discharge. Hospital records of patients were inspected, and patients who had missing information were confirmed by phone calls.

Demographic characteristics, laboratory test results, radiologic imaging study results, treatment regimens for COVID-19, length of hospitalization, and accompanying diseases were recorded. Symptoms, readmission rates, and mortality rates of patients within 30 days of discharge were recorded. Only first readmission data were considered. Data of patients with readmissions and without readmissions were compared.

Complete blood count (i.e., lymphocyte, neutrophil, thrombocyte counts), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, lactic dehydrogenase (LDH), C-reactive protein (CRP), ferritin, D-dimer, and thorax computed tomography (CT) data were inspected. Neutrophil-to-lymphocyte ratio (NLR) was calculated by the division of neutrophil counts to lymphocyte counts. Complete blood counts were analyzed by Sysmex XN-1000 (USA) as 22 parameters. ALT, AST, creatinine, LDH, D-dimer, and CRP tests were analyzed by Roche Hitachi Cobas 501 (Switzerland). Ferritin levels were analyzed by Roche Hitachi Cobas 601 (Switzerland).

Statistical analysis

Data were analyzed using SPSS version 25.0 statistical package (Armonk, NY, USA). Data distributions were evaluated by Shapiro-Wilk test. Data were presented as mean±standard deviation for normally distributed variables and as median, interquartile range, and minimum-maximum for non-normally distributed variables. Comparisons between groups in normally distributed variables were done by independent samples t test and Mann-Whitney U test for not normally distributed variables. The chi-square test was used for the comparison of qualitative data. The Fisher's exact test was used when the chi-square test cannot be used. Logistic regression analysis and odds ratios were determined. A $p < 0.05$ was considered statistically significant.

RESULTS

A total of 437 patients were hospitalized with COVID-19 in the study period at the study hospital. Of these, 22 (5%) died in the hospitalization period, 5 (1.1%) were transferred to other hospitals for various reasons, and 41 (9.4%) patients could not be reached after discharge from the hospital, and these 68 patients were excluded. The remaining 369 (84.4%) patients were included in this study.

Of the 369 patients, 182 were males (49.3%) and 187 (50.7%) were females. The mean age was 52.52 ± 17.90 years (males 54.05 ± 16.73 and females 51.02 ± 18.90). The mean hospitalization period of patients at first admission to the hospital for COVID-19 was 6.10 ± 4.18 days. The mean time for returning to the hospital after discharge was 9.16 ± 7.40 days. Notably, 95 (25.7%) patients were above 65 years of age, and 274 (74.3%) patients were below 65 years of age.

Of the 369 discharged patients, 87 (23.5%) returned to the hospital for symptoms related to COVID-19. Nine (2.4%) of these patients were readmitted to the hospital and only one (0.2%) died. Returning reasons of discharged patients were shortness of breath in 68 (78.1%), cough in 45 (51.7%),

arthralgia in 37 (42.5%), chest pain in 37 (42.5%), headache in 20 (22.9%), high fever in 16 (18.3%), and change/loss of sense of taste or smell in 13 (14.9%).

Demographic characteristics, concomitant diseases, and antiviral treatments of patients with COVID-19 with or without 30-day readmission to the hospital were shown in Table 1. Hydroxychloroquine was used in the treatment of 80 (21.6%) patients, favipiravir was used in 215 (58.3%) patients, and both drugs were used in 74 (20.1%) patients. At first admission to the hospital, 279 (75.6%) patients had pneumonia.

Laboratory test results of patients with COVID-19 with or without 30-day readmission to the hospital were shown in Table 2.

To understand factors affecting patients' return to the hospital, logistic regression analysis was performed. The existence of pneumonia at first hospitalization was associated with a 2.81 times increase in the risk of readmission, and the existence of concomitant malignancy was associated with a 3.45 times increase in the risk of readmission. Logistic regression analysis results for readmission of patients with COVID-19 are shown in Table 3.

Table 1. Demographic characteristics and antiviral treatments of patients with COVID-19 with or without 30-day readmission to hospital.

Property	Total participants (n=369)	With readmission (n=87)	Without readmission (n=282)	p-value
Male/female	182/187	48/39	134/148	0.212
Age	52.52 ± 17.90	53.01 ± 19.21	52.37 ± 17.51	0.769
Length of hospitalization*	5 (IQR=4–7)	6 (IQR=4–9)	5 (IQR=3–7)	0.044
Age above 65 years, n (%)	95 (25.7)	25 (28.7)	70 (24.8)	0.466
Hypertension, n (%)	109 (9.5)	30 (34.4)	79 (28)	0.248
Diabetes mellitus, n (%)	69 (18.6)	17 (19.5)	52 (18.4)	0.818
Coronary artery disease, n (%)	67 (18.1)	21 (24.1)	46 (16.3)	0.098
Chronic obstructive pulmonary disease, n (%)	42 (11.3)	12 (13.7)	30 (10.6)	0.418
Malignity, n (%)	19 (5.1)	9 (10.3)	10 (3.5)	0.012
Chronic kidney disease, n (%)	13 (3.5)	6 (6.8)	7 (2.4)	0.051
Hydroxychloroquine, n (%)	80 (21.6)	17 (19.5)	63 (22.3)	0.581
Favipiravir, n (%)	215 (58.3)	51 (58.6)	164 (58.1)	0.939
Hydroxychloroquine and favipiravir together, n (%)	74 (20.1)	19 (21.8)	55 (19.5)	0.635
Existence of pneumonia, n (%) [†]	279 (75.6)	77 (88.5)	202 (71.6)	0.001

*Median and interquartile range (IQR); [†]At first admission to hospital. Age and length of hospitalization variables were tested using Student's *t*-test, existence of chronic obstructive pulmonary disease, malignity, and chronic kidney disease variable were tested using Fisher's exact test, and all other variables (i.e., gender, age above 65 years, existence of hypertension, diabetes mellitus, coronary artery disease, pneumonia, hydroxychloroquine, favipiravir, hydroxychloroquine, and favipiravir together) were tested using chi-square test. Bold numbers indicate the significant $p < 0.05$.

Table 2. Laboratory test results of patients with COVID-19 with or without 30-day readmission to hospital.

Laboratory results	Mean±standard deviation			p-value
	Total participants (n=369)	With readmission (n=87)	Without readmission (n=282)	
Alanine aminotransferase (IU/L)	30.70±35.68	33.84±36.43	29.71±35.45	0.348
Aspartate aminotransferase (IU/L)	32.11±26.11	37.35±36.65	30.39±21.40	0.033
Lactate dehydrogenase (IU/L)	266.49±210.93	327.06±385.08	248.02±108.73	0.003
C-reactive protein (mg/L)	520.71±670.04	700.82±84.83	470.12±59.59	0.004
Ferritin (µg/L)	370.65±420.03	450.45±499.71	343.82±387.17	0.055
D-Dimer (nmol/L)	4160.94±7255.43	5985.32±9667.93	3594.56±6236.89	0.007
Creatinine (µmol/L)	82.21±50.39	1.02±0.59	0.91±0.56	0.115
Hemoglobin (g/L)	130.19±10.97	13.35±2	13.14±1.96	0.375
Leukocytes (×10 ⁹ /L)	6.96±3.92	8.02±5.65	6.64±3.14	0.004
Platelet count (×10 ⁹ /L)	220.80±81.87	213.95±90.86	222.92±78.94	0.373
Neutrophil count (×10 ⁹ /L)	4.90±0.34	5.79±4.57	4.64±2.92	0.006
Lymphocyte count (×10 ⁹ /L)	1.45±1.53	1.50±2.83	1.42±0.79	0.681
Neutrophil to lymphocyte count ratio	5.07±5.90	6.43±6.24	4.64±5.74	0.013

Alanine aminotransferase, aspartate aminotransferase, ferritin, D-dimer and lymphocyte count were tested using Mann-Whitney *U* test, and any other parameters (i.e., lactate dehydrogenase, C-reactive protein, creatinine, hemoglobin, leukocyte, platelet count, neutrophil count, lymphocyte count and neutrophil to lymphocyte count ratio) were tested using Student's *t*-test. Bold numbers indicate the significant $p < 0.05$.

Table 3. Logistic regression analysis results for readmission of patients with COVID-19.

Characteristics	Readmission odds ratio (95%CI)	p-value
Malignity	3.45 (1.19–10.01)	0.022
Existence of pneumonia at first admission	2.81 (1.37–5.75)	0.005
Male/female	1.01 (0.58–1.76)	0.959
Hemoglobin (g/L)	0.90 (0.77–1.04)	0.152
Length of hospitalization	0.96 (0.91–1.02)	0.187

CI: confidence interval. Bold numbers indicate the significant $p < 0.05$.

DISCUSSION

A total of 369 discharged patients who had treatment for COVID-19 were inspected in this study. Of note, 87 (23.5%) patients returned to the hospital with symptoms related to COVID-19 within 30 days of discharge. Nine (2.4%) of these patients were readmitted to the hospital and only one (0.2%) died. Existence of pneumonia, lengthening of hospitalization period in the first admission, and higher AST, LDH, CRP values, leukocyte and neutrophil counts, and NLR on the first

day of hospital admission were found to be related to increased return risk to the hospital after discharge.

In a study reported from the United States, 275 patients who were confirmed with COVID-19 using PCR test were inspected, 21 (7.6%) of these patients were readmitted to hospital and 6 (2.2%) of them died in 30 days after discharge from the hospital¹⁰. In another study from the United States, 151 discharged patients with COVID-19 were inspected, and 36 (24.0%) of them were readmitted to the hospital within 30 days of discharge; about half of these patients were readmitted with complaints related to COVID-19, but the other half were readmitted with trauma, conditions related to hematological, oncological, and neurological diseases. Sixteen (11%) of these patients were readmitted to hospital¹¹. In a study with 1344 patients, after discharge from the hospital, 216 (16.5%) patients were reported to return to the hospital for reasons related to COVID-19, 132 (9.8%) were readmitted to the hospital, and 32 (2.4%) of these died in hospital after readmission³. In this reported study, return rates were relatively higher but readmissions to the hospital were lower. Since COVID-19 is a mysterious disease that is new to the population, perception and anxiety levels for this disease are higher than expected, and thus, patients may feel

a more serious disease period. In a study, 2897 unconfirmed cases of influenza were inspected, and 409 (14%) of these patients were readmitted to the hospital within 30 days of discharge¹².

When compared with the data of this reported study, these rates are lower. This may be explained by the increased anxiety and perception of COVID-19 as a dangerous disease in the population.

A study with more than 29,000 patients who had confirmed COVID-19 reported the length of hospitalization time as 8 days¹³. A Korean study reported the length of hospitalization due to COVID-19 as 17 days¹⁴. In this study, the mean length of stay for hospitalization for COVID-19 was 6 days. Return rates for patients who stayed in the hospital for more than 6 days were higher. Differences in the length of hospitalization periods are thought to be resulting from different treatments and follow-up recommendations varying from country to country. In addition, patients who had more serious COVID-19 disease course were hospitalized for an increased period, and these patients are expected to have more frequent complaints and return rates to the hospital related to COVID-19 within 30 days of discharge.

Concomitant diseases were found to be more frequent in patients with COVID-19 who were readmitted to the hospital after discharge in a study¹⁵. A total of 154 hospitalized patients who were tested positive using PCR test and who were probable cases (patients who tested negative but had high clinical suspicion of COVID-19) were inspected in a study; patients who were readmitted to the hospital with COVID-19-related symptoms within 30 days of discharge were compared with patients who did not. The readmission rate of patients who had malignancies was found higher⁵. Another study reported that the most frequent concomitant disease in readmitted patients with COVID-19 was hypertension¹⁶. In this reported study, hypertension was also the most frequent concomitant disease in patients returning to the hospital, but when considering comorbidities, the existence of malignancies was found to be the only risk factor for returning to the hospital within 30 days of discharge.

Dyspnea was reported to be the most frequent returning symptom to the hospital that was observed nearly in half of the patients⁷. The most frequent readmission symptom was dyspnea in another study with 1,062 patients². In this reported study, dyspnea was also the most frequent returning symptom that was explained by the high rate of the existence of pneumonia on the first admission.

In a study with 1,368 patients with COVID-19, the return rate of patients who had higher NLR and D-dimer

levels was reported to be higher⁶. This reported study reveals that AST, LDH, CRP levels, leukocyte counts, neutrophil counts, and NLR were higher in patients who returned to the hospital with symptoms related to COVID-19, concordantly. These parameters are usually found elevated in patients with COVID-19 and levels increased as a reflection of the severity of the disease.

This study has limitations. The setup was retrospective, and it was performed in a single center and may not reflect the general population. The relationship of symptoms that resulted in the patient's return to the hospital with other reasons other than COVID-19 was not inspected in detail.

CONCLUSIONS

The returning rate of patients with COVID-19 within 30 days of discharge was found to be increased in patients who had pneumonia in the first admission and who had increases in inflammatory markers such as leukocyte counts and CRP. In addition, patients with increased D-dimer levels, prolonged stay in the hospital, and patients who had concomitant malignancies were more frequently expected to return to the hospital after discharge. These factors will negatively be affecting morbidity rates, and thus, these patients are more likely to have unplanned hospital visits. Although the return rate within 30 days of discharge was high in this study, readmission to hospital and mortality rates were low. Since COVID-19 is a new and enigmatic disease and its long-term effects are not yet elucidated, it may be wise to recommend following these patients for a more prolonged period, from 6 months to 1 year after discharge from hospital.

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AUTHORS' CONTRIBUTIONS

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

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Comparison of two magnetic resonance imaging spectroscopy postprocessing methods

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SUMMARY

OBJECTIVE: The aim of this study was to compare the results obtained using SpectroView® (SV) and J-Magnetic Resonance User Interface (jMRUI) from the same magnetic resonance (MR) spectroscopy of hydrogen data.

METHODS: Data from 23 males with alcohol use disorder (AUD) and 23 healthy non-AUD males were acquired by a 1.5 Tesla MR using a PRESS sequence (TE=30 ms) in four voxels located in the right frontal and left frontal (RF and LF) lobes, and posterior cingulate (AC and PC). The ratio of the signals from both *N*-acetyl-aspartate (NAA) and choline (Cho) over creatine (Cr) was calculated automatically using SV and semiautomatically by an expert neuroradiologist using jMRUI. The software' agreement was calculated by the 95% limits of agreement (LoA) of the ratio of the obtained values.

RESULTS: The standard deviation was greater in jMRUI than in SV. Although there was a correlation between the results from both methods, it was not possible to predict their variance from one another. Additionally, the 95% LoA showed that jMRUI values were expected to vary from 38 to 190% of those obtained using SV for NAA/Cr in RF of AUD subjects and from 48 to 196% for NAA/Cr in CA of non-AUD individuals.

CONCLUSIONS: The difference between the methods may represent clinically significant magnitudes. We suggest the use of the same method when comparing spectroscopic data. We also suggest that in clinical practice, the automatic method should be preferred.

KEYWORDS: Magnetic resonance spectroscopy. Alcoholism.

INTRODUCTION

Proton magnetic resonance spectroscopy (¹H-MRS) is a non-invasive method, which demonstrates brain tissues' biochemistry^{1,2}. Through ¹H-MRS, it is possible to identify the wave frequency from hydrogen protons that characterize specific metabolites and their signals' amplitudes, with the latter used to calculate the relative concentration of metabolites³. Since creatine (Cr) is the most stable metabolite captured by ¹H-MRS

in brain tissue, it is the most adopted as an internal reference to measure the concentration of other metabolites^{4,5}.

Although ¹H-MRS does not show specific disease lesions, it demonstrates general metabolic alterations including neuronal viability, the cellular energy status, and the state of cell membrane⁵. Recently, ¹H-MRS has been used clinically and scientifically to investigate various neurologic alterations, such as metabolic, inflammatory, infectious, and tumoral diseases^{1,2}.

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The signals of metabolites analyzed can be interfered with by many factors, such as the signal of water and the adjacent structures (e.g., the liquor and the skull)^{1,6}. Therefore, the post-processing of data is imperative in order to suppress noises and identify the metabolites of interest^{1,5}. Despite many innovations in data acquiring and processing automatization, there is still a gap between scientific research and clinical application of the ¹H-MRS, partially due to the complexity of the technique and the manual steps of postprocessing^{7,8}.

It is still not much discussed in the literature if the metabolites' ratios in ¹H-MRS vary in different postprocessing methods. The aim of this study was to compare the results obtained by an automatic (J-magnetic resonance user interface, jMRUI) and a semiautomatic (SpectroView® [SV], Philips Medical Systems Nederland B.V., The Netherlands) postprocessing method of ¹H-MRS.

METHODS

Inclusion and exclusion criteria

This study is part of a research project that aimed to evaluate spectroscopic differences in the brain of patients with alcohol use disorder (AUD) and healthy non-AUD subjects with the approval of the Local Ethics Committee. Therefore, this study followed the same inclusion and exclusion criteria of the research project, which may be consulted in the article by de

Souza et al.⁹. The recruitment of both groups was performed between March 2016 and July 2017. Those who were eligible and in agreement to participate in this study signed the Informed Consent Form and were submitted to anamnesis, medical record analysis, magnetic resonance imaging (MRI), and MRS-H.

Data acquisition

¹H-MRS data were acquired by a 1.5 Tesla MRI scanner *Achieva* (Philips Medical Systems Nederland B.V.), with a specific neurovascular coil, model *SENSE Head Coil* with eight channels, without paramagnetic contrast administration.

The MR acquisition was realized by point-resolved spectroscopy sequence (PRESS), in the axial plane, with echo-time of 30 ms, repetition-time of 6 s, and NEX of 2. The total number of acquisitions was 64, with a bandwidth of 4 kHz and a spectral resolution of 4096 points, without suppression of the water signal. The spectroscopic acquisition was performed in four single voxels, each with 8 cm³ (2 cm×2 cm×2 cm), localized in left dorsolateral prefrontal (LF), right dorsolateral prefrontal (RF), anterior cingulate (AC), and posterior cingulate (PC), including both white and gray matter. The estimated time for each sequence was 2 min and 40 s (Figure 1).

Data postprocessing

Data were processed automatically using SpectroView® (Philips, NL), resulting in ratios of the signals' amplitudes

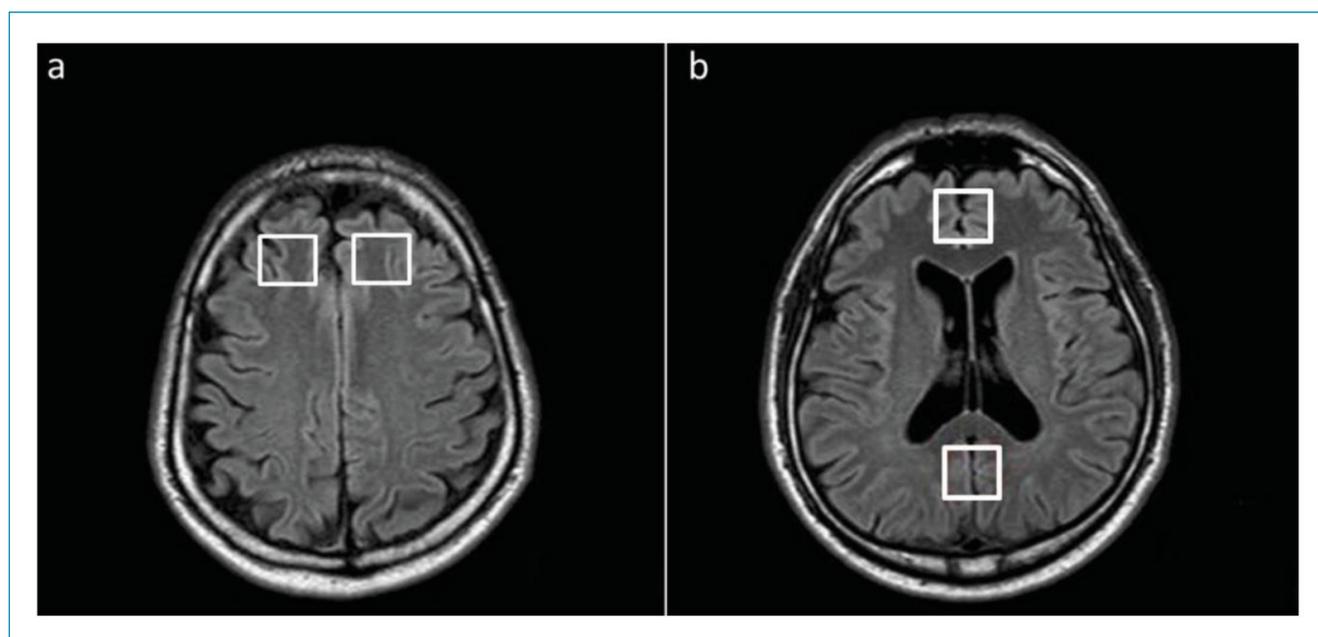


Figure 1. Illustrative magnetic resonance image showing the voxels analyzed (2 cm×2 cm×2 cm) represented in white boxes in (A) (right and left dorsolateral prefrontal) and in (B) (anterior and posterior cingulate).

from *N*-acetyl-aspartate (NAA) and choline (Cho) over Cr. Subsequently, all data were processed by the same examiner, a neuroradiologist (M.R.J.) with 8 years of experience, through the jMRUI program (public-domain MR user-interface software)^{10,11}. In the jMRUI analysis, the Advanced Method for Accurate, Robust, and Efficient Spectral fitting (AMARES) algorithm was used to fit the time-domain data¹², with water peak assigned to 4.68 ppm, NAA to 2.02 ppm, Cr to 3.0 ppm, and Cho to 3.2 ppm. The spectral curves were classified as good quality or inadequate quality for analysis, based on the baseline characteristics (the quality of water suppression and the quality of the separation of metabolite peaks), as well as the frequency, shape, and amplitude of the identified peaks. Inadequate spectroscopies were excluded from the analysis, due to the low reliability of these results and to the already known greater difference between SV and jMRUI when analyzing bad-quality spectroscopies¹³.

Statistical analysis

Initially, the metabolites were analyzed descriptively (mean, lower and upper values, and standard deviation). Subsequently, we executed a linear regression analysis with the SV's results introduced as the independent variable and the jMRUI's results as the dependent variable, separately by group, metabolite, and location analyzed.

To identify the agreement between the programs, we applied the adapted method of Bland and Altman plot¹⁴ as follows: in Y-axis, we located the ratio between the value obtained by jMRUI over the value obtained by SV in each measurement (jMRUI/SV); while in X-axis, we located the mean of the values obtained by the methods in each measurement [(jMRUI+SV)/2].

Then, we calculated and included in this graph the 95% limits of agreement (LoA; -1.96 SD and +1.96 SD of jMRUI/SV), which represent the interval in which we expected to find 95% of the values of the ratio jMRUI/SV.

The statistical analysis was performed using SPSS version 23 software (IBM Corp., USA), and p-value <0.05 was considered statistically significant.

RESULTS

In total, 41 individuals (23 healthy non-AUD subjects and 18 AUD patients) were included in the statistical analysis, after the exclusion of 1 AUD patient due to cerebral lesion and 4 AUD patients who presented spectroscopies with a heterogeneous pattern (low quality). NAA value from AC of one AUD patient and Cho value from CP of one non-AUD subject were also excluded from the analysis due to the low quality of their peaks.

Means and standard deviations obtained by both programs are presented in Table 1. Notably, the standard deviations from jMRUI were greater than those from the SV.

For most of the metabolites, there was a significant correlation between the methods' results ($p < 0.01$). However, in AUD individuals, we could predict at most 69.6% of the variance of NAA/Cr in FE from jMRUI with SV as the independent variable ($p < 0.0001$; 95%CI 0.648–1.348), while at most 44.3% of the variance of NAA/Cr in FE of non-AUD individuals ($p = 0.001$; 95%CI 0.408–1.253). Therefore, although there was a correlation between most of the results, we could not create a linear prediction model between the methods.

Additionally, the 95% LoA were variables for each metabolite and analyzed regions (Figure 2). Among the analyses, the

Table 1. Metabolites' concentration ratios per voxels' locations.

	AUD/Non-AUD	jMRUI				SpectroView®			
		AUD		Non-AUD		AUD		Non-AUD	
	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NAA/Cr (LF)	18/23	1.51	0.58	0.49	0.3	1.53	0.48	1.52	0.24
Cho/Cr (LF)	18/23	0.9	0.21	1.09	0.33	0.96	0.18	1.01	0.08
NAA/Cr (RF)	18/23	1.47	0.31	1.5	0.4	1.32	0.21	1.51	0.26
Cho/Cr (RF)	18/23	0.95	0.17	1.03	0.39	0.94	0.12	1.05	0.17
NAA/Cr (AC)	17/23	1.41	0.44	1.58	0.43	1.35	0.29	1.32	0.16
Cho/Cr (AC)	18/23	0.76	0.18	0.86	0.2	0.84	0.12	0.9	0.11
NAA/Cr (PC)	18/23	1.47	0.43	1.59	0.36	1.49	0.18	1.54	0.14
Cho/Cr (PC)	18/22	0.57	0.11	0.6	0.07	0.61	0.06	0.63	0.08

jMRUI: J-magnetic resonance user interface; AUD: alcohol used disorder; SD: standard deviation; LF: location of voxels (left frontal); RF: right frontal; AC: anterior cingulate; PC: posterior cingulate.

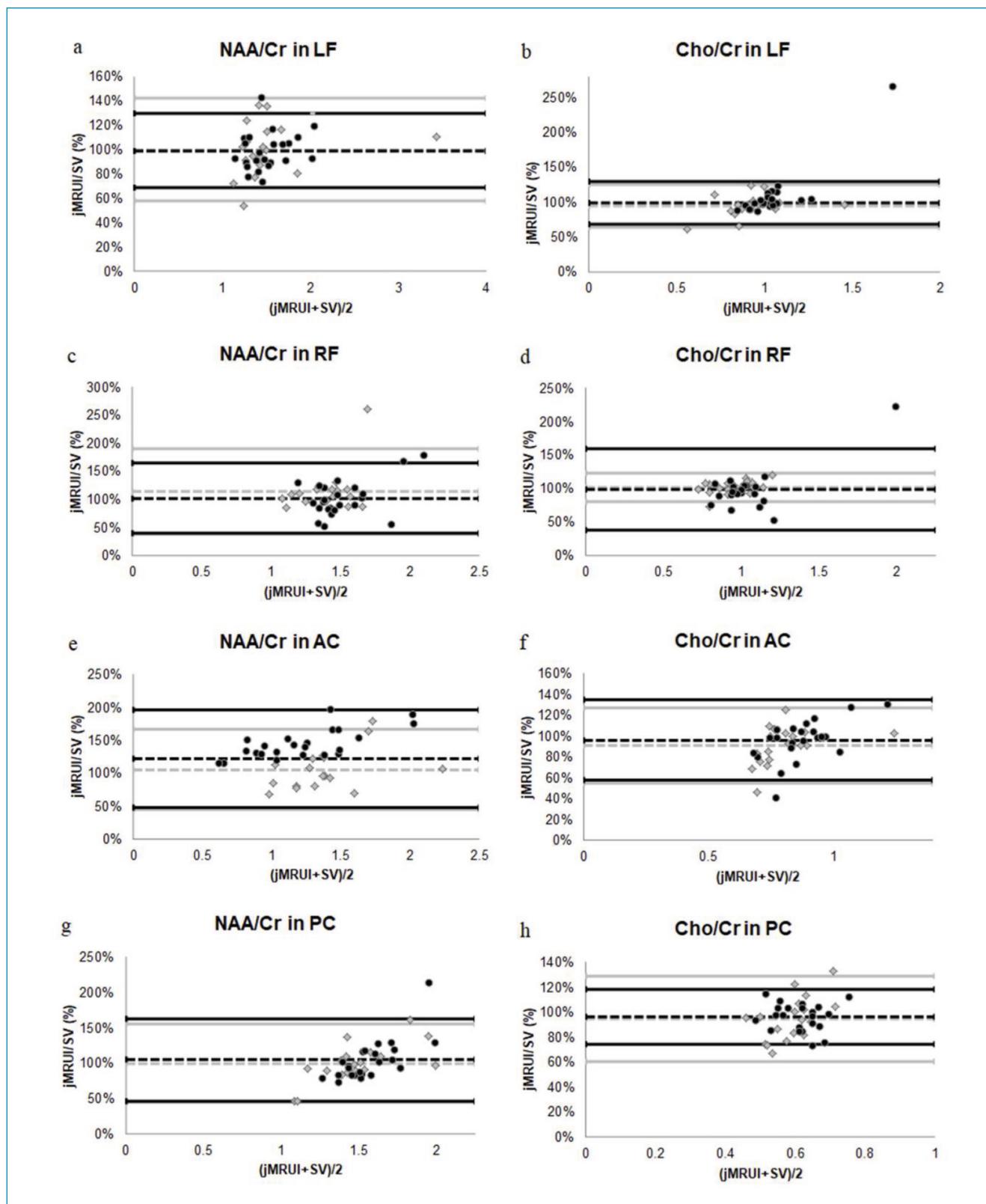


Figure 2. Bland-Altman plots (a-h) showing the ratio of the values obtained by magnetic resonance user interface over the values obtained by SpectroView® (in percentage) in the Y-axis and the mean values between the programs in the X-axis for each individual. The alcohol use disorder group is represented in gray and the healthy non-alcohol use disorder group in black. Mean values for each group are represented as dashed lines and upper and lower 95% limits of agreement are represented as solid lines. Location of voxels is represented as left frontal, right frontal, anterior cingulate, and posterior cingulate.

narrowest LoA were from Cho/Cr in RF from AUD patients (therefore, the most agreeable metabolite), with 95% of jMRUI measurements' values expected to vary between 80% and 122% of SV measurements. In contrast, the broadest LoA were from NAA/Cr in RF from AUD patients, with 95% of the expected results of jMRUI ranging from 38% to 190% in comparison with the same measurements obtained by SV.

DISCUSSION

In this study, we compared the ¹H-MRS postprocessing performed by using an automatic method (SV) and a semiautomatic method (jMRUI), in AUD and non-AUD individuals, since it is expected that different steps followed by each model of postprocessing may result in different measurements of the metabolite ratios¹. The semiautomatic postprocessing model depends on specialized knowledge and is subjected both to the experience of the examiner and to protocols of different hospitals and research centers¹³. In contrast, the automatic model executes standardized and automatic steps, not depending on a specialized examiner.

Standard deviations of the measurements from the semiautomatic method were greater than those from the automatic method in both AUD and non-AUD groups. This finding supports the previous study by Mazzoni et al.¹³, which demonstrated the same tendency in healthy individuals. In addition, this study showed greater standard deviations in jMRUI also when analyzing patients with expected brain metabolic changes, as demonstrated in studies with AUD patients^{15,16}.

Although the methods presented a positive correlation between their results in most metabolites, it was not possible to create a general prediction model to determine the variance of semiautomatic values predicted by automatic results.

Previous studies showed that adjacent structures, such as the liquor and the skull, could influence ¹H-MRS results. Besides, Mazzoni et al.¹³ verified greater LoA between the programs when evaluating regions closer to the liquor, in comparison with more distant regions, and therefore, they hypothesized that the proximity to the water signal could increase the discordance between the software. In contrast, we did not find such a difference when comparing the LoA of the areas closer to the liquors (i.e., AC and PC) with those more distant from the water signals (i.e., RF and LF).

By analyzing the LoA, we estimated that 95% of jMRUI measurements expected for NAA/Cr in RF from AUD patients ranged from 38% to 190% of the value obtained by SV from the same metabolite, representing a non-negligible difference in the spectroscopic analysis. Illustratively, a previous study by Bulakbasi et al.¹⁷ analyzing spectroscopic differences from

malignant tumor, benign tumor, and control tissues identified a mean value for NAA/Cr of 1.05, 1.42, and 1.68, respectively, being this difference of less than 50% of NAA/Cr mean between these tissues significant in the differentiation of tumors ($p < 0.05$)¹⁸. LoA such as these found in our study evidentiate the possible magnitude of difference between the methods and the relevance of adapting reference values to each of them, as well as the importance of specifying the software and the steps of postprocessing adopted in studies.

In spite of the existing studies and data banks that establish reference values¹⁸, there is no gold-standard spectroscopic postprocessing method defined yet. Therefore, a common practice in research evaluating brain alterations through ¹H-MRS is the use of control individuals as parameters of comparison, instead of using a predetermined reference value for identifying brain alterations. Similarly, we considered that in clinical practice, it would be useful to create a spectroscopic data bank in each institution (with its standardized hardware and software), in order to facilitate the clinical application of MRS-H.

As a limitation, our sample size limits the statistical comparison between the software. Besides, the use of ratios of NAA and Cho over Cr does not allow us to determine if the differences in the measurement of metabolites are due to the analysis of Cr, of both NAA and Cho, or of all of them.

CONCLUSIONS

The analyzed methods presented differences that may be clinically significant in both AUD and non-AUD individuals. Due to the smaller dispersion presented by the results from SV and the facility in the method execution, we suggest that the automatic may be preferred in clinical practice.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval was provided by the Conselho Brasileiro de Ética em Pesquisa da Universidade Federal do Espírito Santo (CAAE 19403713.6.0000.5060 and 13528213.2.0000.5060), Brazil.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in this study.

AUTHORS' CONTRIBUTIONS

TMR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft. **TDCE:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. **RSMS:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization,

Writing – review & editing. **EMNP:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **MRJ:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing

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Self-Estimated functional inability because of pain questionnaire for workers: a reliability and construct validity study

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Cid André Fidelis de Paula Gomes⁵ , Daniela Bassi-Dibai¹ 

SUMMARY

OBJECTIVE: The objective of this study was to investigate the reliability and construct validity of the Self-Estimated Functional Inability because of Pain questionnaire for workers in a sample of Brazilian workers with musculoskeletal pain.

METHODS: This is a questionnaire validation study. Workers with musculoskeletal pain were included. Besides the Self-Estimated Functional Inability because of Pain questionnaire for workers, we used the following instruments to perform construct validity: the Work Ability Index and the Numerical Pain Rating Scale. A subsample answered the Self-Estimated Functional Inability because of Pain questionnaire for workers in two moments to calculate reliability by means of the intraclass correlation coefficient and internal consistency by means of the Cronbach's alpha.

RESULTS: A total of 190 Brazilian workers were included. Regarding the construct validity, we observed a correlation with magnitude above 0.50 between the Self-Estimated Functional Inability because of Pain questionnaire for workers and the Numerical Pain Rating Scale, given that these two instruments have similarity in the construct, and correlations above 0.30 between the Self-Estimated Functional Inability because of Pain questionnaire for workers and the domains 2, 3, and 4 of the Work Ability Index. Regarding reliability, we observed adequate reliability (intraclass correlation coefficient=0.864) and internal consistency (Cronbach's alpha=0.807).

CONCLUSION: The Self-Estimated Functional Inability because of Pain questionnaire for workers is a reliable and valid instrument to be used in Brazilian workers with musculoskeletal pain.

KEYWORDS: Pain. Surveys and questionnaires. Reproducibility of results.

INTRODUCTION

Self-report measures are important and routinely used by researchers and health professionals to analyze occupational dysfunctions and gestures¹. Considering pain complaints and musculoskeletal

disorders, the most commonly used instrument for these purposes in research and in the clinical and occupational environments is the Nordic Musculoskeletal Questionnaire (NMQ). This instrument organizes musculoskeletal pain complaints in a regionalized

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manner, considering body parts². However, the limitation of the NMQ is that it does not present a numeric pain or disability score.

In this study, we analyzed a new instrument that was recently created with the aim of measuring musculoskeletal pain and disability in Brazilian workers: the Self-Estimated Functional Inability because of Pain (SEFIP-work)³. This questionnaire allows the regionalized analysis of pain and disability in the body, as well as the NBQ, however, with the positive aspect of generating interpretable numerical scores. The SEFIP was initially developed and validated to investigate the musculoskeletal injuries in dancers (SEFIP-dance)^{4,5}. However, due to its broad structure, this questionnaire has been adapted for athletes (SEFIP-sport)^{5,6} and for workers (SEFIP-work)³.

Considering only the SEFIP-work, the pioneer study conducted by Pinheiro et al.³ evaluated only the content validity. However, other measurement properties need to be investigated to properly support the use of the SEFIP-work in the occupational context⁷. Thus, the present study focuses on this gap in the literature, aiming to investigate the reliability and construct validity of the SEFIP-work in a sample of Brazilian workers. Our hypothesis is that this instrument presents acceptable measurement properties.

METHODS

Ethical aspects and study design

The research was conducted by means of an online platform (Google Forms, Mountain View, CA, USA) with Brazilian workers with musculoskeletal pain and was based at the Universidade Ceuma (São Luís, MA, Brazil). The study procedures were approved by the ethics committee in research of the institution (opinion number: 3.779.579).

This is a questionnaire validation study carried out in accordance with the recommendations of the Consensus-based Standards for the Selection of Health Measurement Instruments (COSMIN)⁷.

Participants

To calculate the sample size, the minimum recommendation of 100 participants were used⁸. The inclusion criteria were workers active for at least 6 months in the same job, aged 18 or above, able to read and write in Brazilian Portuguese, and with musculoskeletal pain. The participants excluded from the study were workers diagnosed with severe cognitive and/or psychiatric illnesses, and workers away from the work environment.

Self-Estimated Functional Inability because of Pain questionnaire for workers-work

The questionnaire consists of 14 items, each item related to a body part, and Likert score for each item (0–4). Thus, the total

score varies between 0 and 56 points; the higher the score, the higher the disability. In addition to this total score, we calculated a separate score for each body region to produce a score from 0 (no disability) to 4 (maximum disability). The version of the SEFIP-work in Brazilian Portuguese and English are available in a previous study³.

Others instruments

The Work Ability Index (WAI) was used to measure the ability to work. This instrument was adapted to the Brazilian Portuguese⁹ and consists of seven domains: domain 1, ability to work at present and compared to the best in life (0–10, score); domain 2, ability to work in relation to the job requirements (2–10, score); domain 3, current number of self-reported and physician-diagnosed illnesses (1–7, score); domain 4, estimated loss for work due to the illnesses (1–6, score); domain 5, absence from work due to the illnesses (1–5, score); domain 6, own prognosis about work ability (1–7, score); and domain 7, mental resources (1–4, score).

The 11-point Numerical Pain Rating Scale (NPRS) was used to measure the highest pain intensity in the body, where 0 means “no pain” and 10 represents “the most excruciating pain.” This instrument has been validated for Portuguese¹⁰.

Statistical analysis

Descriptive analysis was used to characterize the sample, with the presentation of quantitative data using mean and standard deviation and qualitative data using number and percentage.

The test–retest reliability was determined by means of the intra-class correlation coefficient (ICC), standard error of measurement (SEM), and minimum detectable change (MDC). The ICC value was considered adequate when greater than 0.75¹¹. Value between 0.70 and 0.95 was considered adequate for Cronbach’s alpha⁸.

To determine the validity of the construct, Spearman’s correlation coefficient (ρ) was used to determine the magnitude of the correlation between the SEFIP-work, WAI, and NPRS. Our hypothesis is that the SEFIP-work score presents a correlation magnitude greater than 0.50 with the NPRS (similar construct) and between 0.50 and 0.30 with the domains of the WAI (related but dissimilar constructs), as described by the COSMIN⁷.

Data processing was performed using SPSS, version 17.0 (Chicago, IL, USA). All analyses were performed considering a significance level of 5%.

RESULTS

A total of 190 Brazilian workers were included in the study. The characterization of the sample is described in Tables 1 and 2, and it was observed that most workers were young adults, women, slightly overweight, physically active, and nonsmokers.

Table 1. Descriptive analysis of personal and occupational characteristics of a quantitative nature.

	Mean (standard deviation)
Age (years)	37.28 (10.33)
Mass (kg)	72.29 (16.16)
Height (m)	1.66 (0.08)
Body mass index (kg/m ²)	25.81 (4.60)
Total working time (months)	107.46 (97.86)
Weekly workload (h)	33.98 (15.11)
Numerical Pain Rating Scale (score, 0–10)	3.71 (2.88)
Work Ability Index	
Domain 1 (score 0–10)	8.09 (1.43)
Domain 2 (score 2–10)	7.96 (1.44)
Domain 3 (score 1–7)	3.71 (2.34)
Domain 4 (score 1–6)	5.47 (0.72)
Domain 5 (score 1–5)	4.50 (0.78)
Domain 6 (score 1–7)	6.36 (1.56)
Domain 7 (score 1–4)	3.06 (0.77)
SEFIP-work	
Neck (score 0–4)	0.52 (0.72)
Shoulders (score 0–4)	0.39 (0.71)
Elbows (score 0–4)	0.08 (0.40)
Wrists/hands (score 0–4)	0.30 (0.62)
Upper back (score 0–4)	0.61 (0.75)
Lower back (score 0–4)	0.65 (0.75)
Hips (score 0–4)	0.27 (0.60)
Thighs (front) (score 0–4)	0.07 (0.29)
Thighs (back) (score 0–4)	0.12 (0.39)
Knees (score 0–4)	0.31 (0.62)
Legs (front) (score 0–4)	0.19 (0.51)
Calves (score 0–4)	0.14 (0.43)
Ankles (score 0–4)	0.14 (0.44)
Feet (score 0–4)	0.30 (0.64)
Total (score 0–56)	4.14 (4.67)

SEFIP-work: Self-Estimated Functional Inability because of Pain questionnaire for workers. Work Ability Index. Domain 1: ability to work at present and compared to the best in life; Domain 2: ability to work in relation to the job requirements; Domain 3: current number of self-reported and physician-diagnosed illnesses; Domain 4: estimated loss for work due to the illnesses; Domain 5: absence from work due to the illnesses; Domain 6: own prognosis about work ability; Domain 7: mental resources.

Regarding the construct validity, we observed a correlation with magnitude above 0.50 between the SEFIP-work and the NPRS, given that these two instruments have similarity in the construct, and correlations above 0.30 between the SEFIP-work and the domains 2, 3, and 4 of the WAI, as shown in Table 3.

Regarding reliability, a subsample composed of 33 participants answered the SEFIP-work in two moments to analyze the test–retest reliability. Thus, we observed adequate reliability (ICC=0.864) and internal consistency (Cronbach's alpha=0.807), as shown in Table 4.

Table 2. Descriptive analysis of personal and occupational characteristics of a qualitative nature.

Characteristics	Number (%)
Sex	
Female	124 (65.3)
Male	66 (34.7)
Marital status	
Single	87 (45.8)
Married	87 (45.8)
Divorced	16 (8.4)
Scholarity	
Complete primary level	1 (0.5)
Incomplete secondary level	1 (0.5)
Complete secondary level	31 (16.3)
Incomplete higher education	24 (12.6)
Complete higher education	42 (22.1)
Incomplete postgraduate	9 (4.7)
Complete postgraduate	82 (43.2)
Physical activity	
Yes	101 (53.2)
No	89 (46.8)
Posture at work	
Standing	33 (17.4)
Sitting	64 (33.7)
Standing/sitting	90 (47.3)
Standing/sitting/lying down	3 (1.6)
Type of work	
Manual	53 (27.8)
Non-manual	20 (10.6)
Both	103 (54.2)
Others	14 (7.4)
Smoking	
Yes	6 (3.2)
No	184 (96.8)

Table 3. Correlation between the self-estimated functional inability because of pain questionnaire for workers – work and the other instruments used in this study.

Instruments	Self-estimated functional inability because of pain questionnaire for workers	
	rho	p-value
Numerical Pain Rating Scale	0.638	<0.001*
Work Ability Index		
Domain 1	-0.285	<0.001*
Domain 2	-0.344	<0.001*
Domain 3	-0.312	<0.001*
Domain 4	-0.427	<0.001*
Domain 5	-0.199	0.006*
Domain 6	-0.149	0.041*
Domain 7	-0.185	0.011*

*Significant correlation ($p < 0.05$, Spearman's correlation coefficient). Work Ability Index. Domain 1: ability to work at present and compared to the best in life; Domain 2: ability to work in relation to the job requirements; Domain 3: current number of self-reported and physician-diagnosed illnesses; Domain 4: estimated loss for work due to the illnesses; Domain 5: absence from work due to the illnesses; Domain 6: own prognosis about work ability; Domain 7: mental resources.

Table 4. Reliability and internal consistency of the SEFIP-work.

Test	Retest	ICC (95%CI)	SEM	MDC	Cronbach's alpha
2.90 (2.60)	3.57 (2.92)	0.864 (0.667–0.919)	1.02	2.82	0.807

ICC: intraclass correlation coefficient; CI: confidence interval; SEM: standard error of measurement; MDC: minimum detectable change.

DISCUSSION

Our study demonstrated that the SEFIP-work is a reliable questionnaire, with adequate internal consistency, and presents satisfactory correlation with the NPRS and with the following domains of the WAI: domain 2 (ability to work in relation to the job requirements), domain 3 (current number of self-reported and physician-diagnosed illnesses), and domain 4 (estimated loss for work due to the illnesses).

This is the first study carried out verifying the measurement properties of the SEFIP in workers (SEFIP-work). However, validation studies have already been carried out with dancers (using SEFIP-dance) and athletes (using SEFIP-sport).

Similar to our study, the Turkish version of the SEFIP-dance had adequate test–retest reliability (ICC=0.807) and a correlation magnitude of 0.672 with the Visual Analogue Scale (VAS)¹². The Brazilian version of the SEFIP-sport also found adequate measurement properties, with ICC value of 0.91, Cronbach's alpha of 0.83 and a correlation magnitude of 0.481 with the NPRS⁶.

Other occupational health instruments were adapted and validated for Brazilian Portuguese: Rapid Upper Limb Assessment to measure the biomechanical exposure¹³, Rapid Entire Body Assessment to measure the biomechanical risks present in the workplace¹⁴, Quick Exposure Check to assess the perception of workers regarding the task demands and work conditions¹⁵, and Rapid Office Strain Assessment to measure the computer office work with risk levels¹⁶. However, no instrument available for use presents the main characteristic of the SEFIP-work: generating a numerical and interpretable disability score considering 14 parts of the body.

This study has limitations that should be highlighted. Data collection was performed using an online platform. Thus, no clinical assessment was performed to understand musculoskeletal pain and the occupational environment was not assessed. Therefore, the clinical and occupational characteristics were based on self-report.

CONCLUSION

SEFIP-work is a reliable and valid instrument to be used in Brazilian workers with musculoskeletal pain.

AUTHORS' CONTRIBUTIONS

JSPM: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **AVDF:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – review and editing. **CMO:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **CABP:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **DSR:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft. **GNS:** Data curation, Formal Analysis, Methodology, Writing – original draft. **CAFPG:** Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review and editing. **DBD:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Writing – review and editing.

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Advanced ankylosing spondylitis: a multisite, multimodality densitometric analysis for investigation of bone loss in the axial and appendicular skeleton

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SUMMARY

OBJECTIVE: The aim of this study was to investigate if there is a bias in bone mineral density measurements among major densitometric techniques across multiple skeletal sites.

METHODS: In 25 advanced ankylosing spondylitis patients, bone mineral density measurements were acquired in the lumbar spine, the hip, and the forearm.

RESULTS: In total, 60% of patients had a bone mineral density Z-score of -2 or less at one or more skeletal sites. Dedicated loss of cortical bone was identified at the distal forearm (60% of patients). Differences in bone mineral density measurements across all densitometric techniques were highly significant ($p \leq 0.001$). Bone loss was more striking in spinal trabecular bone by three-dimensional quantitative computed tomography [Z-score -2.1] versus dual-energy X-ray absorptiometry [Z-score 0]. A trabecular bone loss quantified by quantitative computed tomography was about twice as much as a cortical bone loss by single-energy X-ray absorptiometry ($p = 0.001$).

CONCLUSIONS: Low bone mineral density is prevalent in advanced ankylosing spondylitis patients, predominating in the spine. Bone mineral density measurements have systematic differences when compared to each other. Knowledge of these offsets is useful for improved diagnosis of regional bone loss that allows for targeted treatment of osteoporosis. Three-dimensional quantitative computed tomography is more suitable for evaluating spinal osteoporosis in advanced ankylosing spondylitis than dual-energy X-ray absorptiometry, which rather underestimates bone loss.

KEYWORDS: Ankylosing spondylitis. Bone mineral density. Osteoporosis. CT X ray. Dual-energy X-ray absorptiometry.

INTRODUCTION

Ankylosing spondylitis (AS) is characterized by spinal ligamentous calcification, osteoporosis, and increased risk for fractures¹⁻³. Reportedly, osteoporosis occurs with an incidence ranging between 18.7 and 62%⁴, and diagnosis becomes important for targeted pharmacologic therapies⁵.

Dual-energy X-ray absorptiometry (DXA) is the bone densitometric technique of choice for evaluating bone

mineral density (BMD) in AS patients⁶⁻¹¹. To acquire accurate BMD measurements, we used three-dimensional quantitative computed tomography (3DQCT) supplanted by DXA and single-energy X-ray absorptiometry (SXA), as well as compared 3DQCT with the other bone densitometric methods. We investigated if there is a bias in BMD measurements across all densitometric techniques when compared to each other.

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METHODS

Patients and spine radiographic assessment

We included 25 patients (20 males, 5 females; 32–80 years) with chronic AS¹² in our study. Prevalent fractures were defined using the grading scheme for vertebral deformity described by Genant¹³.

Bone density measurements

DXA—DXA measurements of bone mass were acquired in the L-spine (L1–L4) and the proximal femur. DXA provides an estimate of integral BMD representing both cortical and trabecular bone¹⁴.

SXA—BMD was measured in the non-dominant forearm, i.e., distal and ultradistal sites, by SXA. The distal forearm measurement is predominantly of cortical (87%) bone, and the ultradistal forearm is of trabecular (65%) bone¹⁴.

3DQCT—A total of 22 (88%) patients underwent BMD measurements in the L-spine (L2–L3) by QCT (i.e., 3DQCT) supplanted with three-dimensional Pro-software (Mindways, San Francisco, CA, USA). QCT allows a selective assessment of cortical and trabecular bone density and a true volumetric assessment of bone mass. BMD was judged reduced if the Z-score was equal to or below -2¹⁵.

Statistical analyses

The Wilcoxon signed-rank test was used to assess the differences between BMD Z-scores across skeletal sites. To test for differences by fracture status, the Mann-Whitney U test was used for median BMD Z-scores at each skeletal site. Correlations between BMD measurements were expressed as Spearman's correlation coefficients. A reduced probability level of 0.002 was accepted as significant in all analyses, except for the two-tailed Spearman's correlation ($p=0.01$).

RESULTS

Patient characteristics

The patient demographic variables are summarized in Table 1.

Fractures: The overall fracture rate was 56% (14/25 patients). The development of fracture was similar between men and women in terms of age and body mass index (BMI). The L-spine was the most common site of fracture (36%; 9/25).

Bone mineral density measurements

Table 1 presents cumulative BMD Z-score measures for our study population. The median Z-scores at all eight sites measured were predominantly negative, indicating an overall trend

toward a significant reduction of the BMD in patients (Figure 1). A BMD Z-score of -2 or less at one or more skeletal sites (osteoporosis) was found in 15 of the 25 AS patients (60%). A further 19 patients (76%) had a Z-score between -1 and -2 at any site (osteopenia). With DXA, spinal BMD [Z-score, 0 (-5.2–3.4)] was decreased in eight patients (32%). Using 3DQCT, spinal trabecular bone loss [Z-score, -2.1 (-5.2 – -0.05)] was identified in 19 patients (86.3%).

Total hip measurements showed decreased BMD in 10 patients (52.6%). BMD was reduced at the femoral neck in 10 patients (43.4%), and trochanteric BMD was reduced in nine patients (39.1%). Measurements at the distal forearm (cortical bone) and ultradistal forearm yielded reduced BMD [Z-score, -1.1 (-4.2–0.8)] in 12 patients (60%) and [Z-score, -0.8 (-4.5–1.1)] in eight patients (40%), respectively. Trabecular bone loss (by QCT) was about twice as much as cortical bone loss (by SXA) ($p=0.001$). Despite statistically not significant differences in BMD between fractured and non-fractured patients ($p<0.002$), the analysis of Z-scores showed a trend toward bone loss across all sites, with the exception of the forearm (Figure 2).

Table 1. Median (range) demographic and bone mineral density Z-scores of patients with advanced Ankylosing spondylitis.

	Ankylosing spondylitis patients (n=25)
Age (years)	55 (32–80)
Weight (kg)	68.1 (49–113)
Height (m)	1.64 (1.4–1.82)
Body mass index (kg/m ²)	24.5 (18.3–35.5)
Z-Distal forearm	-1.1 (-4.2–0.8) n=20
Z-Ultra distal forearm	-0.8 (-4.5–1.1) n=20
Z-Lumbar spine Dual-energy X-ray absorptiometry	0 (-5.2–3.4) n=25
Z-Lumbar spine quantitative computed tomography	-2.1 (-5.2– -0.05) n=22
Z-Femoral neck	-0.9 (-3.3–1.8) n=23
Z-Total hip	-1 (-3.3–1.8) n=19
Z-Trochanter	-0.8 (-2.9–1.6) n=23
Z-Ward's triangle	-0.8 (-3.6–0.9) n=23

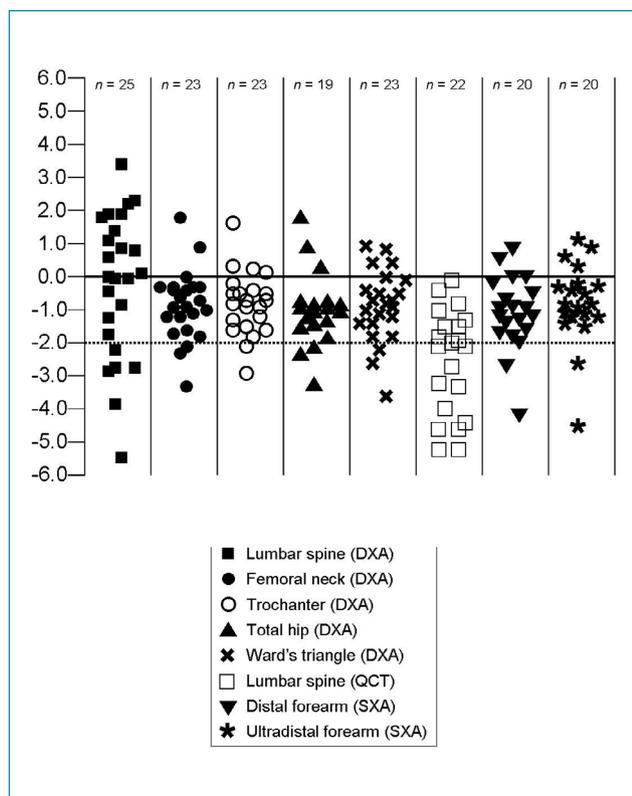


Figure 1. Bone mineral density Z-scores in ankylosing spondylitis patients. The solid horizontal line indicates the mean Bone mineral density Z-score of an age- and sex-matched control population, and the broken horizontal line indicates a Bone mineral density Z-score of -2.

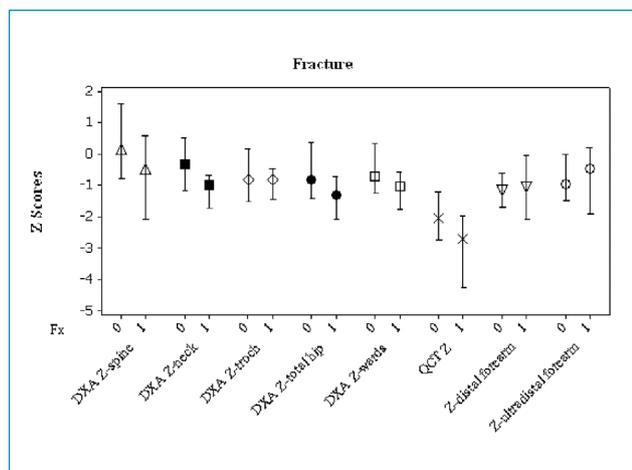


Figure 2. Spinal (Dual-energy X-ray absorptiometry and quantitative computed tomography), femoral neck (Dual-energy X-ray absorptiometry), trochanteric (Dual-energy X-ray absorptiometry), total hip (Dual-energy X-ray absorptiometry), and forearm (single-energy X-ray absorptiometry) Z-scores (median values) for Ankylosing spondylitis patients with (1) and without (0) fractures.

Relationships between BMD Z-scores assessed at multiple sites by various bone densitometric methods

Correlations between the different bone density metrics were analyzed. In comparing the decrease in BMD at the L-spine as measured by DXA and 3DQCT, there was a significant difference between densitometric techniques ($p < 0.001$). We found a moderate, yet significant, correlation between the 3DQCT L-spine BMD measurements and femoral neck DXA-acquired BMD measurements (Spearman's correlation coefficient $r = 0.699$, $p = 0.001$). Significant differences were also found when comparing QCT L-spine measurements of BMD versus SXA-based BMD measurements at the distal and ultradistal forearm ($p = 0.001$). Similar significant differences in BMD Z-scores were recorded between L-spine measurements by QCT and DXA-based measurements at the femoral neck ($p < 0.001$), Ward's triangle ($p < 0.001$), and the trochanter ($p < 0.001$).

Measurements proved poor correlation between Z-scores by 3DQCT at the L-spine and Z-scores by DXA at the L-spine ($r = 0.447$, $p = 0.037$), the trochanter ($r = 0.544$, $p = 0.013$), total hip ($r = 0.6$, $p = 0.011$), and the forearm (by SXA) ($r = 0.365$ – 0.5 , $p = 0.041$ – 0.15). No correlation was found between Z-scores either at the L-spine by DXA or at the distal forearm by SXA ($r = 0.368$, $p = 0.11$).

DXA-acquired hip measurements showed that there was a strong correlation between Z-scores at the trochanter and the femoral neck, total hip, and ultradistal forearm ($r = 0.793$ – 0.887 , $p = 0$). A similar strong correlation was noted between Z-score at the femoral neck and the total hip ($r = 0.794$, $p = 0$). Correlation between Z-scores (by DXA) at the L-spine and the femoral neck ($r = 0.683$, $p = 0$), trochanter ($r = 0.723$, $p = 0$), and total hip ($r = 0.701$, $p = 0.001$) was good.

Distal forearm Z-scores (calculated by SXA measurements) were correlated well with the trochanter ($r = 0.766$, $p = 0$), the femoral neck ($r = 0.744$, $p = 0$), and the total hip ($r = 0.695$, $p = 0.003$). Similarly, the ultradistal forearm measurements (by SXA) were correlated well with the distal forearm ($r = 0.764$, $p = 0$), the femoral neck ($r = 0.754$, $p = 0$), the L-spine ($r = 0.651$, $p = 0.002$), and the total hip ($r = 0.683$, $p = 0.004$). Comparing Z-scores across different sites, with the exclusion of those obtained by QCT, the differences no longer reached the statistical significance (p -values from 0.002 – 0.983).

DISCUSSION

The characteristics of the AS patients are similar to those in other studies with respect to sex, age, BMI, and fractures^{16,17}. Few studies have used dedicated QCT measurements to determine the bone mass in AS, which may have implications for treatment¹⁸.

The major findings in this study indicate that 3DQCT can be effectively used to acquire bone mass measurements that differ significantly from measurements acquired by other bone densitometric methods, in both the trabecular and cortical bone. No prior studies have used QCT in addition to DXA and SXA to evaluate osteoporosis in patients with advanced AS.

In the pathophysiology of bone loss in AS, proposed mechanisms^{1,18,19} have included subclinical inflammatory bowel disease with malabsorption of calcium and vitamin D^{18,20,21} and secondary hyperparathyroidism, which may induce intracortical bone loss. Once there, the appendicular skeleton (rich in cortical bone) is more likely to be offended by changes in parathyroid hormone due to the malabsorption of calcium. Our finding of bone loss in the distal forearm of patients, an area rich in cortical bone¹⁴, lends further support to the coexistence of secondary hyperparathyroidism and AS.

Most previous investigators have focused on bone mass changes at the L-spine and proximal femur and have not reported the changes of bone mass occurring in the appendicular skeleton^{21,22}. Sarikaya et al.¹⁹ concluded that osteoporosis affects the hip sparing the forearm. Unlike these investigators, Will et al.²³ found a 5% decrease in BMD at the carpus, suggesting that bone loss involves both the cortical and trabecular bone. In accordance with Will et al.²³, we found reduced bone density at the radius, as opposed to the previous studies^{2,10,19,24}. Other studies also support our results about reduced cortical BMD at peripheral sites^{2,8}, suggesting a possible link between systemic inflammation and bone resorption. Our measurements show that the trabecular and cortical bone appear to have considerable differences in the rate of bone loss, with the overall difference of trabecular versus cortical bone loss being nearly double ($p=0.001$). Similar findings are reported by other investigators¹⁷ who postulate that trabecular and cortical bone compartments appear to have different biological behaviors to systemic inflammation.

In AS, the frequency of osteoporotic fractures varies from 0 to 40.9%^{3,5,6,11,16,20,22}. Our data indicate a high vertebral fracture rate of 36%, owing probably to advanced AS. Although the decreased bone mass was not statistically significant between fractured and non-fractured patients, in our series, bone loss was trending across all sites with the exception of the forearm. A possible explanation for this noteworthy finding would relate to the non-weight-bearing properties of the forearm as opposed to other sites. Similarly, other investigators²⁵ have reported no significant BMD differences at the L-spine between fractured and non-fractured patients, whereas BMD at the femoral neck⁵ and total hip was significantly lower in fractured patients.

The frequency of bone loss in AS varies, depending on the site used for the measurement of the BMD. A significant decrease in

BMD of the proximal femur has been documented in late AS^{8,25}. Karberg et al.¹⁰ detected a bone loss in 86% of the AS patients at the femoral neck. Bone loss was found in 54.5% of patients at the femoral neck and in 52.3% of patients at the trochanter and total hip, with an overall bone loss recorded in 69% of the patients⁷. Other investigators^{9,11} have reported variably decreased BMD at the femoral neck and L-spine in 13.7–41.2% and 18.7–46.5% of AS patients, respectively. We found bone loss in 39.1% of patients either at the femoral neck or at the trochanter, 52.6% at the total hip, and 32% at the spine by DXA.

As DXA and SXA are the projectional 2D techniques, the discrimination between the cortical and trabecular bone is not feasible. QCT is unique among current bone densitometric techniques, providing separate estimates of trabecular and cortical BMD as a true volumetric mineral density. Indeed, this exquisite capability to distinguish BMD measurements between bone compartments is important because the trabecular bone has a higher turnover rate than cortical bone and greater responsiveness to many metabolic stimuli and drugs. QCT confers the benefit of excluding confounding factors that may falsely elevate BMD such as extraneous calcification, in contrast to DXA or SXA. In patients with late-stage AS, Lee et al.⁸ found decreased BMD at the L-spine when measured by QCT (Z-score -3.85 ± 1.33), whereas BMD at the same site was not low when measured by DXA (Z-score 0.79 ± 2.57). Similarly, another study¹⁰ has indicated that bone loss in the spine measured by QCT (54% of patients) is not equivalent to bone loss measured by DXA (40% of patients). Additional studies in AS patients have validated discrepancies between QCT and DXA measurements of BMD²⁴. Our results determine low bone mass by QCT in 86% of AS patients, as opposed to 32% of the patients by DXA, and are supported by the available literature.

Limitations to this study include an inherent selection bias created because only patients with advanced disease were studied without comparison to a reference standard. Also, a relatively small number of patients with late-stage AS were examined with the various bone densitometric techniques. Finally, not all densitometric techniques were applied in all patients. Notwithstanding its limitations, however, this study suggests that in late AS patients, discrepancies in BMD values are present among all densitometric techniques when compared to each other.

Our measurements indicate that generalized reductions in BMD are present not only at the sites of predominant trabecular bone (spine), or predominant cortical bone (distal forearm), but also at the sites of mixed cortical and trabecular bone (proximal femur), implying that both the trabecular and cortical bone components, in both the axial and appendicular skeletal sites, are offended in advanced AS. Although DXA remains the

most widely accepted clinical tool for the measurement of the BMD, our findings suggest that QCT may play a unique and pivotal role in the accurate assessment of spinal osteoporosis associated with advanced AS. Most importantly, our results show that the true pattern of the demineralization of the axial skeleton may be more accurately and precisely reflected by QCT. It appears that confounding factors related to the state of the AS disease can contribute to spurious results by DXA. Based on our results, we clearly recommend the use of QCT over DXA that will allow clinicians the optimal assessment of the true spinal BMD, in late-stage AS.

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AUTHORS CONTRIBUTIONS

DJT: Conceptualization, Writing – original draft, Writing – review & editing. **SJT:** Data curation, Formal analysis, Writing – review & editing. **YK:** Conceptualization, Writing – review & editing. **ID:** Data curation, Formal analysis. **NT:** Data curation, Formal analysis.

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Atrial fibrillation as a preoperative risk factor predicts long-term mortality in elderly patients without heart failure and undergoing hip fracture surgery

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SUMMARY

OBJECTIVE: Patients with atrial fibrillation (AF) constitute a significant portion of hip fracture patients, and both diseases tend to present more frequently in older age. Our goal was to evaluate the long-term mortality of patients with AF who were free from heart failure undergoing hip fracture surgery.

METHODS: This observational, retrospective study was done in a single research and training hospital setting. Hospital electronic health record data, National Health Registry data, and National Death Registry System data for 233 consecutive patients who were above 65 years of age and were planned to undergo surgery for hip fracture were retrieved and analyzed. An experienced cardiologist evaluated the patients prior to surgery. Each member of the research cohort was categorized into one of the two groups based on their survival status (survivor and non-survivor groups).

RESULTS: Of the 233 cases, 89 (38.2%) who were included in the investigation died during the follow-up period. The median long-term follow-up period was 34 (12–42) months. The frequency of AF was significantly higher in the non-survivor group. In multivariable Cox regression analysis, AF (HR: 2.195, 95%CI 1.365–3.415, $p < 0.001$), advanced age, and blood urea level were determined as independent predictors for all-cause long-term mortality.

CONCLUSIONS: AF is an independent predictor for long-term death in hip fracture cases above 65 years of age who were free from heart failure.

KEYWORDS: Atrial fibrillation. Hip fractures. Mortality. Long-term effects.

INTRODUCTION

The prevalence of atrial fibrillation (AF) significantly increases as people get older, making it the common arrhythmia in people above 65 years of age¹. In those above 65 and 80 years of age, the prevalence is roughly 5% and 10%, respectively. Moreover, approximately 70% of cases suffering from AF are

between the ages of 65 and 85 years². Prior studies reported that AF was linked with a 1.9-fold increase in death rate during the long-term follow-up even after adjusting for coexisting cardiovascular diseases³. Additionally, even though AF is not included in the current perioperative risk prediction models for noncardiac surgery risk assessment, a large database registry clearly

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reported that patients with AF had a higher risk of perioperative and postoperative complications than those with coronary artery disease⁴.

Hip fractures that are commonly observed in elderly people are described as skeletal system injuries. Remarkably, when compared to those without a hip fracture, older people with a hip fracture have a 5- to 8-fold higher chance of dying during the first 3 months⁵, and this increased risk of death persists for around 10 years⁶. Moreover, elderly people operated for hip fractures have a shorter life expectancy than their counterparts in the same age group⁷.

The presence of AF in elderly patients suffering from hip fracture is a common clinical scenario, and AF shares a number of risk factors with hip fracture, including diabetes, elder age, hypertension, and heart failure (HF). Even though some studies show that both AF and HF are associated with hip fracture due to an increase in the incidence of falls resulting from stroke and medication⁸, few studies evaluate the effect of AF on long-term mortality in elderly cases without HF and who underwent surgery for hip fracture. As a consequence, the main goal of this investigation was to evaluate the impact of AF on long-term mortality in elderly patients above 65 years of age with normal left ventricle function and who were recently operated for hip fracture.

METHODS

Study cohort

This study was planned as a single-center, observational, and retrospective cohort investigation. Between January 2017 and December 2019, the data of patients who were above 65 years of age and for whom a preoperative cardiology consultation was requested before the surgery due to hip fracture were analyzed. Patients who died during the index hospitalization and who were diagnosed with paroxysmal AF and HF were excluded from the study. Finally, 233 consecutive elderly cases undergoing surgery for hip fracture were analyzed. The use of medications, the presence of comorbidities, diabetes, hypertension, hyperlipidemia, previous cerebrovascular accident, and demographic features such as age were gathered from the electronic medical files. Additionally, pre-op laboratory values, including hemoglobin level, platelet count, blood creatinine, and thyroid-stimulating hormone levels, were analyzed. AF was defined as the absence of P waves, the presence of fibrillatory waves, and irregularly irregular QRS complexes on surface electrocardiography (ECG) that failed to self-terminate within 7 days. For each patient, the standard surgical procedures, such as closed reduction, open reduction, and hemiarthroplasty, were

applied. Our study design was evaluated and approved by the Local Ethics Commission (21/356).

Preoperative evaluation

All cases were evaluated by an experienced consultant physician before the operation, together with electrocardiography and transthoracic echocardiography within the framework of the recommendations of the relevant guideline of the European Society of Cardiology published in 2014⁹. If required, preoperative treatment was also initiated.

Study outcome

The main purpose of the investigation was the long-term mortality. The median long-term follow-up period was 34 (12–42) months. By using a lifelong and unique national identification number, the data from the National Death Registry System were used to identify long-term mortality.

Statistical analysis

The Statistical Package for Social Sciences 20.0 software (SPSS Inc., IL, USA) was used to conduct statistical analyses. The Kolmogorov–Smirnov test was applied to determine the normality of the data. Continuous variables were displayed as median (interquartile range). Absolute and relative frequencies were used to depict quantitative data. Either independent Student's t test or Mann-Whitney U test was used to compare the continuous variables. To analyze the categorical data, we utilized either the Pearson's chi-square test or Fisher's exact test. The independent predictors of long-term death were first determined using the univariable Cox regression analysis. Later, those parameters with statistical significance in the univariable Cox regression analysis were entered into a multivariable Cox regression analysis to find the independent predictors of long-term death. To estimate the chance of surviving based on the presence of AF, a Kaplan-Meier cumulative survival curve analysis was used. In all statistical analyses, a $p < 0.05$ was deemed significant.

RESULTS

A total of 233 patients who were above 65 years of age and were evaluated by an experienced cardiologist preoperatively were included in the study. Each member of the research cohort was categorized into one of the two groups based on their survival status (survivor and non-survivor groups). During the follow-up period, 89 (38.2%) elderly patients died.

Table 1 displays the demographic properties and previous medications of all cases enrolled in the study. Patients in the non-survivor group were older, and the frequency of AF was significantly higher in this group. However, the distribution

Table 1. Comparison of demographic and clinical characteristics of patients according to long-term mortality after hip fracture surgery.

	Long-term mortality (–), (n=144)	Long-term mortality (+), (n=89)	p-value
Age, year	81 (73–86)	86 (80–89)	<0.001
Male gender, n (%)	33 (22.9)	30 (33.7)	0.074
Hypertension, n (%)	115 (79.9)	64 (71.9)	0.165
Diabetes mellitus, n (%)	465(31.3)	22 (24.7)	0.285
Insulin dependency, n (%)	11 (7.6)	7 (7.9)	0.950
Hyperlipidemia, n (%)	15 (10.4)	7 (7.9)	0.513
COPD, n (%)	14 (9.7)	15 (16.9)	0.114
Dementia, n (%)	30 (20.8)	27 (30.3)	0.104
Cancer, n (%)	27 (18.8)	13 (14.6)	0.411
Coronary artery disease, n (%)	23 (16.1)	14 (15.7)	0.943
CRF, n (%)	13 (9.0)	13 (14.6)	0.194
CVA, n (%)	8 (5.6)	13 (14.6)	0.021
Atrial fibrillation, n (%)	13 (9.0)	26 (29.2)	<0.001
Medical treatment, n (%)			
Psychiatric drug	38 (26.4)	17 (19.1)	0.198
Acetylsalicylic acid	39 (27.1)	19 (21.6)	0.345
Clopidogrel	14 (9.7)	9 (10.1)	0.923
Beta-blocker	47 (32.9)	36 (40.9)	0.216
Calcium channel blocker	37 (25.7)	19 (21.3)	0.448
ACE inhibitor/ARB	69 (47.9)	40 (45.5)	0.715
Spironolactone	1 (0.7)	1 (1.1)	1.000
Furosemide	8 (5.6)	10 (11.2)	0.121
Warfarin	8 (5.6)	10 (11.2)	0.121
NOACs	5 (3.5)	13 (14.8)	0.002
Follow-up, months	24 (14–28)	5 (2–16)	

Continuous variables are presented as median (interquartile range) and nominal variables as frequency (%). COPD: chronic obstructive pulmonary disease; CRF: chronic renal failure; CVA: cerebrovascular accident; ACE: angiotensin converting enzyme; ARB: angiotensin-receptor blocker; NOACs: new oral anticoagulants.

of other comorbid conditions, such as hypertension, diabetes, cerebrovascular accident, chronic obstructive pulmonary disease, coronary artery disease, and cancer, was similar in both groups. In terms of the use of previous medications, consumption of new oral anticoagulants was statistically significantly higher in the non-survivor group.

Table 2 summarizes comparison of the two groups in terms of laboratory and echocardiographic parameters. Except for blood urea and albumin levels, there was no statistically significant difference between the groups in terms of laboratory data. As for the echocardiographic parameters, patients in the non-survivor group had a higher left atrium anteroposterior

diameter. Other echocardiography parameters were similar between the two groups.

According to the univariable Cox regression analysis, advanced age, previous cerebrovascular accident, the presence of AF, blood urea and albumin levels, and left atrium anteroposterior diameter on echocardiography were associated with the long-term all-cause death. In the multivariable Cox regression analysis, the presence of AF (HR: 2.195, 95%CI 1.365–3.415, $p<0.001$), advanced age, and blood urea levels were determined as independent predictors for the long-term all-cause mortality in elderly patients above 65 years of age with hip fracture.

Table 2. Comparison of laboratory and echocardiography parameters of patients according to long-term mortality after hip fracture surgery.

	Long-term mortality (–), (n=144)	Long-term mortality (+), (n=89)	p-value
Laboratory variables			
Hematocrit, %	34.5 (31.6–38.2)	33.6 (30.3–37.2)	0.213
Hemoglobin, g/dL	11.6 (10.4–12.6)	11.3 (10.2–12.3)	0.433
RDW, %	13.4 (12.6–14.0)	13.5 (12.4–15.0)	0.443
WBC count, cells/ μ L	8.7 (7.3–11.0)	8.5 (6.8–10.8)	0.350
Platelet count, cells/ μ L	209 (163–254)	215 (172–282)	0.271
MPV, fL	8.8 (7.8–9.7)	8.5 (7.5–9.7)	0.128
PCT, %	0.19 (0.14–0.24)	0.18 (0.14–0.27)	0.805
Creatinine, mg/dL	1.0 (0.8–1.2)	1.0 (0.8–1.3)	0.339
Urea, mg/dL	42 (34–57)	51 (38–78)	<0.001
TSH, nmol/L	1.3 (0.9–1.7)	1.2 (0.5–1.9)	0.739
T4, nmol/L	0.9 (0.8–1.1)	1.0 (0.8–1.1)	0.292
AST, U/L	22 (17–28)	22 (17–31)	0.854
ALT, U/L	16 (12–22)	16 (12–25)	0.359
Glucose, mg/dL	121 (103–147)	118 (97–142)	0.327
CRP, mg/dL	56 (21–89)	46 (22–90)	0.915
Troponin, ng/mL	9.6 (5.5–27.0)	21.5 (11.0–47.0)	0.003
Albumin, mg/dL	33 (31–37)	30 (27–33)	<0.001
Lymphocytes, cells/ μ L	1.5 (1.1–2.0)	1.4 (0.9–1.8)	0.055
Echocardiography variables			
Ejection fraction, %	61 (60–62)	61 (60–62)	0.582
LAAP diameter, mm	37 (36–39)	38 (36–43)	0.003
LVEDD, mm	48 (45–51)	48 (46–50)	0.701
LVESD, mm	36 (34–38)	36 (34–38)	0.692

Continuous variables are presented as median (interquartile range) and nominal variables as frequency (%). RDW: red cell distribution width; WBC: white blood cell; MPV: mean platelet volume; PCT: plateletcrit; TSH: thyroid-stimulating hormone; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: c-reactive protein; LAAP: left atrium anteroposterior; LVEDD: left ventricle end-diastolic volume; LVESD: left ventricle end-systolic volume.

The Kaplan-Meier cumulative survival curve analysis revealed that AF significantly reduced the long-term survival rate in elderly cases above 65 years of age with hip fracture, as shown in Figure 1 (log-rank chi-square test: 14.408, $p < 0.001$).

DISCUSSION

The incidence of hip fracture increases dramatically in persons above 65 years of age⁵. Surgical treatment is the recommended option for most patients. Even when surgically managed, outcomes are not perfect for these patients^{5,6}. Advanced age in this group is commonly associated with increased frailty and worse surgical and survival outcomes¹⁰. In this age group, comorbidities

such as diabetes mellitus and hypertension are more prevalent. As stated in our study, advanced age and impaired renal function are independent predictors for long-term mortality after hip fracture. However, the presence of diabetes mellitus and hypertension was not found to have an effect on long-term mortality. Besides, HF is a known independent predictor of mortality in hip fracture cases¹⁰. However, there is a scarcity of data for mortality in patients without HF and with AF specifically. To our knowledge, long-term mortality of these patients has not been studied yet.

One of the most common comorbidities in this age group is AF. In our study, 39 (16%) of 233 patients had an AF. This is consistent with the current literature, which puts the prevalence of AF at approximately 12–15%¹¹. Previously, in-hospital

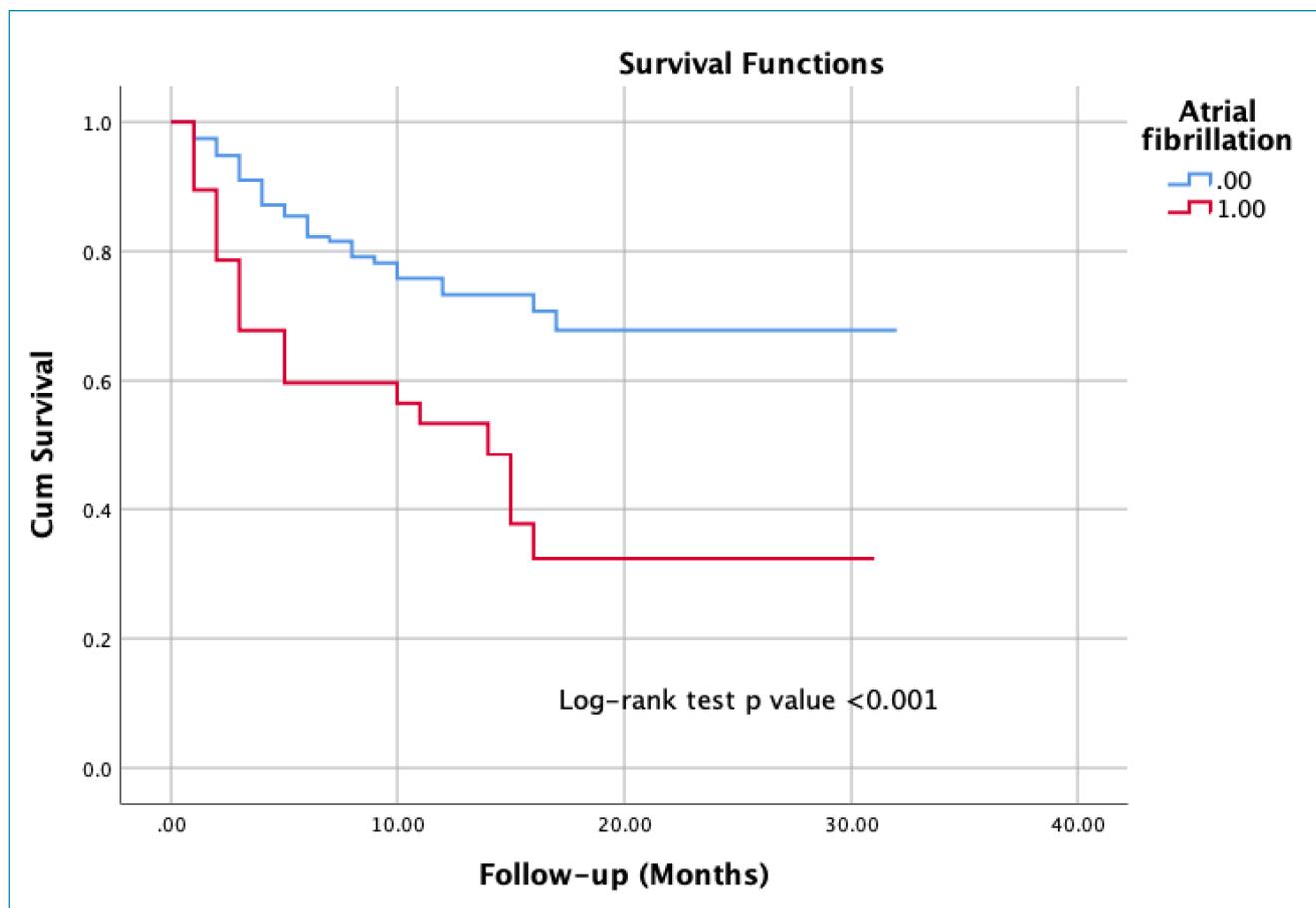


Figure 1. A Kaplan-Meier curve analysis illustrating the cumulative long-term survival function of hip fracture cases based on the presence of atrial fibrillation.

and 1-year mortality for hip fracture in patients with AF had been studied. Neuhaus et al. evaluated the data in the National Hospital Discharge Survey cohort and concluded that AF was a predictor of in-hospital death in hip fracture patients with an odds ratio (OR) of 2.00 ($p < 0.001$), and the presence of AF predicted adverse events with an OR of 1.26 ($p < 0.001$)¹². Adunsky et al. studied 1,114 consecutive hip fracture patients and divided the cohort into three groups: sinus rhythm, paroxysmal AF, and chronic AF. At the end of 1 year, only the chronic AF group had an increased mortality rate, with an HR of 1.786.¹³ In this study, we expanded the current knowledge by demonstrating that AF was also linked with increased risk of death during the long-term follow-up.

Long-term mortality in these patients may be stemming from a myriad of different causes. Orthopedic surgeons may hold back effective anticoagulation in favor of decreasing the hemorrhage risk. However, this decision may be responsible for the increase in mortality by way of ischemic strokes. Additionally, AF increases the risk of stroke 5-fold.¹⁴ Even with effective anticoagulation, thrombi may form in the left atrial

appendix. Microthrombi from the left atrium may cause emboli to the brain. Remarkably, hip fracture patients who have stroke are at an increased risk for long-term mortality.¹⁵

There are multiple guidelines and risk scores designed for preoperative evaluation of patients for noncardiac surgery, the Revised Cardiac Risk Index¹⁶ (RCRI, Lee's Score) and the American College of Surgeons National Surgical Quality Improvement Program (ACS NSQIP) Index¹⁷ being the most prominently known ones. Both these scoring indexes do not include AF as a risk factor. Together with our findings, clinicians using these scoring indexes may modify their interpretation of their results taking AF into account.

Study limitations

Our study was designed in a retrospective manner, conducted in a single center. Even though we conducted multivariate analyses, frailty indices, such as Charleston morbidity index, assessing the patient's frailty specifically, which can have an effect on hip fracture and all-cause mortality, were not assessed. We only recruited patients with chronic AF, and patients with paroxysmal AF were

excluded. Missing physician appointments may play a role in mortality by suboptimal management of both diseases during the follow-up period. Also, some of the patients might even have discontinued their oral anticoagulant regimes by themselves.

CONCLUSIONS

Chronic AF is an independent predictor of long-term death in hip fracture patients who were free from HF. Clinicians responsible for the management of hip fracture patients should also manage AF optimally for further reducing the long-term adverse events.

AUTHORS' CONTRIBUTIONS

ALO: Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **TC:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **MIH:** Formal analysis, Validation, Writing – review & editing. **VÇ:** Data curation, Funding acquisition, Resources. **MS:** Data curation, Funding acquisition, Resources. **SD:** Data curation, Funding acquisition. **SA:** Data curation, Funding acquisition. **SY:** Data curation, Funding acquisition. **SO:** Project administration, Visualization. **NK:** Supervision, Validation.

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Relationship of uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide with acute cerebral infarction

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SUMMARY

OBJECTIVE: The objective was to study the relationship of serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels with acute cerebral infarction.

METHODS: A total of 96 acute cerebral infarction patients were divided into small, middle, and large infarct size groups based on the size of infarct focus and mild, moderate, and severe infarction groups based on the evaluation criteria of nerve defect degree. In addition, 75 healthy people were selected as the control group. The serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels of all subjects were detected.

RESULTS: The serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels in the acute cerebral infarction group were significantly higher than the control group ($p < 0.05$). Compared with the small infarct size group, each index in middle and large infarct size groups was significantly increased ($p < 0.05$). Compared with the middle infarct size group, each index in the large infarct size group was significantly increased ($p < 0.05$). The serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels in moderate and severe infarction groups were significantly higher than the mild infarction group ($p < 0.05$). Compared with the moderate infarction group, each index in the severe infarction group was significantly increased ($p < 0.05$). The serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels were positively correlated with the infarct size and nerve defect degree ($p < 0.05$).

CONCLUSIONS: The serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels are closely correlated with the occurrence and development of acute cerebral infarction. The detection of these indexes has significance for understanding the severity of acute cerebral infarction, guiding the individual treatment scheme, and evaluating the prognosis.

KEYWORDS: Cerebral infarction. Uric acid. C-reactive protein. N-terminal pro-BNP

INTRODUCTION

Acute cerebral infarction (ACI) mainly refers to brain tissue necrosis due to brain blood circulation disorders caused by tissue ischemia, hypoxia, and other factors¹. ACI patients often have varying degrees of dizziness, unclear speech, numbness, and other symptoms, and severe ACI patients may have a disability, or even death². Therefore, the reasonable and effective evaluation of ACI condition is very critical. It is a hot topic

to search for serum markers related to the diagnosis and prediction of ACI. Uric acid (UA) is a purine metabolite, which is excreted by kidney and intestine. The high serum UA level is closely associated with cardiovascular and cerebrovascular diseases³. C-reactive protein (CRP) is a reactive protein produced with tissue damage or inflammation. It is also a major inflammatory factor involved in atherosclerotic disease⁴. B-type natriuretic peptide (BNP) is a kind of hormone

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substance existing in various tissues of the body, especially in brain tissue and heart muscle. It can antagonize the renin-angiotensin-aldosterone system and has the diuretic, sodium excreting, and vasodilating effects⁵. N-terminal pro-B-type natriuretic peptide (NT-proBNP) is the cleavage product of BNP, which has the homology with BNP⁶. In view of this, this study explored the relationship of serum UA, CRP, and NT-proBNP levels in patients with ACI, so as to provide the effective guidance and scientific basis for the clinical diagnosis and treatment of ACI.

METHODS

Subjects

A total of 96 ACI patients treated in the Affiliated Hospital of Zunyi Medical College from March 2018 to May 2020 were enrolled in this study. There were 55 males and 35 females. The age of patients was 46–74 years old, with an average age of 60.45 ± 11.45 years. The time from disease onset to admission was 8–24 h, with an average time of 16.56 ± 6.44 h. Based on the size of infarct focus, the patients were divided into small infarct size group (infarct diameter ≤ 3 cm; 48 cases), middle infarct size group ($3 \text{ cm} < \text{infarct diameter} \leq 5$ cm; 30 cases), and large infarct size group (infarct diameter > 5 cm; 12 cases). Based on the evaluation criteria of nerve defect degree issued by the National Institutes of Health Stroke Scale (NIHSS), the patients were divided into mild infarction group (NIHSS score ≤ 4 points; 45 cases), moderate infarction group (4 points $< \text{NIHSS score} \leq 15$ points; 35 cases), and severe infarction group (NIHSS score > 15 points; 10 cases). In addition, 75 healthy people receiving physical examination in our hospital in the same period were selected as the control group. There were 50 males and 25 females. Their age was 45–74 years, with an average age of 62.16 ± 10.18 years. There was no significant difference in gender or age between the two groups ($p > 0.05$). This study has been approved by the Ethics Committee of the Affiliated Hospital of Zunyi Medical College. All the subjects had signed the informed consent to this study.

Inclusion and exclusion criteria

The inclusive criteria were as follows:

- (i) the age was 30–75 years;
- (ii) the ACI was diagnosed by computed tomography or magnetic resonance imaging;
- (iii) the ACI was the first onset;
- (iv) the time from disease onset to admission was < 24 h; and
- (v) the patients had not received surgical treatment recently.

The exclusion criteria were as follows:

- (i) the patients had incomplete clinical data;
- (ii) the patients had severe diseases of lung, liver, kidney, or other organs;
- (iii) the patients had blood diseases or diabetes;
- (iv) the patients had a history of brain diseases; and
- (v) the patients were pregnant or lactating women.

Study method

In the ACI group, 5 mL of venous blood was collected in the morning after admission. In the control group, 5 mL of venous blood was taken in the morning of the day of physical examination. The blood was centrifuged at 3000 r/min for 10 min. The serum was obtained and was stored at -80°C for detection. The UA was detected by the peroxidase method. The CRP was detected by immunoturbidimetry. The NT-proBNP was detected by quantum dots-based immunofluorescence chromatography. The detection operations were in strict accordance with the instruction of the kits. The comparisons of serum UA, CRP, and NT-proBNP levels between ACI and control groups, among small, middle, and large infarct groups, and among mild, moderate, and severe infarction groups were performed. The correlations of UA, CRP, and NT-proBNP with infarct size and the correlations of UA, CRP, and NT-proBNP with NIHSS score were analyzed.

Statistical analysis

Data were analyzed by SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The enumeration data were expressed as a percentage (%), and the comparison was conducted using the χ^2 test. The measurement data were expressed by mean \pm standard deviation. The comparison between two groups was conducted by the t-test, and that among multiple groups was conducted by the analysis of variance with the SNK-q test. Spearman's correlation analysis was used for the correlations of each serum index with infarct size and each serum index with NIHSS score. $p < 0.05$ was considered statistically significant.

RESULTS

Comparison of serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels between control and acute cerebral infarction groups

As shown in Table 1, the serum UA, CRP, and NT-proBNP levels in the ACI group were significantly higher than those in the control group ($p < 0.05$).

Comparison of serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels among small infarct, middle infarct, and large infarct size groups

Compared with the small infarct size group, the serum UA, CRP, and NT-proBNP levels in middle and large infarct size groups were significantly increased ($p < 0.05$). Compared with the middle infarct size group, the serum UA, CRP, and NT-proBNP levels in the large infarct size group were significantly increased ($p < 0.05$) (Table 2).

Comparison of serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels among mild infarction, moderate infarction, and severe infarction groups

Table 3 showed that the serum UA, CRP, and NT-proBNP levels in moderate and severe infarction groups were significantly higher than those in the mild infarction group ($p < 0.05$). Compared with the moderate infarction group, the serum UA, CRP, and NT-proBNP levels in the severe infarction group were significantly increased ($p < 0.05$).

Table 1. Comparison of serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels between control and acute cerebral infarction groups.

	n	UA (nmol/mL)	CRP ($\mu\text{g/mL}$)	NT-pro BNP (pg/mL)
Control	90	266.61 \pm 45.29	1.08 \pm 0.26	52.04 \pm 11.08
ACI	75	403.32 \pm 88.02	7.16 \pm 1.53	336.60 \pm 84.37
t		12.840	37.084	31.690
p-value		<0.001	<0.001	<0.001

ACI: acute cerebral infarction; t: statistic value; UA: uric acid; CRP: C-reactive protein; NT-pro BNP: N-terminal pro-B-type natriuretic peptide.

Table 2. Comparison of serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels among small infarct, middle infarct, and large infarct size groups.

	n	UA (nmol/mL)	CRP ($\mu\text{g/mL}$)	NT-proBNP (pg/mL)
Small infarct size	48	305.84 \pm 62.09	5.38 \pm 0.78	245.07 \pm 52.05
Middle infarct size	30	473.13 \pm 55.45*	6.81 \pm 0.93*	383.25 \pm 60.27*
Large infarct size	12	543.90 \pm 67.37*#	9.54 \pm 1.34*#	463.72 \pm 72.91*#
F		112.797	102.657	95.228
p-value		<0.001	<0.001	<0.001

UA: uric acid; CRP: C-reactive protein; NT-proBNP: N-terminal pro-B-type natriuretic peptide; F: statistic value. * $p < 0.05$ compared with small infarct size group; # $p < 0.05$ compared with middle infarct size group.

Table 3. Comparison of serum uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels among mild, moderate, and severe infarction groups.

	n	UA (nmol/mL)	CRP ($\mu\text{g/mL}$)	NT-proBNP (pg/mL)
Mild infarction	45	316.45 \pm 45.62	5.65 \pm 1.34	276.27 \pm 34.07
Moderate infarction	35	466.12 \pm 78.17*	7.32 \pm 1.13*	376.82 \pm 54.30*
Severe infarction	10	565.06 \pm 72.04*#	10.16 \pm 1.01*#	441.04 \pm 72.71*#
F		92.914	60.117	70.765
p-value		<0.001	<0.001	<0.001

UA: uric acid; CRP: C-reactive protein; NT-proBNP: N-terminal pro-B-type natriuretic peptide; F: statistic value. * $p < 0.05$ compared with mild infarction group; # $p < 0.05$ compared with moderate infarction group.

Correlation of uric acid, C-reactive protein, and N-terminal pro-B-type natriuretic peptide with infarct size and nerve defect degree

Spearman's correlation analysis showed that, in 90 ACI patients, the serum UA, CRP, and NT-proBNP levels were positively correlated with the infarct size (UA with infarct size: $r=0.683$, $p<0.001$; CRP with infarct size: $r=0.698$, $p<0.001$; NT-proBNP with infarct size: $r=0.564$, $p<0.001$). The serum UA, CRP, and NT-proBNP levels were also positively correlated with the nerve defect degree (UA with nerve defect degree: $r=0.401$, $p<0.001$; CRP with nerve defect degree: $r=0.389$, $p<0.001$; NT-proBNP with nerve defect degree: $r=0.523$, $p<0.001$).

DISCUSSION

ACI is a common and high-incidence cerebrovascular disease. It is commonly found in the middle-aged and elderly population. It has a high disability rate, recurrence rate, and mortality rate. Studies have shown that the most common cause of ACI is atherosclerosis⁷. When atherosclerosis-caused brain ischemia or hypoxia occurs, it will cause a series of local inflammatory reactions, leading to the damage to brain tissue⁸. Therefore, the key to the treatment of ACI is to improve the oxygen supply of brain tissue for promoting the recovery of nerve tissue.

With the continuous development of medical technology, the research on the mechanism of ACI is becoming more and more mature. Study⁹ has shown that hyperuricemia is an important risk factor for cerebral infarction. The mechanism may be that the high level of UA damages the vascular endothelial function by inhibiting oxidative reaction and affects the arterial capillary wall remodeling. This promotes thrombosis and participates in the inflammatory reaction¹⁰. CRP is mainly synthesized by hepatocytes, and the mononuclear phagocytes and fibroblasts can also produce a small amount of CRP. It is found that the content of CRP in the blood is increased sharply when the acute inflammatory reaction occurred¹¹. In addition, CRP participates in the pathological process of thrombosis and arteriosclerosis, which is one of the risk factors of stroke¹². NT-proBNP plays an important role in the progress

of the cerebrovascular disease, which is closely related to the location and size of ACI lesion¹³. The increase in NT-proBNP level can reduce brain edema and protect brain tissue through diuretic and natriuretic effects¹⁴.

This study explored the changes and clinical significance of serum UA, CRP, and NT-proBNP levels in patients with ACI. Results showed that the serum UA, CRP, and NT-proBNP levels in the ACI group were significantly higher than those in the control group. In addition, with the increase of infarct size and nerve defect degree, the serum UA, CRP, and NT-proBNP levels in ACI patients obviously increased. Spearman's correlation analysis showed that the serum UA, CRP, and NT-proBNP levels were positively correlated with the infarct size and nerve defect degree. This indicates that the serum UA, CRP, and NT-proBNP levels are closely correlated with the occurrence and development of ACI. The high level of UA promotes the platelet adhesion and aggregation, weakens the chemotaxis of red blood cells, and aggravates cerebral ischemia and hypoxia, worsening the progress of ACI¹⁵. CRP can activate the complement system and can form many terminal proteins and complexes, which causes the damage of vascular intima resulting in ACI¹⁶. After ACI, the brain tissue ischemia and necrosis causes the abnormal hypothalamus-pituitary secretion, resulting in increased NT-proBNP level¹⁷, so the NT-proBNP level is closely related to ACI.

CONCLUSIONS

The serum UA, CRP, and NT-proBNP levels are closely correlated with the occurrence and development of ACI. The detection of these indexes has significance for understanding the severity of ACI, guiding the individual treatment scheme, and evaluating the prognosis. The limitation of this study is that the sample size is relatively small. In our subsequent studies, a larger sample size will make the results more convincing.

AUTHORS' CONTRIBUTIONS

SY: Conceptualization, Writing – review & editing. **GL:** Data curation. **CH:** Formal analysis. **XX:** Writing – original draft.

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Translation, cross-cultural adaptation, and reliability of the Workplace Sitting Breaks Questionnaire into Brazilian Portuguese

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SUMMARY

OBJECTIVE: This study aimed to translate, adapt, and analyze the reliability of the Workplace Sitting Breaks Questionnaire (SITBRQ) for use in Brazil.

METHODS: This is a cross-sectional study. The translation and cross-cultural adaptation were conducted considering the following six phases: translation, synthesis of translations, back translation, analysis by a committee of experts, test of the pre-final version, and final version. We included workers aged 18 years or above, both genders, and able to understand, read, and write in Brazilian Portuguese. The final version was applied to workers in two moments (i.e., test and retest), with an interval of 7 days, for reliability calculation.

RESULTS: In the translation and cross-cultural adaptation phase, the pre-final version was applied to a sample of 35 workers. For item a of the SITBRQ, there was 100% understanding by respondents, while item b was understood by 94.28%. The reliability phase was conducted with 115 workers. For both items, almost perfect was identified with kappa >0.81.

CONCLUSIONS: The SITBRQ version into Brazilian Portuguese has adequate adaptation and excellent values of reliability.

KEYWORDS: Occupational health. Surveys and questionnaires. Sedentary behavior.

INTRODUCTION

Occupational activities, such as standing or performing household tasks, are considered mild physical activities. It is known that individuals with low levels of light-intensity physical activity are more likely to exhibit sedentary behaviors^{1,2}. Furthermore, it is suggested that high levels of light-intensity physical activity are related to a reduction in the risk of overweight and/or obesity and a consequent reduction in the risk of developing cardiometabolic diseases³⁻⁵.

Understanding this context and relating professional activities that do not allow walking during working hours, remaining

seated for long periods, and favoring a decrease in the willingness to perform simple tasks, it is recommended that workers take few minutes break during their workday. These short breaks favor the reduction of both the biomechanical overload resulting from the posture maintained throughout the workday and the risk of occupational and systemic disorders^{6,7}.

For this reason, in recent years, the development of instruments that aim to analyze the behavior of breaks during the performance of work activities has intensified⁸. Among these, the formulation of the Workplace Sitting Breaks Questionnaire (SITBRQ) stands out⁸. Developed for the English language,

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the SITBRQ presents satisfactory validity and reliability when compared with other self-report instruments related to the study of sedentary behavior. Presenting as positive points the reduced filling time and ease of understanding and interpretation⁸.

In Brazil, although the literature presents numerous instruments that assess physical and labor activity, none of these measure short breaks in working time by sitting. Knowing this, the adaptation and validation of a new self-report instrument capable of evaluating breaks during the sitting time in work activity are justified, facilitating the analysis and implementation of more assertive healthcare strategies for this population.

Our hypothesis is that the SITBRQ, after going through the process of translation and cross-cultural adaptation into Brazilian Portuguese, is reliable for application in this population. The aim of this study was to translate and cross-culturally adapt the SITBRQ into Brazilian Portuguese and to verify the test-retest reliability of this version.

METHODS

Study design

This is a cross-sectional study conducted according to the Guidelines for the Process of Cross-Cultural Adaptation of Self-Report Measures⁹ and the Consensus-Based Standards for the Selection of Health Measurement Instruments (COSMIN)¹⁰. Authorization to perform the cross-cultural adaptation of the SITBRQ into Brazilian Portuguese was granted via email by one of the authors of the original version of the questionnaire (Dr. Željko Pedišić).

This study was approved by the institution's Research Ethics Committee (number 4,555,379). Participants were recruited by means of social media, text messaging, and email. All recruited volunteers gave consent to participate in this study. Data collection took place online. The questionnaire was made available on the Google Forms platform (Mountain View, CA, USA).

Translation and cross-cultural adaptation

The translation and cross-cultural adaptation process of the SITBRQ into Brazilian Portuguese followed the following criteria.

1. Translation: two independent translators, namely, a physiotherapist with above 10 years of experience and an English teacher with above 21 years of experience in translations, however without technical knowledge of matters in the health area. They translated the original version of the SITBRQ into Brazilian Portuguese. It is important to highlight that both had Brazilian Portuguese as their mother tongue and were fluent in English.
2. Synthesis of translations: after discussions and potential revisions, the two translators, under observation by the

responsible researcher, synthesized the two versions of the questionnaire translated independently. Thus, they produced a single version of the SITBRQ in a consensual manner.

3. Back translation: two independent translators, with English as their mother tongue and fluent in Portuguese, translated the Portuguese version of the SITBRQ back into English. It is important to highlight that both did not have technical knowledge in the area of health or even had prior knowledge about the original version of the questionnaire.
4. Analysis by a committee of experts: the four translators involved in the adaptation process met together with four experts in the field with experience in the health field, specifically in occupational health. Together, they reviewed all translated and back-translated versions to correct possible discrepancies. In this way, the pre-final version of the SITBRQ was obtained in an agreed manner among all members of the committee.
5. Test of the pre-final version: the pre-final version of the SITBRQ was applied to 30 Brazilian workers. Participants read and filled out the questionnaire, and at the end of filling it out, they established their understanding of the pre-final version of the SITBRQ by checking a checkbox containing the answers "yes" and "no" for each item in the questionnaire. To be considered as having an adequate degree of understanding, the items must be understood by at least 80% of the participants.
6. After analyzing the pre-final version, the coordinator of the adaptation process thus established the final version of the SITBRQ in Brazilian Portuguese.

Participants

The minimum sample for this study was characterized as 100 participants¹⁰. Specifically, to obtain test-retest reliability, the SITBRQ was applied on two occasions, with an interval of 7 days between assessments¹¹.

For this, the following inclusion criteria were adopted: active workers with at least 6 months in the same job, aged 18 years or above, both genders, and ability to understand, read, and write in Brazilian Portuguese. Exclusion criteria were the presence of diagnosed cognitive diseases and not responding to the retest. The inclusion and exclusion criteria were applied based on the worker's self-report since data collection was performed online.

Workplace Sitting Breaks Questionnaire

The SITBRQ consists of two items (a and b), which assess the frequency and duration of breaks from work in a work environment in which workers usually sit down to carry out tasks. The first item asks about how many breaks the worker takes when leaving the sitting position (whether to drink water, take

a short walk, or even stretch), with seven answer options (0–6 or more up). The second item asks about the time spent by the worker in short-term physical activities, such as getting up from a chair and having a drink, or going to the bathroom, or continuing a task while standing. This item also has seven response options (60 min or more; 30–59 min; 20–29 min; 10–19 min; 5–9 min; <5 min; not applicable). SITBRQ has no score. Items must be analyzed separately⁸.

Statistical analysis

To characterize the sample, descriptive statistics were performed with the presentation of quantitative data by means of mean and standard deviation (SD) and qualitative data by means of absolute number and percentage. The reliability analysis of the SITBRQ was performed using the kappa test with linear weighting. We considered the following interpretation of kappa values: <0, poor; 0.01–0.20, light; 0.21–0.40, reasonable; 0.41–0.60, moderate; 0.61–0.80, substantial; and 0.81–1, almost perfect¹².

Data processing was performed using the SPSS software, version 17.0 (Chicago, IL, USA), and the calculation of kappa with linear

weighting was performed using the website <http://vassarstats.net/kappa.html>. For all analyses, a significance level of 5% was considered.

RESULTS

In the SITBRQ translation and cross-cultural adaptation phase, the expert committee decided to make the following two changes in the questionnaire:

1. The removal of redundant information in the instructions for completing the questionnaire; and
2. The insertion of examples of short physical activities in item b of the questionnaire. Thus, the pre-final version of the SITBRQ was established.

The pre-final version was applied to a sample of 35 Brazilian workers. Of these, 20 (57.1%) were women, with a mean age of 40.94 years (SD=13.75). For item a of the SITBRQ, there was 100% understanding by respondents, while item b was understood by 94.28% of respondents. Thus, the final version of SITBRQ was established (Figure 1).

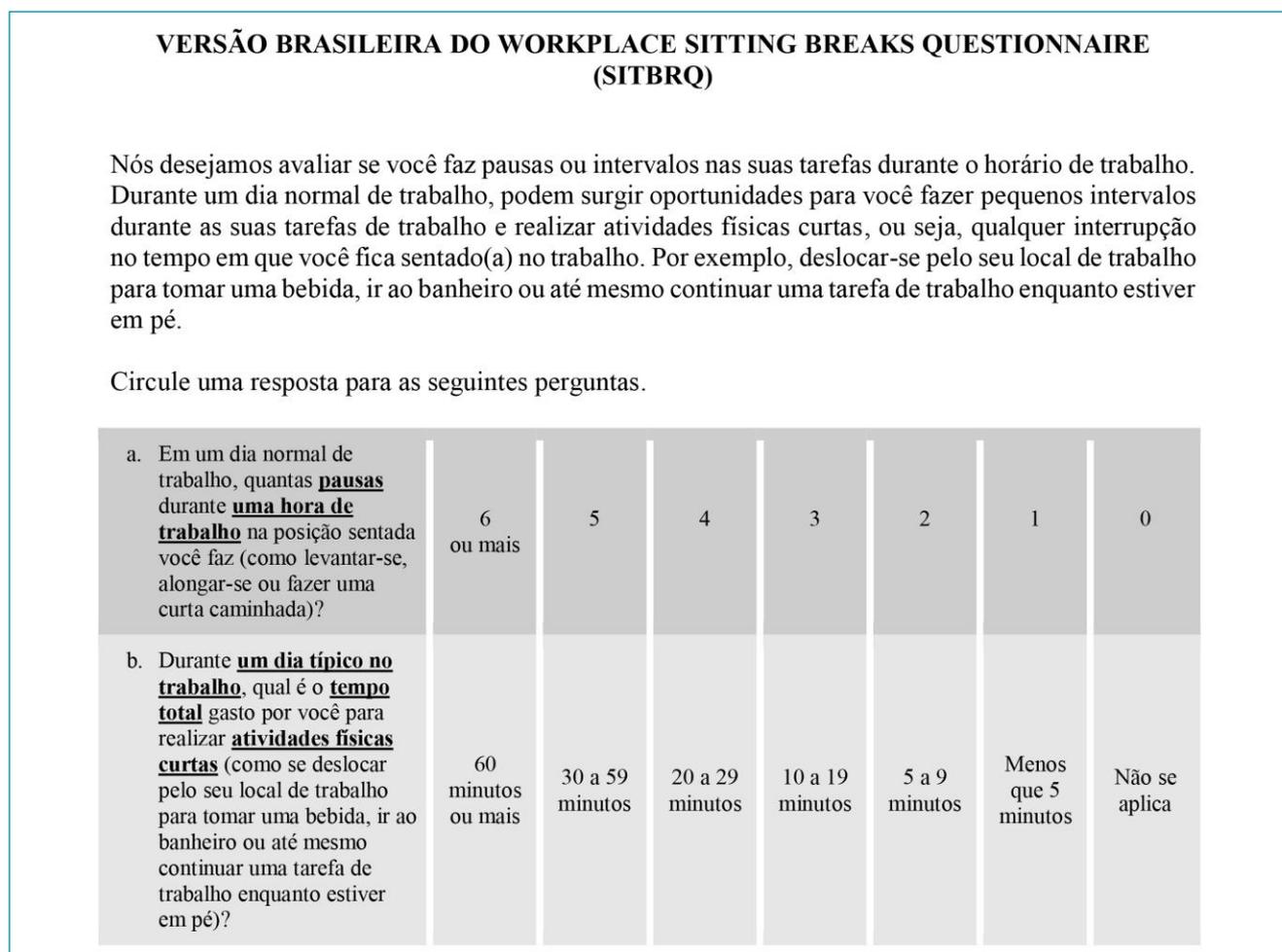


Figure 1. Brazilian version of the Workplace Sitting Breaks Questionnaire.

Therefore, the final version was applied to 115 workers in two moments (i.e., test and retest), with an interval of 7 days between evaluations. As shown in Table 1, most of the sample was made up of men, young adults, singles, and with a weekly workload of more than 35 h. Table 2 shows the reliability values of the SITBRQ. Almost perfect reliability was observed in the two items of the questionnaire, with kappa values >0.81.

Table 1. Characterization of the study sample (n=115).

Variable	Mean (standard deviation) or number (%)
Age (years)*	28.97 (10.84)
Sex (male) [†]	59 (51.3)
Marital status [†]	
Single	75 (65.2)
Married	36 (31.3)
Widower	1 (0.9)
Divorced	3 (2.6)
Education [†]	
Basic education	8 (6.9)
High school	71 (61.8)
University education	36 (31.3)
Weight (kg)*	75.46 (17.92)
Height (m)*	1.68 (0.10)
Body mass index (kg/m ²)*	26.44 (4.73)
Physical activity (yes) [†]	49 (42.6)
Working time (months)*	78.26 (97.19)
Weekly workload (h)*	35.96 (13.65)
Postures at work [†]	
Standing	42 (36.5)
Seated	41 (35.6)
Standing and sitting	32 (27.9)
Kind of work [†]	
Manual	48 (41.7)
Nonmanual	9 (7.8)
Manual and nonmanual	48 (41.7)
Others	10 (8.7)
Ongoing treatments [†]	
Medicative	41 (35.7)
Physiotherapeutic	23 (20.0)
Psychotherapeutic	16 (13.9)

*Values are shown as mean (standard deviation); [†]Values are presented in number (percentage).

DISCUSSION

The translation and adaptation of the SITBRQ into Brazilian Portuguese proved to be adequate for the population studied, resembling the original version applied in the Australian population⁸. However, the Brazilian Portuguese version was actually tested on individuals who performed continuous work activities, with an average weekly workload of 35.96 h, the predominance of standing or sitting posture, associated with a low level of physical activity, similar to office workers¹³.

For the two items that make up the questionnaire, kappa values >0.81 were found, exceeding the values reported in the original version⁸, which presented acceptable values, however lower than those in this study (0.74 and 0.61 for items 1 and 2, respectively). We believed that this difference is related to the better defined profile of the participants included in this study, much closer to the reality of individuals who carry out continuous work activities¹³.

Understanding that individuals with a low level of activities characterized as short have greater sedentary behavior^{1,2}, the SITBRQ appears as a simple, easy-to-understand, quick-filling tool to measure the frequency of breaks, and the total time spent on short physical activities during work⁸. Potentially, it can be used in epidemiological studies and mainly as a facilitator for

Table 2. Test-retest reliability of the Workplace Sitting Breaks Questionnaire (SITBRQ).

SITBRQ items	Test, n (%)	Retest, n (%)	Kappa (95% CI)
Item a			
6 or more	22 (19.1)	19 (16.5)	0.817 (0.665–0.956)
5	6 (5.2)	10 (8.7)	
4	6 (5.2)	7 (6.1)	
3	20 (17.4)	22 (19.1)	
2	21 (18.3)	19 (16.5)	
1	20 (17.4)	23 (20.0)	
0	20 (17.4)	15 (13.0)	
Item b			
60 min or more	22 (19.1)	23 (20)	0.815 (0.730–0.900)
30–59 min	23 (20.0)	23 (20)	
20–29 min	8 (7.0)	11 (9.6)	
10–19 min	14 (12.2)	17 (14.8)	
5–9 min	28 (24.3)	25 (21.7)	
<5 min	17 (14.8)	14 (12.2)	
Not applicable	3 (2.6)	2 (1.7)	

CI: confidence interval.

the development of more assertive strategies in relation to prevention and promotion of healthcare, aiming to reduce sedentary behavior in the work environment¹⁴.

This study is the first cross-cultural adaptation of the SITBRQ, and this fact considerably limited the discussion of the data. Thus, we suggest that further studies should be carried out in other languages and cultures considering the importance of the aspects assessed by the questionnaire for workers' health.

CONCLUSION

The adapted version of the SITBRQ into Brazilian Portuguese has adequate reliability.

AUTHORS' CONTRIBUTIONS

ARS: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **CAFPG:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing. **JEFSJ:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **DSR:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **CABP:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **AVDF:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. **DBD:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing.

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Effect of thyroidectomy in patients with tracheal compression

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SUMMARY

OBJECTIVE: Various therapeutic methods are employed to reduce thyroid gland compression of the trachea. Differences in the amount of shrinkage in the thyroid gland, in the amount of amelioration of tracheal compression, and in the amount of fibrosis after treatment may occur with these different methods. Although the compression of the trachea decreases after thyroidectomy, the number of studies showing the extent of this is limited. The purpose of this study was to investigate the effect of thyroidectomy performed due to tracheal compression, to reveal the extent of improvement using magnetic resonance imaging (MRI), and to evaluate our results.

METHODS: In total, 30 patients, i.e., 24 women and 6 men, with tracheal compression secondary to thyroid gland enlargement and undergoing total thyroidectomy were included in this study. MRI performed before surgery and 6 months after surgery. The amount of deviation from the tracheal midline and the tracheal lateral and anteroposterior (AP) diameters were measured, compared, and subjected to statistical analysis.

RESULTS: Statistical analysis revealed significant differences between pre- and postoperative tracheal deviations, and lateral and AP diameters ($p < 0.001$, $p < 0.001$, and $p = 0.006$, respectively). Histopathologically, benign or malignant pathology caused no significant difference in the postoperative improvement of tracheal anatomy ($p = 0.348$ and $p = 0.148$, respectively).

CONCLUSIONS: Thyroidectomy performed due to tracheal compression provides significant improvement in tracheal anatomy. Due to its rapid and effective results, thyroidectomy should be one of the first options considered in the treatment of thyroid diseases with compression findings.

KEYWORDS: *Goiter*. Thyroidectomy. Tracheal stenosis.

INTRODUCTION

The term “goiter” refers to the abnormal growth of the thyroid gland. Growth of the thyroid gland can lead to symptoms such as swelling in the neck, shortness of breath, hoarseness, and swallowing difficulty by compressing surrounding structures such as the trachea, esophagus, recurrent laryngeal nerve, and the internal jugular vein. Due to slow progression and patient

adaptation, it may often not be diagnosed. Objective findings in the presence of tracheal compression emerge in respiratory function tests when approximately 50% of the trachea is affected¹.

Tests used to evaluate the volume of an enlarged thyroid gland include ultrasonography, computed tomography, magnetic resonance imaging (MRI), and thyroid scintigraphy. Due to its high sensitivity and the greater anatomical detail it

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provides, MRI is regarded as more useful in treatment planning and follow-up compared with other methods^{2,3}.

Total thyroidectomy and radioactive iodine therapy are the therapeutic methods that can be applied in patients with goiter and tracheal compression^{4,5}. Differences may be observed in the extent of shrinkage in the thyroid gland, the amount of tracheal compression amelioration, and the amount of fibrosis after treatment among the different therapeutic methods employed in reducing thyroid gland compression of the trachea. Several studies have shown that thyroidectomy contributes to tracheal airflow^{6,7}. However, the number of studies concerning changes occurring in the structure of the trachea following thyroidectomy is insufficient.

The purpose of this study was to investigate the effect of thyroidectomy performed due to tracheal compression, reveal the extent of improvement using MRI, and evaluate our results.

METHODS

The study commenced following the receipt of Ataturk University Medical Faculty Clinical Research Ethical Committee approval (decision no. 15, session no. 07, dated November 29, 2018). A total of 30 patients with tracheal compression secondary to thyroid gland enlargement and who had undergone total thyroidectomy at the Ataturk University Medical Faculty Ear, Nose, and Throat Department, Turkey, were included in this study. Patients with previous histories of neck surgery, revision cases, who were undergoing lobectomy and hemithyroidectomy, with a history of neck radiotherapy, or receiving radioactive iodine therapy were excluded.

Extracapsular total thyroidectomy was performed on all patients using the same standardized technique. Age, gender, preoperative and postoperative deviation in the trachea and compression regions, and histopathological results were recorded for all the cases included in this study.

MRI of the neck (Siemens Magnetom Avanto 1.5 T MRI Scanner) was performed in the axial, sagittal, and coronal planes from the epiglottis to the carina. This procedure was performed in a mean 30 days before surgery and 180 days postoperatively. T1- and T2-weighted images were obtained with 1.5 T MRI at a section thickness of 5 mm. Coronal plane T1- or T2-weighted MRI was used to measure the degree of deviation of the trachea. Before measurement, following the selection of the section in which the trachea was seen most clearly and in its entirety, the air intensity was enhanced by inclusion in the minimum intensity projection (MinIP) algorithm, and measurements were subsequently performed. At preoperative MRI, the midline of the neck was identified, and the area where the trachea deviated most from the midline was marked.

The distance between the trachea at this level and the midline was then measured manually.

At postoperative MRI, the amount of deviation improvement was determined by measuring the distance between the trachea at the same level and the midline. The amount of tracheal compression and the degree of postoperative improvement were determined using T1- or T2-weighted MRI in the axial plane. At preoperative MRI, the narrowest part of the trachea in the regions where the thyroid gland compressed the trachea was identified, and the lateral and anteroposterior (AP) tracheal diameters were measured manually. At postoperative MRI, the lateral and AP diameters were measured at the same level to determine the amount of improvement in the tracheal lumen.

The study data were recorded and analyzed using SPSS version 22.0 software. Descriptive statistics were shown as mean±standard deviation, median (minimum–maximum), percentage, and frequency distributions. The normality of the distribution of variables was tested using the histogram chart method based on the Kolmogorov-Smirnov test and skewness coefficients. The Wilcoxon test, Mann-Whitney *U* test, and Spearman's Rho correlation test were employed to evaluate variables before and after surgery. The $p < 0.05$ were regarded as statistically significant.

RESULTS

A total of 30 patients were included in this study, i.e., 24 (80%) women and 6 (20%) men. The patients' mean age was 50.23 ± 10.82 years, ranging between 29 and 70. Based on the histopathology results, 53.3% of the removed thyroid tissues were malignant, and 46.7% were benign. The mean specimen weight of the 30 patients was 113.27 ± 54.50 g, ranging between 50 and 262 g.

MRI images of changes in preoperative and postoperative tracheal deviations are shown in Figure 1A, and those of changes in preoperative and postoperative lateral and AP tracheal diameters are shown in Figure 1B. AP and lateral tracheal diameters, levels of deviation, and amounts of improvement measured at pre- and postoperative MRI are summarized in Table 1. Statistically significant differences were observed between pre- and postoperative tracheal deviations, tracheal lateral diameters, and tracheal AP diameters ($p < 0.001$, $p < 0.001$, and $p = 0.006$, respectively) (Table 2).

Benign or malignant thyroid diseases had no significant effect on the levels of deviation and tracheal AP diameter, although lateral tracheal diameters were significantly lower in benign masses ($p = 0.190$, $p = 0.771$, and $p = 0.011$, respectively). Benign or malignant thyroid diseases produced no significant

difference in terms of postoperative improvement of tracheal deviation, lateral tracheal diameter, or AP tracheal diameter ($p=0.348$, $p=0.148$, and $p=0.950$, respectively).

The substernal extension of the thyroid gland was present in 18 cases (60%). The postoperative improvement of tracheal

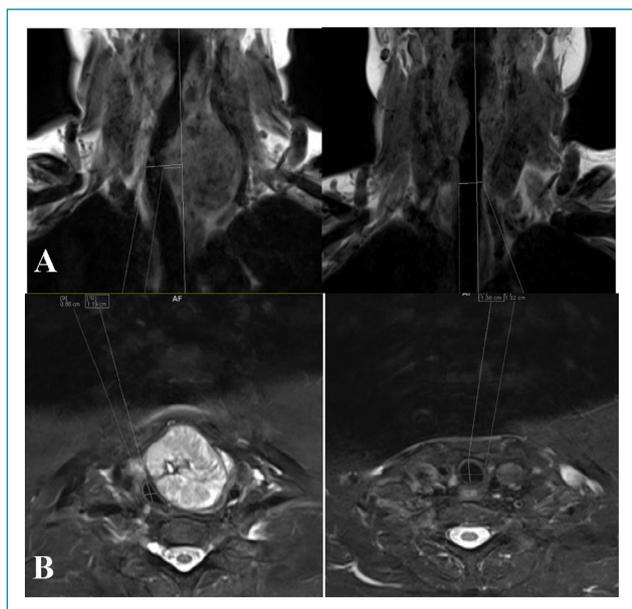


Figure 1. (A) Magnetic resonance image of the change in pre- and postoperative tracheal deviations. (B) Magnetic resonance image of the change in pre- and postoperative tracheal lateral and anteroposterior diameters.

deviation was significantly greater in patients with the substernal extension of the thyroid gland ($p=0.034$). No significant difference was observed in the amount of improvement in lateral or AP tracheal diameters between cervical and substernal goiters ($p=0.241$ and $p=0.226$, respectively).

No significant relationship was observed between age and degrees of improvement in tracheal deviation or lateral tracheal diameter. However, a significant moderate correlation was observed between age and the amount of improvement in AP diameter. Improvement in AP diameter increased with age ($p=0.121$, $p=0.608$, and $p=0.025$, respectively).

The weight of tracheal tissue that was removed had no significant effect on the amount of improvement in tracheal deviation or lateral and AP diameters ($p=0.545$, $p=0.080$, and $p=0.211$, respectively). Histopathological findings did not significantly affect the size of thyroid tissue removed during surgery as a result of pathology ($p=0.371$).

DISCUSSION

Thyroidectomy is the most frequently employed therapeutic technique in goiter patients with compression symptoms. One potential alternative to surgery is radioactive iodine therapy⁵. However, radioactive iodine therapy involves undesirable radiation-associated side-effects, such as thyroiditis, transient hyperthyroidism, Graves-like hyperthyroidism, and sometimes a 15–25% increase in thyroid dimensions. In addition,

Table 1. Anteroposterior and lateral tracheal diameters, deviation, and improvements therein measured using preoperative and postoperative magnetic resonance imaging (minimum, maximum, and mean±standard deviation).

	Minimum	Maximum	Mean±standard deviation
Preoperative deviation (mm)	7.0	28.0	13.96±4.61
Preoperative lateral diameter (mm)	6.0	15.0	10.97±2.42
Preoperative AP diameter (mm)	6.3	30.0	13.26±4.22
Postoperative deviation (mm)	2.5	13.5	7.93±2.86
Postoperative lateral diameter (mm)	12.0	18.0	14.87±1.65
Postoperative AP diameter (mm)	11.0	21.0	14.29±2.48
Deviation improvement (mm)	0.50	19.00	6.03±4.34
Lateral diameter improvement (mm)	0.00	10.00	3.90±2.48
AP diameter improvement (mm)	-9.00	7.00	1.03±2.78

Table 2. Preoperative and postoperative tracheal deviations, as well as lateral and anteroposterior tracheal diameters.

	Preoperative	Postoperative	p-value
Tracheal deviation measure (mm)	13.25 (7.00–28.00)	7.75 (2.5–13.5)	<0.001
Tracheal lateral diameter (mm)	11.00 (6.00–15.00)	15.00 (12.00–18.00)	<0.001
Tracheal AP diameter (mm)	12.50 (6.30–30.00)	14.00 (11.00–21.00)	0.006

AP: anteroposterior.

a decrease in thyroid volume occurs over the long term. The absence of such side-effects and immediate resolution of compression symptoms are the important advantages of goiter patients undergoing thyroidectomy⁸. Significant improvements in tracheal stenosis and deviation were also observed following thyroidectomy performed due to tracheal compression in this study.

The internal diameters of the trachea are of vital importance in the passage of air to the lungs *via* the upper respiratory tracts. According to the Hagen-Poiseuille law, the resistance to flow through a tube is inversely proportional to the fourth power of the radius of the tube if the flow is laminar. At higher rates, the flow can become turbulent and further increase resistance. Deformities arising in the trachea following compression produce a significant increase in airflow resistance by reducing the space of the tracheal airway. A 50% decrease in the internal diameter increases resistance 16-fold and up to 32 times if a turbulent flow develops. Following thyroidectomy, the deformity in the trachea is improved, and the airflow decreases significantly⁹.

The changes in tracheal parameters and respiratory functions which follow other therapeutic methods employed to reduce the effect of thyroid gland pressure have also been investigated. Improvements have been shown in tracheal parameters and respiratory functions 6–12 months after treatment in patients receiving radioactive iodine therapy, either alone or in combination with recombinant human thyroid-stimulating hormone (TSH) (rhTSH)^{5,10,11}. However, the number of studies on the extent of postoperative improvement in areas of tracheal stenosis and tracheal deviation causing preoperative symptoms following thyroidectomy performed due to tracheal compression is limited. Previous studies have evaluated the effect of thyroidectomy on upper airway obstruction using pulmonary function tests^{6,7}.

Sorensen et al. showed that thyroidectomy produced significant improvements in tracheal anatomy and airflow in patients with benign nodular goiter and tracheal compression and that this was also correlated with an improved quality of life. Those authors also reported that the improvement in tracheal parameters was greater in patients with substernal goiter⁶. Similarly in this study, greater improvement was observed in patients with substernal goiter. Additionally, those authors reported a marked relationship between benign thyroid tissue volume and improvement in both tracheal anatomy and also airflow⁶. The amount in line with the weight of the thyroid tissue removed in this study, although no significant relationship was determined between the weight of tissue removed and improvement in tracheal deviation or stenosis.

Greenblatt et al. evaluated swallowing functions in goiter patients after thyroidectomy using the swallowing quality of

life questionnaire and reported significant improvement in swallowing functions in the postoperative period¹². Wang et al. showed that all deviated tracheas returned to their normal positions after 2–3 months postoperatively and that mean tracheal diameters increased significantly on the follow-up radiographs of goiter patients with retrosternal extension¹³.

Ayabe et al. reported that performing hemithyroidectomy due to acute tracheal obstruction resulting from a large benign goiter in an 80-year-old woman and that the tracheal lumen returned to normal postoperatively although malacia was present in the tracheal wall due to prolonged compression¹⁴. Geelhoed reported the subsequent course and outcome in patients undergoing thyroidectomy due to tracheal compression and subsequently developing tracheomalacia¹⁵. Tracheomalacia is a troubling problem that makes thyroidectomy difficult in patients with longstanding or recurrent tracheal compression. No tracheomalacia or associated problem was observed in any patients in this study.

One particular strength of this study is that both benign and malignant diseases were included. The principal limitations are the low patient number, female predominance in terms of gender distribution, the fact that no analysis regarding this could be performed, and the single-center nature of the research. This study should currently be supported by further multicenter studies with larger case numbers.

CONCLUSIONS

Thyroidectomy due to tracheal compression was found to provide significant improvements in tracheal deviation, compression, and deformity. We think that, due to its rapid and effective results, thyroidectomy should represent the first treatment option in thyroid diseases that compress the surrounding tissues and that urgent thyroidectomy will enhance patient comfort and quality of life.

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AUTHORS' CONTRIBUTIONS

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

Validation, Visualization, Writing – original draft, Writing – review & editing. **FA:** Data curation, Formal analysis, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **AK:** Conceptualization, Data curation, Formal analysis, Methodology, Resources, Software, Validation, Visualization, Writing – original draft,

Writing – review & editing. **KT:** Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **MY:** Data curation, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Human fibroblast-like synoviocyte isolation matter: a comparison between cell isolation from synovial tissue and synovial fluid from patients with rheumatoid arthritis

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SUMMARY

OBJECTIVE: Cell culture technology has become a popular method in the field of cell biology, pharmacology, and medical researches. Primary cells represent the normal physiological condition of human cells. Fibroblasts are the most common native cells of connective tissue that play a crucial role in the entire pathogenesis of various disorders, such as rheumatoid arthritis (RA). Fibroblast-like synoviocytes (FLSs), which overlie the loose connective tissue of the synovial sublining, are known to be the central mediators of joint damage. The most routine approach for the isolation of FLS is an enzymatic digestion of synovial tissue. This experimental study is designed to introduce an easy, fast, and high-throughput method compared with enzymatic digestion for isolation of FLS.

METHODS: The synovial tissue and synovial fluid (SF) samples were collected from eight patients with RA who underwent routine knee replacement surgery. Synovial tissue was incubated with collagenase VIII enzyme, while SF was washed with a similar volume of phosphate-buffered saline. The cells were further subcultured and stored based on the standard protocols. The purity of isolated synoviocytes was confirmed using flow cytometry analysis.

RESULTS: Isolation of FLS from SF was more successful with a faster rate, 3–5 days after culture. The morphological assessment and flow cytometry analysis confirmed the purity of SF-derived cells in passage 4.

CONCLUSIONS: SF could be a more accessible source of FLS than synovial tissue. Obtaining primary FLS from SF is a simple, fast, and cost-effective way to have a large-scale cell during a short time.

KEYWORDS: Cell culture. Cell isolation. Fibroblast-like synoviocyte. Synovium. Synovial fluid.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex and multisystem disorder that primarily affects the synovial joints^{1,2}. Fibroblast-like synoviocytes (FLSs) and macrophage-like synoviocytes (MLSs) are two main resident cells in the intimal layer of the synovial membrane, which play a central role in the joint pathology

of RA³. Nevertheless, FLSs are the most common cell at the bone–pannus interface with a more abundant population than MLSs^{4,5}. The migration and invasion of FLS into cartilage and bone are a key event in synovial hyperplasia, resulting in cartilage destruction in patients with RA^{6,7}. Activated FLSs produce a wide range of inflammatory mediators, which promote

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the recruitment and activation of circulating and resident immune cells. Also, the migration of arthritis to unaffected joints has been attributed to the transmigration of FLSs^{8,9}. Although there is still much to be learned about their resolution in RA disease, FLSs have sparked a lot of attention in recent studies. The culture and growth of FLSs is the first step for the investigation and analysis of these cells. At present, the synovium is the main and traditional source of FLS, and access to the synovium is feasible only during arthroplasty or arthroscopy surgery. In advanced RA, total joint arthroplasty (TJA) has been suggested as a successful intervention when optimal medical and supportive therapies have failed^{10,11}. It has been reported that early and continuous use of biological and disease-modifying antirheumatic drugs (DMARDs) delays TJA^{12,13}. Given the importance of FLS in RA pathology, there is a great need to find a reliable and repeatable source for quick access to them. As we know, the extraction of FLSs from synovium obtained during surgery is time-consuming and prone to contamination with microorganisms as well as needs a lot of materials. So, the establishment of an optimized procedure with low cost and shorter time compared with an enzymatic method is essential. In this study, for comparing the efficiencies of different isolation, we aimed to compare two currently being used FLS isolation methods to find a simple and effective procedure.

Materials and reagents

Dulbecco's modified Eagle medium: nutrient mixture F-12 (DMEM/F12)+PSF (penicillin, streptomycin, and fungizone) (Biosera) (for the digestion mixture), fetal bovine serum (FBS) (Biosera), 0.25% trypsin-EDTA (Biosera), collagenase type VIII (Sigma C-2139), fluorochrome-labeled antibodies, cell culture flasks, dishes and tubes, and surgical blade (NO.17) were used.

METHODS

Patients and tissue samples

Human synovial tissue and synovial fluid (SF) were obtained from eight female patients with RA who underwent total knee arthroplasty in the Department of Orthopedics, Shafa Hospital, Sari, Mazandaran, with an average age of 58.8 ± 9.66 years, ranging from 48 to 77 years. All patients fulfilled the 2010 RA classification criteria¹⁴. This study was approved by the Institutional Medical Ethics Committee of Mazandaran University of Medical Sciences (MAZUMS; IR.MAZUMS.REC.1398.1364), and informed consent was acquired from all patients before surgery.

Cell culture

Synovium

The synovial tissues were carried from the surgery department to the cell culture laboratory in a 50-ml falcon tube containing 15-ml DMEM culture medium with 100 U/ml penicillin, 100 U/ml streptomycin, and 1% amphotericin B as transport media and stored at 4°C before processing. Then, the collected tissues were, respectively, washed with phosphate-buffered saline (PBS) (pH 7.3–7.4), alcohol (75%), and finally with PBS+100 U/ml penicillin, 100 U/ml streptomycin, and 1% amphotericin B, each for a period of 2 min. Tissues were minced into 1 mm×1 mm pieces with the help of sterile BP blade No. 17 in a sterilized Petri dish containing DMEM media and then incubated with collagenase VIII for 2 h at 37°C in a shaker incubator to promote isolation of FLS. After incubation time, cell pellets were harvested by centrifugation at 1000 g for 10 min. Then, pellets were suspended in DMEM supplemented with 10% (vol/vol) heat-inactivated FBS, 100 U/ml penicillin, and 100 U/ml streptomycin and placed in a T-25 flask at 37°C with 5% CO₂ in a humidified atmosphere. After 48 h, the medium was changed, and the isolation of cells from synovial tissues was checked every day. The medium was changed twice a week.

Synovial fluid

After collection of SF in a 15-ml falcon tube containing anti-coagulant, it was diluted twice with sterile PBS and centrifuged at 400g for 10 min. Harvested pellets were suspended in DMEM with 10% FBS and antibiotics containing 100 U/ml penicillin and 100 mg/ml streptomycin. The cells were placed in T-25 flasks and incubated under the standard condition at 37°C with 5% CO₂ with saturated humidity. Following the 48 h incubation, fresh DMEM was added to the cells. The culture medium was refreshed every 3–4 days.

SF and synovial tissue culture processing steps are shown in Figure 1.

Identification of FLS by flow cytometry

FLSs at passage 4 were identified by flow cytometry. The purity of FLS was checked for the presence of MLS. The cells were stained with the following antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-CD68, phycoerythrin (PE)-conjugated anti-CD14 antibody, and allophycocyanin (APC)-conjugated anti-CD90.

RESULTS

The results showed that three of eight primary cultures of FLS by enzymatic digestion method were successful. The median

time to the presence of the first cells was 15 days, and they reached 70–80% of confluency around 30–35 days of culture. The remaining tissue samples were discarded if any cells were observed after 30 days. In contrast, cell isolation of all SF samples was successful, and the first cells were seen after about 3–4 days of culture. Getting 70–80% of confluency was dependent on the initial volume of SF.

Morphology of FLS cells

The cells were monitored for morphological assessment after the first day of their presence in the T-25 flask. Most of the cell population were spindle-shaped fibroblast, even in passage 0; however, few number of round to spherical shape, stellate-shaped, and epithelioid-shaped cells were also

observed, especially in initial passages (Figure 2). Overall, isolated FLSs from synovium showed more similarity in shape in passages 1–3 compared with those from SF. It should be mentioned that the homogeneity of cells was different from one sample to another but all get to purity from passages 3 and 4 onward.

Percentage of FLS

Isolated synovial cells from synovium and SF were evaluated by flow cytometry at passage 4. We found a similar percentage of positive cells in specific markers in both procedures used after passage 4.

Overall comparison between the two methods is summarized in Table 1.

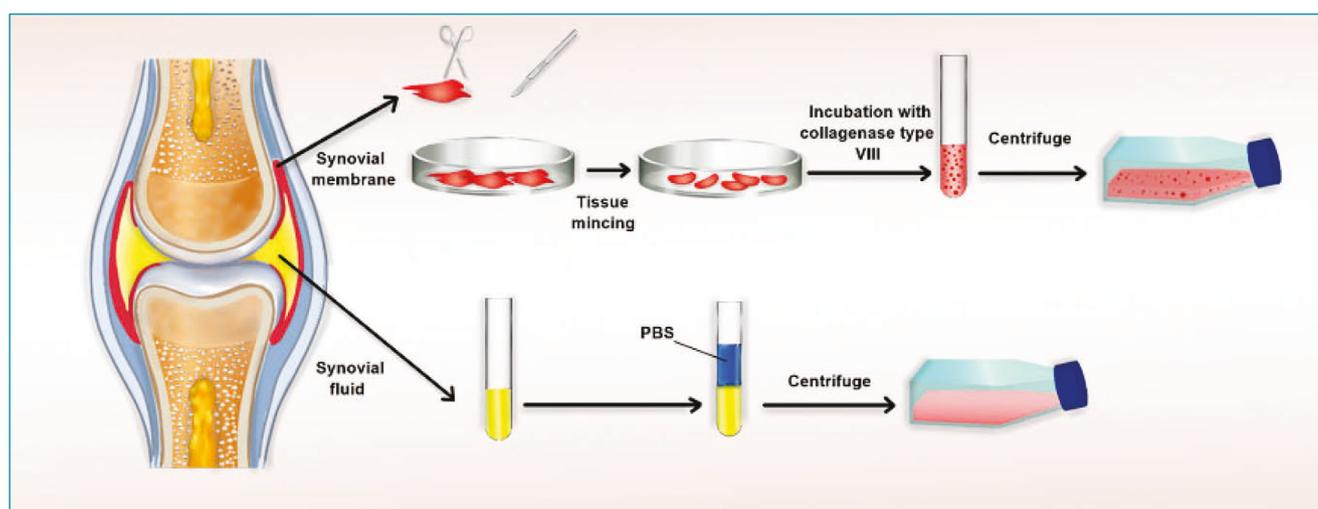


Figure 1. Schematic illustration of synovial fluid and synovial tissue culture processing steps.

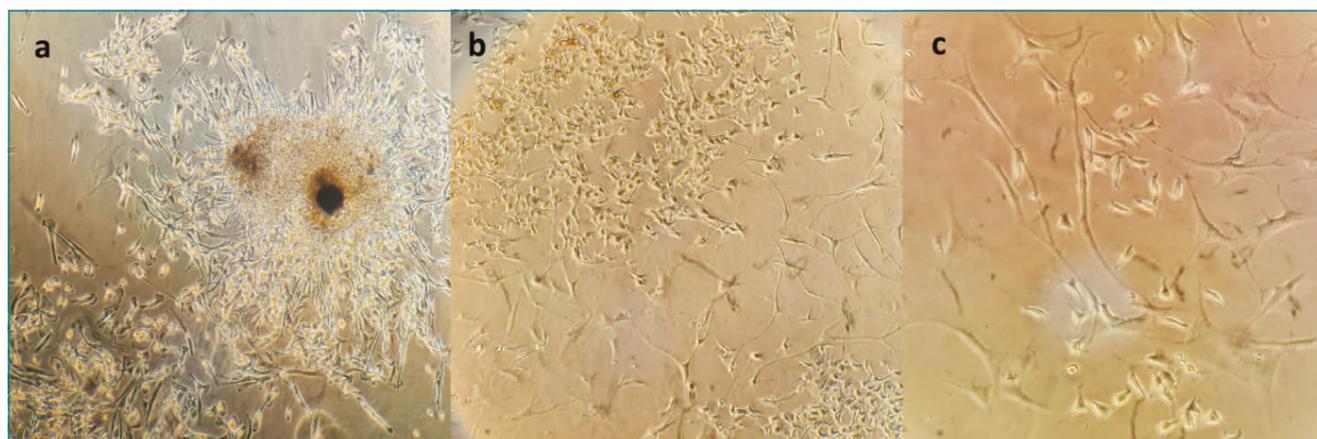


Figure 2. Light microscopic features of synovial fluid and synovial tissue culture. Cell outgrowth from synovial tissue on days 15–25 (a) (100×), a mixture of spindle-shaped and spherical shape fibroblasts with different size in passage 0 from synovial tissue (b) (100×) and synovial fluid (b) (400×).

Table 1. Comparison of important characteristics between two isolation methods.

FLS source	Volume	Sample preparation time	Primary incubation	Required materials	Number of days to see the first cell	Getting to 95% purity
Synovial tissue	1 cm ² (1 mg)	3.5–4 h	+	Digestion enzyme, PBS, alcohol	15–25 days	Passage 3
Synovial fluid	500 µl (minimum)	20 min	–	Anticoagulant, PBS	3–5 days	Passages 4 and 5

PBS: phosphate-buffered saline.

DISCUSSION

Isolation of primary cell lines is required for researchers to investigate the morphological, functional, and cellular characteristics of a special tissue^{15,16}. Nevertheless, a single-standard protocol has not yet been optimized for the isolation of many cells, especially recently discovered ones. FLSs were recently proven to be key players of observed inflammation in RA context^{17,18}. These cells locate inside joints in the synovium and are involved in pannus formation, a hallmark pathological change in patients with RA¹⁹⁻²¹. Recent advances in the treatment of rheumatologic disorders have resulted in a reduction of access to synovial tissues by investigators²². Accordingly, it is essential to find a more proximal source of FLSs than the replaced joints during arthroplasty. In this study, we readily extracted adherent fibroblast cells from SF compared with synovial tissues. Also, flow cytometry analysis of the SF-derived cells confirmed the phenotype of FLS cells. We found that FLS does not migrate from all synovial tissues, but FLS extraction from all SF samples was successful. However, extracted cells from SF were more heterogeneous in initial passages than those from synovial tissue, but in the following, they reach to a similar phenotype like synovial tissue-derived cells. These results are in the same direction as those obtained by Stebulis et al. who indicated that FLS isolation from SF can facilitate the study of synovial cells when synovial tissues are not available. They found that both SF- and tissue-derived FLS are the same in functional and cellular measures such as the presence of some specific enzymes and production of inflammatory mediators²³. Moreover, Ahn et al. reported that there is no significant difference between the invasive capacity of the two FLS types and that both of them are identical in surface markers²⁴. Neidhart et al.

demonstrated that SF-derived FLSs mediate cartilage destruction similar to tissue-derived ones²⁵. These reports are in agreement with our results regarding the identical characteristics of extracted FLSs from both methods.

CONCLUSIONS

We found that the extraction of FLS from synovial tissues is not 100%, and FLS isolation from SF is the most convenient and effective method. The use of 500-µl SF is sufficient to get a higher number of FLS with a shorter time and lower cost of cultivation than synovial tissues. These data may support the selection of FLS isolation from SF for downstream analysis.

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AUTHORS' CONTRIBUTIONS

PZ: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **AR:** Investigation, Writing – review & editing. **FF:** Formal analysis. **SG:** Investigation, Methodology. **AH:** Investigation, Methodology. **MT:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Does large breast problem and macromastia surgery affect a woman's body image, depression level, sexual life, and quality of life? A prospective study

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SUMMARY

OBJECTIVE: This study was designed to evaluate the change in the body perception, sexual life, quality of life, and depression levels in women undergoing a reduction mammoplasty due to breast hypertrophy problem.

METHODS: This descriptive and comparative study was carried out prospectively with 34 women who were diagnosed with breast hypertrophy in a public hospital in the province of Kayseri between 1 April and 31 December 2019 and agreed to participate in the study. The data were collected via the self-reporting method using the Introductory Information Form prepared in line with the literature, Preoperative Data Collection Form, Postoperative Data Collection Form, Female Sexual Function Index (FSFI), Quality of Life Questionnaire, Body Perception Scale (BPS), and Beck Depression Inventory (BDI).

RESULTS: In the preoperative period, 97.1% of the women with breast hypertrophy reported health complaints related to breast hypertrophy, 91.2% need to take a shower frequently, 85.3% social problems like shame and stigma, 85.3% exercise intolerance, and 79.4% sense of dislike. It was determined that general health complaints of these women decreased in the postoperative third month and they obtained significantly high scores from the FSFI, BDI, BPS, and all subscales of the SF-36 Quality of Life Questionnaire, except from the mental subscale ($p < 0.05$).

CONCLUSIONS: It was observed that there were remarkable improvements in the mental, physical, social, and sexual areas of women with breast hypertrophy after the reduction mammoplasty.

KEYWORDS: Body perception. Depression. Reduction mammoplasty. Sexual behavior. Quality of life.

INTRODUCTION

Breast hypertrophy is a benign enlargement of breast caused by the hyperplasia of the hormone-sensitive breast tissue and glandular epithelial tissue. It is also considered an excessive overgrowth of breast^{1,2}. Although the presence of factors such as genetic predisposition and oversensitivity to estrogen is mentioned in the etiology of breast hypertrophy, this issue remains

a mystery². Breast hypertrophy is a disease that is encountered frequently in clinics, affects women's life multidimensionally, impairs the quality of life, and has mentally, physically, and socially negative effects¹⁻⁶.

On the other hand, it has been reported that breast hypertrophy is an important factor affecting the body perception and is associated with negative body image^{4,7-11}. In addition,

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it has been stated that the increase in breast size in women is associated with depression level⁵. It has been asserted that conditions (such as being disliked, anxiety, body image dissatisfaction and health issues, being unable to find appropriate clothes, and participating in sportive activities) caused by large breasts or breast hypertrophy lead to a psychological breakdown in women^{5,12}. Psychological impairments or health issues caused by breast hypertrophy may directly trigger sexual dysfunctions, thus leading to sexual anxiety and avoidance of sexual activity^{13,14}.

The purpose of this study was to address the breast hypertrophy problem and reduction mammoplasty from the perspective of women's health and determine the effect of reduction mammoplasty on women's body perception, sexual life, quality of life, and depression levels.

METHODS

Type of the study

The study was designed and carried out prospectively as a descriptive and comparative study.

Population and sample of the study

The population of the study consisted of all women with the diagnosis of breast hypertrophy who underwent a reduction mammoplasty in the Plastic and Reconstructive Surgery Clinic of a public hospital in the province of Kayseri. In this clinic, the total number of patients per year is 14,387 and the number of women undergoing a reduction mammoplasty due to breast hypertrophy problem is 41. The sample of the study consisted of 37 women who applied to the hospital between April 19, 2019, and December 30, 2019, were diagnosed with breast hypertrophy, were over the age of 18, had no communication obstacle or identified heavy mental disorder, and agreed to participate in the study after being informed. In the follow-up process, three patients, who withdrew from the study willingly and could not be reached for the post-test, were excluded. Finally, only 34 women completed the study. Data were collected from women admitted to the hospital through face-to-face interviews before (on the first day before the surgery – first interview) and after (at the third month after the surgery – second interview) surgery.

Data collection tools

The data were collected using Preoperative (on the first day before the surgery – first interview) and Postoperative (at the third month after the surgery – second interview) Data Collection Forms, which were prepared by the researchers

and contain questions about women's sociodemographic characteristics (such as age, marital status, marriage duration, and educational background), gynecological-obstetrical characteristics (such as gravidity, parity, the number of miscarriages, the number of abortions, and state of menopause), and breast hypertrophy. The SF-36 Quality of Life Questionnaire (SF-36)^{15,16}, Female Sexual Function Index (FSFI)^{17,18}, Beck Depression Inventory (BDI)^{19,20}, and Body Perception Scale (BPS)²¹ were also used.

SF-36 Quality of Life Questionnaire (SF-36)

It has eight subscales and 36 questions, with score ranging from 0 to 100. Higher score is directly proportional to the quality of life^{15,16}.

Female Sexual Function Index (FSFI)

The index has six subscales and 19 questions, with score ranging from 2 (minimum) to 36 (maximum). Higher score indicates a better sexual function^{17,18}.

Beck Depression Inventory (BDI)

It is a four-point Likert scale comprising a total of 21 items. Higher total score indicates a higher depression severity^{19,20}.

Body Perception Scale (BPS)

The score of this scale ranges from 40 to 200. Higher scores are directly proportional to the degree of satisfaction²¹.

Ethical Approval and Consent to Participate

Before the study, permission (No. E.15211) was obtained from the ethics committee of the hospital where the study was conducted (IRB: 2020/25) and from the hospital administration. Prior to the interviews, the participants were informed about the purpose and method of the study and their oral consent was obtained.

Data analysis

The collected data were evaluated using the Statistical Package for Social Science for Windows, version 21.0, package program. Descriptive measurements of all the variables were calculated. Categorical data were compared using the chi-square test and Fisher's exact test. The dependent-sample Student's t test was used to compare the scores related to repeated measurements and the Mann-Whitney U test was used for comparison between two independent groups. The multiple regression analysis was performed to examine the correlation between one dependent variable and two or more independent variables. A p-value <0.05 was considered statistically significant.

RESULTS

When examining the sociodemographic characteristics of the women participated in the study, it was determined that their mean age was 41.47 ± 10.0 years (min: 22, max: 64), mean marriage duration was 18.26 ± 10.70 years (min: 3, max: 45), and average body mass index (BMI) was 26.37 ± 3.43 (min: 20.93, max: 35.38). Notably, 55.9% had a BMI of ≥ 25 . It was found that women had a large breast problem for an average of 13.55 ± 6.95 (min: 3, max: 25) years (Table 1). Of the women with breast hypertrophy problem, 91.2% frequently took a shower due to malodor and sweating, and 94.1% had to choose large size cloths due to breast hypertrophy. Also, 79.4% of the women thought that they were not liked by people and 11.8% were subjected to verbal abuse (by their partners). On the one hand, the vast majority of women (85.3%) reported that they felt embarrassed because their breasts looked too big and that they had problems in their social lives. On the other hand, more than half of the women (85.3%) stated that they experienced limitation of movement (such as not being able to run, not be able to walk fast, and not be able to jump) due to large breast problems.

In this study, it was determined that the women with breast hypertrophy problem obtained significantly higher scores from all subscales of the SF-36 Quality of Life Questionnaire, except for the mental subscale in the postoperative third month, compared to preoperative period ($p < 0.001$, Table 2).

The mean scores of the FSFI and subscales, as well as those of desire, arousal, lubrication, orgasm, general satisfaction, pain,

and FSFI, were found to increase significantly compared to the first day before surgery and at the third month after surgery ($p < 0.001$, Table 2).

The preoperative BDI mean scores of the women who underwent a surgery due to breast hypertrophy problem were significantly higher compared to the postoperative third month ($p < 0.001$, Table 2).

There was a statistically significant difference between the BPS scores of the women in the first interview conducted in the preoperative period and the second interview conducted in the postoperative third month, and the postoperative BPS scores significantly increased ($p < 0.001$, Table 2).

In the multivariate analysis, it was found that 32% of the variance of the FSFI in women with breast hypertrophy problem was associated with the SF-36 Quality of Life Questionnaire — role limitations due to physical problems ($\beta = -0.008$, $p < 0.05$), duration of breast hypertrophy problem ($\beta = 0.25$, $p < 0.05$), and Body Perception Scale ($\beta = 0.007$, $p < 0.05$), which were the determinants for the sexual functions of women with breast hypertrophy problem (Table 3).

DISCUSSION

In this study, the women's mean age was 41.47 ± 10.00 years, which is compatible with the study by Emami and Sobhani¹³. Emami and Sobhani suggested that women undergoing a reduction mammoplasty have a variation in age¹³. Some research studies found that most of the women were married and this

Table 1. Comparison of the participants' characteristics related to marriage and sexual life in the preoperative (first day) and postoperative (third month) periods (n=30).

Characteristics	Preoperative (first day, first interview)		Postoperative (third month, second interview)		t	p
	Mean±SD		Mean±SD			
Number of sexual intercours (week)	1.96±0.88		2.46±1.07		-3.330	0.002
	Number	%	Number	%	χ^2	p
Relationship status with the partner						
Very good/good	17	65.4	1	25.0	-	0.274*
Medium	9	34.6	3	75.0		
Satisfaction with sexual life						
Satisfied	20	74.1	2	66.7	-	0.172*
Dissatisfied	7	25.9	1	33.3		
Sexual problems						
Available	8	88.9	8	38.1	-	1.000*
NA	1	11.1	13	61.9		

*Fisher's exact test.

Table 2. Comparison of the women's SF-36 Quality of Life Questionnaire, female sexual function index subscale beck depression inventory, and body perception scale total scores in the preoperative (first day) and postoperative (third month) periods.

Scales SF-36 Quality of Life Questionnaire (n=34)	Preoperative (first day, first interview)	Postoperative (third month, second interview)	t	p
	Mean±SD	Mean±SD		
Physical functioning	41.17±28.04	81.25±26.69	-5.982	0.000*
Role limitations due to physical problems	47.05±42.97	94.11±23.88	-4.961	0.000*
Role limitations due to emotional problems	51.96±43.57	95.09±18.59	-5.216	0.000*
Vitality	36.63±18.75	64.43±23.40	-5.554	0.000*
Mental health	60.47±27.76	67.84±27.12	-1.530	0.135
Social functioning	43.27±27.12	81.17±26.02	-5.640	0.000*
General health	43.11±24.39	70.77±17.59	-5.594	0.000*
Pain	49.01±26.53	69.11±32.01	-2.822	0.000*
FSFI (n=30) [†]				
Desire	2.27±1.16	3.95±0.82	-5.092	0.000*
Arousal	2.26±1.14	4.24±0.91	-6.143	0.000*
Lubrication	2.84±1.45	4.91±0.80	-5.396	0.000*
Orgasm	2.32±1.38	4.57±0.92	-6.215	0.000*
General satisfaction	2.53±1.29	5.00±0.79	-5.082	0.000*
Pain	4.89±1.40	5.24±0.94	-3.387	0.002*
FSFI total	17.12±6.29	27.93±3.96	-6.806	0.000*
BDI (n=34)	19.23±9.07	6.73±4.68	8.735	0.000*
BPS (n=34)	108.76±23.32	165.75±22.46	-10.407	0.000*

SF-36: Short Form-36; FSFI: Female Sexual Function Index; BDI: Beck Depression Inventory; BPS: Body Perception Scale. *p<0.05; [†]Sexually inactive people were excluded.

Table 3. Multiple regression analysis for the variables related to the women's female sexual function index scores (n=30)*

Variables	B	Standard error	b	t	p
Constant	0.909	0.572		1.588	0.125
SF-36 Quality of Life Questionnaire- Role limitations due to physical problems	-0.008	0.003	-0.441	-2.625	0.015 [†]
Duration of breast hypertrophy problem (year)	0.025	0.010	0.371	2.394	0.024 [†]
Body Perception Scale	0.007	0.004	0.345	2.152	0.042 [†]

*Sexually inactive people were excluded; [†]p<0.05. Dependent variable: Female Sexual Function Index cutoff point. R: 0.411, adjusted R²: 0.317, F: 4.362, p=0.008, Durbin Watson: 1.971.

may be due to the fact that they wished to have a positive body perception and to be liked by their partner or spouse^{22,23}. In this study, it was determined that 55.9% of the participants had a BMI of ≥ 25 and there are also similar results in the literature^{4,24}.

Several studies reported that breast hypertrophy affects all dimensions of the quality of life negatively^{1,7,23}. Çeber et al.¹ revealed that the quality of life of women with symptomatic

macromastia was affected positively in the postoperative period. In this study, it was determined that the women with breast hypertrophy problem had lower quality of life and increased self-care needs in the preoperative period. In the reevaluations conducted for these women in the postoperative third month, it was determined that the women had significantly higher scores from all subscales of the SF-36 Quality of Life Questionnaire, except for the mental subscale.

In contrast, body perception affects women both psychosocially and psychosexually⁷. Recent studies stated that women with breast hypertrophy problem have lower self-respect^{4,5,10}. Janik et al.³ reported that body image dissatisfaction was a great problem for women with breast hypertrophy and shame, depression, and anxiety were associated with weak body image. In a study on women with breast hypertrophy problem, Pérez-Panzano et al.⁴ determined that there were improvements in not only body satisfaction but also mental state of mind and social relations in the postoperative period. Brunetti et al.⁷ found that there were significant improvements in body perception in the postoperative period. Also in this study, the women stated that their body became more proportional and their breasts became more upright, and as a result they felt more attractive, perceived themselves “just like a woman,” looked fitter, and had a positive self-respect and higher self-confidence. In the follow-up conducted with the women with breast hypertrophy problem using the body perception scale in the postoperative third month, it was determined that the women obtained higher scores and their body perception improved at a positive level. Female breasts are an important part of sexual activity and self-confidence, and breast hypertrophy causes women to have lower self-confidence, sexual anxiety, and psychosexual problems¹³. Singh and Losken²⁵ reported that 80% of women with breast hypertrophy problem had sexual problems, felt shame, and disliked being touched on their breasts (by their partner). Likewise, in this study, the women indicated that they felt shame when changing clothes and stripping their breasts in front of their partner, wanted to hide their breasts, and had a sense of being disliked (79.4%). More than half of the women (53.3%) stated that they had sexual problems in the preoperative period. In this context, the studies revealed that during the interviews carried out with the women with breast hypertrophy after reduction mammoplasty, there were improvements in their psychosexual functions^{3,10,14}. As a matter of fact, in this study it was also determined that there were significant improvements in sexual functions of the women with breast hypertrophy problem in the postoperative third month.

Nonproportional, heavy, and large breasts are an effective factor for the cause of intense depressive symptoms^{5,10}. Young women and adolescents, for whom body perception is more important, are more inclined to showing depressive symptoms. It is suggested that breast hypertrophy problem affects the bilateral relations or social relations negatively as a result of serious mental exposures in young women and adolescents^{5,6,12}. Indeed, it is indicated that breast hypertrophy

problem leads to risk in some adolescents such as eating disorder, bulimia nervosa, obesity, intentional vomiting, laxative and diuretic drugs, and suicidal thought^{1,8,12,25}. It is stressed that reduction mammoplasty reduces negative mental effects in women with breast hypertrophy problem and thus provides multidimensional improvements in the quality of life and sexual life^{12,14,23,24}. Rogliani et al.¹² stated that reduction mammoplasty made permanent positive improvements in the quality of life and body image of women having breast hypertrophy problem and undergoing a surgery and significantly developed psychological well-being by removing the breast weight and ptosis. In this study, it was determined that 26.5% of the women ate less to have smaller breasts, felt shame, had low self-confidence, and their depressive symptoms decreased during the follow-up conducted in the postoperative third month compared to preoperative condition, which is compatible with the results of the studies.

CONCLUSIONS

Finally, it was determined that the women with breast hypertrophy problem had lower scores from all subscales of the SF-36 Quality of Life Questionnaire in the preoperative period, had a lower body perception, had lower mean scores from the FSFI total and all subscales, and showed depressive symptoms according to the BDI. It was found that the participants obtained higher scores from the SF-36 Quality of Life Questionnaire in the postoperative third month, had a positive body perception, had a positive effect in their sexual functions according to the FSFI, and decreased depressive symptoms according to the BDI.

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AUTHORS' CONTRIBUTIONS

SC: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review and editing. **MD:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – original draft, Writing – review and editing.

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Nonalcoholic fatty liver disease: scintigraphy in the diagnosis of steatohepatitis

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SUMMARY

OBJECTIVE: Nonalcoholic fatty liver disease is the most prevalent cause of chronic liver disease worldwide. Nonalcoholic steatohepatitis is associated with increased mortality rates due to the liver and cardiovascular diseases. The gold standard for discriminating nonalcoholic fatty liver disease activity and staging is the anatomopathological examination, which is an invasive method. In this regard, noninvasive methods, such as scintigraphy, have been under investigation. This study investigated the role of scintigraphy in the diagnosis of nonalcoholic steatohepatitis in obese patients with nonalcoholic fatty liver disease undergoing bariatric surgery.

METHODS: Patients undergoing bariatric surgery and liver biopsy were prospectively included. ^{99m}Tc-phytate scintigraphy was performed to assess liver/spleen, spleen/heart, and liver/heart uptake ratios, while ^{99m}Tc-isonitrile scintigraphy assessed liver/heart ratio. To evaluate the presence of nonalcoholic steatohepatitis, the results of ^{99m}Tc-phytate scintigraphy and ^{99m}Tc-isonitrile scintigraphy were compared with the anatomopathological examination.

RESULTS: Sixty-one patients with nonalcoholic fatty liver disease were allocated into two groups, namely, nonalcoholic steatohepatitis (n=49) and non-nonalcoholic steatohepatitis (n=12). The results of scintigraphic images obtained after the infusion of radiopharmaceutical ^{99m}Tc-phytate in liver/spleen, spleen/heart, liver/heart ratios and ^{99m}Tc-isonitrile liver/heart ratio presented no difference between groups with and without nonalcoholic steatohepatitis with an accuracy of 47.5, 37.7, 50.8, and 52.5%, respectively.

CONCLUSION: Scintigraphy was not proven to be a useful method to differentiate patients with and without nonalcoholic steatohepatitis.

KEYWORDS: Non-alcoholic fatty liver disease. Nonalcoholic steatohepatitis. Diagnosis. Radionuclide imaging.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most frequent cause of chronic liver disease worldwide^{1,2}. The clinical presentation of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH). NASH may progress to fibrosis, cirrhosis, and ultimately to hepatocellular carcinoma (HCC)³. Furthermore, NAFLD is currently considered the hepatic manifestation of metabolic syndrome (MetS), which has well-documented associations with cardiovascular diseases,

type 2 diabetes, and chronic kidney disease⁴. Patients with NASH present a higher mortality risk due to both liver and cardiovascular causes⁵.

Histological examination of the liver is the gold standard method for discriminating between the forms of NAFLD and for the diagnosis and staging of NASH. However, this is an invasive method. Additionally, due to the high prevalence rates of NAFLD, it is not suitable to perform a liver biopsy in most of the patients^{6,7}.

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The available usual noninvasive methods do not allow discrimination between steatosis and steatohepatitis⁸⁻¹², and since the currently existing study on the use of scintigraphy to assess NASH is scarce^{13,14}, we aimed to evaluate the role of this method in the detection of inflammation in patients with NAFLD.

METHODS

Patients with clinical indications for bariatric surgery attended in Irmandade Santa Casa de Porto Alegre Hospital, a tertiary level hospital in southern Brazil, were prospectively included from October 2016 to March 2019.

Patients who underwent bariatric surgery according to formal indications¹⁵ were included.

All included patients underwent liver biopsy during surgery.

Patients with positive serological markers for hepatitis B virus and hepatitis C virus, patients with significant alcohol consumption (>30 g/day for men and 20 g/day for women), and patients with chronic liver disease of other etiologies were not eligible for this study.

Information on gender, age, weight, height, body mass index (BMI), and the presence of comorbidities was recorded. The diagnosis of diabetes mellitus (DM)¹⁶, systemic arterial hypertension (SAH)¹⁷, and dyslipidemia¹⁸ followed the international recommendations. Additionally, the platelet count and biochemical parameters were evaluated. All laboratory tests were performed according to institutional standards and manufacturers' recommendations.

Histological analyses were performed by a pathologist blinded to clinical data. For the NASH diagnosis, the presence of steatosis associated with ballooning and the presence of inflammatory infiltrate was considered^{19,20}.

Liver scintigraphy was performed on SIEMENS equipment (i.e., model Symbia T2, SPECT/CT technology, and 2-channel tomography), and the images were obtained using the Somaris/5 software, version 8.5.10.30.

After an 8-h fasting period and 20 min after the intravenous administration of 8 mCi of Technetium-99m-phytate (^{99m}Tc-phytate), the patients underwent planar static image acquisition, including liver, spleen, and the cardiac area in the field of view, until 2100 million counts were reached, in a gamma™ 2-detector camera equipped with a low-energy, high-resolution collimator with parallel holes, 512×512 matrix, zoom 1.23. Regions of interest (ROIs) were designed in the liver, spleen, and heart, obtaining the average number of counts in these organs to calculate the liver/spleen, spleen/heart, and liver/heart ratios, according to the institutional protocol.

In the protocol with Technetium-99m-methoxy-isobutyl isonitrile (^{99m}Tc-MIBI), after fasting for 8 h and 10 min after

the intravenous administration of 16 mCi of the radiopharmaceutical, patients underwent planar static image acquisition including the liver and the heart in the field of vision for 5 min, until reaching 1500 million counts in gamma-chamber. ROIs were drawn with the liver, right lobe, and heart in the field of view, obtaining the number of counts in these organs, to calculate the liver/heart ratio, following the institutional protocols. Liver scintigraphy was performed up to 1 month before surgery, and both protocols were performed on the same day.

In the statistical analyses, quantitative data were described as mean±standard deviation (SD). Median and minimum–maximum intervals were presented if the variables did not show normal distribution. Categorical data were presented by counts and percentages. Quantitative data were compared using the Mann-Whitney U test and categorical data using the Fisher's exact test. Diagnostic performance measures, such as sensitivity, specificity, and predictive values, were obtained. Statistical significance was set at $p < 0.05$.

RESULTS

Sixty-one patients were eligible for this study. Following the histopathological assessment of liver biopsies, patients with NAFLD were divided into two groups as follows: with NASH (n=49) and without NASH (steatosis only) (n=12).

Clinical and demographic characteristics were compared between groups (Table 1). The NASH group showed higher mean alanine aminotransferase (ALT) levels in comparison with the group without NASH. Regarding MetS, patients with NASH presented greater insulin resistance (IR). Although triglyceride levels were higher in patients with NASH, there was no difference in the frequency of dyslipidemia between groups. Likewise, despite the differences observed in the mean blood glucose values between groups, there was no difference in the distribution of DM between the groups with and without NASH.

The scintigraphic images obtained after infusion of the radiopharmaceutical ^{99m}Tc-phytate to assess the liver/spleen, spleen/heart, liver/heart ratios and ^{99m}Tc-MIBI for the liver/heart ratio were not different between groups (Table 2). After assessing sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy, the results did not reach the desired levels to validate scintigraphy as a method for the detection of NASH (Table 3).

DISCUSSION

Since NAFLD is the most common cause of liver disease and bearing in mind the different natural histories of patients with NASH and those with steatosis, a differential diagnosis is of the highest importance.

Table 1. Distribution of demographic, clinical, and laboratory data of groups with and without steatohepatitis.

	All-cases (n=61)	Without NASH (n=12)	With NASH (n=49)	p-value
Age* (years)	39.4±9.1	36.1±8.4	40.2±9.2	0.159 ^a
Female**	49 (80.3)	9 (7.5)	40 (81.6)	0.689 ^a
BMI* (kg/m ²)	42.5±5.5	44.5±3.0	42.1±5.7	0.050 ^a
ALT* (U/L)	36.3±21.2	24.6±10.3	39.1±22.0	0.009 ^a
AST* (U/L)	26.4±11.1	22.3±6.3	27.4±1.8	0.167 ^a
Bilirubin* (mg/dL)	0.4±0.3	0.4±0.3	0.4±0.3	0.956 ^a
Triglycerides* (mg/dL)	177.3±160.8	105.6±34.8	198.9±74.5	0.011 ^a
TC* (mg/dL)	193.9±38.6	174.8±30.4	198.5±39.3	0.059 ^a
HDL* (mg/dL)	47.1±10.4	53.6±11.2	45.6±9.7	0.038 ^a
LDL* (mg/dL)	114±29	100±26	117±28	0.106 ^a
Glucose* (mg/dL)	104.5±33.0	87.2±8.8	108.8±35.3	0.004 ^a
Insulin* (U/L)	20.7±9.1	16.8±5.8	21.7±9.5	0.037 ^a
HOMA-IR*	5.5±3.3	3.8±1.5	6.0±3.5	0.013 ^a
Platelets* (mm ³)	274573±67274	272000±68747	275204±67616	0.949 ^a
Ferritin* (ng/mL)	240.9±226.6	214.6±157.2	247.3±241.5	0.986 ^a
Albumin* (g/dL)	4.2±0.3	4.2±0.3	4.2±0.3	0.964 ^a
Hypertension**	28 (45.9)	3 (25.0)	25 (51.0)	0.122 ^b
Diabetes**	14 (23.0)	1 (8.3)	13 (26.5)	0.264 ^b
Dyslipidemia**	12 (19.7)	0 (0)	12 (24.5)	0.100 ^b

NASH: nonalcoholic steatohepatitis; BMI: body mass index; HOMA-IR: homeostasis model assessment-estimated insulin resistance; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TC: total cholesterol. *Data presented as mean±SD; **Data presented as number of patients (% of all patients). ^aMann-Whitney U test. ^bFisher's exact test.

Table 2. Comparison of scintigraphic ratios between patients with and without nonalcoholic steatohepatitis.

	All-cases (n=61)	Without NASH (n=12)	With NASH (n=49)	p-value
^{99m} Tc-phytate liver/spleen				
Mean±SD	5.73±2.60	7.43±4.46	5.32±1.73	0.095
Median	5.35	7.05	5.31	
(min-max)	2.67-19.49	3.8-19.49	2.67-10.59	
^{99m} Tc-phytate spleen/heart				
Mean±SD	2.25±0.65	2.03±0.62	2.30±0.65	0.207
Median	2.17	1.99	2.26	
(min-max)	1.01-4.23	1.01-3.33	1.30-4.23	
^{99m} Tc-phytate liver/heart				
Mean±SD	11.90±3.08	13.21±3.89	11.58±2.80	0.179
Median	11.85	12.84	11.56	
(min-max)	6.10-19.84	6.81-19.84	6.10-17.96	
^{99m} Tc-MIBIliver/heart				
Mean±SD	2.08±0.71	2.32±0.84	2.02±0.67	0.171
Median	1.92	2.19	1.92	
(min-max)	1.11-4.31	1.51-4.31	1.11-4.26	

NASH: nonalcoholic steatohepatitis; MIBI: methoxy-isobutyl isonitrile. *Mann-Whitney U test.

Table 3. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of scintigraphy in the diagnosis of nonalcoholic steatohepatitis.

	Sensitivity (%)	Specificity (%)	PVV (%)	NPV (%)	Accuracy (%)
^{99m} Tc-phytate/liver/spleen≤5	44.9	58.3	81.5	20.6	47.5
^{99m} Tc-phytate/spleen/heart≤2	34.7	50.0	73.9	15.8	37.7
^{99m} Tc-phytate/liver/heart≤11.5	46.9	66.7	85.2	23.5	50.8
^{99m} Tc-methoxy-isobutylisonitrile/liver/heart≤1.9	49	66.7	85.7	24.2	52.5

PVV: positive predictive value; NPV: negative predictive value.

The identification of inflammation by the assessment of serum biomarkers is expensive, poorly available, and presents a low accuracy. In turn, imaging methods are a recognized approach for the detection of only steatosis and fibrosis but are limited for the diagnosis of NASH¹². Among them, scintigraphy may represent a low-risk and relatively low-cost method, thus favoring its employment for the identification of inflammation in patients with NAFLD. It has been demonstrated that the reticuloendothelial dysfunction of Kupffer cells contributes to the pathogenesis of steatohepatitis. As ^{99m}Tc-phytate accumulates in the liver followed by phagocytosis by Kupffer cells, the reduction of this radioisotope uptake of during the scintigraphic exam could be a sensitive marker of steatosis to NASH progression¹³. Similarly, due to the hepatic mitochondrial abnormalities described in the pathogenesis of NASH, the decreased uptake of ^{99m}Tc-MIBI could also reveal clinical significance, as it indicates progression to a more advanced disease stage¹⁴.

To the best of our knowledge, only two studies have compared the role of scintigraphy between patients with NASH and simple steatosis to the present time^{13,14}. Kikuchi et al.¹³ evaluated 37 patients with suspected NAFLD at the time of liver biopsy (8 patients with simple steatosis and 29 with NASH). A reduced ^{99m}Tc-phytate liver–spleen ratio was demonstrated in the NASH group in relation to the group with simple steatosis. Importantly, the decrease in the liver/spleen ratio was observed in all NASH stages, and it was identified as an independent predictor to distinguish NASH and steatosis. Masuda et al.¹⁴ employed the radiopharmaceutical ^{99m}Tc-MIBI to evaluate 26 patients with biopsy-proven NAFLD (four with simple steatosis, 11 with borderline NASH, and 11 with NASH). The authors reported a significantly lower liver–heart ratio in the NASH group compared with the steatosis group. In contrast to these results, which support the use of scintigraphy in the diagnosis of NASH, we did not identify a difference in ^{99m}Tc-phytate or ^{99m}Tc-MIBI uptake between the groups with and without NASH. Unlike the above-mentioned

studies, despite having assessed the ROIs in order to include the liver tissue to a greater extent, our results did not provide the expected superior accuracy.

Furthermore, in contrast to the population included in this study, whose mean BMI was 42.5 kg/m², the patients included in the studies of Kikuchi et al.¹³ and Masuda et al.¹⁴ presented an average BMI of 25–30 kg/m². In fact, the patients included in this study were obese who underwent bariatric surgery, which could possibly have a higher degree of inflammation, thus favoring a more precise distinction between groups.

Despite this study having been prospectively performed and all included patients having undergone histological evaluation, the main limitation was the small sample size, particularly in the group of patients without NASH. However, this study included 61 patients, while the studies by Kikuchi et al.¹³ and Masuda et al.¹⁴ have evaluated 37 and 26 patients, respectively.

CONCLUSION

We believe that it was not possible to demonstrate the usefulness of scintigraphy to discriminate groups of patients with or without NASH.

AUTHORS' CONTRIBUTION

ADS: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **AAM:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **CVT:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **LFD:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **OEA:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **GPC:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **AAM:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

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Association between thrombocytopenia and platelet profile with morbidity/mortality of severe and non-severe COVID-19 patients

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SUMMARY

OBJECTIVE: Coronavirus disease 2019 (COVID-19) is a novel infectious viral disease that can be associated with changes in platelet counts. Thrombocytopenia is a risk factor for increased mortality and morbidity among these patients. In this study, we aimed to measure the platelet count of COVID-19 patients and find the association with morbidity and mortality after following up.

METHODS: This study was conducted on 1,320 confirmed COVID-19 patients who were admitted to the Ayatollah Taleghani and Shohada Tajrish Hospital in Tehran, Iran. The diagnosis of COVID-19 was confirmed by standard protocols. The data on the platelet profile were retrospectively extracted from patients' electronic medical records consisted of platelet counts on admission, the next 7 days during the hospital stay, and on discharge. Patients were categorized into two groups, namely, "non-severe presentation" and "severe presentation" based on clinical signs.

RESULTS: There was no significant difference in platelet counts and thrombocytopenia between severe and non-severe, survivors and non-survivors, and severe survivors and severe non-survivors groups at the time of admission to the hospital. After 7 days, a trend toward an increase in platelet counts was seen in non-severe patients, survivors, and severe compared with severe patients, non-survivors, and severe non-survivors, respectively.

CONCLUSIONS: Thrombocytopenia and thrombotic complications in COVID-19 patients are common and lead to a higher mortality rate.

KEYWORDS: COVID-19. Morbidity. Mortality. Platelet. Thrombocytopenia.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) started with an outbreak in Wuhan, China, in December 2019 and rapidly evolved into a global pandemic within a few months, resulting in 15,099,548 confirmed cases and 619,605 deaths worldwide till date¹, and

the numbers are growing promptly. Although the mortality rates are low compared to other diseases caused by the coronavirus family, the fatality rate of 10% in severe acute respiratory syndrome (SARS) and 37% in the Middle East respiratory syndrome (MERS) due to the alarming rates of transmission,

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the pandemic is causing a huge burden on countries' resources and economy². The rising mortality and morbidity rates are worrisome particularly in resource-restricted countries with health care systems already facing shortages³.

COVID-19 was primarily regarded as respiratory disease, but the emerging data revealed that it should rather be recognized as a systemic illness, involving not only the respiratory system but also multiple organs such as the cardiovascular, neurological, gastrointestinal, hematopoietic, and immune system⁴⁻⁶. Numerous studies have focused on hematological and hemostatic abnormalities — common and significant among severe cases — in COVID-19, highlighting their capacity to predict disease progression and prognosis⁷⁻⁹. Among the hematological findings, lymphopenia, neutrophilia, thrombocytopenia, leukocytosis, and/or leukopenia have been observed¹⁰⁻¹²; of those, the platelet count is an available and affordable factor with evident association with COVID-19 severity and possibly mortality^{13,14}. Considering the lack of effective therapy and the heavy burden on the health care system, having convenient tools for predicting the prognosis can play a vital role. Thrombocytopenia was previously identified and used as a major prognostic factor in SARS with fair accuracy^{15,16}. It can be effectively used in COVID-19 likewise, but to measure the extent, further investigations are in demand and according to the rapidly evolving situation, soon. In this study, we described the epidemiology of thrombocytopenia and platelet profile alterations in a relatively large population of 1,019 confirmed COVID-19 patients and explored the possible link to morbidity and mortality among them.

METHODS

Participants and procedures

A total of 1,320 confirmed COVID-19 patients who were admitted to the Ayatollah Taleghani and Shohada Tajrish Hospital in Tehran, Iran, was recruited in a retrospective double-center study from February 20 to May 20, 2020. The diagnosis of COVID-19 was confirmed by:

1. Polymerase chain reaction (PCR) analysis; and
2. Chest computed tomography (CT) inspection. SARS coronavirus 2 (SARS-CoV-2)-contaminated swab specimens from the upper respiratory tract were obtained, maintained in a virus-transport medium, and detected by real-time reverse transcriptase PCR (RT-PCR) analysis.

The amplification arrangements were as followed: 50°C for 15 min, 95°C for 3 min, 45 cycles of 95°C for 15 s, and 60°C for 30 s. CT scan was requested for all admitted patients

with common COVID-19 signs and symptoms such as fever, cough, dyspnea, pleuritic chest pain, decreased O₂ saturation, and also abnormal lung auscultation and evaluated by an adaptive radiologist with precise consideration of all imaging features including pure consolidation, pure ground-glass opacity (GGO), mixed GGO and consolidation, reversed halo, intralobular traction bronchiectasis, crazy-paving, intralobular vascular enlargement, linear opacities, pleural effusion, and pericardial effusion. The affected lung lobes were counted, and lesions were categorized as peripheral or central based on lesion location. A thin-section CT involvement was calculated based on the extent of lung involvement. This study was approved by the Ethics Committee of the Shahid Beheshti University of Medical Sciences.

Vital signs of all patients, including blood pressure, respiratory rate, heart rate, and temperature on admission, were measured and demographic baseline information were recorded. The data on the platelet profile were retrospectively extracted from patients' electronic medical records of the Ayatollah Taleghani and Shohada Tajrish Hospital. The profile for each patient consisted of platelet counts on admission, the next 7 days during the hospital stay, and on discharge. For this matter, 301 patients with an incomplete laboratory profile were excluded from this study, and the participants' count with complete baseline and laboratory information dropped to 1,019 cases. Thrombocytopenia was defined as the platelet count under 150,000 platelets per microliter of blood.

Patients were categorized into two groups, namely, "non-severe presentation" and "severe presentation." They were defined as severe if admitted to ICU or died during the course of disease progression or had severe hypoxia (O₂ saturation ≤85%) on admission or had respiratory rate ≥30 on admission and non-severe otherwise. The severe group was also divided into two subgroups of severe survivors and non-survivors.

Statistical analysis

The continuous variables were examined to determine the normality of the distribution using histograms, measures of skewness and kurtosis, and Kolmogorov-Smirnov test. The normally distributed variables were described as the mean ± standard deviation (SD), and the skewed distributed variables were expressed as the median and interquartile range (25–75%). Categorical variables were summarized as frequencies (percentage). The normally distributed continuous variables were compared between non-severe and severe groups using the two independent sample *t*-test and non-normally distributed variables with the Mann-Whitney *U* test. The comparisons of categorical variables between groups were conducted using the chi-square test of independence.

All tests were two-sided, and a $p < 0.05$ was considered to indicate a statistically significant difference. All the statistical analyses were performed using the IBM SPSS version 24.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline characteristics of patients with COVID-19

A total of 1,320 patients with confirmed COVID-19 diagnosis was enrolled in this study. Patients were categorized into two groups, namely, “non-severe presentation” and “severe presentation” accounting for 74.16 and 25.75% of total patients. Of note, 13.9% of patients died during the study while most of them (85.98%) survived, and 60.9% of all patients were males with similar distribution in both non-severe and severe groups (61.69 and 57.9%). The similar distribution in both groups was also true for the female sex (38.1% non-severe and 42.1% severe groups). Most non-severe participants were at the younger end of the age spectrum, and controversially, most participants in the severe group were older. Notably, 52.8% of all patients were 50 years old and higher, and while this applied to a significant count of 79.4% of the severe group in this age range, this highlights the significant effect of age in severe and non-severe groups. In contrast, while men were the most

diagnosed patients overall, the role of sex-affecting severity was not significant (Table 1).

The admission and discharge platelet counts and thrombocytopenia

There was no significant difference in platelet counts and thrombocytopenia between severe and non-severe, survivors and non-survivors, and severe survivors and severe non-survivors groups at the time of admission to the hospital; however, this difference dramatically changed while discharge. Regarding the platelet counts, these alternations were so impressive in the case of survivors and non-survivors as well as severe survivor and severe non-severe groups. Also, severe patients, non-survivors, and severe non-survivors showed higher thrombocytopenia and lower platelet counts compared with non-severe patients, survivors, and severe survivors, respectively, at the time of discharge (Figure 1).

Alteration in the platelet counts and thrombocytopenia during a 7-day follow-up

The platelet count did not show a significant difference between severe and non-severe, survivors and non-survivors, and severe survivors and severe non-survivors groups on the admission day. After 7 days, a trend toward an increase in platelet counts was seen in non-severe patients, survivors, and severe compared with severe patients, non-survivors, and severe non-survivors,

Table 1. Baseline characteristics of admitted COVID-19 patients.

Variables	All patients n (%); mean (±SD)	Non-severe* n (%); mean (±SD)	Severe n (%); mean (±SD)	p-value	Survivors n (%); mean (±SD)	Non-survivors n (%); mean (±SD)	p-value
Sample size	1,320 (100)	979 (74.16)	340 (25.75)		1,135 (85.98)	184 (13.93)	
Age (years)	52.15 (±19.22)	47.84 (±17.58)	64.59 (±18.32)	0.000	49.29 (±18.147)	69.82 (±15.98)	0.000
≤39	406 (30.8)	371 (37.9)	35 (10.3)		401 (35.3)	5 (2.7)	
40–49	216 (16.4)	181 (18.5)	35 (10.3)		204 (18.0)	12 (6.5)	
50–59	201 (15.2)	156 (15.9)	45 (13.2)		178 (15.7)	23 (12.5)	
60–69	196 (14.9)	131 (13.4)	65 (19.1)		161 (14.2)	35 (19.0)	
≥70	301 (22.7)	140 (14.3)	160 (47.1)		191 (16.8)	109 (59.2)	
Sex							
Female	516 (39.1)	373 (38.1)	143 (42.1)	0.193	442 (38.9)	74 (40.2)	0.736
Male	804 (60.9)	607 (61.69)	197 (57.9)		694 (61.1)	110 (59.8)	
Platelet count	n (%); median (IQR)	n (%); median (IQR)	n (%); median (IQR)	0.100	n (%); median (IQR)	n (%); median (IQR)	0.055
	176.5 (133.0–246.5)	177.0 (139.0–224.0)	176.5 (133.0–246.5)		177.0 (139.0–224.0)	181.0 (122.0–271.0)	

*Severe patients are defined as cases (i) admitted to intensive care unit or (ii) died during the course of disease progression or (iii) had severe hypoxia (O_2 saturation $\leq 85\%$) on admission or (iv) had respiratory rate ≥ 30 on admission. SD: standard deviation; IQR: interquartile range.

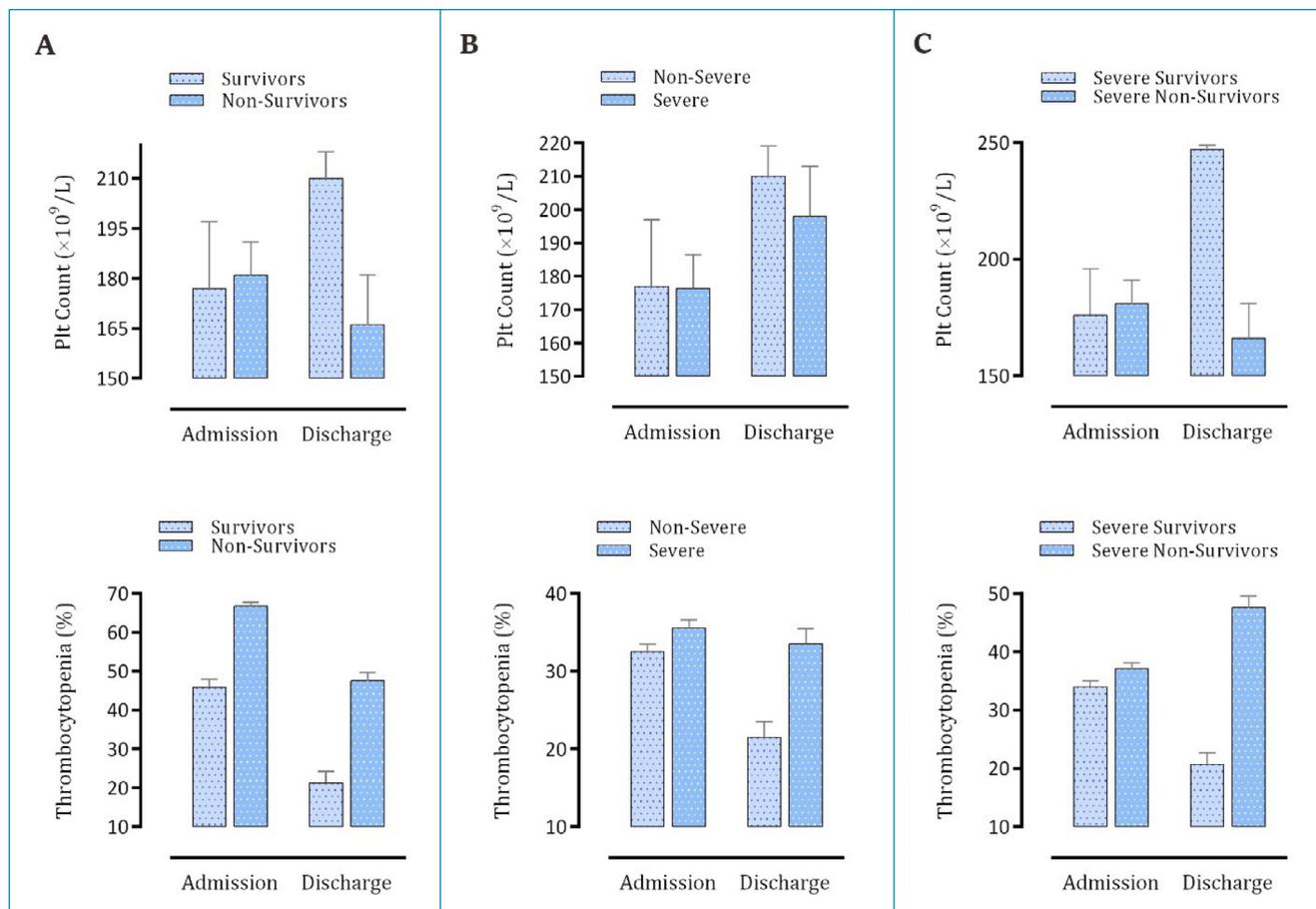


Figure 1. The admission and discharge platelet counts and thrombocytopenia divided by mortality and morbidity.

respectively. Thrombocytopenia proportions did not show the same pattern in all the three groups on the admission day. After following up, the maximum difference of thrombocytopenia occurred between severe survivors and severe non-survivors with a higher magnitude in the non-survivors. Also, thrombocytopenia was higher in severe patients and non-survivors rather than non-severe and survivors after 7 days, respectively (Figure 2).

DISCUSSION

Mounting evidence has shown that thromboembolic complications resulting from COVID-19 disease are one of the main reasons for sudden deterioration and death¹⁷. Platelets play a pivotal role in thrombogenesis. An increased platelet activation leading to platelet aggregation, platelet spreading, α -granule secretion, and dense granule release is related to thrombosis in COVID-19¹⁸. It has been shown that the incidence of thromboembolic events in severe patients and non-survivors is much higher compared with their non-severe counterparts and survivors^{19,20}. This is in harmony with our results showing a significantly

decreased platelet count in severe and non-survivor patients rather than non-severe and non-survivor patients, respectively. Although the difference was not significant in admission, it was shown that the platelet count can predict the mortality rate and disease orientation. Also, the platelet count can be considered as an indicator of disease severity. During the disease course, the platelet count showed more change in non-survivors compared with severe and non-severe groups. Although thrombocytopenia is often believed to be an indicator of bleeding, no history of bleeding was reported in COVID-19 patients during hospitalization. Bowles et al. reported that the frequency of bleeding events was 0 in 35 COVID-19 patients who suffered from a prolonged activated partial-thromboplastin time (aPTT)²¹ which may result from the nature of severe hypercoagulable rather than a hypocoagulable state of COVID-19 coagulation pattern. The possible mechanisms by which COVID-19 leads to thrombocytopenia might be that:

1. The suppression of hematopoiesis caused by an impaired hematopoietic microenvironment resulting from systemic inflammation or cytokine storm²²;

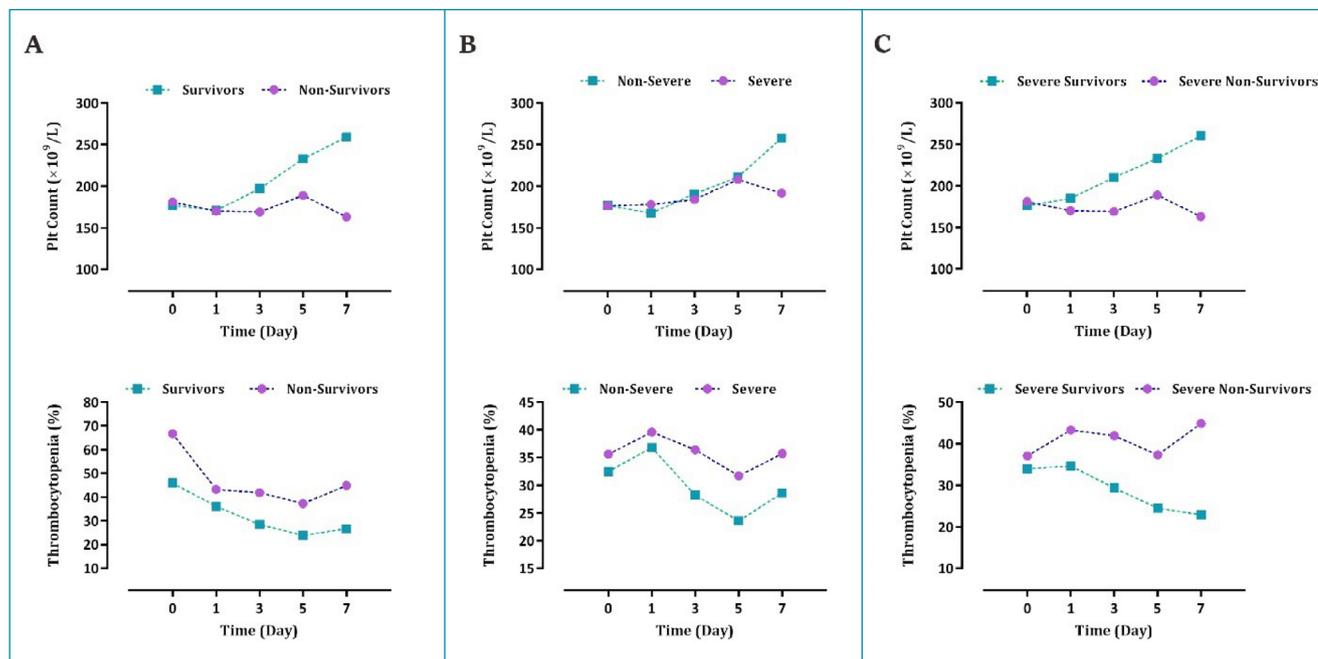


Figure 2. Platelet counts and thrombocytopenia during a 7-day follow-up course divided by mortality and morbidity.

- The infection of hematopoietic stem cells or megakaryocytes through angiotensin-converting enzyme 2 (ACE2), CD13, or CD66a like other coronavirus infections²³;
- The cross-reaction of antiviral antibodies with hematopoietic cells and platelets like anti-adenovirus antibody reaction with platelet integrin GPIIb/IIIa²³;
- The scavenging of activated platelet by splenic/hepatic macrophages; and
- The increased consumption of platelets caused by thrombotic microangiopathy and disseminated intravascular coagulation²⁴. Also, Chen et al. indicated the delayed-phase thrombocytopenia resulting from the impaired maturation of megakaryocytes in COVID-19 patients²⁵. Following up patients after 7 days revealed that higher thrombocytopenia is related to severe patients and non-survivors rather than non-severe and survivors, respectively. It seems that thrombocytopenia and thrombotic complications in COVID-19 patients are common and lead to a higher mortality rate, but significant bleeding

reports are rare among these patients. Taken together, dynamic monitoring, early detection, and thromboprophylaxis can help us to control the platelet count in patients not contraindicated for treatments.

CONCLUSIONS

Thrombocytopenia and thrombotic complications in COVID-19 patients are common leading to a higher mortality rate.

AUTHORS' CONTRIBUTIONS

LJK: Investigation, Writing – review & editing. **PZ:** Investigation, Writing – review & editing. **AP:** Formal analysis. **SS:** Conceptualization, Data Curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **AB:** Formal analysis. **MEA:** Investigation, Writing – review & editing. **DB:** Conceptualization, Data Curation, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Has external banding become a historical technique during venous valve repair?

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SUMMARY

OBJECTIVE: In deep venous valve repair, transcommissural external valvuloplasty (TEV) is the commonly used technique. In some cases, external banding (EB) is combined with this procedure to improve the patency and durability of the surgical procedure.

METHODS: We retrospectively analyzed patients who underwent deep venous valve repair from 1998 through 2018. Patients were divided according to the surgical procedure: Group A: TEV alone and Group B: TEV+EB. Early postoperative outcomes of the procedure were compared between the groups.

RESULTS: There were 265 patients in Group A and 165 patients in Group B. The mean follow-up period was 4.2±3.7. The rate of recurrence of venous reflux, ulcer, and reoperation were 31.9 versus 30.9, 21.2 versus 21.8, and 16.7 versus 13.9 in Group A and Group B, respectively. There were 67 reoperations in the follow-up period. At reoperation, external valvuloplasty was performed in 64% of the reoperations in Group A, while this rate was 13% for Group B.

CONCLUSIONS: There is no more need for EB during the venous valve repair with the increased experience of valvuloplasty techniques. TEV might be enough with acceptable long-term outcomes during deep venous reconstruction.

KEYWORDS: Venous insufficiency. Venous valves. Venous thrombosis.

INTRODUCTION

In recent times, the importance of deep venous insufficiency, which threatens patient comfort, has been better understood. Even in the earlier stages of the disease, it affects a patient's daily life with disturbing symptoms. In patients with deep venous insufficiency, superficial venous surgery with/without perforator surgery may not be enough to relieve symptoms. Deep venous valve repair is indicated in symptomatic patients as a consequence of conservative treatment failure and superficial/perforator surgical procedures¹⁻³. In symptomatic patients with severe deep venous insufficiency combined with superficial venous insufficiency, both systems may undergo surgery in the same session.

Deep venous valve reconstruction techniques improve patients' quality of life and decrease the Venous Clinical Severity

Score (VCSS). However, valvuloplasty procedures should be performed by highly skilled physicians at specialized centers². Different surgical techniques including internal valvuloplasty, external valvuloplasty, external banding (EB), neovalve, valve transposition, and valve transfer have been reported for deep venous valve reconstruction^{1,4-8}. External valvuloplasty has the advantage of not needing venotomy with acceptable mid- and long-term results^{1,2}. This technique closes the wide angle between the valve attachment lines⁸. However, its results are related to the surgeon's experience because it is performed without direct vision. To improve the outcomes of external valvuloplasty, angioscopic guidance has been reported, but its use has not widened since external valvuloplasty has similar outcomes in experienced centers. In the early terms, external valvuloplasty was combined

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with EB to improve valve competence and to avoid further dilatation^{1,2}. Currently, as a result of increased surgical experience, external valvuloplasty alone has recently been reported to have similar results with internal valvuloplasty or EB combined with external valvuloplasty with a 5-year success rate of 70%².

In symptomatic patients with recurrent deep venous insufficiency, reoperation might be needed in the long term, especially in the presence of venous ulcers. During reoperation, the dissection of the vein wall might be difficult due to the inflammatory response to the synthetic material in the patients with the external band. Choices of venous reconstruction techniques might be restricted related to the condition of the vein and valves. In this study, we present our outcomes with external valvuloplasty alone and with EB and our experience of their reoperations.

METHODS

We retrospectively analyzed patients who underwent transcommissural external valvuloplasty (TEV) alone or TEV combined with EB (TEV+EB) due to deep venous insufficiency from 1998 through 2018. This study was initiated after the approval of the institutional review board. Demographic and clinical information including long-term results, reoperations, and ultrasonographic reports were collected from a review of the medical records, which included the surgical database.

Patients with primary venous insufficiency and who underwent TEV at a single vein site (common femoral vein (CFV)) were included in this study. Patients who underwent other venous reconstruction techniques (i.e., internal valvuloplasty, neovalve, and valve transposition) or had surgery at a different venous site, patients with secondary venous insufficiency, and patients with the peripheral arterial disease were excluded from this study. The history of venous thrombosis and major trauma was the other exclusion criteria.

Patients were evaluated using the Clinical, Etiologic, Anatomic, and Pathophysiologic (CEAP) classification, VCSS, and Doppler ultrasonography. The Doppler ultrasonographic examinations were performed both preoperatively and postoperatively while patients were standing. A reflux time >0.5 s was considered as venous insufficiency. Venous valve reconstruction was planned in patients with venous ulcers or severe symptoms. External valvuloplasty, which is a less invasive procedure, does not require venotomy, does not directly interfere with the vascular endothelium, and was preferred as the first option for the venous valve reconstruction.

We divided patients into two groups according to their surgical procedures. Patients who underwent TEV alone were assigned to Group A, and patients with TEV+EB were in Group B. All patients underwent surgery by the same surgical

team under spinal or epidural anesthesia. In patients with superficial venous insufficiency, ankle-to-groin stripping of the greater saphenous vein was performed. The CFV and superficial femoral vein (SFV) were then dissected free around their circumference, and the side branches were divided. Topical papaverine solution was applied to induce vasodilatation, and the patient was then asked to perform the Valsalva maneuver in a reverse-Trendelenburg position to detect venous deformity or dilatation. Diameters of the CFV and SFV adjacent to the dilated venous segment were measured using calipers⁵. Intravenous heparin (5,000 IU) was introduced prior to the valve reconstruction. External valvuloplasty was performed using the technique we previously described⁵. We performed valvuloplasty with a continuous suture using 7.0 polypropylene to strengthen the lines of the valves on the wall⁹. In Group B, additional external wrapping using a Dacron patch was performed. The same approach was performed during reoperations, but the surgical procedure was decided according to the condition of the venous wall and the durability of the valvuloplasty techniques.

All patients were ambulated on postoperative day 1 and wore 30–40 mmHg compression stockings. The patients were administered oral warfarin treatment, which began on the day of the operation and continued for 6 months to maintain international normalized ratio (INR) levels between 2.0 and 2.5.

The early postoperative outcomes of the procedure including ulcer healing, ecchymosis, lymphatic leakage, wound infection, hematoma, and paresthesia were compared. The patients' symptoms were evaluated using the VCSS. Patients were followed up through the Doppler ultrasonographic examinations before discharge, and 2 months, 6 months, and 1 year postoperatively, and annually thereafter by the same investigator. Failure of the valve repair was accepted as >2-s reflux at a repaired site⁴. Reoperation was performed in patients with recurrent venous ulcers or severe symptomatic patients with recurrent venous insufficiency.

Categorical variables are presented as the number of patients and percentage; continuous variables are presented as mean (SD). Groups were compared with a two-sample *t*-test and chi-square test.

RESULTS

A total of 428 patients underwent deep venous valve reconstruction with external valvuloplasty. Of these, 165 patients had additional EB. All patients underwent superficial venous valve surgery during the same period or prior to the venous valve reconstruction. The mean age was 45.4 years, and 146 were men. The patients' primary symptoms at admission are shown in Table 1.

All patients in both groups had superficial and deep venous insufficiency. The patients' characteristics were classified in accordance with the CEAP classification and VCSS (Table 2).

Table 1. Patient’s characteristics at admission.

	Group A (n=263)	Group B (n=165)	p
Age	46.1±8.5	44.4±9.4	0.023
Male (%)	91 (34.6)	55 (33.3)	0.788
Primary complaint			
Ulcer	160 (60.8)	105 (63.6)	0.562
Pain	199 (75.7)	124 (75.6)	0.904
Presence of dermatitis	63 (24.0)	39 (23.6)	0.940
Leg swelling	231 (87.8)	146 (88.5)	0.84

Table 2. Clinical findings of patients.

	Group A (n=263)	Group B (n=165)	p
Clinical			
C3	49 (18.6)	24 (14.5)	0.029
C4	55 (20.9)	38 (23.0)	
C5	71 (27.0)	45 (27.3)	
C6	88 (33.5)	58 (35.2)	
Etiology			
Primary	263 (100)	165 (100)	NA
Secondary	–	–	
Anatomic	S and D reflux	S and D reflux	NA
Pathophysiology	S and D reflux	S and D reflux	NA
VCSS	6.6±2.6	6.2±2.7	0.0627

VCSS: venous clinical severity score.

The mean follow-up period was 4.2±3.7. In the follow-up period, the rate of recurrence of venous reflux and ulcers was 30% and 20%, respectively. In the comparison of the groups, recurrence rates were similar (Table 3).

There were 67 reoperations in the follow-up period. Surgical procedures in the reoperation were related to the prior reoperation. In Group A, external valvuloplasty was performed in almost two-third of the patients. However, in Group B, the rates of valvuloplasty procedures were lower (Table 3).

Table 3. Rates of recurrence and reoperation techniques.

	Group A (n=263)	Group B (n=165)
Venous reflux recurrence (%)	84 (31.9)	51 (30.9)
Venous ulcer recurrence (%)	56 (21.2)	36 (21.8)
Reoperation		
External valvuloplasty	28	3
Internal valvuloplasty	6	2
Valve transposition	3	11
Neovalve	5	3
External banding	2	4

DISCUSSION

To our knowledge, this is the first study to examine the outcomes of external valvuloplasty alone and the combination of external valvuloplasty with EB. In the comparison of the groups, preoperative characteristics were similar. Although there were similar rates of recurrence of postoperative venous insufficiency, venous ulcers, and reoperation, the reoperative procedures were more complex in patients with EB. Dissection of the venous wall was difficult due to the inflammatory response to the EB tissue. In most cases, the effort to relieve the femoral vein around its circumference and external band was not enough to perform second valvuloplasty procedures. In these situations, we performed alternative venous reconstruction procedures, which were more complex and have lower long-term results than valvuloplasty procedures.

Primary deep valve incompetence is the second cause of deep venous insufficiency². It occurs due to prolapsed valves, dilatation of the valve ring, and asymmetric insertion of the cusps; these anomalies can easily be repaired with valvuloplasty procedures². Many reconstruction techniques have been reported in the treatment of deep venous insufficiency. Valvuloplasty techniques have more durable results than the other venous reconstruction techniques⁴. Lehtola et al.⁴ reported that external valvuloplasty was a more durable technique with a 71% durability rate; however, there was a selection bias in their study because internal valvuloplasty procedure was mostly performed in post-thrombotic patients.

Direct angiography might be used during external valvuloplasty; but, it is unnecessary because widened valve attachment lines will easily get closer in a certain way in experienced centers¹⁰. We did not use angiography in any of our patients. EB in addition to external valvuloplasty might be preferred to restore valve competence by reducing the caliber of the vein.

Dacron, Venocuff, polytetrafluoroethylene (PTFE), and bovine pericardium might be used for EB¹¹. If external sleeves increase the rigidity of the vein wall, this can limit the motion of the valve cusps⁷. Fibrotic/thrombotic occlusion of the vein station might develop in cases with external cuffs³. Also, the inflammatory response to these foreign materials might cause a more complex second procedure. In that situation, the alternatives of re-valvuloplasty techniques are restricted. Autologous valve transfer or cryoinsertion might be performed in this clinical condition. In our reoperated patients, the rates of valvuloplasty procedures were 77% and 21% in Groups A and B, respectively. In Group B, almost half of the patients had undergone valve transposition.

Cryoinsertion has poor mid-term outcomes with high occlusion rates and are recommended only as a secondary choice¹². Neoinsertion is an alternative technique for deep valve surgery with high competence and ulcer healing rate⁶. This technique might be used in secondary valve insufficiency and reoperation as an alternative to external valve repair. Recently, neoinsertion procedures have become our first option in cases where we cannot perform valvuloplasty techniques. In our cases, we performed neoinsertion reconstruction in eight patients with acceptable results.

Less than 10% of chronic venous insufficiency is caused by deep venous insufficiency alone⁹. In the patients with combined superficial and deep venous insufficiency, saphenous vein ablation solves the deep venous problem in one-third of patients¹³. In addition to superficial venous surgery, subfascial endoscopic perforator ligation might be effective to reduce deep venous insufficiency and to improve patients' outcomes¹⁴. After superficial venous surgery, segmental deep venous valve insufficiency is more likely to become competent¹³. It is impossible to identify whether surgery of the superficial venous system will be enough to improve the symptoms of patients with superficial insufficiency combined with moderate-severe deep venous insufficiency¹⁵. Deep venous valve repair is needed, especially if they have venous ulcers and severe symptoms. A surgery of at least one valve of the femoral vein in addition to superficial venous surgery relieves patients' symptoms and improves long-term outcomes⁹. In our patients, we performed deep venous reconstruction in addition to the superficial venous surgery due to pain, swelling, and venous ulcer in patients with severe deep venous insufficiency. In the follow-up period, patients' symptoms were relieved without any complications.

The main treatment methods for leg ulcers are compression therapy and surgical correction of superficial venous incompetence¹⁶. These procedures relieve symptoms and heal leg ulcers in 80% of patients¹⁷. Remaining 20% of patients were refractory to conservative treatment and superficial venous surgery; deep venous valve repair should be considered in this group

of patients¹⁷. Treatment of the deep venous insufficiency in experienced centers increases the success of treatment strategies and decreases recurrence rates. In patients with mild-moderate deep venous insufficiency, a combination of ligation of the incompetent superficial vein and valvuloplasty has moderate and sustained improvement for 7–10 years¹⁸. The recurrence of venous ulcers may develop due to patients' poor compliance with compression therapy or an underlying deep venous defect¹⁶. In our study, the rate of freedom from venous ulcers was 80% in the long term.

Valvular reconstruction was reported to be more effective in primary valvular reflux than in secondary reflux³. The reoperation rate was 4% after valve reconstruction⁸. Joh et al.¹⁹ reported 19.4% recurrence after EB valvuloplasty of the greater saphenous vein. In our long-term results, the reoperation rate was 15%, and we could not find any potential advantage of EB in deep venous reconstruction.

The coronavirus disease 2019 (COVID-19) pandemic, which the world has been struggling with recently, might cause thromboembolic complications such as deep venous thrombosis and pulmonary embolism. Thromboembolic complications have been reported in 15–25% of COVID-19 patients^{20,21}. Venous thrombosis caused by COVID-19 might damage the valve structure and will lead to the emergence of more deep venous insufficiency patients in the future. Therefore, the follow-up and treatment of patients with deep venous insufficiency will gain more importance over time.

In this retrospective study, the data were gained from the medical records; thus, it has inherent limitations of retrospective studies. As a nature of retrospective studies, we could not give the exact rate of the reoperations or recurrence. We could only comment on our medical records. Before 2009, we usually prefer EB. However, due to the difficulty of reoperation of EB and increased experience with deep venous valve repair, we started to prefer first the external valvuloplasty alone. It could be advocated to compare these groups, but we aimed to share the long-term results of our perspective after the progress about venous valve repair.

CONCLUSIONS

In experienced centers, the combination of superficial venous surgery with deep venous surgery improves the long-term outcomes⁹. External valvuloplasty might be preferred with acceptable long-term results. In the earlier period of the procedure, external valvuloplasty was combined with external bands to improve outcomes. However, with surgeons' increased experience of external valvuloplasty, the need for the external band has been decreased. In addition, synthetic tissue may increase

the complexity of second procedures, if necessitated. The venous valve repair operations should be performed in experienced centers, and external valvuloplasty should be kept in mind as a first option with the advantages of easy to perform, durability, and decreased risk of reoperation.

AUTHORS CONTRIBUTIONS

MHU: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Validation, Writing – reiveur & editing. **MU:** Conceptualization, Formal analysis, Visualization, Writing – original draft.

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Comparison of outcomes of the patients with acute cholecystitis treated in the COVID-19 pandemic and pre-pandemic period

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SUMMARY

OBJECTIVE: The coronavirus disease 2019 (COVID-19) pandemic has affected the health care system in an unpredictable way. In this study, we aimed to analyze the effects of the pandemic process on the disease severity on admission, management strategies, and outcomes of patients.

METHODS: The medical records of the patients who applied to the emergency department and consulted to the general surgery clinic from March 2020 until January 2021 were retrospectively reviewed as the pandemic period. For the control group, patients' medical records in the same time interval of 2019 were evaluated as the pre-pandemic period.

RESULTS: A total of 88 patients in the pre-pandemic period and 89 patients in the pandemic period were treated for acute cholecystitis. There was no statistically significant difference between the two groups in terms of the treatment strategies and length of hospital stay between the two periods ($p=0.087$ and $p=0.587$, respectively).

CONCLUSIONS: In the pandemic period, it is thought that postponing and bridging treatments may replace surgery for reducing the risk of contamination of both patients and health care workers.

KEYWORDS: Acute cholecystitis. SARS-CoV-2. COVID-19. Cholecystostomy.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) pandemic, has affected globally the health care system in an unpredictable way¹. First COVID-19 case recorded in Turkey on March 10, 2020. Thereafter, the World Health Organization (WHO) declared the COVID-19 outbreak as a global pandemic on March 11². While the adaptation processes in the health care systems and social life are being presented worldwide, studies for the standardization of approaches have rapidly started to take place in the literature³.

Although elective surgical procedures, except cancer cases, were largely cancelled or delayed to preserve hospital resources and mitigate disease transmission, there are not enough data yet on how this approach affected the emergency surgical cases and interventions in the pandemic period⁴. In this period, as recommended for some surgical emergencies, nonoperative, medical, interventional radiological or endoscopic interventions for the treatment of acute cholecystitis cases have been suggested by the scientific communities in the early stages of the pandemic^{5,6}. As with all hospital admissions, excluding COVID-19 cases, there was a decrease in the emergency department admissions. Although this can be explained as

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a decrease in the number of unnecessary emergency department visits, it is also thought that individuals may hesitate to apply to the hospital and, therefore, delays in real emergencies may occur⁷. This situation has raised concerns regarding the risk of surgical emergencies becoming more complicated at presentation⁸. Due to the rapid progress in the pandemic period, the health care services continued in practice without completely eliminating the contradictions by the devotion of the health care workers. Therefore, in our study, we aimed to analyze the effects of the pandemic process on the disease severity on admission, management strategies, and outcomes of patients with acute cholecystitis by comparing with the cases treated in the previous year.

METHODS

Ethical approvals were obtained from both the Ethics Committee of Gulhane Training and Research Hospital (approval no: 2020-449) and Ministry of Health Sciences Committee. The medical records of the patients who applied to the emergency department and consulted to the general surgery clinic from March 11, 2020, until December 31, 2020, were retrospectively reviewed as the pandemic period. For the control group, patients' medical records in the same time interval of 2019 were evaluated in the same way as the pre-pandemic period. Patients younger than 18 years, positive COVID-19 test within 7 days before or 7 days after the admission, or reoperated due to the previous complication were excluded from the study.

Demographic characteristics of the patients; laboratory tests, including C-reactive protein (CRP) levels and leukocyte counts; previous history of endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous cholecystostomy; American Society of Anesthesiologists (ASA) scores; treatment strategies; operation time in those who had surgery; intraoperative complications; length of hospital stay; Clavien-Dindo Classification⁹ for surgical complications; and 30-day rehospitalization rate were examined. Tokyo Guidelines 2018/2013 severity grading was used to assess the severity of the acute cholecystitis¹⁰. Besides, the Parkland Grading Scale for Cholecystitis was used to stratify gallbladder disease severity during cholecystectomy¹¹.

All data were presented as median with interquartile range (IQR) or frequency (%). The chi-square test was used for categorical variables and the Mann-Whitney U test for continuous variables. A value of $p < 0.05$ was considered statistically significant. The statistical analysis was performed using the RStudio statistical software (version 1.0.136; RStudio Inc., Boston, MA, USA).

RESULTS

Notably, 88 patients in the pre-pandemic period and 89 patients in the pandemic period were hospitalized and treated for acute cholecystitis. No statistically significant difference was found in the comparison of the two groups in terms of demographic characteristics. Median leukocyte values of the patients at admission were $13.5 \pm 4.9 \times 10^9/L$ in the pre-pandemic period and $13.9 \pm 6.1 \times 10^9/L$ in the pandemic period. No statistically significant difference was found between the groups with regard to leukocyte and CRP values at admission ($p=0.668$ and $p=0.571$; respectively) (Table 1).

According to the Tokyo Guidelines 2018/2013 severity grading for acute cholecystitis classification, 59.1% of the patients were grade I, 36.4% grade II, and 4.5% grade III in the pre-pandemic period. During the pandemic period, these rates were 47.2, 47.2, and 5.6%, respectively. In the comparison of the two groups, no statistically significant difference found in severity grading ($p=0.284$). Laparoscopic or open technique cholecystectomy was performed to 20.5% of the patients in the pre-pandemic period; this rate was 15.7% in the pandemic period. However, the rate of patients undergoing percutaneous cholecystostomy was 17% in the pre-pandemic period and 28.1% in the pandemic period, which means no statistically significant difference between the two groups in terms of the treatment strategies ($p=0.087$) (Table 1).

Patients undergoing urgent cholecystectomy were also evaluated separately. However, we did not find significant differences in demographic features such as age ($p=0.896$), gender ($p=0.530$), ASA score ($p=0.680$), severity grading ($p=0.475$), previous ERCP history ($p=0.685$), type of surgery ($p=1.000$), Parkland grading scale ($p=1.000$), CRP ($p=0.442$), and leukocyte ($p=0.180$) between the patients who underwent surgical treatment in the pre-pandemic period and the pandemic period. Two patients had intraoperative complications as the common bile duct injury during the pandemic period. Besides, there was no significant difference in the length of hospital stay and 30-day rehospitalization rate between the two periods ($p=0.587$ and $p=0.295$, respectively) (Table 2).

DISCUSSION

After WHO declared COVID-19 a pandemic in early March 2020, many scientific publications regarding the approaches to both elective and emergency surgical cases in general surgery practice have been published at national and international levels globally¹². In the recent study, we aimed to analyze the

Table 1. Comparison of the outcomes between the pre-pandemic and pandemic periods.

Outcomes	Pre-pandemic period (n=88)	Pandemic period (n=89)	p-value
Age (year)	57.5 (IQR, 48–72)	54.0 (IQR, 47–66)	0.132
Sex, n (%)			
Female	43 (48.9)	37 (41.6)	0.330
Male	45 (51.1)	52 (58.4)	
Laboratory tests			
Leucocyte ($\times 10^9/L$)	13.5 (IQR, 10–18)	13.9 (IQR, 10–18)	0.668
C-reactive protein (mg/L)	114 (IQR, 33–199)	131 (IQR, 16–232)	0.571
TG18/TG13 severity grading, n (%)			
Grade I	52 (59.1)	42 (47.2)	0.284
Grade II	32 (36.4)	42 (47.2)	
Grade III	4 (4.5)	5 (5.6)	
Treatment strategies, n (%)			
Medical treatment	49 (55.7)	49 (55.1)	0.087
Percutaneous cholecystostomy	15 (17.0)	25 (28.1)	
ERCP	6 (6.8)	1 (1.1)	
Cholecystectomy	18 (20.5)	14 (15.7)	

IQR: interquartile range; TG18/TG13: Tokyo Guidelines 2018/2013 severity grading for acute cholecystitis; ERCP: endoscopic retrograde cholangiopancreatography.

effects of the pandemic on the volume, disease severity, management strategies, and outcomes of patients.

Acute cholecystitis has an important place in general surgery practice. Although it has surgical, medical, and interventional treatment options, laparoscopic cholecystectomy is now considered a standard treatment in optimal conditions with its increasing scientific studies in recent years. However, due to some factors such as comorbidities, age, and hospital admission time; open cholecystectomy, percutaneous or tube cholecystostomy, and medical treatment strategies can be considered an option¹³. We applied these treatment strategies in our clinic in both pre-pandemic and pandemic period groups in a similar way.

It is a well-known fact that nonsurgical strategies such as medical treatment or percutaneous cholecystostomy have a lower success rate and increase recurrence of the disease in the treatment of acute cholecystitis¹⁴. For this reason, it increases the popularity of the surgical treatment strategies, especially the laparoscopic approach¹⁵. In our study, no significant difference was found in treatment strategies and surgical technique between the pre-pandemic and pandemic periods.

In the COVID-19 pandemic period, as a disease severity laboratory test for many inflammatory diseases such as acute

cholecystitis and acute appendicitis, acute-phase reactants including leukocyte and CRP are expected to increase because of possible delayed hospital admissions of patients¹⁶. Also, in the COVID-19 disease, it is known that the laboratory findings of leukopenia and high CRP are seen, but in this situation for the differential diagnosis, the clinical presentation of the patient is much more important¹⁷. In our study, no significant difference was observed in leukocyte and CRP values between the pre-pandemic and pandemic periods. In addition, we did not find a significant difference in these laboratory markers between patients operated in the pre-pandemic and those operated in the pandemic period.

Tokyo Guidelines 2018/2013 severity grading scale is a grading system using local and systemic signs of inflammation and imaging findings in acute cholecystitis. In this scale, acute cholecystitis is classified into grade I (mild), grade II (moderate), and grade III (severe)¹⁸. When we separated our patients according to this scale that determines the treatment strategies, we did not find a significant difference between the periods in the surgical and nonsurgical patients. Parkland grading scale is a scale ranging from grades I to V, which is revealed by intraoperative evaluation of adhesions from the gallbladder and evaluation of other inflammatory findings¹⁹. This scale is used to evaluate the difficulty of laparoscopic cholecystectomy and

Table 2. Comparison of patients undergoing urgent cholecystectomy between the pre-pandemic and pandemic periods.

Outcomes	Pre-pandemic period (n=18)	Pandemic period (n=14)	p-value
Age (year)	53.0 (IQR, 46–63)	55.0 (IQR, 42–63)	0.896
Sex, n (%)			
Female	7 (38.9)	7 (50.09)	0.530
Male	11 (61.1)	7 (50.0)	
Laboratory tests			
Leucocyte ($\times 10^9/L$)	14.3 (IQR, 10–19)	12.4 (IQR, 7–18)	0.180
C-reactive protein (mg/L)	55 (IQR, 25–152)	17 (IQR, 2–188)	0.442
ASA score, n (%)			
ASA 1	1 (5.6)	1 (7.1)	0.680
ASA 2	16 (88.9)	11 (78.6)	
ASA 3	1 (5.6)	2 (14.3)	
TG18/TG13 severity grading, n (%)			
Grade I	11 (61.1)	7 (50.0)	0.475
Grade II	7 (38.9)	6 (42.9)	
Grade III	0	1 (7.1)	
Previous ERCP history, n (%)	5 (38.5)	5 (50.0)	0.685
Type of surgery, n (%)			
Open cholecystectomy	3 (16.7)	2 (14.3)	1.000
Laparoscopic cholecystectomy	15 (83.3)	12 (85.7)	
Parkland grading scale, n (%)			
Grades 1–2	11 (61.1)	8 (57.1)	1.000
Grades 3–5	7 (38.9)	6 (42.9)	
Intraoperative complication, n (%)	0	2 (14.3)	0.183
Operation time (minute)	75 (IQR, 59–103)	59 (IQR, 55–100)	0.338
Length of hospital stay (day)	5 (IQR, 4–8)	5 (IQR, 4–8)	0.587
30-day rehospitalization, n (%)	1 (5.6)	3 (21.4)	0.295

IQR: interquartile range; ASA: American Society of Anesthesiologist; TG18/TG13: Tokyo Guidelines 2018/2013 severity grading for acute cholecystitis; ERCP: endoscopic retrograde cholangiopancreatography.

possible complications such as converting the open technique¹⁹. When we divided this scale into two subgroups as grades I–II and grades III–IV–V, we showed that there was no significant difference between the two periods.

Percutaneous cholecystostomy is a treatment strategy that can be used in patients with acute cholecystitis, especially in patients with high ASA scores, and can also be used as a bridge to elective treatment. Despite its low morbidity and mortality rates, it includes risks such as hemorrhage, liver abscess, and recurrence of symptoms^{20,21}. Especially during the pandemic period, it may be thought that the risk of suffering from morbid and mortal complications of

COVID-19 disease for elderly people has increased, and the tendency to this treatment strategy may have increased in order to mitigate disease transmission²². However, our study did not show an increased tendency in terms of percutaneous cholecystostomy.

Laparoscopic and open technique cholecystectomy is one of the surgical treatment strategies for acute cholecystitis. Converting to the open technique may be considered after intraoperative Parkland grading scale evaluation²³. Although the preference of laparoscopy during the pandemic period is questioned in recent studies²⁴, no significant difference was found with the pre-pandemic period in our study.

To reduce the increased risk of contamination during the pandemic period, there is a general recommendation to shorten the length of hospital stay^{4,25}. Researches showed that there is a tendency in this direction²⁵. However, in our study, it was showed that hospitalization periods were similar in the two periods.

CONCLUSIONS

In the pandemic period, it is thought that more complicated acute cholecystitis cases may be seen due to the increased burden of the health care system and late admission to the hospital. Additionally, in this period, it is thought that postponing and bridging treatments such as medical treatment and percutaneous drainage may replace surgical interventions in order to reduce the risk of contamination of both patients and health

care workers. In our study, there was no statistically significant difference between the groups in terms of the disease severity on admission and patients' outcomes. However, prospective randomized studies and reviews with larger population are needed on this subject.

AUTHORS' CONTRIBUTIONS

EL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MZB:** Data curation, Investigation, Methodology, Resources, Software, Writing – original draft, Writing – review & editing. **YSP:** Data curation, Methodology, Resources, Writing – review & editing.

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Superiority of endoscopic transsphenoidal pituitary surgery to microscopic transseptal pituitary surgery for treatment of Cushing's disease

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SUMMARY

OBJECTIVE: This study aims to compare the efficacy and safety of endoscopic transsphenoidal pituitary surgery and microscopic transseptal pituitary surgery in the treatment of Cushing's disease (CD).

METHODS: A total of 46 patients with CD were randomized into endoscopic group and microscopic group, with 23 cases in each group. The endoscopic group received the endoscopic transsphenoidal pituitary surgery, and the microscopic group received the microscopic transseptal pituitary surgery. The retrospective data, surgical outcomes, surgical cure rates, and complications in two groups were compared.

RESULTS: Compared with microscopic group, the operative time was significantly shorter ($p < 0.05$), the estimated blood loss was significantly less ($p < 0.05$), and the hospital stay was significantly shorter ($p < 0.05$) in endoscopic group. The surgical cure rate in endoscopic and microscopic groups was 69.56% and 60.86%, respectively, with no significant difference between the two groups ($p > 0.05$). The incidence of complications in endoscopic group was significantly lower than that in microscopic group ($p < 0.01$).

CONCLUSIONS: For the treatment of CD, the efficacy of endoscopic transsphenoidal pituitary surgery is basically the same as that of traditional microscopic transseptal pituitary surgery. However, the endoscopic surgery can further shorten the operative time, reduce the estimated blood loss, shorten the hospital stay, and reduce the complications.

KEYWORDS: Cushing's disease. Pituitary adenomas. Trans-sphenoidal. Endoscopic. Microscopic.

INTRODUCTION

Adrenocorticotrophic hormone (ACTH)-secreting adenoma is a particularly challenging subtype for solid tumors. Incomplete tumor resection can lead to late postoperative recurrence and surgical failure. Persisting Cushing's disease (CD) after unsuccessful operation is associated with a four-to five-fold increased standardized mortality rate and a larger number of morbidity¹. Therefore, successful surgical treatment of CD is critical. Since Hardy² used the microscopic approach to selectively excise ACTH-secreting pituitary

adenomas through the transsphenoidal route in 1960s, transsphenoidal surgery has become a standard treatment method for CD. Over the decades, there have been many reports about the results of microscopic transsphenoidal surgery in cases with CD³. It is demonstrated that, even by the hands of experienced neurosurgeons, the effectiveness is not always satisfactory with a microscopic technique⁴. Besides, remission rates are lower in patients with recurrent tumors and magnetic resonance imaging (MRI) negative CD⁵. In addition, the lateral extensions of mass may be beyond the surgical

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field achievable with the microscopic approach, which may make it hard to achieve complete resection. Consequently, it has been noted that transsphenoidal surgery is the optimal primary treatment for a specific subset of individuals with CD⁶. Thus, further treatment procedures should be applied to achieve the remission of CD. In the 1990s, Gamea et al.⁷ started using the pure endoscopic technique of transsphenoidal surgery to replace the traditional microscopic technique. The main advantage of endoscopic technique is that it adds panoramic illumination and ensures a visual field of the pituitary region and related structures. This study compared the efficacy and safety of endoscopic transnasal transsphenoidal pituitary surgery and traditional microscopic transseptal pituitary surgery for patients with CD.

METHODS

Patients

Forty-six patients undergoing pituitary adenomas excision for CD in The Characteristic Medical Center of PLA Rocket Force from January 2017 to July 2020 were enrolled in this study. They were randomly divided into endoscopic group and microscopic group, with 23 cases in each group. The endoscopic group received the endoscopic transsphenoidal pituitary surgery, and the microscopic group received the microscopic transseptal pituitary surgery. This study was approved by the Ethics Committee of the Characteristic Medical Center of PLA Rocket Force. Consent form was obtained from all patients.

Diagnosis of Cushing's disease

At presentation, all cases had symptoms and signs of active hypercortisolism. The clinical diagnosis of CD was made by two specialists based on a combination of the presence of detectable plasma ACTH concentrations in patients with elevated 24 h free cortisol excretion in urine, loss of circadian plasma cortisol pattern, and failure to suppress plasma cortisol secretion with low-dose (1 mg) dexamethasone overnight.

Pituitary imaging and tumor grading

All preoperative MRI scans were reviewed by two independent neurosurgeons. To evaluate these results, the adenomas were divided into four grades as follows: grade I: microadenomas without cavernous sinus invasion (≤ 10 mm); grade II: non-invasive macroadenomas (≥ 10 mm); grade III: local tumor invasion; and grade IV: diffuse tumor invasion. Invasion in the cavernous sinus space was subclassified according to the criteria proposed by Knosp et al.⁸

Perioperative treatment

Seventy-eight percent of all subjects underwent cortisol-lowering treatment (metyrapone in 14 patients, ketoconazole in 18 patients, and a combination of both drugs in 4 patients) before transsphenoidal surgery for 3.1 ± 1.2 months. One hour before surgery, operators started controlling the level of glucocorticoids (prednisolone, 25 mg intravenously every 8 h), then after operation the dose was tapered rapidly.

Endoscopic transsphenoidal pituitary surgery

After infiltration of the posterior septum, middle turbinate, and the area of the sphenopalatine artery foramen with a local anesthetic, a vertical incision was made in the right side. Then, the surgeons elevated the septal perichondrium toward the maxilla inferiorly, the skull base superiorly, and the sphenoid sinus in the anterior wall posteriorly. During this procedure, endoscopic septoplasty can be conducted to widen the exposed area, if septal deviation was seen. After elevating the right side mucoperichondrium, an incision was made anterior to the perpendicular plate. Then, the perichondrium of the left side was elevated toward the anterior sphenoid sinus wall. During elevation of the sphenoid sinus ostium, the mucosa was carefully elevated and the sphenoid sinus ostium was enlarged on both sides. The sphenoid sinus mucosa was elevated laterally; thus, it can be used as a flap at the end of procedure. Next, the anterior wall of the sphenoid sinus was excised so that the sella base was revealed. The surgeon excised the sella base and performed dural incision. Then, resection of the pituitary adenoma was achieved using the routine procedure. Finally, operators excised the tumor, closed the dural with the sphenoid sinus mucosa, and performed transseptal suturation and/or nasal packing to prevent the formation of septal hematomas.

Microscopic transeptal pituitary surgery

The transeptal approach was carried out through a standard sublabial or hemitransfixion incision. The septum was removed with care to preserve the mucosa, and a wide sphenoidotomy was created. Then, the surgeon resected the mass using an operating microscope. After removing the mass, the sella was reconstructed and wounds were closed. Finally, the surgeon used a through-and-through quilting stitch to approximate the septal mucosa and closed the incision with absorbable suture. Both nostrils were compressed with a nasal packing for 3–5 days.

Criteria of remission and recurrence

Remission of clinical symptoms was defined as disappearance of hypercortisolism, with basal plasma cortisol level ≤ 50 nmol/l after discontinuation of glucocorticoid withdrawal for 24–48 h

and/or suppression of plasma cortisol level ≤ 50 nmol/l after a 1 mg overnight drug sensitivity test within the first 3 months after surgery⁹. Recurrence after initial remission was defined as a lack of inhibition of plasma cortisol levels after a 1 mg overnight drug sensitivity test (>50 nmol/l) accompanied by an elevated midnight salivary cortisol level and/or elevated 24-h UFC levels¹⁰.

Statistical analysis

Continuous variables were represented by mean and range, and categorical variables were represented by frequency. Analyses were carried out using SPSS version 11.0. Independent-group t test was used for statistics of means and the Mann-Whitney U test was used to analyze nominal variables. Statistical analyses of categorical variables were conducted by χ^2 and Fisher's exact tests. A p-value <0.05 was considered statistically significant.

RESULTS

Comparison of demographics data between the two groups

The demographics data of patients in two groups are provided in Table 1. There was no significant difference in terms of age,

Table 1. Demographics data of patients in two groups.

Variable	Endoscopic group	Microscopic group	p
n	23	23	
Age (years)	26–58 (55.6)	23–60 (53.2)	>0.05
Gender, n (%)			
Male	13 (57)	12 (52)	>0.05
Female	10 (43)	11 (48)	
Tumor shape, n (%)			
Macroadenomas	4 (17)	5 (22)	>0.05
Microadenomas	19 (83)	18 (78)	
Tumor size (cm)	0.5–2.5 (0.82)	0.4–2.8 (0.89)	>0.05
Invasion site, n (%)			
Cavernous sinus invasion	2 (9)	2 (9)	>0.05
Suprasellar invasion	2 (9)	2 (7)	
Sphenoidal invasion	1 (4)	1 (4)	

gender, tumor shape, tumor size, or invasion site between the two groups ($p>0.05$).

Comparison of surgical outcomes between the two groups

The surgical outcomes in two groups are presented in Table 2. Compared with microscopic group, the operative time was significantly shorter ($p<0.05$), the estimated blood loss was significantly less ($p<0.05$), and the hospital stay was significantly shorter ($p<0.05$) in endoscopic group.

Comparison of surgical cure rates between the two groups

The surgical cure rates in two groups are presented in Table 3. The surgical cure rate in endoscopic group and microscopic

Table 2. Surgical outcomes in two groups.

Variable	Endoscopic group	Microscopic group	p
n	23	23	
Operative time (h)	1–3.2 (1.8)	1.6–4.3 (2.9)	<0.05
Estimated blood loss (mL)	64–100 (95)	120–173 (159)	<0.05
Incomplete resection, n (%)	2 (9)	3 (13)	>0.05
Hospital stay (days)	2–5 (2.8)	4–8 (5.1)	<0.05
Visual field improvement, n (%)	2/3 (67)	3/4 (75)	>0.05
Remission, n (%)	16 (70)	14 (61)	>0.05
Complications, n (%)	3 (13)	8 (35)	<0.01

Table 3. Surgical cure rates in two groups.

	Endoscopic group	Microscopic group	p
n	23	23	
Grade I (n)	12/14	8/12	>0.05
Grade II (n)	3/4	5/6	>0.05
Grade III (n)	1/2	1/2	>0.05
Grade IV (n)	0/3	0/3	>0.05
Cure (n)	16/23	14/23	>0.05
Cure rate (%)	69.56	60.86	>0.05

group was 69.56% and 60.86%, respectively, with no significant between the two groups ($p>0.05$).

Comparison of complications between the two groups

In endoscopic group, there were two cases of sinusitis and one case of massive nasal bleeding. In microscopic group, there were two cases of sinusitis, two cases hypopituitarism, one nasal septum perforation, one wound disruption, one cerebrospinal fluid (CSF) rhinorrhea, and one case of massive nasal bleeding. The incidence of complications was 13.04% (3/23) in microscopic group, which was significantly lower than 34.78% (8/23) in endoscopic group ($p<0.01$).

DISCUSSION

Endoscopy has been advocated to be a promising treatment for pituitary adenomas on the basis of its panoramic improved visualization and mobility¹¹. Rather than tunnel vision, the endoscope is positioned directly in the sphenoid sinus, only 1–2 cm away from the surgical region. A better field of view is favorable in resecting tumor and preserving normal structures. Conversely, for endocrine-active tumors, such as growth hormone or ACTH-secreting adenomas, endoscopic examination facilitates wider resection and higher hormonal remission rates. Our study compared the efficacy of endoscopic transsphenoidal pituitary surgery and microscopic transseptal pituitary surgery in the treatment of CD. Results showed that 69.56% of patients returned to normal plasma cortisol levels in the endoscopic group and 60.86% of patients in the microscopic group. This indicates that the endoscopic technique appears to improve the remission than microscopic, but the surgical cure rate has no statistically significant difference between the two groups.

Patients' comfort is another significant factor in determining the value of a given surgical operation. The rapid spread of endoscopy in clinical surgery was largely due to its ability to achieve the same surgical outcomes and improve patients' wound healing and postoperative comfort simultaneously. Endoscopy-based endonasal pituitary procedure shows similar results. Because this approach does not use a submucosal excision of nasal tissues, subjects generally suffer less pain, bruising, and postoperative rhinological dysfunction than microscopic procedures. In a study by Koren et al.¹², the endoscopic procedure allowed a shorter

operative time (by about 40 min), and half shorter hospitalization time than the microscopic approach. Consistently, Sheehan et al.¹³ showed that endoscopic approach provided a markedly shorter operative time (2.7 h in endoscopic group vs. 3.4 h in microsurgery group) without compromising the extent of tumor removal. In our study, the endoscopic procedure also reduced the operative time and hospital stay. This may be due to the fewer intraoperative and postoperative complications and the fewer need for wound management.

The use of endoscopes can also improve patient safety. The most common complications of pituitary procedure include CSF leak, pituitary hormone dysfunction, diabetes insipidus, and critical artery injury. Koren et al.¹² showed that there was no recurrence of epistaxis or denture problems, and the incidence of septal perforation, synechia, and crust formation was decreased by the endoscopic technique. In a study by Cooke and Jones¹⁴, only 5.8% of patients exhibited major postoperative complications. There was no long-term nasal, septal, or dental complication. In our study, the complications of endoscopic procedure were significantly fewer compared to microscopic approach. The reason may be that, once operators are familiar with the wide view of the surgical region, they quickly learn how to use the improved degree of visualization to completely and safely excise tumors and visualize the structures to be preserved. Therefore, there is less wound trauma by applying endoscopic technique.

CONCLUSIONS

For the treatment of CD, the efficacy of endoscopic transsphenoidal pituitary surgery is basically the same as traditional microscopic transseptal pituitary surgery. However, the endoscopic surgery can further shorten the operative time, reduce the estimated blood loss, shorten the hospital stay, and reduce the complications. This approach seems to be a good choice for minimally invasive surgery for patients with CD.

AUTHORS' CONTRIBUTIONS

TZ: Conceptualization, Writing – review & editing. **BZ:** Data curation, Writing – original draft. **LY:** Formal analysis, Writing – review & editing. **YS:** Data curation. **FW:** Formal analysis, Writing – review & editing.

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Disability and pain in capoeira practitioners

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SUMMARY

OBJECTIVE: This study aims to analyze the level of disability and pain in capoeira practitioners.

METHODS: This is a cross-sectional study. Data collection took place at the training sites of participants. The Self-Estimated Functional Inability because of Pain questionnaire for athletes (SEFIP-sport) was used to assess the pain and disability. Descriptive analysis was performed with the presentation of variables through mean and standard deviation (SD) or absolute number and percentage.

RESULTS: The sample consisted of 65 capoeira practitioners. Of these, 42 (64.61%) reported pain or discomfort. The total score of the SEFIP-sport presented an average of 2.28 points (SD=2.65). The body regions with the highest reports of pain and disability were the knees, lower back, and wrist/hands. However, we observed a mild degree of disability measured by SEFIP-sport.

CONCLUSIONS: The knees, lower back, and wrists/hands were the regions of the body with the highest reports of pain and disability. However, the disability presented by capoeira practitioners was slight.

KEYWORDS: Martial arts. Musculoskeletal injury. Pain.

INTRODUCTION

Musculoskeletal injuries and pain are the possible adverse effects that can affect practitioners of various sports, from light activities, such as walking, to more intense sports, such as combat sports and fights¹. Fights, in general, have high rates of sports injuries and are directly associated with the sports characteristics and gestures of each modality. Muscle contusion is the most frequent type of injury in medium-distance fights that use punches and kicks, and ligament injuries, dislocations, fractures, and muscle strains are mainly related to short-distance fights that use projections and twists^{2,3}.

Capoeira is a Brazilian cultural manifestation that presents itself with different faces, such as fight, dance, art, sport, leisure, game, and folklore. It is characterized by being a game/fight of body dexterity practiced in pairs with the use of attacks such as punches, kicks, headbutts, elbows, dodges, and insinuations

that focus on the partner, although there is not always contact. In addition, several turns are used, especially on the support of just one foot, just one arm or head, sudden changes in direction, throws, releases, and acrobatics, combined with attacks and dodges⁴.

Literature presents few studies on the characteristics of injuries in capoeira practitioners. From the previously implemented scientific initiatives, a case study points to the development of chronic subdural hematoma, supposedly due to translational, rotational, or angular movements of the head, as it creates an impulse load (acceleration or deceleration) on the surface of the brain, which can cause tension, compression, or shear effects⁵.

However, some studies show a significant percentage of injuries in capoeira practitioners, especially in the ankle, foot, and knee region^{1,6-9}. In complement, Mariconda et al.¹⁰ observed that the hip region has great potential to be affected, and of the

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17% of individuals reported hip pain, 91.7% had radiographic confirmation of femoroacetabular impingement.

In this context, despite the importance of previous studies observing the anatomical location of lesions in capoeira, functional factors were not properly reported. Thus, this study aimed to investigate the level of disability and pain in capoeira practitioners.

METHODS

Study design and ethical aspects

This is a cross-sectional study. Nonprobability sampling method was used. The study procedures were approved by the research ethics committee of the institution, in accordance with protocol number 3,641,542. All participants signed an informed consent form.

Participants

The inclusion criteria were age ≥ 14 years, minimum practice time of 6 months, and should be a member of a Capoeira Group or Association in the city of São Luís (Maranhão, Brazil).

The exclusion criteria were the practice of another fighting, martial art, or combat sport modality besides capoeira; diagnosis of cognitive, neurological, degenerative, or rheumatologic disease; pregnancy; and the presence of amputations.

Data collection

Data collection took place at the training sites of participants. Initially, the collection of sociodemographic, personal, clinical, and related to the practice of the sport was carried out.

In addition, the Self-Estimated Functional Inability because of Pain questionnaire (SEFIP-sport) was applied. This is an adapted and validated questionnaire for the Brazilian population^{11,12}, composed of 14 items, each relating to one body part, and it is possible to mark one of the five answers for each item, which correspond to scores from 0 to 4 (total score ranges between 0 and 56 points). The higher the score, the higher the disability.

Data analysis

We performed descriptive analysis with the presentation of variables through mean and standard deviation (SD) or absolute number and percentage. Data were processed in SPSS software, version 17.0 (Chicago, IL, USA).

RESULTS

A total of 69 capoeira practitioners were initially recruited, and four were excluded, two of them for practicing another fighting, martial art or combat sport modality; one for not having

minimum practice time; and one for not answering all the items in the questionnaire, totaling a final sample of 65 participants, aged between 14 and 53 years (mean=25.71 years, SD=11.03), 40 (61.54%) males and 25 (38.46%) females. The average time of practice of the fighting modality was 10.13 years (SD=9.55), ranging from 1 to 36 years.

Of the 65 capoeira practitioners, 42 (64.61%) reported pain or discomfort. The total score of the SEFIP-sport presented an average of 2.28 points (SD=2.65). According to Table 1 with the SEFIP-sport analysis, we observed that the most affected body regions with pain were the knees, lower back, and wrists/hands. Similarly, the body regions with the highest mean disability scores (ranging from 0 to 4) were the knees, lower back, and wrist/hands. However, we observed a mild degree of disability measured in the SEFIP-sport by the body part, with a score ranging from 0.05 (1.25%) to 0.40 (10%).

DISCUSSION

This study is a pioneer in reporting pain and disability in capoeira practitioners. Of the 65 participants, 42 (64.61%) reported musculoskeletal pain or discomfort. We observed that the body regions with the greatest pain and disability were the knees, lower back, and wrist/hands. However, the pain and disability observed in the sample were mild ($\leq 10\%$ of the maximum score per item of the SEFIP-sport).

Table 1. Report of pain and disability of capoeira practitioners (n=65).

Body parts	Pain or discomfort report	SEFIP-sport score (0–4)
	n (%)	Mean (SD)
Neck	5 (7.69)	0.08 (0.27)
Shoulders	8 (12.30)	0.17 (0.49)
Elbows	3 (4.61)	0.05 (0.21)
Wrists/hands	11 (16.92)	0.22 (0.54)
Upper back	9 (13.84)	0.15 (0.40)
Lower back	13 (20.00)	0.23 (0.49)
Hips	8 (12.30)	0.17 (0.52)
Thighs (front)	9 (13.84)	0.20 (0.56)
Thighs (back)	6 (9.23)	0.14 (0.50)
Knees	14 (21.54)	0.40 (0.90)
Legs (front)	3 (4.61)	0.06 (0.30)
Calves	3 (4.61)	0.06 (0.30)
Ankles	10 (15.37)	0.15 (0.36)
Feet	11 (16.92)	0.20 (0.47)

Considering the knee, the pain can be directly linked to the overload imposed on this joint by the sudden change of direction, hyperextension caused by the execution of kicks and dodges, jumps, and landings common in the practice of capoeira that can accentuate the frictional forces and make this region more susceptible to injury and pain¹³. In the study conducted by Zucca and Grüninger⁹, 56.1% of the volunteers had injuries in the knee region and the authors listed acrobatics as the most harmful movements in the practice of capoeira, being responsible for 49.1% of the injuries.

It is necessary to draw attention to the fact that the lower back has the second highest percentage in relation to disability in our study. The study conducted by Moraes et al.¹⁴ with 45 capoeira practitioners of both sexes pointed out the presence of pain and/or lumbar discomfort in 36.6% of the individuals. This fact may be directly related to preexisting or undiagnosed injuries. In addition, some blows and acrobatics present in capoeira, such as mortals, screws, falls, and flourishes, can cause compressive force in the lower back due to the impact on the ground¹⁴. Furthermore, overuse is pointed out by Sanchez et al.¹⁵ as the mechanism responsible for most of the injuries reported during the practice of capoeira.

The wrists and hands play a very important role during the capoeira game, as many punches and dodges are performed with at least one hand on the ground. At other times, the body of practitioner is fully supported by the hands and suffers from the frequent falls inherent in the sports practice. Bonfim and Gomes¹⁶ showed that 66% of practitioners who participated in the study reported pain in the wrists/hands and 65.22% of respondents did not stop the training, even if they were feeling pain or injured.

However, despite the reduced value of disability, attention to complaints of pain and discomfort on the part of practitioners should always be considered by health professionals, as even low-intensity pain may indicate possible musculoskeletal injuries¹⁷. Therefore, clinical professionals and trainers should turn their attention to the regions most affected by pain and disability in capoeira practitioners. We hypothesize that preventive measures focused on the joint, muscle, and gestures of the fighting modality can be beneficial to capoeira practitioners. However, clinical trials must be conducted to confirm this hypothesis.

CONCLUSIONS

Of the capoeira practitioners in our sample, 64.61% reported musculoskeletal pain or discomfort. The knees, lower back, and wrists/hands were the parts of the body with the highest reports of disability. However, the disability presented by capoeira practitioners was slight.

AUTHORS' CONTRIBUTIONS

JWSC: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **AVDF:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. **MECC:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **ERM:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **SARS:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing.

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Comparison of the effects of articaine, tetracaine, and lidocaine application on pain and hemorrhage during and after nasal pack removal

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SUMMARY

OBJECTIVE: We purposed to compare the effects of certain local anesthetic applications on pain and hemorrhage caused by nasal pack removal.

METHODS: Design: Prospective, placebo-controlled study. Setting: Ataturk University Medical Faculty Hospital. This study was done in 90 patients who applied nasal packing after septoplasty. All patients were divided randomly into four groups. Each group was applied 2% lidocaine, 2% tetracaine, 4% articaine or 0.9% sodium chloride (NaCl) into their Merocel packs 15 min before removing. Verbal analog scale (VAS) score was registered from all patients, and the amount of hemorrhage was noted during the removal of the nasal packs and then for 30 min.

RESULTS: The study groups had significantly better pain scores than the control group during nasal pack removal and after 5 min ($p<0.001$). The articaine and the lidocaine groups had also better pain scores than the control group at 15th min after the removal of the nasal packs ($p<0.05$), but the tetracaine group had no better pain scores than the control group, which is statistically significant at $p>0.05$. Analysis of bleeding scores after the removal of packs showed that the articaine and the lidocaine groups had better bleeding scores than the control group ($p<0.004$ and $p<0.033$, respectively).

CONCLUSION: Topical articaine application into nasal packs just before removing in the patients who underwent septoplasty can be safely used for less pain, less hemorrhage, and more patient tolerance.

KEYWORDS: Hemorrhage. Local anesthetic. Removal. Intranasal surgery. Pain.

INTRODUCTION

Septoplasty is one of the most commonly applied surgical procedures in the ear, nose, and throat clinics. Nasal packs are commonly used following septoplasty. This application prevents nasal bleeding and supports the septal mucoperichondrial flaps, thus preventing the risk of septal hematoma and intranasal adhesion in the post-operative period¹. However, the removal of the nasal packs is a discomfort and painful procedure. The majority of patients who have undergone septoplasty express that the removal of the nasal pack was the most painful section of the septoplasty procedure².

Different methods, such as the use of the packs with gel foam, blocking the sphenopalatine ganglion, and wetting the packs with local anesthetics before the removal of the nasal packs for the solution of this problem, have been reported in the literature³⁻⁵.

Lidocaine is an amide group local anesthetic and is effective in infiltration anesthesia and nerve block. It also penetrates into soft tissues rapidly. The commercial lidocaine form is 2% of lidocaine with adrenaline. The effect of this preparation is the medium duration and quick start. Its mean plasma half-life is 2 h⁶.

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Tetracaine is an ester group local anesthetic and is effective in spinal anesthesia and nerve block. In addition, it has good mucosal absorption. The effect of tetracaine is fast-acting, long term, having low risk of methemoglobinemia, and strong compared to other local anesthetics. The mean dose is 0.1–0.2 mg/kg, and the mean effect duration is 90–200 min⁷.

Articaine is both an amide group and an ester group local anesthetic due to its chemical structure. Articaine can easily cross the lipid-rich nerve membrane to access target receptors since it has greater lipid solubility compared to other amide group local anesthetics. It can be easily metabolized in the plasma and excreted from the primary kidney since it contains an ester group. It is effective in infiltration anesthesia, spinal anesthesia, and nerve block and penetrates into tissues, rapidly. Also, it does not negatively affect wound healing. Articaine is a local anesthetic, which has an intermediate potency, short-term effectiveness, and rapid onset of action. The onset of action is between 1 and 3 min, and the average duration is 70 min⁸.

We aimed to investigate the effectiveness of these local anesthetics (i.e., lidocaine, tetracaine, and articaine) injected in nasal packs to reduce the pain, bleeding, and discomfort at the time of the removal of the packs and just after in patients who underwent a septoplasty. In this way, we purposed to correct the discomfort of patients and increase the tolerance of patients during the removal of the packs.

METHODS

This prospective, placebo-controlled study was performed at the Otorhinolaryngology Clinic of Ataturk University Medicine Faculty for 1 year. This study was approved by the Ethical Committee of the same faculty with approval number B.30.2.ATA.0.01.00/110, and informed consent was obtained from all patients.

This study included 90 patients aged 18–65 years and applied nasal packing after septoplasty. The exclusion criteria included nasal polyposis, allergic rhinitis, chronic sinusitis, bleeding disorders, renal or hepatic disease, systemic infection, known allergy to the trial drugs, pregnancy, cardiac problems, and neurological or psychiatric disease.

Septoplasty operations were performed under general anesthesia by the same surgeon. Merocel intranasal packs were applied to both nasal cavities of all patients following septoplasty operation. Merocel nasal pack (Medtronic-Xomed, Jacksonville, FL, USA) is a hydroxylated polyvinyl acetate pack, which expands on contact with the fluid.

The patients were divided into four groups, and they were randomly included in the groups. Three of the four groups received one of the local anesthetics studied in our trial, and

the control group received 0.9% NaCl. All packs were removed 48 h after the operation. The local anesthetic solutions and NaCl solution (2.5 mL for each Merocel) were prepared by the clinic nurse. Both the patient and the surgeon were unaware of the used solution. Both the Merocel nasal packs of each patient were infiltrated with 2.5 mL either 2% lidocaine+0.0012% adrenaline (Lidofast®, VEM, Turkey) or 2% tetracaine (Pantocaine) or 4% articaine+0.0012% adrenaline (Ultracain®, Sanofi, Turkey) or 0.9% NaCl before the removal of the nasal packs. Nasal packs were removed 15 min after this application. Nasal pain and bleeding were evaluated during and after the removal of the nasal packs.

The patients were asked to mark the visual analog scale (VAS) at the 0–5–15–30th min after the removal of the nasal packs. The VAS values were evaluated using a ruler with a range of 0–10, 0 being no pain and 10 representing the worst pain imaginable. After the removal of the nasal packs, bleeding from both sides was recorded by the physician, who was blinded to the solution used, according to the following scale: 0=no bleeding, 1=mild bleeding just after the removal of the packs requiring no intervention, 2=moderate bleeding in the form of leakage for 0–5 min, 3=moderate bleeding in the form of leakage for 15 min, and 4=significant bleeding requiring repacking or continuous bleeding for 30 min. All outcomes were compared between the four groups, and the results were evaluated statistically.

Statistical analysis was performed using SPSS for Windows version 23.0 software program (IBM Corp., Armonk, NY, USA). The Student's t test for continuous variables and the chi-square test for categorical variables were used, and a p value <0.05 was considered to be statistically significant.

Ethics Committee approval was obtained, and the written informed consent was obtained from all patients. This study was conducted adhering to the Declaration of Helsinki.

RESULTS

A total of 90 patients were enrolled for this study. All the patients completed the trial. The mean age of the patients was 33.05±11.10 (range 18–65). The number of male patients was 63 (70%) and female was 27 (30%). The mean length of the patients was 172.03±7.5 (range 154–192), and the average weight of the patients was 73.48±13.36 (range 50–110).

Each of the study groups included 20 patients and the control group included 30 patients. The patients in the four groups were similar in terms of demographic parameters such as age, gender, race, geographical region, and culture.

Mean VAS values of the three study groups and the control group are provided in Table 1 and Figure 1.

Table 1. Mean visual analog scores of groups according to time.

	Groups	n	Mean±standard deviation	p
0 min	Control	30	6.73±1.782	–
	Lidocaine+A	20	4.20±2.262	<0.001
	Tetracaine	20	4.05±1.191	<0.001
	Articaine+A	20	3.05±1.572	<0.001
5 min	Control	30	3.77±1.870	–
	Lidocaine+A	20	0.90±1.294	<0.001
	Tetracaine	20	0.30±0.979	<0.001
	Articaine+A	20	0.00±0.000	<0.001
15 min	Control	30	0.43±0.817	–
	Lidocaine+A	20	0.00±0.000	0.022
	Tetracaine	20	0.10±0.447	0.103
	Articaine+A	20	0.00±0.000	0.022
30 min	Control	30	0.00±0.000	–
	Lidocaine+A	20	0.00±0.000	–
	Tetracaine	20	0.10±0.447	0.224
	Articaine+A	20	0.00±0.000	–

+A: plus adrenaline. Statistically significant at $p \leq 0.05$.

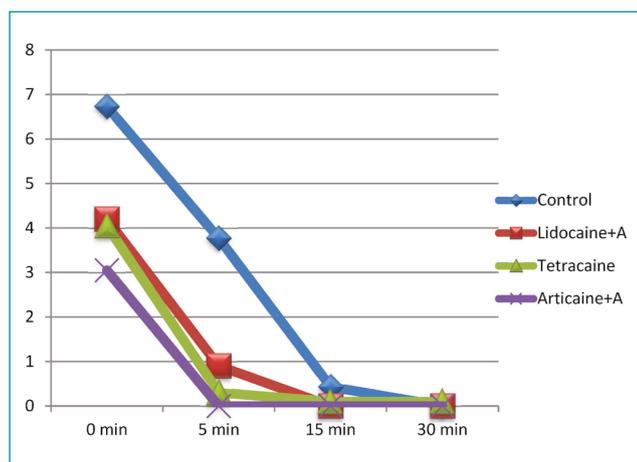


Figure 1. Mean visual analog scores of each group according to time points during and just after the removal of the nasal packs. The control group pain scores started from a high level, dropped rapidly in the first 15 min, and then dropped slowly in the second 15 min. Pain scores of lidocaine plus adrenaline group dropped rapidly in the first 5 min, then descended slowly until 5th min, and then remained stable. Tetracaine group pain scores showed a rapidly dropping during the first 5 min and then slowly a drop. Articaine plus adrenaline pain scores decreased quickly in the first 5 min, after which they remained stable until the end of the observation period. +A: plus adrenaline.

All study groups had significantly better pain scores than the control group at 0 and 5th min ($p < 0.001$). The lidocaine plus adrenaline and the articaine plus adrenaline groups had better pain scores than the control group at 15th min after the removal of the nasal packs ($p = 0.022$). Although the tetracaine group had a better pain score than the control group, this result was not statistically significant ($p = 0.103$). There was not a significant difference in terms of pain at 30th min. Almost all patients did not feel pain at 30th min. While the articaine plus adrenaline group had better pain scores than both study groups and the control group during 15 min after the removal of the packs, the lidocaine plus adrenaline group had the second best pain scores (Figure 1).

Mean hemorrhage values following the removal of the packs of three study groups and the control group are provided in Table 2.

The articaine plus adrenaline group had a less bleeding rate compared to the both study groups and the control group ($p = 0.004$). The lidocaine plus adrenaline group had the second less bleeding score ($p = 0.033$). There was no statistically significant difference in terms of hemorrhage after the removal of the nasal packs between the tetracaine and the control groups ($p > 0.05$).

There were no side effects due to the use of medications in this study.

DISCUSSION

The most common problem after septoplasty is pain, and discomfort is seen during nasal packing and especially at the time of the removal of the packs and just after the procedure⁹. In this study, we found that certain local anesthetics (i.e., lidocaine, tetracaine, and articaine), especially the articaine application to nasal packs, reduce the pain, bleeding, and discomfort formed during the removal of the packs of patients who underwent a septoplasty.

Table 2. Distribution of patients in the groups according to the Bleeding Scale.

Groups	n	Distribution of patients according to Bleeding Scale					p
		0	1	2	3	4	
Control	30	8	12	9	1	0	–
Lidocaine+A	20	10	10	0	0	0	0.033
Tetracaine	20	8	9	3	0	0	0.470
Articaine+A	20	15	5	0	0	0	0.004
Total	90	41	36	12	1	0	

+A: plus adrenaline. Statistically significant at $p \leq 0.05$.

Removal of the nasal packs is often the most uncomfortable and painful application of the septoplasty process for patients². The cause of the pain may be due to the displacement of the blood clot and adherent tissues, following adherence of nasal tampons to the bleeding site and the mucosa in the nasal cavity¹⁰. Different applications have been described for reducing the effect of this discomfort and painful procedure. There are applications such as wrapping the packs with gel foam, blocking the sphenopalatine ganglion, use of preemptive analgesia, use of different packs, and wetting packs with topical local anesthetics in the literature^{3-5,11}. There are also few studies reporting that nasal packing should not be used due to the discomfort that occurred while removing¹².

The efficacy of some local anesthetics on pain and bleeding management during the removal of the packs has been reported before in the literature. In one study, the authors applied sphenopalatine ganglion block by using topical 1% lidocaine and found better analgesia versus the control group during the removal of the packs. However, this application required an invasive procedure⁴. Researchers have evaluated the efficacy of prilocaine and levobupivacaine on reducing pain and discomfort during the removal of nasal packs in a study. They included 72 patients who had undergone septoplasty or endoscopic sinus surgery in this trial. They found that the levobupivacaine or the prilocaine infiltration into the packs before the removal of the nasal packs can decrease discomfort and improve patient tolerability during the removal of the packs¹³. In another study, researchers have investigated the effect of local anesthetics, such as ropivacaine, lidocaine plus adrenaline, and bupivacaine, and found that bupivacaine provides better analgesia and does not cause increased bleeding during the removal of the packs⁶. In a study conducted using topical 2% lidocaine and 0.9% saline on the packs, the authors found no significant difference in terms of the pain scores between the groups¹⁴. In another study conducted to compare the effect of ropivacaine, bupivacaine, prilocaine, and lidocaine on reducing pain and hemorrhage during the removal of the nasal packs, the authors found that topical lidocaine application provides better pain and bleeding score compared to the other drugs².

In this study, we investigated the effectiveness of the topical application of lidocaine plus adrenaline, tetracaine, and

articaine plus adrenaline into nasal packs to reduce pain and bleeding during the removal of nasal packs. We found that the articaine had the best pain and bleeding scores, and the lidocaine had the second best pain and bleeding scores during the removal of the nasal packs.

The limitation of this study is a small sample size. Further studies with a large number of patient groups should be conducted.

CONCLUSIONS

In this study, local anesthetics, especially the articaine plus adrenaline topical applied into the packs before the removal of the nasal packs, provide better analgesia and less hemorrhage during the removal of the packs. In other words, topical articaine plus adrenaline application into nasal packs before removing in patients who underwent septoplasty can be safely used for less pain and bleeding and more patient tolerance during the removal of the packs.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the Ethical Standards of the Institutional and/or National Research Committee (name the institution/committee) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in this study.

AUTHORS' CONTRIBUTIONS

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

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High serum 8-hydroxy-2'-deoxyguanosine levels predict DNA damage and aging in professional divers

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SUMMARY

OBJECTIVE: Reactive oxygen species and oxygen free radicals cause oxidative damage to lipids, proteins, and cell DNA in the cell membrane. Although many DNA products are produced during oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most common one, since it can be produced in *in vivo* environment. In recent years, diving has been done quite frequently for business and sports purposes all over the world. Increased environmental pressure in diving leads to hyperoxia and causes oxidative stress.

METHODS: The acute effects of diving on DNA damage were evaluated by comparing 8-hydroxy-2'-deoxyguanosine values of 15 professional diver groups before and after diving. In addition to the demographic characteristics, the serum 8-hydroxy-2'-deoxyguanosine levels of these 15 divers were compared with the control group consisting of nondiving medical students to examine the chronic effect of diving on DNA damage.

RESULTS: After deep dive, the amount of 8-hydroxy-2'-deoxyguanosine increased significantly in the diver group and acute DNA damage was observed (T1: 38.86±4.7; T2: 51.77±4.53; p<0.05). In the control group, the amount of 8-hydroxy-2'-deoxyguanosine was insignificant (C1: 47.48±3.73; T1: 38.86±4.7; p>0.05).

CONCLUSIONS: It was found that air dives caused an increase in serum 8-hydroxy-2'-deoxyguanosine levels, leading to acute oxidative stress and aging. However, there is no chronic side effect, according to the study of samples taken from the control group. This was thought to be due to the relative sedentary life of the control group. The duration of the effect or the ability to return to normal values should be investigated with further studies planned with large populations.

KEYWORDS: 8-Hydroxy-2'-deoxyguanosine. Air pressure. Naval medicine. DNA damage. Diving.

INTRODUCTION

Exposure of some workers and healthcare workers to hyperbaric environment means breathing air at high pressure of more than 10% of 1 atm. Operations in hyperbaric environments can take place in wet environments such as tunnels, tunnel scraping and pressure chambers, and search-rescue and military purposes¹.

According to Henry's gas law, limited depth and time of the dive is suggested to avoid oxygen toxicity during diving². Witte et al. reported DNA damage increases with

depth and bottom time during diving³. These partial pressure changes of oxygen and nitrogen in body tissues are responsible for many biochemical reactions that cause the formation of reactive oxygen and reactive nitrogen species⁴. These radicals cause a decrease in the activities of antioxidant enzymes, leading to lipid peroxidation in the cell membrane with DNA damage.

Some metabolomics have been used successfully in assessing the health status of workers at environmental exposures⁵. 8-Hydroxy-2'-deoxyguanosine (8-OHdG), which is the most

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common metabolomics in the oxidative damage of DNA, is a sensitive biomarker that can show even low-level DNA damage of oxidative stress⁶. It is formed when the hydroxyl radical damages the guanidine nucleoside at the C8 position on the DNA or when the hydroxyl radical binds to deoxyguanine with receiver electron⁷.

The studies on DNA damage in the hyperbaric environment remain challenging. In fact, this study is the first to examine 8-OHdG levels after air diving. In a previous study, we showed that single deep diving in the dry hyperbaric environment can cause thiol-disulfide imbalance in divers⁸. In addition to the high oxygen levels, the increasing number of workers in pressurized environments and the water temperature can also cause increase oxidative DNA damage⁹. Therefore, this study aims to investigate and identify the negative impact of environmental factors on health and cellular aging of individuals exposed to hyperbaric environment throughout their professional life.

METHODS

The experimental protocol was approved by the local ethics committee of the Harran University (2018/09/12) and was planned in accordance with the Helsinki Declaration. The study was planned with the control group, and the samples were taken before and after the application.

Study population

A total of 35 healthy volunteers (34 males and 1 female) participated in this study. Based on the demographic characteristics, participants were divided into study and control groups. The study group consisted of 17 volunteer professional divers (16 males and 1 female, 20.6 ± 0.8 years) graduated from professional underwater diving school and were exposed to hyperbaric hyperoxic environment. The control group consisted of 18 volunteer male medical students (19.4 ± 0.3 years) who did not do any diving and were included as the control group. Informed consent with personal information and health status was obtained from all volunteers and was kept confidential. Inclusion criteria of participants were nonsmokers, nonalcoholics, without any chronic disease (e.g., orthopedic, heart, kidney, liver, autoimmune, and metabolic diseases), and no history of any medication use. A diet and sleep plan was prepared for all volunteers 7 days prior to the study. All participants should not have any travel history during this period. Before the dive, only light breakfast was given to the participants (17 divers). Volunteer divers had 162 h of self-contained scuba apparatus, 192 h surface supplied breathing apparatus, and 28 h pressure room diving experience.

Study design and diving session

Fifteen minutes before the hyperbaric environment exposure, a blood sample was taken from all divers to examine whether the chronic hyperbaric exposure caused DNA damage. Then, blood samples taken 1 h after diving and before diving were compared to examine whether the dive caused acute damage to DNA.

Hyperbaric hyperoxic exposure was planned inside the decompression chamber as a dry (Barotech Multi DK 1700, Istanbul, Turkey). The Dive Plan was planned according to the U.S. Navy Air dive profile with zero deco limits. The divers were planned as a group of quaternaries and the first dive was made at 40 meter sea water (msw) at 11:00 a.m. with 5 min bottom time. The ascent rate was set at 10 m/min, at the end of the dive; a 3-min safety deco was applied at 5 msw. The first dive was completely ended at 11:15 a.m. The other groups were taken to the chamber in turn according to the plan.

During the dive, the temperature was kept constant at 25°C depending on the temperature in the pressure chamber and the indoor air was continuously analyzed and ventilated during the dive. A light and tempo walking and on-site movement were planned for volunteer divers during the chamber dive to imitate the stress factors in wet diving.

Sample collections and 8-hydroxy-2'-deoxyguanosine analysis

The antecubital vein was selected for sample collection from all participants. Samples were taken from the divers 90 min before the dive (T1) and 90 min after the dive (T2). The samples of the control group (C1) are the same as the time of T1 samples. Immediately after the blood was taken into tubes, it was centrifuged at 2,000 g (Hettich, Universal 320R, Tuttingen, Germany) and the sampling serum was obtained. It was stored at -80°C for commercial kits application.

To measure the levels of 8-OHdG in samples, the commercial enzyme-linked immunosorbent assay (ELISA) (Lot No: WXCJYEGD8D, Elabscience, Houston, TX, USA) commercial kit procedure was applied.

The sera stored in suitable storage conditions were dissolved to room temperature (+18–25°C). After the samples were loaded into 50 µl wells, 50 µl of biotin was added and incubated at +37°C for 45 min. After aspirating and washing three times, 100 µl of conjugate liquid was added and incubated for 30 min at +37°C. The sera were aspirated again, washed three times, and finally incubated with 90 µl of the substrate at +37°C for 15 min. In the final stage, 50 µl of stop solution was added to the sera and the results were calculated by measuring at 450 nm.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows software version 22.0 (IBM Inc., Chicago, IL, USA). Data are expressed as mean and standard deviation. Laboratory findings were evaluated with the “Shapiro-Wilk” test to determine whether they were normally distributed.

RESULTS

The demographic and anthropometric characteristics of the volunteers participated in the study are given in Table 1. There was no statistically significant difference in the demographic and anthropometric values between the two groups ($p>0.05$).

To examine the chronic effects of hyperbaric environment exposure on DNA damage, the 8-OHdG levels in serum taken from the C1 group and the T1 group were compared and found to be statistically insignificant (C1: 47.48 ± 3.73 ; T1: 38.86 ± 4.7 , $p>0.05$) (Table 2).

To examine the acute effects of hyperbaric environment exposure on DNA damage, the 8-OHdG levels in serum samples taken from the T1 group and the T2 group were compared. The levels after the dive were higher than those before the dive, and this difference was statistically significant (T1: 38.86 ± 4.7 ; T2: 51.77 ± 4.53 , $p<0.05$) (Table 3).

DISCUSSION

The aim of this study is to show whether hyperbaric environment exposure causes damage to DNA in divers. We planned a single

air dive to a depth of 40 msw to assess DNA damage during acute hyperbaric exposure in divers. The reason for choosing 40 msw is to measure the partial pressure of oxygen in the inhaled air as 1 atm. Also, 40 msw depth is the upper limit of recreational diving. We also aimed to make inferences about sport, recreational, and professional diving. According to the results, although single-session deep diving caused acute damage to DNA, this damage did not continue when divers were not involved in diving.

Factors causing increased DNA damage include stress factors such as increased environmental pressure, compressed gas breathing, and claustrophobia in dry hyperbaric environment exposure, and stream-cloudy water and hot-cold water in wet diving. Similar to underwater exercise, during the dive, the divers do a light walk and on-site movement in the pressure chamber. Tranfo et al. found that hypoxanthine levels were higher in the wet environment than in the dry hyperbaric environment¹. Bosco et al. showed that the exposure to wet hyperbaric environment caused stress with hot diving water and underwater cycling, and increased urinary 8-OHdG levels¹⁰. As mentioned previously, the exercise given to the divers in a single-session hyperbaric environment exposure caused an increase in 8-OHdG levels, leading to DNA damage.

8-OHdG is a biomarker of oxidative stress and aging which can be analyzed through urea, organs, and leukocyte DNA¹¹. 8-OHdG is the most common product of oxidative DNA damage¹². In addition, increased levels of 8-OHdG are responsible for cardiovascular risk and endothelial dysfunction⁶. High levels of 8-OHdG have been shown to impair renal function, with inflammatory markers⁶.

Table 1. Demographic and anthropometric characteristics of study population.

Subject	Age	Height (m)	Mass (kg)	BMI (kg/m ²)
Control group (medical students) n=18	19.4±0.3	1.74±10.6	66±12.5	23.7±2.2
Study group (professional diver group) n=17	20.6±0.8	1.71±11.8	63±11.9	22.8±1.6
p-value	0.081	0.057	0.61	0.066

Values are presented as mean±SD. Statistically significant at $p>0.05$.

Table 2. Comparison of serum 8-hydroxy-2'-deoxyguanosine levels before the dive (T1) of control group and study group.

Subject	Control group (C1)	Study group (T1)	p-value
8-Hydroxy-2'-deoxyguanosine serum level	47.48±3.73	38.86±4.7	0.20

T1/C1= $p>0.05$. Values are presented as mean±SD.

Table 3. Comparison of serum 8-hydroxy-2'-deoxyguanosine levels of study group 1 h before diving and 1 h after diving.

Subject	Study group (T1 before exposure)	Study group (T2 after exposure)	p-value
8-Hydroxy-2'-deoxyguanosine serum level	38.86±4.7	51.77±4.53	0.02

T1/T2= $p<0.05$. Values are presented as mean±SD.

It has been shown that homocysteine increases due to oxidative stress in saturation dives where exposure to a hyperbaric environment lasted for days and weeks¹³, and circulating vitamins B6 and B12 and folate levels decrease¹⁴. This hyperoxic environment causes increased iron stores and decreased erythropoietin levels¹⁵. The decrease in folate acts as an antioxidant in the oxidative stress environment¹⁶. A previous study showed that oxidative stress and DNA damage return to normal values with the end of the dive¹⁷. Our study shows that the 8-OHdG levels of C1/T1 were statistically insignificant, which is consistent with the results of the previous study.

Oxidative stress causes damage by disrupting the structure of enzymes in the mitochondrial membrane and the tertiary structure and functions of proteins¹⁸. Brain aging occurs with the formation of 8-OHdG and increased oxidative damage to mtDNA¹⁸. In addition, mitochondrial damage due to oxidative stress in brain tissue has been associated with aging and Alzheimer's disease¹⁹.

Aerobic exercise in a hyperbaric environment has been shown to increase serum lactate levels²⁰. In another study, oxidative DNA damage has been shown to cause pulmonary injury²¹. It has also been shown that DNA damage and subsequent serum 8-OHdG levels increase chronic fatigue syndrome²². As can be seen, DNA damage caused by oxidative stress results in aging that affects the whole body.

Some restraints should be considered in the assessment of our study. In this regard, our study was mostly conducted on male samples. To simulate warm water diving, oxidative DNA damage could be increased by turning off the air conditioner

in the pressure chamber. Finally, since it is a cross-sectional study, no conclusion can be drawn between 8-OHdG levels and long-term hyperbaric exposure.

CONCLUSIONS

We studied the effect of occupational and environmental hyperbaric exposures on the formation of 8-OHdG. From a practical point of view, it may be suggested that DNA damage occurs in the early stages of diving and its effects are seen in many tissues in our body. Although there is no DNA damage in daily life in divers with high dive time record in this study, there is a need for multicenter studies with wider interpretation.

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AUTHORS' CONTRIBUTIONS

AEG: Conceptualization, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. **OY:** Conceptualization, Methodology, Resources, Supervision. **CE:** Investigation, Supervision. **SND:** Data curation, Formal analysis, Writing – review & editing. **HC:** Data curation, Formal analysis, Resources, Software, Validation, Visualization.

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Morbidities and medications used by practicing nurses during the climacteric

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SUMMARY

OBJECTIVE: This study aimed to identify the morbidities and medications used by nurses practicing in São Paulo, Brazil, during the climacteric period to raise awareness and contribute to future preventive health measures in this population.

METHODS: A descriptive, cross-sectional study of 218 nurses aged 46–65 years based on data collected between January 2018 and July 2019 using a self-administered questionnaire was conducted.

RESULTS: Respondents had a mean age of 53.9±5.1 years, mean weight of 71.3±13.6 kg, mean waist circumference of 91.6±14.5 cm, and mean body mass index of 27.8±4.9 kg/m². Notably, 70.5% did not menstruate, had a mean age at last menstruation of 47.5±6.2 years and mean total score (Menopause Rating Scale) of 10.8±8.1, and reported the following morbidities: dyslipidemia (35.5%), subarachnoid hemorrhage (31.0%), rheumatologic diseases (14.8%), coronary heart disease (10.3%), diabetes (10.2%), malignant tumors (5.5%), deep venous thrombosis (3.2%), stroke (2.8%), and pulmonary embolism (0.5%). Medications used are antihypertensive agents (28.7%), antidyslipidemic agents (20.6%), antidiabetic agents (10.2%), antidepressants (9.6%), and anxiolytic agents (8.9%).

CONCLUSIONS: The most prevalent climacteric symptoms were somatic and psychological, most notably physical and mental exhaustion and muscle and joint issues. The most common morbidities were anxiety, dyslipidemia, arterial hypertension, depression, arthrosis, arthritis and rheumatologic diseases, osteoporosis, diabetes mellitus, and coronary heart disease. The most frequently used medications were antihypertensive agents, antidyslipidemic agents (statins), antidiabetic agents, antidepressants, and calcium supplements. The study results furnish information on the health of practicing nurses during the climacteric period to inform preventive measures aimed at the main risk factors for morbidities to which nurses are exposed during this period.

KEYWORDS: Climacteric. Menopause. Morbidity. Pharmaceutical preparations. Nurses. Nursing.

INTRODUCTION

The climacteric period marks the transition from the reproductive to nonreproductive phase and, although physiological, manifests differently for each woman^{1,2}.

Menopause is a point within the climacteric, diagnosed following 12 consecutive months of amenorrhea and sera levels of follicle-stimulating hormone (FSH) ≥ 25 UI/mL, after ruling out pathological causes. Statistically speaking, menopause

occurs, on average, at 50 years, whereas the climacteric begins at around 40 and extends up to 65 years of age^{3,4}.

Another issue to consider is the metabolic risk factors comprising the metabolic syndrome (MetS), which are precursors of cardiovascular diseases (CVDs), given that the prevalence of MetS rises between premenopausal and postmenopausal periods. MetS is a complex disorder characterized by a group of cardiovascular risk factors usually associated with the central

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deposition of fat and insulin resistance. The association of MetS with CVD is important, increasing general mortality 1.5-fold and cardiovascular mortality 2.5-fold⁵⁻⁷.

CVD is the leading cause of death worldwide. In Brazil, this pattern is no different, and despite a declining trend, the disease still accounts for 34.2% of deaths. Women develop the disease later than men, with a typical lag of 10 years. This marked increase in CVD is seen after menopause, supporting the hypothesis that the steady decline in hormone production during the menopausal transition period increases cardiovascular risk⁶.

The nursing profession in Brazil is regulated by Law n° 7.498, of June 25, 1986, and Decree n° 94.406, of June 8, 1987⁸. Nursing is the profession with the second largest number of professionals, over 2.2 million, comprising predominantly women. In São Paulo state, there are 506,846 registered nursing professionals overall, of which 134,518 are nurses and 86.47% women⁹.

Therefore, the objective of this study was to identify the morbidities and medications used by nurses practicing in São Paulo, Brazil, during the climacteric. The results can raise awareness of and contribute to the adoption of educational and preventive measures targeting the main risk factors of morbidities to which nurses are exposed during this period.

METHODS

A cross-sectional study involving a descriptive analysis of 218 nurses aged 46–65 years practicing at public and private health institutions in São Paulo, Brazil, was conducted.

Ethical aspects

This study was approved by the Research Ethics Committee of the Irmandade da Santa Casa de Misericórdia de São Paulo, under permit n° 3.728.222/2019. The following institutions were co-participants in this study: Conjunto Hospitalar do Mandaqui (CHM), permit n° 3.782.883/2019; Hospital do Servidor Público Municipal (HSPM), permit n° 3.787.697/2019; and Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), Permit n° 3.803.718/2019. Data collection was also approved at the facilities of the São Paulo Headquarters of the Regional Board of Nursing (Coren-SP), Administrative Process n° 2882/2017.

The objectives of this study were explained to all participating nurses both verbally and via an explanatory leaflet. All participants signed the free and informed consent form, in accordance with Resolution n°. 466/2012 of the National Board of Health¹⁰.

Data collection

A self-report questionnaire was devised collecting data on socio-demographics, professional qualifications, lifestyle habits, and general aspects of health. Symptoms specific to the climacteric were investigated using the Menopause Rating Scale (MRS) incorporated in the questionnaire under the following inclusion and exclusion criteria:

Inclusion criteria

Nurses aged 46–65 years, practicing in the health services.

Exclusion criteria

Nurses not practicing in health services.

Statistical methods

The sample size was calculated by performing a pilot study based on the MetS variables, which encompassed the main morbidities that are risk factors for CVD. An initial estimated prevalence of 21.1% was determined. The sample size calculation was based on this estimate, for a 95% confidence interval and 6% error (higher or lower), yielding a total sample of 178 nurses to be interviewed.

The data collected by the questionnaire were entered into an Excel® spreadsheet designed for this purpose. The following variables were summarized using the pertinent descriptive measures: mean, standard deviation, median, quartiles, and minimum and maximum values for the quantitative variables, whereas results were expressed as absolute (n) and relative (%) frequencies for categorical variables.

RESULTS

Sociodemographic data

The age of patients ranged from 46 to 65 years, with mean age of 53.9±5.1 years, and 49 (22.5%) individuals were between 46 and 49 years of age, 129 (59.2%) between 50 and 59 years, and 40 (18.3%) were between the ages of 60 and 65 years.

Lifestyle habits

Of note, 18 (8.3%) participants were smokers and 33 (15.2%) former smokers; 83 (38.2%) were alcohol users and 134 (61.8%) were non-users; 99 (45.6%) engaged in regular physical activity and 118 (54.4%) performed no regular physical exercise.

Anthropometric data

Nurses had a mean height of 1.60 m, ranging from 1.37 to 1.80 m. Bodyweight ranged from 43 to 127 kg, with a mean

of 71.3 kg (± 13.6); waist circumference range was 58–167 cm, with a mean of 91.6 cm (± 14.5); body mass index (BMI) was in the range of 16.7–43.9 kg/m², with a mean of 27.8 kg/m² (± 4.9), where 60 (27.9%) had BMI < 25 kg/m², 97 (45.1%) had 25–29.9 kg/m², and 58 (27.0%) had ≥ 30 kg/m².

Menstrual pattern

A total of 153 (70.5%) respondents no longer menstruated, while 64 (29.5%) still menstruated. Of the group still menstruating, 35 (54.7%) had a regular cycle, 28 (43.7%) had an irregular cycle, and 1 did not answer this question. Of the group not menstruating, 34 (22.2%) ceased menstruating after surgery, 68 (44.5%) naturally, and the remaining 51 (33.3%) reported no information for this variable. Age at last menstruation ranged from 19 to 57 years, with a mean of 47.5 years (± 6.2). Time in menopause ranged from 1 to 26 years, where half of the nurses reported < 6 years since menopause and the other half ≥ 6 years.

Menopausal symptoms

The presence and intensity of the symptoms related to the climacteric were determined using the MRS (Table 1).

Each symptom is rated on a 5-point Likert-type scale of rising intensity values, where 0=no and little, 1=mild,

2=moderate, 3=severe, and 4=very severe. Total score ranges from 0 to 44, where scores on the somatic and psychological symptom domains range from 0 to 16 points and on the urogenital domain 0 to 12 points. Higher scores indicate greater symptom intensity^{11–13}.

Among nurses who reported at least one of the symptoms for the respective domains, results were as follows:

- Somatic: 140 (65.1%) had joint and muscle complaints; 122 (56.7%) hot flushes or sweating; 113 (52.6%) sleeping disorders; 69 (32.1%) cardiac complaints.
- Psychological: 156 (72.6%) physical and mental exhaustion; 120 (55.8%) irritability; 106 (49.3%) anxious; 102 (47.4%) depressed;
- Urogenital: 128 (59.5%) sexual problems; 73 (34.0%) urinary complaints; 115 (53.5%) vaginal dryness.

Domain and total scores were tallied for the 196 study participants who provided answers for all symptoms on the scale (Table 2).

Morbidity and medications used

The nurses answered questions based on the presence of morbidities and the use of medications for their treatment (Table 3).

Table 1. Intensity of climacteric-related symptoms, as measured on the Menopause Rating Scale (MRS), for participants reporting at least one symptom (n=215), age 46–65 years, January 2018 to July 2019.

Symptom domain	None (0)		Mild (1)		Moderate (2)		Severe (3)		Very severe (4)		No response	
	n	%	n	%	n	%	n	%	n	%	n	%
Somatic												
Hot flushes	91	42.3	61	28.4	33	15.3	20	9.3	8	3.7	2	0.9
Heart discomfort	145	67.4	44	20.5	21	9.8	3	1.4	1	0.5	1	0.5
Sleep problems	100	46.5	28	13.0	49	22.8	27	12.6	9	4.2	2	0.9
Joint and muscle discomfort	73	34.0	52	24.2	42	19.5	33	15.3	13	6.0	2	0.9
Psychological												
Depressive mood	111	51.6	51	23.7	35	16.3	14	6.5	2	0.9	2	0.9
Irritability	90	41.9	55	25.6	43	20.0	18	8.4	4	1.9	5	2.3
Anxiety	105	48.8	45	20.9	36	16.7	17	7.9	8	3.7	4	1.9
Physical and mental exhaustion	58	27.0	73	34.0	42	19.5	36	16.7	5	2.3	1	0.5
Urogenital												
Sexual problems	86	40.0	45	20.9	47	21.9	27	12.6	9	4.2	1	0.5
Bladder problems	142	66.0	42	19.5	23	10.7	7	3.3	1	0.5	0	0.0
Vaginal dryness	97	45.1	41	19.1	41	19.1	27	12.6	6	2.8	3	1.4

n=absolute number of responses per item.

Table 2. Climacteric symptom scores, as measured on the Menopause Rating Scale (MRS), for participants answering all items of respective domains (n=196), age 46–65 years, January 2018 to July 2019.

Scores	Domains and symptoms			
	Somatic	Psychological	Urogenital	Total
Mean (SD)	4.0 (3.2)	4.1 (3.7)	2.7 (2.6)	10.8 (8.1)
Median (Q1; Q3)	4.0 (1.0; 6.0)	3.0 (1.0; 6.0)	2.0 (0.0; 4.0)	9.0 (5.0; 16.5)
Minimum; maximum	0; 16	0; 15	0; 10	0; 32

SD: standard deviation; Q1: first quartile; Q3: third quartile.

Table 3. Morbidities present and medications used by participant nurses, age 46 to 65, January 2018 to July 2019.

Morbidity	Total responses	Morbidity present		Medications used	
		n	%	n	%
Anxiety	214*	88	41.1	19	8.9
Dyslipidemia	214*	76	35.5	44	20.6
Systemic arterial hypertension	216*	67	31.0	62	28.7
Depression	209*	41	19.6	20	9.6
Rheumatologic diseases	216*	32	14.8	12	5.6
Osteoporosis	213*	24	11.3	16	7.5
Coronary heart disease	214*	22	10.3	7	3.3
Diabetes mellitus	215*	22	10.2	22	10.2
Malignant tumor	217*	12	5.5	7	3.2
Deep vein thrombosis	217*	7	3.2	2	0.9
Stroke	217*	6	2.8	2	0.9
Pulmonary embolism	217*	1	0.5	0	0.0

*Total de respostas por item; n: número absoluto de respostas por item; %: porcentagem.

DISCUSSION

This is the first cross-sectional descriptive study of nurses from São Paulo, Brazil, practicing in the climacteric period at public and private health institutions, exploring the morbidities and medications used by this group of professionals.

Nursing in Brazil is the largest category in the health field employing the highest number of women⁹. Thus, this study group was selected based on the predominance of women in the nursing team, participant experience in the health area, and the ability to furnish information on health changes. Moreover, to ensure a homogeneous sample, only individuals holding nursing degrees were selected.

In the State of São Paulo, 46.0% of women are in the climacteric period, indicating that the remaining 53.2% will go on to experience this phase, where 0.8% have already gone through this stage⁹.

In this study, 72.1% of participants had a BMI >25 kg/m² and thus classified as overweight or obese. This rate proved higher

than that for the Brazilian general population. We believed that these long working hours, operational demands, and non-observance of mealtimes are additional risk factors for obesity in the study group¹⁴⁻¹⁶.

The results revealed that the proportion of non-smokers was greater than current and former smokers. Similarly, more nurses were non-drinkers than drinkers. For physical activity, over half of the sample did not engage in regular exercise. Studies show that regular exercise reduces the risk of CVDs and osteoporosis, besides promoting esthetic and mood benefits¹⁷.

The mean age at last menstruation was 47.5 years (± 6.2). This value is low compared with the first population-based study of Brazilian women conducted in 2003, which found a mean age at natural menopause of 51.2 years¹⁸. In contrast, a more recent study of 749 women in the Southeastern region of Brazil found a mean age of 46.5 (± 8.5), a result similar to that of this study¹⁹. In a health study involving Chinese nurses, the results were again similar, identifying a mean age at natural menopause

of 48.6 years (± 3.61)²⁰. Data published by the World Health Organization state that the most common age range for natural menopause is between 45 and 55 years, where this study result lies within this expected age range⁴.

Regarding the symptoms assessed by the MRS, the total score corresponds to a moderate symptom rating. These study results are in line with those reported by an investigation of Chinese nurses and women in general, conducted in the Southeast of Brazil, reporting a mean score of 9.8 (± 7.9). However, these scores vary depending on the country, culture, and socioeconomic and educational background of the population studied. Understanding and controlling these factors can help reduce climacteric symptoms and target those groups most needing assistance^{13,19,20}.

Psychological and somatic symptoms proved more severe and frequent than urogenital symptoms, most notably physical and mental exhaustion. Also, hot flushes, irritability, anxiety, and depression were reported by around 50% of the nurses, relevant results that may impact the performance of work activities by these professionals.

In the study of Japanese nurses, the authors concluded that health professionals should be aware that menopausal symptoms are associated with job-related stress factors²¹.

In the United Kingdom, 45% are registered experiencing the host of symptoms that menopause can cause²².

Besides the specific symptoms of the climacteric in this study, 75.7% of participants reported more than one morbidity, including, in decreasing order of incidence: dyslipidemia, systemic arterial hypertension, arthrosis, arthritis or rheumatological diseases, coronary heart disease, diabetes mellitus (DM), malignant tumors, and risk factors for CVD.

In fact, data from the Ministry of Health show that CVDs account for 3 of the 10 most common causes of death in the female population in Brazil². A study based on the presence of multimorbidity and associated factors in Brazilian women found that sedentarism, obesity, irritability, and postmenopause were associated with multimorbidity⁵.

Preventing CVD in postmenopausal women is critical, given the incidence of this disease rises with age. The condition is of multifactorial origin and can be influenced by modifiable risk factors such as smoking, sedentarism, poor diet, and elements of MetS²⁻⁵.

With regard to the use of medications for treating the morbidities reported by the nurses, good adherence in the study group was noted for diseases requiring strict medication control, such as subarachnoid hemorrhage and DM. All of those nurses who reported having diabetes and 92.5% of those with arterial hypertension were in use of medications for treatment, possibly due to their knowledge of the health area. However, rates for the other conditions were lower.

CONCLUSIONS

The most prevalent climacteric symptoms were somatic and psychological, most notably physical and mental exhaustion and muscle and joint issues. The most common morbidities were anxiety, dyslipidemia, arterial hypertension, depression, arthrosis, arthritis and rheumatologic diseases, osteoporosis, DM, and coronary heart disease. The most frequently used medications were antihypertensive agents, antidyslipidemic agents (statins), antidiabetic agents, antidepressants, and calcium supplements.

These results can help guide the development of health strategies aimed at health prevention among women in nursing. This group represents a large workforce with a key role in providing essential health services at all levels of health care, and such strategies can promote better working conditions, mechanisms for reducing occupational stress, as well as educational and preventive measures targeting the main risk factors for morbidities to which nurses are exposed in this period.

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AUTHORS' CONTRIBUTIONS

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

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Increase in fertility rate before the age of 14 in Brazil from 1996 to 2018

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SUMMARY

OBJECTIVE: This study aimed to assess live birth frequency and age-specific fertility rates (ASFR) in the period 1996–2018 and the number of pregnancies at <14 years old in the period 2012–2018.

METHODS: This was a cross-sectional study conducted by search on Live Births Data System (SINASC/DATASUS) database.

RESULTS: There was a variation in ASFR in Brazil of 0.78‰ in 1996 to 0.87‰ in 2018 (+11.5%). In the north region, it increased from 1.28‰ to 1.66‰ in 2018. In the northeast region, it increased from 0.72‰ to 1.66‰ (+131%) in 1996–2011, but decreased to 1.31‰ in 2018 (-21% in relation to 2011). When comparing 1996 and 2018, in the southeast region, there was a 22% decrease; in the south region, it was 48.2%; and in the Center-West region, it was 34%; but in the north region, there was a 29.7% increase, and in the northeast region, it was 81.9%. When adding girls who became pregnant aged 13 years and gave birth at 14, there was a threefold increase in the rate.

CONCLUSIONS: The increase of pregnancies in <14 years old in less developed regions of Brazil shows an association with socioeconomic factors and reveals the severe problem of rape of vulnerable persons in the country.

KEYWORDS: Adolescent. Pregnancy. Rape. Sex offenses.

INTRODUCTION

Although the fertility rate in Brazil has decreased since 1960, it increased in the 1990s among adolescents, and from 2001 onward, there is a slow decrease.

The birth rate reduction was from 21.5% in 2006 to 18.1% in 2015, motivated by the age group of 15–19 years in all the

country's regions, with a stability tendency among mothers aged 10–14 years and a 5% increase in the north region¹.

Pregnancy in adolescence is more frequent in populations with low socioeconomic level, such as sub-Saharan Africa (28%)², and Latin America and the Caribbean present the second high-rate (15%) of the total number of births³.

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This situation is aggravated when pregnancy occurs until 14 years old, with higher risks of maternal-fetal complications and social and psychological sequelae⁴. It is estimated that 2% of women in Latin America-Caribbean have their first childbirth before 15 years of age³.

On the other hand, data on pregnancy in girls aged <13 years are limited. Souto et al.⁵ studied the period 2011–2015 and observed that 31,611 girls aged ≤13 had children, especially in the northeast region (39.4%), with a higher rate of unfavorable gestational outcomes in the group of rape notification⁵.

These pregnant girls are more susceptible to stress, emotional insecurity, preeclampsia, premature birth, postnatal complications, repeated pregnancies, and less prenatal consultations. Their children present a higher risk of low weight, mortality, hospitalizations, and emergency care^{3–5}.

The objectives of this study were to evaluate the frequency of live births (LB) of mothers in the age group of 10–13 years and the age-specific fertility rate (ASFR) in the period 1996–2018. There was also a calculation of the number of pregnancies initiated at <14 years old in the period 2012–2018, since pregnancy at this age constitutes a crime of rape of vulnerable⁶.

METHODS

A cross-sectional epidemiological study was conducted through search on Live Births Data System (SINASC/DATASUS) database in the period 1996–2018. Using the application TabWin and the scripts developed in Python language 3.8 version, 68.8 million registers were imported to the Database Management System (DBMS) opensource PostgreSQL 11.8 version.

Since SINASC registers all LB in the country, two groups of registers were selected for this study:

Group 1 (G1): Adolescents who had LB aged 10–13 years between 1996 and 2018.

Group 2 (G2): Adolescents who started pregnancy at 13 years old but delivered at 14 years old between 2012 and 2018 (selection from mother's age minus the number of full gestational weeks until childbirth).

It is noteworthy that SINASC database had its fields altered through time, more substantially between 2010 and 2012⁷. From 2012 onward, over 90% of LB registers received the attribute *semagestac* to calculate the mother's age at the start of pregnancy for G2.

The sum of occurrences of G1 and G2 results in the quantity of registers on SINASC that characterize rape of vulnerable, according to Art. 217-A of Federal Law nr. 12015⁶.

Although G1 represents girls <14 years at childbirth, the actual number of pregnancies starting at <13 years is underestimated due to the large ratio of adolescents who gave birth at 14 years but became pregnant at 13 years.

This study was developed using the following variables: birth according to mother's place of residence, birth according to region, and year of birth.

For the analysis of births in this age group in relative numbers, ASFR for G1 was calculated using as reference mother's age at childbirth. ASFR represents the mean number of LB/woman in a specific age interval, presented per group of 1,000 women for each age group, according to Equation 1:

$$ASFR\%_{10-13;reg;year} = G1_{reg;year} \cdot 1000 / FemPop_{10-13;reg;year} \quad (1)$$

where $year \in \{1996, 1997, \dots, 2018\}$ is the reference year for data; $reg \in \{BR, CW, NE, N, SE, S\}$ corresponds to Brazil (BR) or one of the five regions given as follows: Center-West (CW), northeast (NE), north (N), southeast (SE) and south (S). $ASFR\%_{10-13;reg;year}$ is the age-specific fertility rate for the interval 10–13 years old for a given region (reg) in a given year; $G1_{reg;year}$ is the frequency of G1 for a given region and year; and $FemPop_{10-13;reg;year}$ is the total female population aged 10–13 years in the region and year of reference.

For the sum G1+G2, a similar rate was calculated to $ASFR\%_{10-13;reg;year}$, denominated rape-related pregnancy rate according to legislation (RPRAL). Equation 2 shows the calculation, where $year \in \{2012, 2013, \dots, 2018\}$:

$$RPRAL\%_{10-13;reg;year} = (G1_{reg;year} + G2_{reg;year}) \cdot 1000 / FemPop_{10-13;reg;year} \quad (2)$$

For the variable $FemPop_{10-13;reg;year}$, the sources used were two population databases:

- Period 1996–2009: Demographic data available at DATASUS⁸ website.
- Period 2010–2018: IBGE provides annual population projections considering the latest demographic census (2010)^{9,10}.

To obtain the measures to follow the evolution of ASFR and RPRAL in Brazil and its regions, the option was to calculate two percentage variations. The first is the percentage variation of $ASFR\%_{10-13;reg;year}$ and $RPRAL\%_{10-13;reg;year}$ in relation to the same region (reg) in the first year of the calculation of the respective rate, i.e., 1996 for ASFR and 2012 for RPRAL. Equation 3 exemplifies the calculation. For example, if a region A presents $ASFR\%_{10-13;A;1996} = 0.6$ and $ASFR\%_{10-13;A;2010} = 0.9$, then $VarASFR\%_{10-13;A;2010} = 50\%$.

$$\frac{VarASFR\%_{10-13;reg;year}}{ASFR\%_{10-13;reg;year}} * 100 / ASFR\%_{10-13;reg;1996} - 100 \quad (3)$$

The second percentage variation also relates ASFR and RPRAL rates of a given region and year in the function of the respective rate of the same year for Brazil. Thus, it measures the percentage difference of the rate of a region (CW, NE, N, SE, and S) in a given year in relation to the same period in Brazil. Equation 4 exemplifies the calculation of $ASFR\%_{10-13;reg;year}$:

$$\frac{VarASFRBR\%_{10-13;reg;year}}{ASFR\%_{10-13;reg;year}} * 100 / ASFR\%_{10-13;BR;year} - 100 \quad (4)$$

The research project was approved under the number CAAE 04209418.1.0000.5259.

RESULTS

In the period 1996–2018, there were 130,778 births from mothers aged 10–13 years in Brazil. In 1996, there was a variation on $ASFR\%_{10-13;BR;1996}$ from $0.78\%_{10-13;BR;1996}$ to $0.87\%_{10-13;BR;2018}$, resulting in a percentage variation of +11.5%.

In the period 1996–2018, the ASFR in the N region varied from $1.28\%_{10-13;N;1996}$ to $1.66\%_{10-13;N;2018}$ with a peak of $1.99\%_{10-13;N;2011}$ in 2011. The ASFR in the NE region increased from $0.72\%_{10-13;NE;1996}$ to $1.66\%_{10-13;NE;2011}$, showing a variation of +131%, but receding at the end of the series to $1.31\%_{10-13;NE;2018}$ (21% reduction in relation to 2011). CW and S regions presented the highest ASFR at the beginning of the series, respectively, $1.44\%_{10-13;CO;1996}$ and $0.83\%_{10-13;S;1996}$. The SE region had the highest ASFR in 2003 ($0.64\%_{10-13;SE;2003}$), ending the series with $0.46\%_{10-13;SE;2018}$.

When comparing 1996 and 2018 ($VarASFR\%$), there was a 22% reduction in the SE region, 48.2% in the S region, and 34% in the CW region; but there was a 29.7% and 81.9% increase in the ASFR in the N and NE regions, respectively (Table 1)⁸⁻¹⁰.

Figure 1 shows the temporal evolution of $ASFR\%_{10-13;reg;year}$ in Brazil and regions between 1996 and 2018. In the last years of the temporal series, it was verified that the SE and S regions composed the group with ASFR below the country's average; the N and NE regions presented an opposite relation; and the CW region was nearer to the country's rates.

Figure 1 also shows $ASFR\%_{10-13;reg;year}$ and the evolution of this rate with $VarASFR\%_{10-13;reg;year}$. It was verified that the CW and S regions presented the most significant percentage reduction regarding 1996, whereas the NE region surpassed the N region, more than doubling ASFR in relation to 1996 between 2011 and 2013, but presented a reduction from then until 2018.

Data used in this study show a heterogenous population distribution across the territory. In 2018, Brazil had a population of 5,638,556 girls aged 10–13 years, with 2,234,368 (39.6%) in the SE region; 1,528,044 (27.1%) in the NE region; 747,171 (13.3%) in the S region; 664,832 (11.4%) in the N region; and 484,141 (8.6%) in the CW region. There was a decrease of $VarASFR\%_{10-13;reg;2018}$ in the three regions, including the most populated, but an 11.5% increase in ASFR in relation to 1996.

When adding to ASFR results from 2012 onward the number of girls who became pregnant at 13 years of age and gave birth at 14 years (RPRAL), a 203.5% increase was observed in relation to the data presented in Figure 1. On average, the rate was more than tripled when observing age 10–13 at the start of pregnancy, instead of the mother's age at childbirth.

Table 2 shows the total number of adolescents in G1 and G2. When adding G2 to the analysis, it was verified that the number of pregnant girls aged 13 years was much higher, since in G2 over 60% of them became pregnant at 13 years, although in the official statistics they were counted as pregnant at 14 years old.

DISCUSSION

This is the first study on the temporal assessment of fertility rate in Brazil among the age group of 10–13 years. Outcomes show 11.5% increase in ASFR in the past 23 years. Another originality was the assessment of maternal age at conception, since statistics use a mother's age at childbirth as a criterion.

ASFR evaluation is a challenging task due to data scarcity and the country's development level^{4,5,11}. This could be the main reason for the reduced number of publications about research on populations in the age group of 10–14 years, especially up to 13 years old.

On the other hand, data in this study show that ASFR increase in the age group of 10–13 years in Brazil is heterogenous and is concentrated in the N and NE regions. As the two regions present the country's lowest Human Development Index (HDI) 0.73 and 0.71¹², respectively, there is an evident association with poverty and underdevelopment, as well as racism and low education level¹¹. The association of inequalities and sexual violence is also verified worldwide, with the highest rates of pregnancy in adolescence in Africa, Central America, and South America³. In Colombia, pregnancy as a consequence of violence reached 21.5%¹¹.

In the United States, declines in natality rates in the age group of 10–14 years were observed in the period 2000–2016, with greater falls after 2008. There was a greater reduction among 13 years old, from 7.08 births/10,000 in 2000 to 1.80 in 2016¹³.

Table 1. Distribution of age-specific fecundity rate in group 1 (10–13 years) per year and region of residence per 1,000 adolescents ($ASFR\%_{10-13,reg;year}^{8-10}$).

Year	North		Northeast		Center-West		Southeast		South		Brazil	
	B 10–13	ASFR‰	B 10–13	ASFR‰	B 10–13	ASFR‰	B 10–13	ASFR‰	B 10–13	ASFR‰	B 10–13	ASFR‰
1996	727	10.28	1,617	0.72	670	1.44	1,568	0.59	777	0.83	5,359	0.78
1997	809	10.38	1,825	0.81	585	1.22	1,525	0.57	702	0.74	5,446	0.78
1998	823	10.37	2,063	0.9	647	1.33	1,595	0.59	730	0.76	5,858	0.83
1999	892	10.46	2,110	0.91	587	1.18	1,631	0.59	671	0.69	5,891	0.82
2000	909	10.52	2,175	1.00	598	1.26	1,508	0.58	668	0.72	5,858	0.86
2001	948	10.54	2,228	1.01	596	1.23	1,476	0.55	671	0.71	5,919	0.86
2002	868	10.38	2,438	1.09	544	1.1	1,426	0.53	646	0.68	5,922	0.85
2003	995	10.56	2,183	0.97	557	1.11	1,740	0.64	600	0.62	6,075	0.86
2004	1,072	10.64	2,063	0.91	557	1.09	1,237	0.45	608	0.62	5,537	0.77
2005	1,081	10.59	2,182	0.94	560	1.05	1,519	0.53	600	0.60	5,942	0.80
2006	1,096	10.58	2,333	0.99	589	1.08	1,445	0.50	584	0.58	6,047	0.81
2007	1,224	10.92	2,427	1.23	577	1.16	1,442	0.57	575	0.63	6,245	0.95
2008	1,232	10.97	2,448	1.24	566	1.15	1,516	0.61	636	0.71	6,398	0.99
2009	1,180	10.89	2,369	1.20	534	1.09	1,425	0.57	556	0.63	6,064	0.94
2010	1,192	10.75	2,397	1.37	523	1.01	1,34	0.51	595	0.66	6,047	0.93
2011	1,354	10.99	2,867	1.66	585	1.14	1,448	0.56	543	0.61	6,797	1.06
2012	1,329	10.98	2,624	1.55	576	1.14	1,571	0.62	571	0.66	6,671	1.07
2013	1,291	10.94	2,524	1.53	596	1.20	1,526	0.62	554	0.67	6,491	1.07
2014	1,150	10.75	2,181	1.35	567	1.15	1,425	0.60	509	0.63	5,832	0.98
2015	1,224	10.88	2,249	1.41	545	1.11	1,355	0.58	455	0.58	5,828	1.00
2016	1,203	10.86	2,124	1.34	519	1.06	1,261	0.55	458	0.59	5,565	0.96
2017	1,016	10.57	1,965	1.26	484	0.99	1,153	0.51	368	0.48	4,986	0.87
2018	1,069	10.66	2,002	1.31	459	0.95	1,027	0.46	325	0.43	4,882	0.87

ASFR: Age-specific fecundity rate. Total LBs from mothers between 10 and 13 years/total population of resident adolescents of this age group, multiplied by 1,000.

Romania has the highest fertility rates in Europe. In the age group of 10–14 years, in 2011 the pregnancy rate was 2.64/1,000 and the birth rate was 1.41/1,000¹⁴.

The CLADEM¹⁵ study conducted in 14 countries in Latin America and the Caribbean demonstrated that specific data on pregnancy or abortion in girls aged <14 years existed only in El Salvador, with childbirths in 2/3 of pregnancies. In the other countries, statistics were extracted from childbirths data and reflected only one subset of pregnancies in this age group¹⁵.

Pregnancy in girls aged 10–13 years is considered a crime of rape of vulnerable by Brazilian legislation⁶, regardless of consent. By adding the total number of cases, this study verified

the existence of a contingent of sexual violence victims several times higher than shown in the official notification data.

Various motives can be inferred for the increase in the number of childbirths until the age of 13 years: difficulty to access public legal abortion services, late discovery of pregnancy, threats from the family or the rapist, religious beliefs, among others⁷. Furthermore, public health services that conduct elective pregnancy interruption are scarce and face barriers related to conscientious objection by health professionals and managers⁴.

In 2020, the total number of sexual violence cases was 66,123 with 57.9% corresponding to the rape of vulnerable. Gender inequality is evident, with 85.7% of female victims¹⁶.

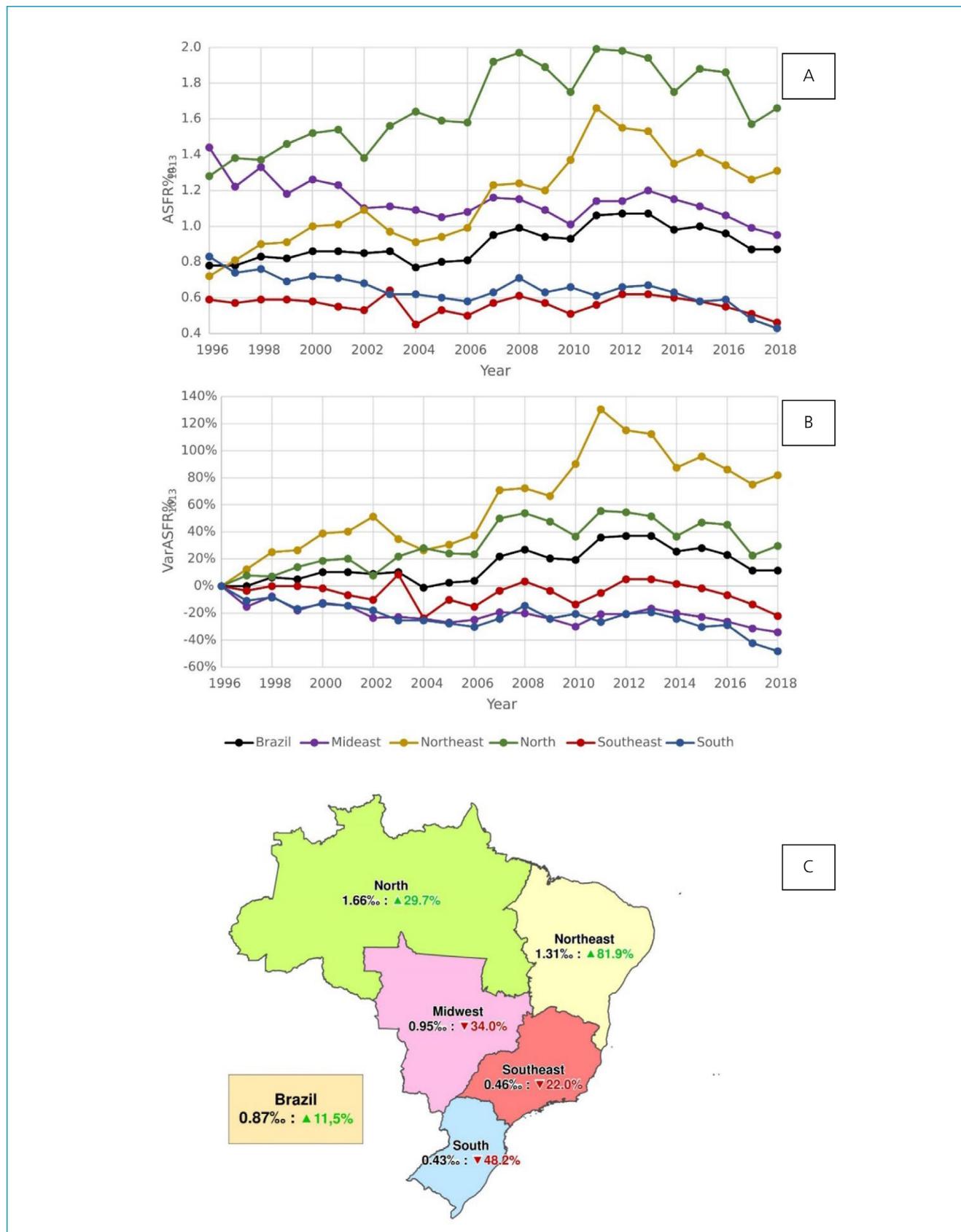


Figure 1. (A) Age-specific fecundity rate for interval 10–13 years old per region of Brazil ($ASFR\%_{10-13,reg,year}$); (B) percentage variation of $ASFR\%_{10-13}$ from 1996 to 2018 ($VarASFR\%_{10-13,reg,year}$); and (C) both rates 2018 for Brazil and its regions.

Table 2. Annual distribution of the number of girls who became pregnant under 14 years of age and fertility rate (2012–2018).

Year	Female population aged 10–13 years (IBGE)	Mother <14 years at birth (Group 1)	Mother <14 years at start of pregnancy (Groups 1+2)	ASFR‰	RPRAL‰	RPRAL percentage not included in ASFR
2012	6,240,822	6,671	18,348	1.07	2.94	63.61
2013	6,086,944	6,491	18,908	1.07	3.11	65.59
2014	5,958,212	5,832	18,840	0.98	3.16	68.99
2015	5,852,614	5,828	18,008	1.00	3.08	67.53
2016	5,789,131	5,565	16,355	0.96	2.83	66.08
2017	5,712,535	4,986	15,230	0.87	2.67	67.42
2018	5,638,556	4,882	14,496	0.87	2.57	66.15

ASFR: Age-specific fecundity rate. Total LBs from mothers between 10 and 13 years/total population of resident adolescents of this age group, multiplied by 1,000; RPRAL: Rate-related pregnancy rate according to legislation. Total LBs from mothers between 10 and 13 years added to total number of mothers who became pregnant aged 13 years but gave birth aged 14 years/total population of resident adolescents aged between 10 and 13 years, multiplied by 1,000.

Souto et al.⁵ compared the registers of rape of vulnerable with that of births from mothers aged <14 years and verified that only 4% were notified as sexual violence. A similar study¹⁷ showed notifications only in 1.3% in the age group of 10–13 years.

The low notification by health professionals reveals that pregnancy in girls aged <14 years is not identified as resulting from sexual abuse, which highlights the invisibility of the problem^{5,17}.

As limitations to our study, we highlight the following: insufficient filling in of official data available on SINASC/DATASUS; impossibility to obtain the true number of adolescents who suffered abortion; difficulty to obtain the total number of stillbirths in adolescents; and utilization of two different data sources to estimate the resident population per age group (DATASUS and IBGE). This suggests that our data could be underestimated and the number of pregnancies at age <14 years could be even higher.

CONCLUSION

The ASFR increased 11.5% among girls aged <14 years in the less-developed regions of Brazil, which shows an association with socioeconomic factors and reveals the invisibility of rape of vulnerable in the country.

AUTHORS' CONTRIBUTIONS

DLMM: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **FRDM:** Conceptualization, Writing – original draft, Writing – review & editing. **IMSL:** Data curation, Formal analysis, Writing – original draft. **SRT:** Data curation, Formal analysis, Methodology, Writing – original draft. **JASR:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **FMS:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **NCPR:** Conceptualization, Data curation, Formal analysis, Methodology.

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Evaluation of magnetic resonance imaging findings in adhesive capsulitis: which quantitative findings are most valuable?

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SUMMARY

OBJECTIVE: This study aims to investigate magnetic resonance imaging (MRI) findings in adhesive capsulitis (AC) and determine the most valuable MRI finding in diagnosis using easily applied quantitative methods.

METHODS: Shoulder MRI was performed on 193 patients who were diagnosed with AC by clinical examination and 116 controls. Axillary pouch thickness (APT), superior and inferior glenohumeral ligament (SGHL and IGHL) thickness, coracohumeral ligament (CHL) thickness, fluid increase and soft-tissue thickness in the rotator interval (RI), and increases in the fluid and signal in the localization of biceps tendon attachment were evaluated. MRI examinations were assessed by three radiologists blinded to the clinical findings of the patients, and the results were obtained based on consensus and records.

RESULTS: There were 119 women and 74 men in the AC group and 80 women and 36 men in the control group. IGHL, SGHL, RI, and CHL thicknesses were measured thicker in AC patients than in the control group. When IGHL=4, RI=3.6, SGHL=2.0, CHL=4.6-mm cutoff, the area under the receiver operating characteristic (ROC) curve (AUC) values were 0.700, 0.922, 0.972, and 0.783, respectively.

CONCLUSIONS: According to the results obtained in this study, IGHL=4 mm, RI=3.6 mm, SGHL=2.0 mm, and CHL=4.6 mm can support the diagnosis of AC. Using the quantitative values in diagnosis can provide objective criteria and prevent variability among interpreters.

KEYWORDS: Adhesive capsulitis. Pain. Shoulder. Frozen shoulder. Musculoskeletal diseases. Magnetic resonance image.

INTRODUCTION

Adhesive capsulitis (AC) is a clinical condition of uncertain etiology. Diagnosis is mostly clinical, and the role of radiology is mostly secondary to confirm or deny the presence of contributory causes and perhaps the extent of disease¹. AC is a diffuse inflammatory disease involving the scapulohumeral joint, joint capsule, joint synovial tissue, glenohumeral ligaments, periarticular tendon and bursae (especially the subacromial bursa), and biceps tendon sheath².

There are various studies on the magnetic resonance imaging (MRI) findings of AC, examining signal changes

and morphological changes in different anatomical structures in the shoulder joint³⁻⁵. However, a clear consensus has not yet been achieved among the findings of studies on the diagnosis of this condition. Therefore, interobserver variability among physicians interpreting MRI may be very high. This study aimed to quantitatively evaluate the changes in the MRI signal and morphological changes including the thickness of the capsuloligamentous structures and considered to be affected by AC and to compare the diagnostic values of these findings.

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METHODS

This retrospective study was approved by our institutional ethical committee and carried out in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines. The requirement for informed consent from the patients was waived due to the retrospective nature of the study.

Patients who were diagnosed with AC at the Physical Therapy and Rehabilitation Clinic between January 2018 and April 2020 and underwent a shoulder MRI examination were included in this study. The diagnosis of AC was made by the clinician based on the clinical examination. The control group was formed with patients that were referred to MRI by the same clinic for the exclusion of conditions other than AC, such as fibromyalgia and myofascial pain syndrome, and were determined to have no morphological and signal changes in the structures affected by AC. The patient and control group list was determined by the clinician. The radiologists who performed the evaluation had no clinical knowledge of the patients.

MRI examinations were performed using a 1.5 T device (Philips Ingenia, Best, The Netherlands) and a phased array shoulder dedicated coil. A 160-mm field of view (FOV), 3-mm slice thickness, 0.3-mm interslice gap, and 256×256 matrix were used for all sequences. The parameters used for the oblique coronal proton density (PD) sequence were TR/TE 3,700/30 ms, for the oblique coronal T1W sequence were 608/9 ms TR/TE, for the axial PD sequence were TR/TE 4,200/30 ms, for the oblique sagittal PD sequence were TR/TE 4,600/25 ms, and for the sagittal T1W sequence were TR/TE 608/9 ms.

MRI evaluation

All images evaluated were non-contrast shoulder MRIs taken with the same device. Fluid increase and soft-tissue thickness in the rotator interval (RI) were shown in oblique sagittal PD images. The presence of increased fluid and signal at the attachment site of the biceps tendon was shown in oblique sagittal PD images. Coracohumeral ligament (CHL) thickness and obliteration of the subcoracoid fat triangle were shown in oblique sagittal T1W images (Figure 1). The study was conducted retrospectively, and the evaluation was undertaken based on the consensus of three radiologists with 30, 16, and 4 years of experience in radiology, who examined the images obtained from the Picture Archiving and Communication System of our hospital.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) package program v. 23.0 was used for the statistical analysis of the data. The chi-square and Fisher's exact tests were used for the comparison of categorical variables. The Shapiro-Wilk test was conducted to determine whether the parameters investigated

in this study showed a normal distribution. In the comparison of continuous measurements between the groups, the normality assumption was checked, and the independent Student's *t*-test was used for normally distributed parameters while the Mann-Whitney *U* test was used for the parameters that did not conform to a normal distribution. The statistical significance level was accepted as 0.05 in all tests. The areas under the receiver operating characteristic (ROC) curves (AUCs) were calculated by using the method that DeLong et al.⁶ found. For each reader, the highest Youden index ($J = \text{sensitivity} + \text{specificity} - 1$) was calculated to select the optimal threshold to discriminate between, and the corresponding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed. AUC was calculated according to the median values for inferior glenohumeral ligament (IGHL), superior glenohumeral ligament (SGHL), CHL, and RI thicknesses (Table 1).

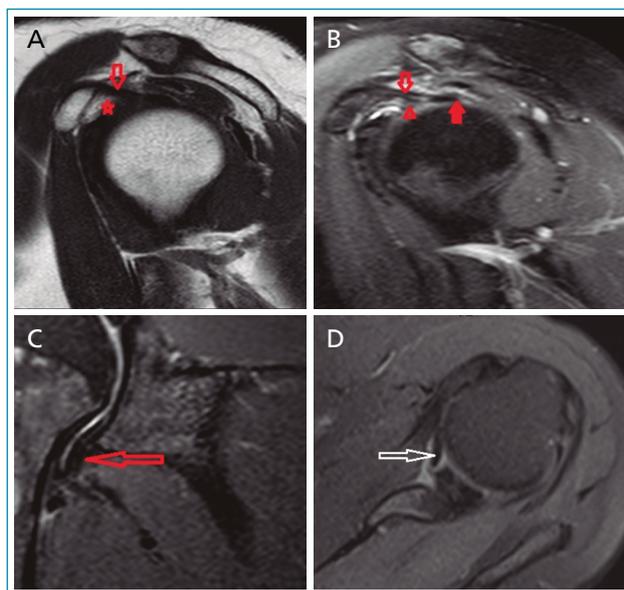


Figure 1. (A) Sagittal oblique magnetic resonance T1-weighted examination on the left shows slight thickening of the coracohumeral ligament (area marked with a hollow arrow) and mild obliteration in the subcoracoid fat tissue (marked with an asterisk). (B) Proton density-weighted sequence examination shows thickened coracohumeral ligament (area marked with a hollow arrow), fluid and soft-tissue increase in the rotator interval (area marked with an arrowhead), and signal increase at the biceps attachment point (area marked with a solid arrow). (C) In the coronal oblique plane right shoulder magnetic resonance imaging proton density-weighted sequence, thickened inferior glenohumeral ligament is seen (marked with arrow). (D) Proton density-weighted magnetic resonance imaging in the axial plane of the left shoulder showed significantly thickened superior glenohumeral ligament (marked with arrow).

RESULTS

The mean age of the AC group was 51.48 ± 12.41 years and that of the control group was 50.09 ± 11.68 years. There was no statistically significant difference between the patient and control groups in terms of age and gender (p-values 0.023 and 0.194, respectively).

There was no statistically significant difference between the two groups in terms of the obliteration of the subcoracoid fat triangle (p=0.568). Concerning the evaluation of signal increase at the attachment site of the biceps tendon, there was a statistically significant difference between the two groups (p<0.05) (Table 2).

Table 1. Cutoff values and statistical analysis of parameters evaluated in this study.

	IGHL thickness	RI soft-tissue thickness	SGHL thickness	CHL thickness
AUC	0.700	0.922	0.972	0.783
95%CI	0.621–0.771	0.868–0.959	0.932–0.992	0.709–0.845
Cutoff	≤4.0	≤3.60	≤2.0	≤4.6
Sensitive (%)	100	100	100	100
95%CI	59–100	73.5–100	47.8–100	29.2–100
Specificity	53.74	75.18	95.86	62.25
95%CI	45.3–62	67.2–82.1	91.2–98.5	54.0–70.0
PPV	9.3	25.5	45.5	5
NPV	100	100	100	100
p	0.001	<0.001	<0.001	0.001

IGHL: inferior glenohumeral ligament; RI: rotator interval; SGHL: superior glenohumeral ligament; CHL: coracohumeral ligament; AUC: area under the receiver operating characteristic curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

Table 2. Comparison of the mean values of the investigated parameters between the study groups.

		Control	Adhesive capsulitis	Total	p
		(n=116)	(n=193)		
		n (%)	n (%)	n (%)	
		Mean±SD	Mean±SD	Mean±SD	
Obliteration of the subcoracoid fat triangle	Absent	72 (62.1)	126 (65.3)	198 (64.1)	0.568
	Present	44 (37.9)	67 (34.7)	111 (35.9)	
Increased fluid and/or signal at the attachment site of the biceps tendon	Absent	58 (50.0)	42 (21.8)	100 (32.4)	0.001
	Present	58 (50.0)	151 (78.2)	209 (67.6)	
Axillary pouch thickness		3.93±0.72	12.17±2.09	9.08±4.34	0.027
		(3–6.1)	(6.1–21.2)	(3–21.2)	
Inferior glenohumeral ligament thickness		2.68±0.63	3.79±0.66	3.37±0.84	0.034
		(1.2–4.1)	(2.3–6.1)	(1.2–6.1)	
Rotator interval soft-tissue thickness		0.84±1.03	3.72±2.24	2.64±2.34	0.016
		(0–4.4)	(0–9.2)	(0–9.2)	
Coracohumeral ligament thickness		2.81±0.49	4.66±1.13	3.97±1.30	0.029
		(2.1–5.2)	(1–8.1)	(1–8.1)	
Superior glenohumeral ligament thickness					0.044
		0.041	0.042	0.043	

SD: standard deviation.

The mean IGHL thickness was 3.79 ± 0.66 (2.3–6.1) and 2.68 ± 0.63 (1.2–4.1) mm, respectively; the mean SGHL thickness was 2.58 ± 0.99 (2.8–6.7) and 1.33 ± 0.25 (0.8–2.1) mm, respectively; the mean CHL thickness was 4.66 ± 1.13 (1–8.1) and 2.81 ± 0.49 (2.1–5.2) mm, respectively; and the mean soft-tissue thickness in the RI was 3.72 ± 2.24 (0–9.2) and 0.84 ± 1.03 (0–4.4) mm, respectively, between the two groups. There was a statistically significant difference between the two groups in terms of all these parameters ($p < 0.05$) (Table 1).

IGHL thickness, RI soft-tissue thickness, SGHL thickness, CHL thickness, AUC values that are determined according to median values, corresponding sensitivity, specificity, PPV, and NPV are shown in Table 1.

DISCUSSION

Adhesive capsulitis progresses with thickening, contraction, and adhesion of the glenohumeral capsule and shoulder joint ligaments, followed by decreased capsular enlargement³⁻⁵. Gokalp et al.⁷ measured axillary pouch thickness (APT) using contrast-enhanced shoulder MRI and compared it between the AC and control groups, noticing the presence of a significant difference. However, Petchprapa et al.⁸ reported that while there was no difference in the axillary pouch width between the patients with AC and the control group, the capsule-synovium thickness in the axillary pouch was increased in the former. Sofka et al.⁹ showed that the APT ranged from 2 to 13 mm among these patients. In this study, using non-contrast shoulder MRI, the APT was measured as 12.17 ± 2.09 (6–21) mm for the AC group and 3.93 ± 0.72 (3–6) mm for the control group, and the difference between the two groups was statistically significant. However, it is noteworthy that in cases with suspected AC, the thickness of the axillary pouch increased compared with the controls. In this respect, considering the APT when evaluating shoulder MRI in patients with suspected AC will be useful for diagnosis.

In this study, the difference between the two groups in terms of signal changes at the biceps tendon attachment site was statistically significant ($p < 0.05$). These findings are consistent with those reported by previous studies. However, the presence of an increased signal at the attachment site of the biceps tendon can be seen in many synovitis and synovitis-like conditions, and it seems insufficient to support the diagnosis of AC when used alone.

Connell et al.¹⁰ did not provide numerical measurements, and they stated that the SGHL thickness increased and showed signal changes in patients diagnosed with

AC. Petchprapa et al.⁸ reported that the SGHL thickness increased in AC cases, although the amount of thickening was not quantitatively specified. In this study, SGHL thickness was evaluated in the diagnosis of AC, and the AUC value was calculated as 0.972 when a 2-mm cutoff was accepted according to the median value. The results we obtained from this study show that an SGHL thickness of 2 mm and above can help diagnose AC.

The measurement of IGHL thickness is another valuable method in the diagnosis of AC. Teixeira et al.¹¹ observed that signal enhancement in IGHL on T2 sequences had high sensitivity and specificity. Zappia et al.¹² showed an increase in IGHL signal with high sensitivity and specificity in T2W sequences in patients diagnosed with AC. Bang et al.¹³ measured the mean IGHL anterior band thickness as 4.52 ± 1.02 mm for the 54 AC patients and 3.47 ± 0.99 mm for the control group. When the information obtained from this study and the information obtained from similar studies are evaluated together, an IGHL value of 4 mm and above can be considered as a favorable criterion for the diagnosis of AC.

Emig et al.¹⁴ suggested that there was no statistically significant difference between the AC patients and the control group in terms of the CHL thickness measured on conventional shoulder MRI. In a more recent conventional shoulder MRI study, Li et al.¹⁵ measured the CHL thickness as 3.99 ± 1.68 mm for the AC group and 3.08 ± 1.32 mm for the control group. In this study, the CHL thickness was measured as 4.66 ± 1.13 (1–8) mm, and the difference between the two groups was statistically significant ($p < 0.05$). According to the data obtained, CHL being over 4.6 mm can be considered as a finding supporting the diagnosis of AC.

As far as we know, although there are current studies examining signal changes in the RI, no quantitative studies are investigating soft-tissue thickness in the RI^{16,17}. In this study, the soft-tissue thickness of the RI was found to be 3.72 ± 2.24 (0–9) mm in the patients with AC, and the difference between the two groups being statistically significant ($p < 0.05$). This result is a statistically significant value and shows that the soft-tissue thickness in the RI must be evaluated in shoulder MR examination for the diagnosis of AC. There is a need for an increase in large series of studies on this subject.

The limitations of the study include the retrospective evaluation of the shoulder MRI examinations and the study being conducted in a single center. Another limitation can be considered as the inability to histopathologically confirm the presence of inflammation due to the retrospective study design.

CONCLUSIONS

Clinicians frequently refer to shoulder MRI to confirm the diagnosis of AC and exclude other possible diagnoses. For this reason, there is a need to clarify the diagnostic criteria for AC. According to the data obtained in this study, the thickness of SGHL over 2 mm or the soft-tissue thickness in the RI of 3.6 and above can be used to support the diagnosis of AC diagnosis. In addition, CHL thickness of 4.6 mm and above and the thickness of IGHL over 4 mm can be counted among the criteria that support the diagnosis of AC.

AUTHORS' CONTRIBUTIONS

HA: Conceptualization, Methodology, Validation, Software, Writing –original draft. **ES:** Resources, Investigation, Formal analysis, Validation, Writing –original draft. **OD:** Project administration, Supervision, Visualization, Investigation. **GS:** Data curation, Methodology, Software, Supervision, Writing –original draft. **BG:** Methodology, Resources, Supervision, Visualization, Writing –review & editing.

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Shear-wave elastography evaluation of adrenal glands in healthy newborns: a preliminary study

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SUMMARY

OBJECTIVES: Establishing standard shear-wave elastography (SWE) values for healthy newborns can help distinguish normal conditions of the adrenal gland (AG) from pathological conditions. We aimed to establish a reference data set for AG stiffness values using SWE in healthy newborns.

METHODS: The quantitative stiffness of the AG was measured in the coronal plane in kilopascal (kPa). The quantitative descriptive statistics were presented as mean with standard deviation and median with range. The relationship between the quantitative variables was calculated using "Spearman's rank correlation coefficient test." The intraclass correlation coefficient (ICC) test was used to analyze intraobserver reliability. A p-value <0.05 was considered statistically significant.

RESULTS: A total of 120 AGs of 60 healthy newborns (30 females and 30 males) was examined. The mean stiffness values of the right AG for the first and second visits were 7.51 ± 2.45 and 7.54 ± 2.49 kPa, respectively, and those of the left AG for the first and second visits were 7.60 ± 2.03 and 7.42 ± 1.97 kPa, respectively. There was no statistically significant difference between the mean values of adrenal stiffness and the length and width of AG and weight, height, and age ($p > 0.05$). The ICC values for mean stiffness values of each AG were >0.80 – 0.90 , indicating good intraobserver agreement.

CONCLUSIONS: This study is the first SWE study to evaluate the AG in healthy newborns. Our study's data can be used as a reference for future research.

KEYWORDS: Adrenal gland. Newborn. Reference data. Sonoelastography. Stiffness.

INTRODUCTION

Adrenal glands (AGs) are organs with an endocrine function that are located retroperitoneally in the abdomen. Unlike adults, the AG is easier to examine in newborns due to its large size and low amount of retroperitoneal fat. With the introduction of ultrasound (US), several studies have researched the sonographic appearance and measurement of the typical AG in neonates¹⁻⁴. The AG size is a harbinger of preterm labor in fetal life and a predictor of postpartum glucocorticoid-responsive

circulatory collapse in neonates⁵⁻⁷. The AGs may show involvement secondary to diseases such as bleeding, hyperplasia, and tumor in the newborn, as well as due to storage diseases such as Wolman's disease^{8,9}.

The stiffness of tissues can be measured quantitatively using the shear-wave elastography (SWE) technique. With the stiffness changes in the tissues, the SWE can help diagnose diseases; however, to compare with pathological tissues, normal values must first be determined. There exists no

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research on the normal stiffness of the AG in the literature. In this study, we aimed to determine normal values of adrenal stiffness in term newborns and their relationship with age, height, and weight.

METHODS

Study population and design

A total of 60 healthy newborns were included in this prospective study conducted between October and December 2020. An SWE for the right AG (RAG) and left AG (LAG) was performed on newborns between 0 and 28 days who came for routine control US. Babies with adrenal thickening, difficult labor history, and abnormal metabolic laboratory parameters were excluded from this study. In addition, crying or very mobile babies could not be included in this study, since only respiratory movements were accepted during the SWE examination. Premature babies were included in this study, considering their corrected ages. All subjects were noted for age in days, gender, height, and weight. This study was approved by the Local Ethics Committee and complied with the Helsinki Declaration (2019-1901). Informed consent was obtained from parents of newborns before the SWE examinations.

US and SWE imaging

All US and SWE examinations were performed via a digital US device (LOGIQ P9, GE Healthcare, Chicago, IL, USA) and a 9-MHz frequency linear transducer. This study involved a single observer (IA with 11 years of experience in abdominal radiology). The examinations were first started using the grayscale US. Both AGs were evaluated for mass, contour irregularity, hyperplasia, and echogenicity abnormality. The length of the AG was defined as the maximum cephalocaudal dimension. The width was defined as the maximum thickness of one of the limbs. Both AGs were observed as triangular structures near the superior and medial sides of the kidneys. The grayscale US and SWE examinations were performed with the same probe in the same session. In the meantime, care was taken that the baby is as immobile as possible. Images were retaken until they were acquired without artifacts. The evaluation of the stiffness of both AGs was also performed in the coronal plane (Figure 1). A 2–4 mm region of interest was placed in both AGs with minimal pressure on the skin to avoid external compression. The stiffness measurements were obtained in kilopascal (kPa). The examiner assessed the adrenal stiffness values five times and obtained the median of these consecutive measurements on the same SWE images in kPa. All subjects were examined consecutively

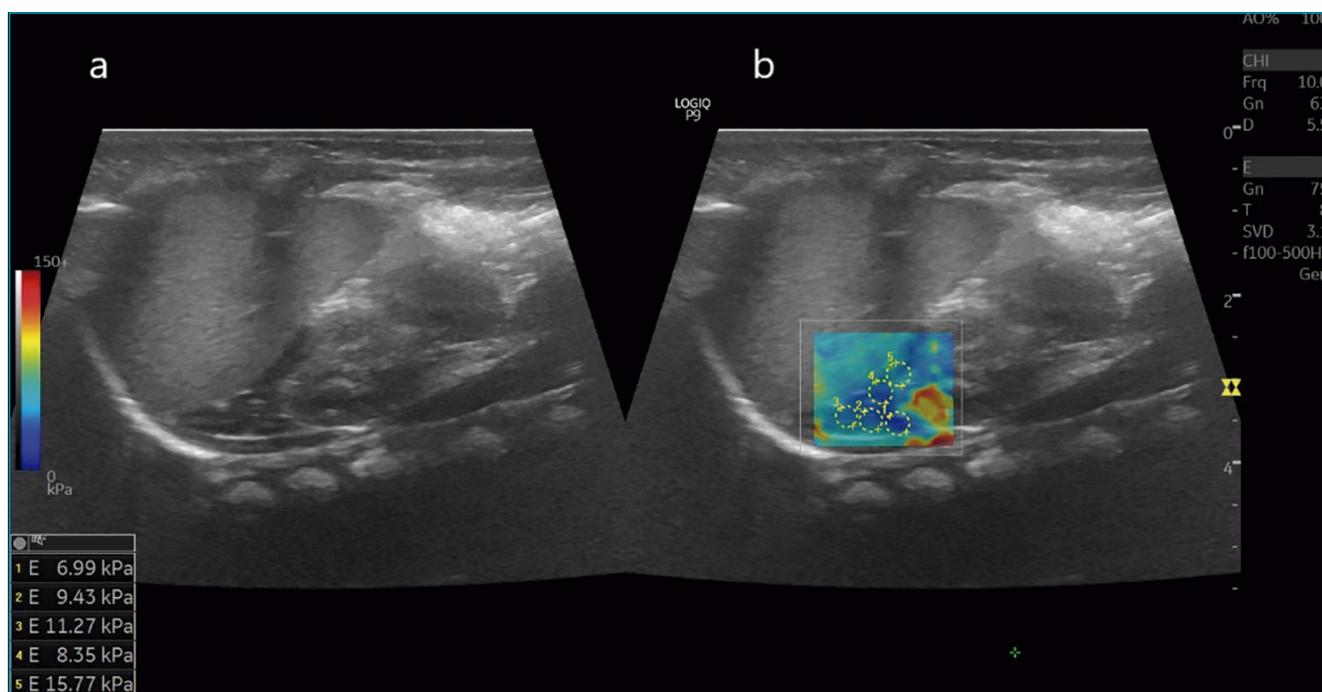


Figure 1. A 14-day-old male healthy newborn. Normal right adrenal gland was shown in B-mode ultrasound (a) and standard shear-wave elastography (b) imaging, superomedial to the kidney and inferior to the liver. (a) B-mode ultrasound: triangular shape right adrenal gland is depicted, and (b) standard shear-wave elastography imaging: a square region of interest with a color elastogram map is depicted along with the smaller circular region of interest with elastogram measurements.

by the examiner during the same visit. For intraobserver reliability, two measurements were made by the examiner at 1-h intervals in two separate settings.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences version 22 package program. The quantitative descriptive statistics were presented as mean with standard deviation (SD) and median with range. The distribution of data was analyzed using the Kolmogorov-Smirnov test. A non-parametric test (Mann-Whitney U test) was used to evaluate the relationship between the mean stiffness, length, and width measurements of the AG and gender. In addition, the Mann-Whitney U test was used to assess the differences between the mean elasticity of the RAG and LAG.

The relationship between the mean stiffness and length and width measurements versus age, height, and weight was calculated using the “Spearman’s rank correlation coefficient test.” The intraclass correlation coefficient (ICC) test was used to analyze intraobserver reliability with their 95% confidence intervals (CI) of repeated measurements in the absolute-agreement, two-way mixed-effect model. The ICC was defined as follows: values less than 0.5 indicate poor reliability, values between 0.5 and 0.75 indicate moderate reliability, values between 0.75 and 0.9 indicate good reliability, and values higher than 0.90 indicate excellent reliability. A p-value <0.05 was considered statistically significant.

RESULTS

In this study, 70 participants (140 AGs) were initially recruited, and 10 patients were eventually excluded (4 participants with adrenal thickening, 4 participants had insufficient SWE evaluation due to inadaptability, and 2 participants with a difficult labor history). As a result, a total of 60 subjects (120 AGs; 30 males and 30 females; age range: 1–28 days) were included in this study. Thus, the success rate of performing SWE was 85.7% (60/70). The demographic data of the subjects are presented in Table 1.

There was no statistically significant relationship between the mean stiffness, length, and width measurements of both AG and gender ($p>0.05$). We also measured the mean stiffness and length and width of AG on the right and left sides of each subject and found no statistically significant differences ($p=0.544$). There was no correlation between length and width measurements and stiffness values of AG ($p>0.05$). The mean stiffness values of the RAG for the first and second visits were 7.51 ± 2.45 and 7.54 ± 2.49 kPa, respectively, and those of the LAG for the first and second visits were 7.60 ± 2.03 and 7.42 ± 1.97 kPa, respectively. The mean stiffness of AG and the

length and width of each AG during the two visits are given as mean and SD in Table 2.

The ICC for intraobserver reliability of mean stiffness values of RAG and LAG was 0.898 (0.838–0.930) and 0.897 (0.863–0.918), respectively, at a 95% CI. The ICC values for mean stiffness values of each AG were >0.80–0.90, indicating good intraobserver agreement (Table 3).

Table 1. Descriptive statistics for age, height, weight, and gender distributions of all subjects.

Clinical characteristic	Total (n=60)
Age (in days)	15.03±9.04 (1–28)
Height (cm)	51.5±5.11 (49–56)
Weight (g)	3509.63±482.65 (2480–4450)
Gender (M/F)	30/30

Data are expressed as n (number) or the mean± standard deviation (range). F: female, M: male.

Table 2. Measurements of adrenal gland’s stiffness and length and width.

Parameters	First visit	Second visit
RAG		
Mean±SD (range)	7.51±2.45 kPa (4.44–12.71)	7.54±2.49 kPa (4.52–12.82)
Median	6.76 kPa	6.84 kPa
RAGL and RAGW	17.48±3.58 mm and 2.98±0.52	17.16±3.52 mm and 2.96±0.50
LAG		
Mean±SD (range)	7.60±2.03 kPa (4.49–11.72)	7.42±1.97 kPa (4.34–10.94)
Median	7.19 kPa	7.08 kPa
LAGL and LAGW	17.13±2.83 mm and 3.03±0.47	17.18±2.85 mm and 3.05±0.48

RAG: right adrenal gland; RAGL: right adrenal gland length, RAGW: right adrenal gland width, LAG: left adrenal gland; LAGL: left adrenal gland length, LAGW: left adrenal gland width.

Table 3. Intraclass correlation coefficient for intraobserver measurement of adrenal gland mean stiffness values.

	ICC value	95%CI	p-value
RAG	0.898	(0.838–0.930)	<0.001*
LAG	0.897	(0.863–0.918)	<0.001*

RAG: right adrenal gland, LAG: left adrenal gland, ICC: intraclass correlation coefficient; CI: confidence interval. *p-value significant at 0.05.

There was no statistically significant difference between the mean values of adrenal stiffness and the length and width of AG and weight, height, and age ($p>0.05$). In addition, there was no statistically significant correlation between mean length and width of AG and mean stiffness values of AG ($p>0.05$).

DISCUSSION

The US is the ideal modality for the initial evaluation and follow-up of newborns because it is accessible, rapid, safe, noninvasive, and free of ionizing radiation. Computed tomography and magnetic resonance imaging are useful for the evaluation of the AGs. However, these imaging modalities do not provide information about quantitative mechanical properties, such as tissue stiffness.

Elastography is a noninvasive technique used to determine the stiffness or elasticity of the tissue in a specific area. Strain elastography and SWE are the two types of elastography methods¹⁰. The SWE provides real-time quantitative information on tissue elasticity in kilopascals (kPa). Operator independence, repeatability, and quantitative analysis are the advantages of SWE. As a result, SWE outperforms strain elastography in terms of repeatability, objectivity, and quantification¹¹.

Normative measurements of adrenal elasticity in newborns may facilitate the early detection of the adrenal abnormalities mentioned above. In addition, it may be beneficial to use standardized stiffness values to distinguish ectopic or accessory adrenal tissue from other tissues¹²⁻¹⁵. We believe that the results of this preliminary study will facilitate the characterization of adrenal tissue, especially in cases of aberrant localization.

In this study, 120 AGs from 60 healthy newborns were assessed with SWE. The preliminary results show the elasticity of the adrenal tissue and give information about the average stiffness of the AGs. The relationships between elasticity and height, weight, and gender were also studied.

In the literature, there are grayscale US studies of the AGs in newborns rather than SWE studies. In their study of 156 healthy newborns, Karagüzel et al.¹⁶ determined the length of the RAG to be 17.6 ± 3.6 mm and its width to be 2.1 ± 0.5 mm, and the length of the LAG to be 16.8 ± 3.1 mm and its width to be 2.1 ± 0.5 mm¹⁶. In a study of 39 diabetic pregnant women conducted by Garcia-Flores et al.¹⁷, a statistically significant correlation was observed between birth weight and adrenal length. In a study conducted by Karsli et al.¹ on 99 infants, AG volume was found to be significantly related to prenatal factors and postnatal outcomes.

SWE values for the AGs in normal newborns are needed as reference data, and to the best of our knowledge, this study is the first report on this topic. Fernandez et al.¹⁸ conducted an acoustic radiation force impulse study of adrenal elastography in 30 healthy dogs and found no significant relationship between

RAG and LAG shear velocity values. In an adrenal SWE study, Slapa et al. measured the elasticity values of 16 nonmalignant adrenal lesions in 13 patients. According to the results, no statistically significant difference was found between the stiffness of adenomas and hyperplastic nodules, whereas nonmalignant neoplastic myelolipomas were found to be harder than adenomas¹⁹. In another study, Slapa et al.² demonstrated the importance of US and SWE in the evaluation of the AGs. In this study, the mean stiffness values of the RAG for the first and second visits were 7.51 ± 2.45 and 7.54 ± 2.49 kPa, respectively. For LAG for the first and second visits, the mean stiffness values were 7.60 ± 2.03 and 7.42 ± 1.97 kPa, respectively. There were no significant differences between the absolute values of the intraobserver measurements. This significant intraobserver agreement suggests that SWE is a repeatable method for assessing the elasticity of AG and that the stiffness values from normal AG can be used as reference data to distinguish between different pathological conditions.

This study has several limitations. First, we did not assess interobserver reproducibility because all repeated measurements were performed by a single observer. Second, it is a single-center study. Third, this study had a relatively small number of subjects. Fourth, although SWE measurements were taken using the breath-holding technique in adult studies, we were only able to measure the resting breathing position for newborns. Finally, repeated follow-up measurements should be performed with the same machine to avoid reference value discrepancies between different elastography machines.

CONCLUSIONS

There are some SWE studies on intra-abdominal organs in the newborn age group in the literature, yet there are no SWE studies on the AG. This study is the first SWE study to assess AG in healthy newborns. We described the SWE method and reported normal values for AG in healthy newborns. Establishing standard SWE values for healthy newborns can help distinguish normal conditions of AG from pathological conditions. The data from our study can be used as a reference for future research. Multicenter and prospective studies with a larger number of subjects are needed to establish the reference values and repeatability of this imaging technique.

ETHICAL APPROVAL

This study was approved by the Local Ethics Committee (2019-1901). 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.

INFORMED CONSENT

Verbal and written informed consent was obtained from the parents of patient.

AUTHORS' CONTRIBUTIONS

EG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration. **IA:** Data curation, Investigation, Methodology, Project administration.

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Lung ultrasound: an opportunity to increase the accuracy of the physical examination by the nephrologist

Marcus Gomes Bastos¹ 

INTRODUCTION

Point-of-care ultrasound (POCUS) performed at the bedside by the attending physician is primarily meant to provide answers to focused questions to narrow the differential diagnosis and guide clinical therapy practically in all medical specialties, ranging from the emergency department to ambulatory clinics^{1,2}. One of the main applications of POCUS is lung ultrasound (LUS). Initially used by intensive care and emergency physicians, LUS was soon embraced by cardiologists. However, among Brazilian nephrologists who already use POCUS, only 31% use LUS routinely³.

The main indications of LUS are for the diagnosis of pneumothorax, acute pulmonary edema, pleural effusion, infectious pathologies (bacterial and viral pneumonia), acute respiratory distress syndrome, asthma, and chronic obstructive pulmonary disease. In contrast, the main limitations of using LUS are subcutaneous emphysema (the sound wave cannot pass through subcutaneous air), large pneumothorax, large surgical dressings, and body fat in morbidly obese individuals^{4,5}. With the recent disponibility at low cost and highly portable handheld ultrasound, the most important barrier to implementing LUS into routine clinical practice is still the scarcity of well-trained faculty able to conduct training and apply LUS at the bedside.

In this minireview article, we discussed some technical aspects of LUS and how the lung artifacts generated can be used by nephrologists to diagnose pneumothorax, pleural effusion, and pulmonary congestion in patients with renal diseases.

TECHNICAL ASPECTS OF LUS

Lichtenstein et al.⁴ was the first to demonstrate that interpretation of artifacts makes it possible to diagnose pleuro-pulmonary

pathologies with LUS. However, it is necessary to warn that LUS is not anatomical; it only identifies pathologies that “touch” the pleura and that the lung parenchyma, when viewed at LUS, is always pathological. In contrast, we can infer that the lung is normal if we observe lung sliding, A-Lines, and the curtain sign at the lung bases⁵.

Figure 1 (left and right) shows the structures that make up the chest wall anatomically and their corresponding US images. The top of the US viewing screen is always where the transducer touches the patient’s skin. The transducer should be held like a pen, with its orientation marker pointing toward the head. When using lung or abdominal exam presetting, the screen marker will appear on the viewing screen’s upper left side. As the transducer’s orientation marker and the viewing screen marker point in the same direction, the screen’s left side will be cephalad and the right side will be caudad. Comparing both figures and looking from top to bottom, we identify the skin and subcutaneous tissue, then the pectoral and intercostal muscles, the ribs (with posterior acoustic shadow), and the pleural line (parietal and visceral pleura). Below the pleural line and between the ribs, anatomically is the lung, but in the

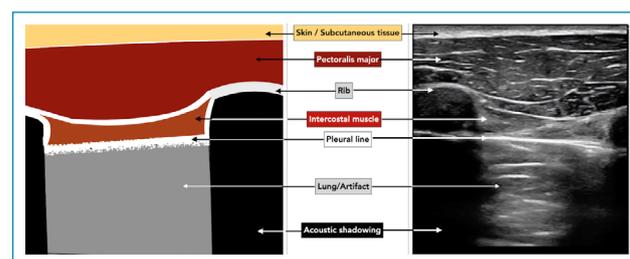


Figure 1. Drawing (left) and ultrasound image (right) showing the structures that make up the chest wall.

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LUS, the corresponding images are artifacts produced by the sound waves' scattering.

To obtain the images in the LUS, we can use high- and low-frequency transducers (or probes). However, depending on the pathology assessed, we might select a determined transducer. For instance, if the suspicion is pneumothorax, a high-frequency transducer (with higher image resolution) allows a more detailed analysis of the pleura line. In contrast, the convex and phased-array probes (longer wavelengths that penetrate deep structures) are most often used to identify pleural effusion and pulmonary congestion^{5,6}.

Understanding the equipment controls is essential to optimize and interpret the images obtained with the LUS. The most significant adjustments are:

1. Gain control increases brightness by increasing the amplitude of returning US waves;
2. Time gain compensation controls are intended to compensate for the attenuation of sound waves that occur with depth;
3. Depth control alters the scanning depth displayed on the screen; and
4. The focal point indicates where maximum resolution occurs. In contrast, tissue harmonic imaging, a tool for improving contrast resolution and lateral resolution, and the multi-beam, features that enhance the image's quality should be disabled to facilitate the recognition of the pulmonary artifacts, of paramount importance in LUS⁵.

In practical terms, a simple, accurate, and efficient lung exam protocol divides the chest into three zones per hemithorax:

1. Anterior, superior, and inferior;
2. Lateral, superior, and inferior; and
3. Posterior, superior, and inferior.

The choice of a specific region in the evaluation with LUS depends on the pretest probability. For instance, if the diagnostic suspicion is pneumothorax, the scanning should begin in the upper anterior region in a patient in the supine position. In contrast, if the diagnostic suspicion is pleural effusion, the examination should start in the lower lateral region⁶.

LUNG ARTIFACTS

As mentioned, LUS is based on the interpretation of artifacts^{4,6}; the main ones are:

1. Lung or pleural sliding;
2. A-lines;
3. B-lines;
4. The mirror image of the liver or spleen;
5. The thoracic spine sign; and
6. The curtain sign^{5,6}.

To obtain an adequate acoustic window for LUS, the transducer should be positioned as perpendicular as possible to the pleura and the images are obtained in the sagittal, oblique, or parallel to two ribs⁷.

The lung or pleural sliding artifact is a normal finding and appears as a shimmering or sliding of the visceral pleura against the parietal pleura during the respiratory cycle. It is identified as a hyperechoic (white) line located a few millimeters below the ribs and can be evaluated using linear, convex, and phased-array transducers (Figure 1). A health pleura should be uniformly thin (<0.3 mm) without irregularities^{5,7}.

In case of difficulty to confirm lung sliding by B-mode, an alternative is to use the M-mode, which depicts the tissues' movement along a single scan line over time. The chest wall is less mobile in normal conditions, appearing as a series of horizontal lines, whereas the lung parenchyma is more mobile; it moves back and forth, giving it a grainy appearance by M-mode.

The A-lines are artifacts that originate from the reverberation of sound waves between two highly reflective surfaces, i.e., the US transducer and the pleural line^{5,6}. They appear as horizontal lines deep to the pleural line. A-lines are identified as repetitive hyperechoic horizontal lines equidistant from the pleural line and each other (Figure 2A). The A-lines mean air-filled lungs⁵⁻⁷.

The B-lines have a complex and incompletely understood pathophysiology. The normal interlobular and intralobular septa are below the resolution of standard US frequencies, and US waves cannot propagate in air-filled lungs. As soon as the septa are widened or distended with interstitial fluid, fibrous tissue, collagen, or cellular deposition, US waves can propagate into the lung and are seen as B-lines⁷. The B-lines are vertical hyperechoic lines, which emanate from the pleural line, move with the breathing cycle, erase the A-lines, and extend to the viewing screen's lower limit (Figure 2B). The finding of three or more B-lines between two ribs indicates interstitial pulmonary syndrome, such as pulmonary congestion^{8,9}.

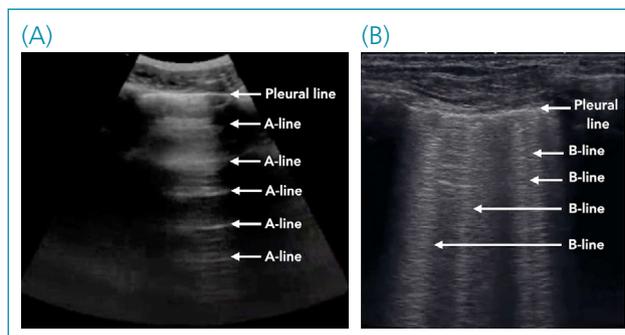


Figure 2. (A) Lung ultrasound showing the pleural line and the artifact A-line and (B) Lung ultrasound displaying the artifact B-line.

The comet tail is a short reverberation artifact, hyperechoic, move with lung sliding, whose appearance is obviously different from B-lines and can be seen in normal lungs^{10,11}.

The mirror image artifact of the liver or spleen corresponds to these organs' supposed presence above the diaphragm. US images originate from the intensity and the time when the sound beams take back to the transducer. When the sound waves encounter a highly reflective structure like the diaphragm, the sound beams suffer multiple reflections on the way back to the transducer⁵. However, the US machine's processor interprets that the sound waves were obtained along a straight line. Two images, i.e., the actual image below the diaphragm (liver or spleen) and the mirror image above, are generated, resulting in a mirror artifact. In Figure 3, the image obtained in the upper right quadrant region, at the middle axillary line, one

can identify the liver, the diaphragm, and, above them, the mirror image of the liver⁷.

The vertebral bodies correspond to a wavy hyperechoic (white) line posterior to the liver (or spleen) and the kidney (Figure 3). Under normal conditions, the vertebral bodies are visualized only up to the diaphragm limit. Above the diaphragm, the air-filled lungs block the passage of sound, preventing vertebral bodies' visualization^{5,7,12}. The thoracic spine sign is the spine's visualization above the diaphragm level, most commonly due to pleural effusion; however, it can also occur with hemothorax and lung consolidation.

The curtain sign is seen in healthy and aerated lungs. An air-filled lung is like a "curtain" that sweeps down and over the other organs, momentarily obscuring them during the respiratory cycle. Thus, the diaphragm, liver, or spleen disappears during inspiration and reappears during expiration.

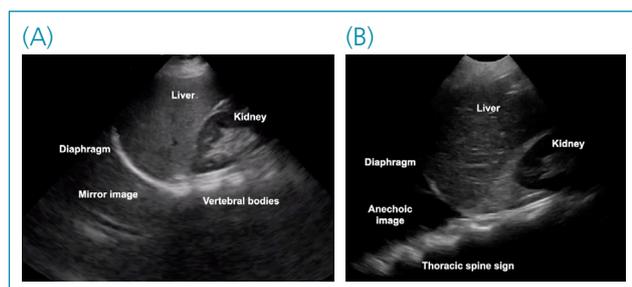


Figure 3. The ultrasound image obtained in the right upper quadrant. (A) Normal findings and (B) Pleural effusion is characterized by an anechoic image above the diaphragm and thoracic spine sign.

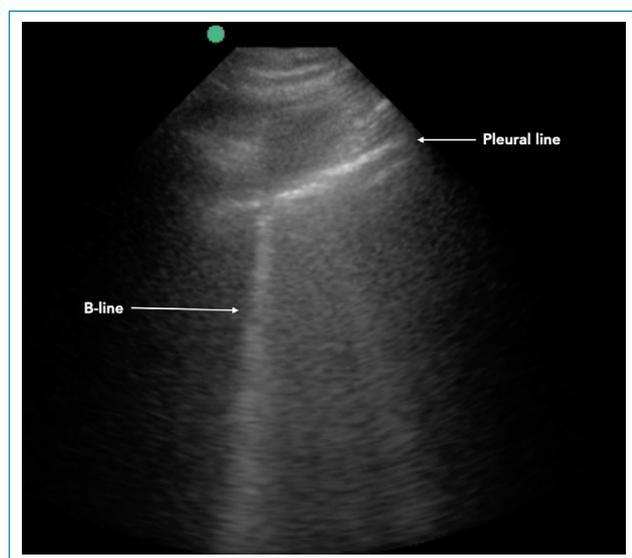


Figure 4. Lung ultrasound from a patient with COVID-19 showing an irregular and fragmented pleural line from which a B-line originates.

MOST IMPORTANT CLINICAL USE OF LUNG ULTRASOUND IN NEPHROLOGY

Diagnosis of pneumothorax

Central venous access is a routine procedure in nephrology but often proves to be challenging. Although US was first used to facilitate vascular access almost 40 years ago¹³, most Brazilian nephrologists still perform the procedure based on anatomical landmarks³. This procedure would be much easier and safer if guided by ultrasonography, which allows the visualization of the internal jugular vein, the carotid artery, and the needle in the same path, thus minimizing the possibility of puncture accidents. Cannulation of the internal jugular vein with real-time US guidance is now standard practice and is highly recommended by many societies and supported by evidence¹²⁻¹⁶. Thus, US-guided central venous access makes the procedure safer for the patient and the nephrologist.

The pneumothorax assessment is usually done in the patient in the supine position and scanning the anterior and upper chest wall. The three transducers commonly used to obtain images in internal medicine can be used. The high-frequency linear probe with a frequency between 5 and 13 MHz offers excellent image resolution but a maximum useful imaging depth of approximately 6 cm. Convex probe (frequency between 2 and 5 MHz) and phased-array probe (frequency between 1 and 5 MHz) provide a deeper penetration to depths around 22 cm but at the expense of lower resolution. Several different sonographic signs can be used to rule in or rule out pneumothorax, specifically, lung sliding, B-lines, lung point, and the findings on M-Mode⁵⁻⁷.

As previously mentioned, when the visceral and parietal pleura are opposed and normal respiration occurs, a shimmering of the visceral on parietal pleura will be observed, essentially ruling out any air between both pleurae with nearly 100% sensitivity^{17,18}. The other indications that the visceral and parietal pleura are in touch is observing the artifact B-lines, which emanate from the pleural line. The visualization of the lung sliding and/or the B-lines rules out pneumothorax diagnosis in the examined area in most circumstances. However, when air interposes between the two pleurae, as in the pneumothorax, it is only possible to see the parietal pleura; therefore, the lung sliding and/or B-lines are no more seen. The absence of lung sliding may indicate pneumothorax in an appropriate clinical context but is also seen in other clinical conditions, such as shallow and rapid breathing, apnea, pneumonia, atelectasis, pleural adhesions, previous pleurodesis, and selective orotracheal intubation.

Alternatively, pneumothorax can be assessed using the M-mode^{6,7}. Typically, the near field, which is superficial to the pleural line, is not moving and appears in straight parallel lines. The far-field, deep to the pleural line, is shimmering back and forth and appears grainy, known as the seashore sign. In contrast, when air interposes between the visceral and parietal pleurae, the last structure seen is the visceral pleura, which will appear as a fixed, white, hyperechoic line. Thus, the chest wall still appears as straight parallel lines on M-mode, but since no lung sliding is visualized, the area deep to the pleural line also appears as straight parallel lines; this is known as the barcode sign.

However, the findings at LUS that rule in the diagnosis of pneumothorax is the lung point sign^{5,7,19}. The lung point marks the location on the chest wall where the collapsed lung meets the parietal pleura, seen as inspiratory presence and expiratory absence of lung sliding in a determined point of examination. On M-mode, seashore and barcode signs are seen, which vary with the respiratory cycle. The lung point identification is made by moving the transducer around the chest wall, from areas of lung sliding to areas without lung sliding, until both are seen in the same location. The lung point sign is highly specific²⁰ but presents relatively low sensitivity in the diagnosis of pneumothorax²¹.

In a systematic review and meta-analysis (1,048 participants) to compare the diagnostic accuracy of US with that of chest radiography in patients with suspected pneumothorax secondary to trauma (767 participants) and iatrogeny (281 participants), it was found that pooled estimates of sensitivity were 90.9% (95%CI 86.5–93.9%) for the LUS and 50.2% (95%CI 43.5–57.0%) for chest radiography. Pooled estimates of specificity were 98.2% (95%CI 97.0–99.0%) for LUS and 99.4% (95%CI 98.3–99.8%) for chest radiography^{21,22}.

Diagnosis of pleural effusion

Another use of LUS is in diagnosing pleural effusion^{5-7,20}. Pleural effusion may be classified into a transudate or an exudate. The majority of the pleural effusion seen in renal patients is transudate secondary to conditions that cause an increase in the pulmonary capillary hydrostatic pressure or a decrease in the oncotic capillary pressure (e.g., nephrotic syndrome and end-stage renal disease)^{23,24}. Less frequently, pleural effusion can be due to the retroperitoneal leakage of urine via the diaphragmatic lymphatics (urinothorax)²⁵ or originate from the movement of dialysate from the peritoneal to the pleural cavity across the diaphragm in a patient undergoing peritoneal dialysis²⁶.

Pleural fluid accumulates in the posterolateral costophrenic recesses, the most dependent portions of the thorax in upright patients. Thus, in ambulatory patients, the US examination is usually performed with the patient in an upright position. In contrast, in hospitalized or critically ill patients, the examination is in a supine or semi-recumbent position. The transducers used are of low frequency and longer wavelengths (convex or phased-array) that penetrate deep structures. The transducer is positioned on the chest with the marker point cephalad; consequently, the patient's head is always toward the left of the viewing screen.

The three most critical sonographic findings in pleural effusion diagnosis are the presence of the thoracic spine sign, the absence of the mirror artifact, and the absence of the curtain sign^{5,7}. Unlike a normal air-filled lung that blocks any sound waves from passing, in pleural effusions, sound waves can pass through the pleural fluid, allowing the spine's visualization also above the diaphragm (the thoracic spine sign). Additionally, the mirror artifact is substituted by an anechoic image of fluid-filled material above the diaphragm (Figure 3). Finally, the fluid-filled pleura does not allow the aerated lung to descend into the scanning field at the diaphragm level during inspiration, precluding the visualization of the curtain sign^{5,7,27}.

In a systematic review with meta-analysis on the accuracy of sonography for detecting pleural effusion using computed tomography or thoracic drainage as a reference, LUS showed consistently high average sensitivity (93%), specificity (96%), and accuracy for detecting pleural effusion²⁸. Besides, it allows the identification of pleural effusion as less as 20 mL²⁹.

Diagnosis of pulmonary congestion

LUS is a simple, noninvasive, and semiquantitative tool to assess interstitial lung syndrome. B-lines are a surrogate for the alveolar–interstitial syndrome, with pulmonary congestion being the most frequent pulmonary complication found in patients with kidney disease⁵⁻⁷. Volume overload in hemodialysis patients is an independent risk factor for death from

cardiovascular events³⁰. Notably, the number of B-lines has an excellent correlation with the severity of fluid accumulation in the lung. In hypervolemic patients who present a low glomerular filtration rate, the number of B-lines increases as pulmonary extravascular water accumulates³¹. Eventually, they converge into vertical sheets (white lung) seen in both lungs. Another interesting finding is that B-lines are highly dynamic, decreasing in number with fluid removal during a hemodialysis session³². They are also more sensitive than auscultation of pulmonary crackles in the diagnosis of asymptomatic pulmonary congestion, in addition to being a strong and independent predictor for fatal and nonfatal cardiac events and mortality from all causes³³.

The scanning technique uses a low-frequency convex or phased-array transducer to scan the thoracic cavity to get a good sense of interstitial lung syndrome distribution^{6,7}.

Various protocols have been used to assess lung congestion by B-lines. A 28-zone protocol for both hemithorax is used mainly for research purposes^{32,33}. A more practical approach is the four-zone per hemithorax scanning method in the semi-quantitative evaluation of interstitial lung syndrome. However, for diagnosis, a two-zone protocol is sufficient. The presence of interstitial syndrome due to extravascular lung water is defined as three or more B-lines between two rib spaces seen in two or more positive interspaces bilaterally^{5,7}. This approach presents a high sensitivity of 85.7% and specificity of 97.7% for alveolar-interstitial syndrome compared with chest radiography^{6,34,35}.

B-lines show high sensitivity as a manifestation of pulmonary congestion but low specificity; they are also seen in other

interstitial lung syndromes, such as pulmonary fibrosis, infection (pneumonia and COVID-19), atelectasis, pulmonary contusion, pulmonary infarction, or neoplasia^{5-7,36}. At present, in the context of the COVID-19 pandemic, B-lines characteristics may help distinguish COVID-19 pneumonia from pulmonary congestion in dyspneic patients. In COVID-19, the B-lines originate from a pleural line often irregular, fragmented, and are patchy, non-gravity related in distribution, more often coalescent, and with defined spared lung areas (Figure 4). In contrast, in cardiogenic pulmonary edema, the B-lines originate from a pleural line usually thin, regular, and homogeneous and are gravity-related distributed bilaterally, more frequently separated or coalescent in more severe cases, and with no pulmonary spared areas (Figure 2B)³⁶.

CONCLUSIONS

LUS allows the nephrologist to evaluate pulmonary pathologies that can occur in different nephrological scenarios and reduce other imaging methods (X-ray and computed tomography) that use ionizing radiation. The portability, broad availability, and improved technology of US devices and their practical utility as diagnostic, monitoring, and procedural guidance tools allow to carry out LUS in different practice environments such as nephrological offices, dialysis rooms, and intensive care units. Proficiency in LUS increases the physical examination accuracy in diagnosing pneumothorax, pleural effusion, and pulmonary congestion at the bedside and enables better nephrological practice.

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Progress and application on severe combined immunodeficiency mouse model for rheumatoid arthritis: a literature review

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disease¹ that can affect many tissues and organs, such as the heart and kidneys. Its pathogenesis is not completely stated. Previous studies^{2,3} have shown that it may be related to immune cells, autoantigens, and cytokines, and it is related to the intestinal flora. Some studies have reported that it may be related to genetic polymorphisms^{4,5}. At present, the number of cases is increasing year by year, and the number of female patients is significantly higher than that of male patients⁶. If it is not treated or intervened in time, the disease has the risk of disability and teratogenesis, and in severe cases, it can endanger the life of the patient. To better understand the disease, it is particularly important to build good and easy-to-operate animal model for experimental. Pathology, immunology, and ethical regulations as well as technical limitations put forward higher requirements for animal models. Animals, such as mice, are good subjects for human disease models that summarize human symptoms and reactions. In addition, the genetic background of mice can be artificially altered to suit different situations⁷. Severe combined immunodeficiency (SCID) mice can provide an organizational environment that removes the effects of the immune system, and when incorporated into human RA synovial tissues, it is protected from the interference of the mouse immune system. Therefore, it is convenient for experimental research and quantitative analysis of the results.

The process and methods for the preparation of HuRag-SCID mouse models

The history of application in SCID mice dates back to the 1990s, when Geiler et al.⁸ implanted human RA synovial tissues and

human normal cartilage into the kidney sacs of SCID mice for the first time. They also found that the transplanted synovial tissues survived well and was constantly growing. At the same time, they found that cartilage in contact with RA was significantly damaged and that the synovial tissues at the erosion site were more active in fibroblast proliferation. They successfully demonstrated that RA, in an environment away from human tissue, can grow in SCID mice and produce a large quantity of tissue factors and matrix degradation enzymes that erode cartilage. Consequently, the environment in SCID mice simulated the proliferation of human RA membrane cells, cartilage erosion, and degradation process. It also established a new humanized RA animal model. It is true that this method is free from the effects of the human tissue environment on RA erosion of cartilage. However, due to the limitation of scientific and technological research progress at that time, Geiler et al.⁸ did not transplant simple RA synovial tissue cells, but simply implanted a whole piece of synovial tissue. Although this method is simple to operate, it is inevitable that inflammatory factors and lymphocytes of local tissue in the human body will be transplanted into mice together. Therefore, the role of RASFs in cartilage erosion is not highlighted.

In response to the above problems, Lehmann et al.⁹ selectively isolated and cultured RASFs *in vitro*, purified, then directly injected them into the knee cavity of SCID mice. Finally, they found knee swelling, erosion, and destruction of articular cartilage surface, as well as a large number of Aschoff bodies in the synovial cavity. This method directly proves that RASFs' erosion of cartilage can be affected by human tissue environment and tissue factors. It provides a new direction for the follow-up

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experiment. Lehmann et al.⁹ *in vitro* separation culture RASFs, injected into sterile gelatin sponge, and then the two with normal cartilage implanted in the kidney sacs of mice, found that RASFs in mice can still maintain the transformation and secretion activity, express the vascular cell adhesion molecule 1 (VCAM-1), and secrete cathepsin B and L to erode cartilage. On the basis of Lehmann et al.⁹, Pap et al.¹⁰ constructed three-dimensional cartilage-like matrix *in vitro*. This method of operation is to send RASFs, sterile gelatin sponge, and the body's normal synovial tissue *in vitro* suitable pH and ion concentration in suspension culture. Subsequently, Lefèvre et al.¹¹ innovatively transplanted the RASFs-cartilage-sterile sponge gelatin complex into one side of the mouse subcutaneously, and the other side was simultaneously transplanted with normal human articular cartilage. RASFs were injected subcutaneously, intraperitoneally, and intravenously into human mice. RASFs were detected in the blood and spleen of mice, which confirmed that RASFs migrated through the blood circulation in the body and could erode the cartilage of the contralateral or distant knee joint.

In recent years, scholars have continuously enriched and expanded the application of SCID mouse models in the field of RA research. Serrati et al.¹² found that *in vitro* and SCID mouse models, the decrease in urokinase plasminogen activator receptor (UPAR) expression in RASFs reduced the damage of RASFs to cartilage and bone. Frey et al.¹³ isolated fibroblast-like synoviocytes (FLSs), expanded them *in vitro*, and transferred them to the knee joint cavity of SCID mice. They found that FLSs from AIA mice were able to transfer arthritis to recipient SCID mice. FLSs metastasis induces chronic arthritis and can find inflammatory cell collection and significant cartilage damage. They also observed in a single joint arthritis model that FLSs isolated from the contralateral non-arthritis joint have the potential to cause arthritis. FLSs metastasis induces chronic arthritis and can observe inflammatory cell collection and significant cartilage damage. They also observed arthritis potential in FLSs, which is separated from the opposite side of the non-arthritis joint, in the monoarthritis model. The transformation of these cells into arthritic cells occurs early in the development of arthritis. This challenges current assumptions about the role of FLSs in the pathogenesis of arthritis and opens the way for further mechanism research.

Application of SCID-HuRAg mouse model

Application of the SCID-HuRAg model in virology and oncology and RA disease progression studies

The pathogenesis of RA is extremely complex. As far as the current scientific research is concerned, its pathogenesis may be related to autoimmunity and digestive system. In recent

years, there have been continuous reports in the scientific literature that the incidence of RA may also be related to viral infections and tumor attacks. Among them, the most extensive research is the relationship between Epstein-Barr virus (EBV) and RA and the functional changes or mutations of the tumor suppressor gene p53.

Many human viruses, including EBV, do not infect mice, which is challenging for biomedical research. The research team of Nagasawa et al.¹⁴ found that human osteoclasts mainly induce erosive arthritis during EBV infection. They cultivated bone marrow cells from EBV-infected SCID-HuRAg mice and analyzed their characteristics. Multinucleated cells cultured from bone marrow cells stained positive for human cathepsin K and human MMP-9, indicating that bone marrow cells of SCID-HuRAg mice could differentiate from human osteoclast progenitors into human osteoclasts, the human immune response to EBV infection may induce the activation of human osteoclasts and cause erosive arthritis in this mouse model. This study is also the first to demonstrate human osteoclast genesis in humanized mice. This model can be used to study the relationship between EBV infection and RA and human bone metabolism.

Application of SCID-HuRAg model in pharmaceutical research and drug screening

The special immune environment provided by SCID-HuRAg can highlight the erosion and degradation of cartilage and bone by RASFs. RASFs can secrete more cytokines, such as IL-1, IL-6, IL-18, and TNF- α ^{15,16}. Through these tissue factors, it plays its role in erosion and diffusion. In recent years, the focus of RA treatment programs has shifted from simply controlling pain and other characteristics to more effective antirheumatic treatments that control synovial hyperplasia and cartilage erosion and protect joint function. The SCID-HuRAg model has gradually begun to play an important role in pharmacological research and drug screening processes.

Therapies successfully tested in mice with a functional human immune system include the antiviral drug poly Pegylated Interferon Alpha-2a (Peg-IFN α 2a), which shows signs of hepatitis C virus suppression, such as human interferon- γ production decrease, serum alanine aminotransferase level, hepatitis C virus ribonucleic acid copy number, and no leukocyte infiltration or fibrosis in the liver. Close to the clinical situation, humanized mice administered with ipilimumab developed autoimmune diseases, showing signs of weight loss, antinuclear antibodies, and increased adrenaline, which would aggravate the erosion and destruction effects of RASFs on cartilage and bone. In addition, theralizumab, a biological agent highly specific to human CD28, was tested in humanized mice implanted with

polybrominated diphenyl ethers. These mice showed severe reduction in CD45⁺ human cells, rapid decline in body temperature, and elevated cytokine levels. They were given treatment 6 h after antibody administration. The adverse effects in the clinic can be observed, which will also exacerbate the negative effect of RASFs on the body. Taking into account the advantages, limitations, and potential development of humanized mice, the current data¹⁷ show that these models are useful tools for researchers to investigate short-term and long-term studies of therapeutic interactions and toxicity *in vivo* to reduce risks and ensure safety of healthy volunteers and patients exposed to drug candidates during clinical trials.

CONCLUSIONS

In recent years, SCID-HuRAg model mice have played an important role in the pathogenesis and pathological research of RA. At the same time, this model is also continuously applied to the research and development of new drugs, as well as pharmacodynamics and pharmacokinetics. Based on SCID-HuRAg model mice, human RA synovial cells or tissues combined with normal cartilage tissue or cells of the human body are embedded in mice in different ways, and the embedding positions are also different, it can be selected at the joints of mice or subcutaneously in mice. In the case of complete lack of B cells and T cells, study the changes of RASFs and the different responses of surrounding tissue

factors and cytokines, explore the pathological process of cartilage and bone tissue erosion and destruction, and explore the possible pathogenesis of RA. The SCID-HuRAg model mouse highlights the relationship between RASFs and cartilage. At present, research on RA has gradually adopted this model and has begun to develop transgene therapy, RA, and other diseases on this model platform. It has an irreplaceable role in the treatment of cartilage erosion and degradation, as well as various body manifestations caused by inflammation. The application of this model mouse is to further improve and explore the pathogenesis of RA. With the deepening of research on the mechanism of RA disease, the quality of life of RA patients is expected to gradually improve.

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AUTHORS' CONTRIBUTIONS

ZL: Conceptualization, Data curation, Formal analysis, Writing – original draft. **RY:** Data curation. **YY:** Data curation. **JQ:** Data curation. **YS:** Data curation, Writing – original draft. **LG:** Data curation, Formal analysis. **QY:** Data curation, Formal analysis. **XL:** Conceptualization, Writing – review & editing.

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Comment on “Assessment of left atrial function by strain in patients with acute ischemic stroke left atrial function and acute stroke”

Li, Zhipeng¹ , He, Lianping^{1*} 

Dear Editor,

We were glad to read the interesting study published by Unal Ozturk et al.¹ They found that left atrial longitudinal strain is associated with stroke severity during admission in patients with acute ischemic stroke. Although they found a series of evidence about the relationship between left atrial strain function and ischemic stroke, there are some issues that should be further discussed.

To begin with, the purpose of this study was to investigate the relationship between left atrium myocardium and tissue function in patients with acute ischemic stroke, so the title “Assessment of left atrial function by strain in patients with acute ischemic stroke left atrial function and acute stroke” should be changed to “Assessment of left atrial function by strain in patients with acute ischemic stroke and acute stroke.” In the method part of the summary, the statistical analysis should be described in detail, including the statistical software SPSS version 12.0 used in this study and the corresponding statistical methods, such as Mann–Whitney U test and the chi-square test, which were used appropriately for comparing data.

Furthermore, the normal distribution test of the National Institutes of Health Stroke Scale and the longitudinal strain of

the left atrium should be conducted. The distribution of the data is an important base for the selection of statistical methods; however, the author does not give the normality test of the above score. In Table 1 of the results section, the authors should offer the exact statistics value, for example, Student’s *t*-test value or chi-square value. In addition, the authors did not estimate the sample size and directly used clinical cases as the research object, which may lead to selection bias in the results which in turn affects the accuracy of the conclusions.

Finally, the general demographic characteristics of the subjects in the second paragraph of the discussion section, such as age, gender, and place of residence should be described. The study aimed to evaluate the relationship between left atrial longitudinal strain and acute ischemic stroke; however, the conclusion section failed to give the applicable age. Therefore, we suggest adding the scope of application of this conclusion in the discussion section.

AUTHOR CONTRIBUTION

ZL: Formal Analysis, Writing – Original Draft. **LH:** Conceptualization, Writing – Review & Editing.

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Comment on “Comparison of two pandemics: H1N1 and SARS-CoV-2”

Fangbin Weng¹ , Xiaofei Li^{1*} 

Dear Editor,

We read with great interest the article “Comparison of two pandemics: H1N1 and SARS-CoV-2” by Kant et al.¹ In this study, the authors demonstrated that the experience in the HAmg of A/Brisbane/59/2007 (H1N1) pandemic may be the guide to prevent the coronavirus disease 2019 (COVID-19) pandemic from a worse end. This study offers new ideas for the prevention of COVID-19 pandemic. However, some ideas should be put forward from our point of view.

First, the two pandemics occurred at different times; hence, it is not clearly comparable.

Since the average age of 143 patients with H1N1 diagnosed during the H1N1 pandemic in 2009 was lower than that of 309 COVID-19 patients in the same centers in the 2020 SARS-CoV-2 pandemic, the calculated duration of symptoms (fever, cough, sputum, sore throat, myalgia, weakness, headache, and shortness of breath) in the two groups are not directly comparable.

Second, there are significant differences between their conditions, clinical courses, and preventive measures. Thus, experience in H1N1 pandemic cannot be fully replicated in COVID-19 pandemic. If measurements are based only on the comparisons of body temperatures, pulses, respiratory rates, and laboratory tests between the two groups, the findings may be misleading.

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Comment on “Limited cardiopulmonary capacity in patients with liver cirrhosis when compared to healthy subjects”

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Dear Editor,

We have perused with interest your article entitled “Limited cardiopulmonary capacity in patients with liver cirrhosis when compared to healthy subjects” in which Nasser et al.¹ found that liver cirrhosis can compromise the quality of life of patients, mainly by inducing metabolic alterations, which can impair functional capacity and lead to a sedentary lifestyle. However, a number of issues were raised from our point of view.

So far, we do not know how to select patients with liver cirrhosis and healthy subjects for this study. For the chronic liver disease-matched analysis, for which three groups were required, control patients with liver disease were matched with the patients with liver cirrhosis and control patients without liver disease were matched with the patients with liver disease.

How to define inactive lifestyle, consisting of daily routine activities and never having been engaged in exercise programs for a long time? The study should provide a means for quantitative measurement of inactive lifestyle. Physical activity level could be evaluated by the Physical Activity Scale. The references for the evaluation of physical activity should be given in this study.

AUTHORS' CONTRIBUTION

MS: Writing – original draft, Writing – review & editing. **BZ:** Data curation, Formal analysis, Writing – original draft. **BH:** Data curation, Formal analysis, Writing – original draft. **YM:** Conceptualization, Writing – review & editing.

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In the manuscript “Hypofractionated radiotherapy recommendations for localized prostate cancer in Brasil”, DOI: 10.1590/1806-9282.67.01.002, published in the Rev Assoc Med Bras. 2021;67(1):7-18.

Page 7, title:**Where it reads:**

Hypofractionated radiotherapy recommendations for localized prostate cancer in Brasil

It should read:

Hypofractionated radiotherapy recommendations for localized prostate cancer in Brazil

Page 7, summary**Where it reads:**

There are many benefits of hypofractionation, including a more convenient schedule for the patients and better use of resources, which is especially important in low- and middle-income countries like Brasil. Based on these data, the Brazilian Society of Radiotherapy (Sociedade Brasileira de Radioterapia) organized this consensus to guide and support the use of hypofractionated radiotherapy for localized prostate cancer in Brasil. METHODS: The relevant literature regarding moderate hypofractionation (mHypo) and ultra-hypofractionation (uHypo) was reviewed and discussed by a group of experts from public and private centers of different parts of Brasil.

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Page 7, institutional bond**Where it reads:**

¹Hospital Sírio-Libanês – Brasília (DF), Brasil.

²Hospital Felício Rocho – Belo Horizonte (MG), Brasil.

³Hospital Mãe de Deus – Porto Alegre (RS), Brasil.

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⁵Hospital São José – Criciúma (SC), Brasil.

⁶Hospital da Providência – Apucarana (PR), Brasil

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¹⁶Hospital Alemão Oswaldo Cruz – São Paulo (SP), Brasil.

¹⁷Sociedade Brasileira de Radioterapia, Grupo Oncoclínicas – São Paulo (SP), Brasil.

It should read:

- ¹Hospital Sírrio-Libanês – Brasília (DF), Brazil.
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- ¹⁶Hospital Alemão Oswaldo Cruz – São Paulo (SP), Brazil.
- ¹⁷Sociedade Brasileira de Radioterapia, Grupo Oncoclínicas – São Paulo (SP), Brazil.

Page 8, Introduction, first column, first paragraph**Where it reads:**

Prostate cancer (PCa) is one of the most incident cancers in Brasil and worldwide, representing more than 30% of all cases in men^{1,2}.

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Page 8, Introduction, second column, first paragraph**Where it reads:**

In order to support radiation oncologists and physicists to implement hypofractionation in the clinical practice, this consensus aimed to guide indications and the minimum requirements to safely conduct hypofractionation RT for localized PCa patients in Brasil.

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Page 8, Methods, second column, second paragraph**Where it reads:**

Sixteen radiation oncologists from different areas of Brasil, from both public and private institutions, with known expertise in the topic, attended the meeting and composed the panel.

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Page 11, Table 3**Where it reads:****Table 3.** Techniques of moderate hypofractionation treatment.

Treatment techniques	Yes, adequate (%)	No, inadequate (%)	Consensus achieved
Planning: conventional or bidimensional radiotherapy	0	100	Yes
Planning: conformal or tridimensional radiotherapy	88	12	Yes
Planning: intensity modulated techniques (IMRT or VMAT)	100	0	Yes
Modality of IGRT: tridimensional imaging (cone beam computed tomography; ultrasonography) with or without fiducials	100	0	Yes
Modality of IGRT: online bidimensional imaging (portal) with fiducials (onboard imaging, EPID)	100	0	Yes
Modality of IGRT: online bidimensional imaging (portal) without fiducials (onboard imaging, EPID)	69	31	Yes

It should read:**Table 3.** Techniques of moderate hypofractionation treatment.

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Page 16, Conclusions, first column, second paragraph**Where it reads:**

As a consequence, patient's access to treatment can be increased, which is especially important in LMIC like Brasil.

It should read:

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Page 16, Acknowledgments, second column, second paragraph**Where it reads:**

The SBRT thanks Varian Medical Systems Brasil for their financial support used to hold a consensus meeting, which took place on October 11, 2019 in São Paulo.

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In the manuscript “Quantitative evaluation of computed tomography findings in patients with pulmonary embolism: the link between D-Dimer level and thrombus volume”, DOI: 10.1590/1806-9282.67.02.20200539, published in the Rev Assoc Med Bras. 2021;67(2):218-223.

Page 219, Methods, Study population, second paragraph:

Where it reads:

Patients between 18–65 years of age with optimum CTPA image quality and breathing, patients clinically and radiologically diagnosed with PE, were enrolled in the study.

It should read:

Patients aged 18 and over with optimum CTPA image quality and breathing, patients clinically and radiologically diagnosed with PE, were enrolled in the study.

