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Palliative Medicine: 10 years as an area of medical practice in Brazil

Ricardo Tavares de Carvalho^{1*} , Tulio Loyola Correa¹ 

The World Health Organization (WHO) first defined Palliative Care (PC) in 1990, involving the practice as one of the focuses of cancer care (prevention, diagnosis, treatment, and PC). Since then, the concept was broadened in 2002 to include any life-threatening disease throughout its entire course, especially, but not only, in advanced and terminal diseases and end-of-life care¹.

In 2014, WHO qualified this practice so that its principles of action should be guiding for any good health practice², leading to a new definition in 2017³. Since then, the concept has been broadened even more, involving aspects of human care in situations of suffering related to social vulnerability conditions and pandemics⁴. In 2020, a group of experts gathered by the International Association of Hospice and Palliative Care (IAHPC) formulated the most recent and comprehensive definition involving this practice³.

Despite the successive and recent reformulations of its definition and the large increase in the number of scientific publications in the field, PC has not been recognized as a medical specialization in several countries around the world in spite of the UK and other countries' recognition since 1987⁵.

Development in the Brazilian context

In Brazil, the practice of PC is described from individual initiatives and in a punctual way since the 1980s⁶.

In 1997, with the creation of the Brazilian Palliative Care Association (ABCP), an entity with a multiprofessional character, the professionals involved in the practice started to organize themselves. They discussed their actions and preliminary aspects of the so-called "Brazilian Palliativist Movement", holding the first National Congress in the field in 2004 at the headquarters of the National Cancer Institute, in Rio de Janeiro.

Then, a group of professionals, understanding that the regulation of this practice was necessary and that this depended on articulation with the medical entities in the country, founded the National Academy of Palliative Care (ANCP) on February 26, 2005. The association was

composed of multiple health professionals but directed only by physicians, a mandatory requirement for this articulation at the time.

This allowed greater visibility and insertion in the then recently created Technical Chamber of Terminality of Life and Palliative Care of the Federal Council of Medicine (CFM), active in the preparation of important documents such as the resolution 1.805/2006⁷ and the first version of the New Code of Medical Ethics (2009/2010), which was the first to textually mention the term "Palliative Care" in the Fundamental Principle number XXII and the articles 36 and 41. The text was maintained in its entirety in the most recent edition of the Code of Medical Ethics⁸.

Palliative Medicine: a new area of medical practice

In Brazil, the inclusion of an area of knowledge as a recognized specialization is a process that involves specific rules and prerequisites regulated by competent medical bodies, especially the CFM.

In the context of the growing practice and gradual representation in decisive medical regulatory boards in the country, it was time, in mid-2010, for the official recognition of Palliative Medicine as an area of medical practice.

As a first step, the National Commission on Palliative Medicine was created by the Brazilian Medical Association (AMB). The first meeting of the commission took place on March 30, 2010 with the representation of the medical specialties who understood that Palliative Medicine was an area of practice related to their practice, after an open consultation, by letter, made by the AMB to the boards of all medical specialties. On this occasion, the societies that initially manifested themselves were Oncology, Internal Medicine, Geriatrics, Family and Community Medicine, and Pediatrics, besides ANCP itself. Later, the Anesthesiology Society started to compose the group of specialties considered prerequisites to obtain the title of Palliative Medicine as an area of practice.

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This Commission was responsible for requesting to the Mixed Committee of Specialties, constituted by the CFM and AMB, the approval, in August 2011, of the request so that Palliative Medicine could integrate the list of recognized areas of practice in Brazil, which, according to the recent update, resolution 2221/2018 that ratifies the CME 01/2018 ordinance, lists the existence of 57 areas of medical practice in the country⁹.

On May 18, 2012, by the letter OF/AMB/0117/2012, the AMB informed the six societies of the first call for the selection of candidates, by curriculum analysis, requiring that the candidates had documented experience in Palliative Medicine for at least 5 years and that they were certified by the AMB in one of the areas listed as prerequisites at the time. In this initial selection process, 45 physicians (20 anesthesiologists, 9 pediatricians, 7 geriatricians, 7 internists, and 2 family physicians) were qualified.

From 2013, upon request to the AMB, the ANCP became part, with two representatives, of the board responsible for preparing the exam for new candidates, now by written test and curriculum analysis, along with the other six societies that had participated in the previous selection process. That year, the National Commission of Palliative Medicine decided to hold the sufficiency exams for the area of Pediatrics separately from the other areas.

From that moment, the exam started to be held periodically by the AMB in a place predefined by the entity or during the ANCP Palliative Care Congresses, according to the entity's calendar.

The number of medical specialties considered prerequisites to obtain the title has increased progressively, so that it currently amounts 12 (Anesthesiology, Internal Medicine, Head and Neck Surgery, Oncological Surgery, Geriatrics, Mastology, Family and Community Medicine, Intensive Care Medicine, Nephrology, Neurology, Clinical Oncology, and Pediatrics). In the year 2021, Brazil had 389 medical professionals certified by the AMB in this area of practice¹⁰.

Medical residency in Palliative Medicine: a requirement

As part of the providences required for the regulation of a new area of medical practice in Brazil, guidelines were elaborated for the registration of Medical Residency Programs in Palliative Medicine with the duration of one additional year to the residencies in the prerequisite areas previously listed¹¹. These guidelines oriented the characteristics of the programs and the distribution of the workload among the different assistance modalities of Palliative Medicine in diverse scenarios of medical practice.

Based on this regulation, the registration of the programs with the Ministry of Education and Culture (MEC) began in 2012. Currently, there are 17 active residency programs in Brazil (only one in Pediatric Palliative Medicine) having already trained around 200 physicians in 2022¹².

Palliative Medicine as a specialty in Brazil: the state of the art

Palliative Medicine refers only to the body of knowledge concerning the medical doctor. By concept, the adequate and competent application of PC practice requires an appropriate organization and training of all health care professionals. In this sense, some representative councils of different health professions are already beginning to recognize, from their criteria, that their professionals may be considered specialists or have an area of practice in PC.

Nevertheless, regarding medical doctors, one cannot, by force of concept, speak of a professional specialty.

Palliative Medicine is an area of practice for which, in Brazil, the candidate for the so-called "title" has only two options: either to undergo a written test prepared by the AMB or to complete 1 year of Medical Residency in Palliative Medicine in one of the programs duly registered and recognized by the MEC in the country.

However, with the increasing visibility and growth of the area in the country, following the MEC norms¹¹, a training alternative for any health professional has been configured with the emergence of *lato sensu* postgraduation courses in the area. The norms refer to on-site training with a minimum of 360 h and certified by a Higher Education Institution (HEI) registered with the MEC. In times of the COVID-19 pandemic, the transition to online training modalities, whether in hybrid-live format or distance learning with recorded classes, is being considered valid by the MEC as long as the minimum workload is respected and duly certified by a registered HEI.

In the *lato sensu* postgraduation modality, there are a rapidly increasing number of Medical and Multiprofessional Specialization courses in Brazil. However, it is fundamental to understand that these courses confer to the professional the title of Academic Specialist, which is not recognized by the AMB and, therefore, is not equivalent to the title of Professional Specialist.

More recently, the AMB has recognized that these specialization courses and other education courses (for physicians), with a minimum length of 1 year, may be computed in a more expressive way among the prerequisites to register for the sufficiency exam for obtaining the title of area of practice in Palliative Medicine.

Palliative Medicine as a medical specialty: perspectives

Considering the reality of Palliative Medicine as an area of medical practice in the country, there is an open perspective for the recognition of the area as a medical specialty.

Although this is already a reality for several countries in the world, this process is still under discussion in Brazil. In this aspect, it is worth clarifying that Palliative Medicine comprehends a set of competencies and skills that all physicians must carry, as occurs with all other medical specialties, in graduation.

However, the advances in technical and scientific knowledge in the area, the increasing number of publications and scientific events worldwide, and the already existing Brazilian medical ethic normative⁸ regarding that PC must be offered in the context of advanced and terminal diseases highlight Palliative Medicine as an area of its own and with demands for a large number of specific technical and attitudinal competencies, which were recently recognized in Brazil¹³.

The recognition of this situation and a compilation of specific competencies required of the physician in this practice, which cannot be contemplated in only 1 year of residency, are essential for the acknowledgment that Palliative Medicine has the requirements for its establishment as a new medical specialty in Brazil. Contributing to that are the recent establishment of

the Ministerial Resolution 41/2018, of October 31, 2018¹⁴ and four State Laws¹⁵⁻¹⁸ that begin to provide the basis for the development of specific Health Policies aimed at the adequate provision of PC in Brazil, especially in the public context. In this sense, it was necessary to re-register all residency programs and to elaborate new pedagogical projects in order to start, in 2023, the already expanded 2-year residencies.

CONCLUSION

The process of increasing the organization of Palliative Medicine in Brazil is accelerated. However, the inclusion of PC training in the graduation of future health professionals, as well as the development of National Health Policies with universal access to PC are challenges of vital importance for the upcoming years.

AUTHORS' CONTRIBUTIONS

RTC: Conceptualization, data curation, investigation, methodology, supervision, writing – original draft, writing – review & editing. **TLC:** Conceptualization, investigation, validation, writing – original draft.

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Comparison between ceramic-on-polyethylene versus metal-on-polyethylene prostheses in Total Hip Arthroplasties: a systematic review and meta-analysis

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The Guidelines Project, an initiative of the Brazilian Medical Association, aims to combine information from the medical field to standardize how to conduct, and to assist in the reasoning and decision-making of doctors. The information provided by this project must be critically evaluated by the physician responsible for the conduct that will be adopted, depending on the conditions and the clinical condition of each patient.

INTRODUCTION

Total hip arthroplasty (THA) is considered one of the most successful orthopedic interventions worldwide. The technological evolution of this technique began at the end of the 19th century, when studies began to evaluate the tolerance of the human body to foreign bodies.

Since then, the hunt for improvement and better long-term results has continued with the search for new surfaces, greater material biocompatibility, and less aggressive surgical techniques¹.

There are several models of prostheses available for this procedure, which differ from each other in various ways, ranging from the fixation method used (cemented or not) to the type of material that composes them, the most widely used being metallic, ceramic, or plastic (polyethylene) components.

These differences directly affect the survival period and replacement of each prosthesis and are considered contributing factors to prosthesis wear, osteolysis around it, and its aseptic loosening.

In view of the above, we performed a systematic review with meta-analysis in order to compare the hip prosthesis with a femoral head and acetabulum composed of ceramic and polyethylene, respectively, with the prosthesis composed of metal and polyethylene. The reviews, wear rates, and clinical outcomes were considered.

METHODS

The methodology will address the following information: the clinical question, structured question (PICO), eligibility criteria of the studies, sources of information consulted, search strategies used, critical evaluation method (risk of bias), follow-up greater than 24 months, quality of evidence, data to be extracted, measures to be used to express the results, and the method of analysis.

Clinical question

For THA, is the use of ceramic-on-polyethylene prostheses more efficient than metal-on-polyethylene prostheses?

Structured question

P (population): Adult patients undergoing THA

I (intervention): Ceramic-on-polyethylene prostheses

C (comparison): Metal-on-polyethylene

O (“outcome”): Reviews, wear rates, and clinical outcomes.

Eligibility criteria

- PICO components
- Randomized clinical trials (RCTs)
- No time restriction
- Languages English, Spanish, and Portuguese
- Full text or abstract with necessary data
- Outcomes expressed in absolute number of events or mean/median with variation

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Exclusion criteria

- Observational and noncomparative studies
- In vitro and/or animal studies
- Case series or case reports
- Narrative or systematic reviews

Sources of information consulted and search strategies

Medline via PubMed, manual search

(Arthroplasty, replacement, Hip OR Hip Prosthesis OR Hip Prostheses) AND (Metal OR Metals OR Ceramic OR ceramics OR Polyethylene OR Polyethylenes OR Polythene OR LDPE OR HDPE OR Polymers OR Polypropylenes) AND random*

Risk of bias and quality of evidence

For RCTs, the following risks of bias will be evaluated: focal question, randomization, blinded allocation, double blinding, evaluator blinding, losses, analysis by intention to treat (ITT), definition of outcomes, and sample size calculation.

Data extracted

Author, year of publication, study design, characteristics and number of patients, intervention, comparison, and outcomes (reviews, wear rate, and clinical outcomes)

Outcome measures

For categorical variables, we use absolute numbers, percentage, absolute risk, risk reduction or increase, number needed to treat (NNT) or harm (NNH), AND 95% confidence interval (95%CI). For continuous variables, means or difference between means with standard deviation are utilized.

Expression of the results

If there is the possibility of aggregating the results of the included studies regarding one or more common outcomes, a meta-analysis will be performed using the RevMan version 5.3 software (Cochrane)².

To calculate the mean and standard deviation, when not presented in the work, the VassarStats software is used: Website for Statistical Computation³.

Analysis of the quality of evidence

The quality of the evidence is assessed using the GRADEpro software⁴.

RESULTS

The results presented include diagram of study retrieval (Figure 1) and selection, risk of bias (Table 1), results by outcome, quality of evidence (grade, Table 2), and evidence synthesis.

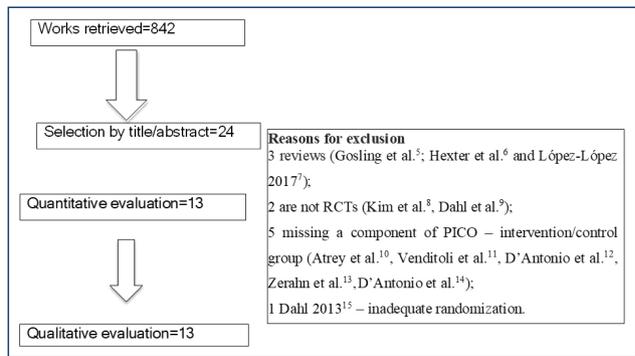


Figure 1. Flowchart of the selected works (CONSORT).

In total, 842 studies were retrieved (Medline via PubMed). After applying the eligibility criteria, 24 studies were selected, of which 13 studies were included for full-text evaluation (Figure 1).

Characteristics of the included studies

Bjorgul et al.¹⁶

A total of 399 hips were selected, of which 376 patients aged below 73 years with hip osteoarthritis (OA) undergoing THA. All prostheses had a femoral head of 28 mm; those composed of ceramic-on-polyethylene were compared with others with metal-on-polyethylene according to the Harris Hip Score (HHS) outcomes and the number of surgical reviews; the follow-up time was 84 months.

Ise et al.¹⁷

Participants included 77 patients aged over 60 years with hip OA undergoing THA. The ceramic-on-polyethylene prosthesis (with three different types of ceramic in the femoral head) was compared with the metal-on-polyethylene prosthesis, all with a 22.225-mm head. The follow-up time was 36 months, and the outcome linear wear rate per mm/year was analyzed through radiographic examinations.

Jassim et al.¹⁸

A total of 401 patients aged over 18 years with hip OA were analyzed. For the THA, prostheses with a 32-mm femoral head, composed of ceramic-on-polyethylene or metal-on-polyethylene, were used. Patients were followed up for 60 months, and the outcome analyzed was linear wear rate in mm/year.

Jonsson et al.¹⁹

Participants included 120 patients aged 59–80 years with hip OA who underwent THA. The size of the femoral head was 28 mm, and a comparison was made between the prostheses made of ceramic-on-polyethylene and prostheses made of metal-on-polyethylene. HHS outcomes were analyzed along with linear wear rate in mm/year through radiographic examinations. The follow-up time was 60 months.

Table 1. Risk of bias and quality of evidence.

Author/Year	Randomization	Blinded allocation	Double blind	Evaluator blinding	Losses	Prognostic characteristics	Outcomes	ITT	Sample calculation	Early interruption
Bjorgul, et al. ¹⁶										
Ise, et al. ¹⁷										
Jassim, et al. ¹⁸										
Jonsson, et al. ¹⁹										
Kadar, et al. ²⁰										
Kawate, et al. ²¹										
Kim ²²										
Kraay, et al. ²³										
Morison, et al. ²⁴										
Nakahara, et al. ²⁵										
Zaoui, et al. ²⁶										
Bergvinsson, et al. ²⁷										
Kayani, et al. ²⁸										

ITT: Intention to treat.

Absence of bias	Absence of information	Presence of bias
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Table 2. Grade table for evaluating the level of evidence.

Certainty assessment							Nº of patients		Effect		Certainty	Importance
Nº of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Intervention (%)	Comparison (%)	Relative (95%CI)	Absolute (95%CI)		
1. Linear wear (mm/y) XLPE												
9	Randomized trials	Serious ^{a,b}	Not serious	Not serious	Not serious	None	448	443	-	MD 0 (0.01 minor to 0)	⊕⊕⊕○ moderate	
2. Linear wear (mm/y) UHMWPE												
4	Randomized trials	Serious ^{a,b}	Not serious	Not serious	Not serious	None	67	71	-	MD 0 (0.04 minor to 0.05 higher)	⊕⊕⊕○ moderate	
3. Surgical reviews												
7	Randomized trials	Serious ^{a,b}	Not serious	Not serious	Serious ^c	None	2/400 (0.5)	5/400 (1.3)	Not estimable	10 more for 1.000 (from 10 less to 20 more)	⊕⊕○○ low	
4. Head size												
11	Randomized Trials	Very serious ^{a,b,d}	Very serious ^e	Very serious ^e	Serious ^c	None	518	536	-	SMD 0.75 higher (0.52 lowest to 2.03 highest)	⊕○○○ very low	
5. Harris hip score (HHS)												
5	Randomized trials	Very serious ^{a,b,d}	Not serious	Not serious	Serious ^c	None	147	155	-	MD 5.02 smaller (7.3 minor to 2.73 minor)	⊕○○○ very low	

CI: confidence interval; SMD: standardized mean difference. ^aAbsence of analysis by intention of treatment; ^bAbsence of blinding; ^cLong confidence interval; ^dAbsence of sample calculation; ^eHigh heterogeneity.

Kadar et al.²⁰

In total, 77 patients aged 59–80 years with hip OA who underwent THA were selected; the ceramic-on-polyethylene hip prosthesis (with three different types of ceramic in the femoral head) was compared with the metal-on-polyethylene hip prosthesis, using femoral heads of 22, 22.5 and 28 mm. The linear wear rate in mm/year was evaluated by radiographs. The follow-up time was 24 months.

Kawate et al.²¹

This was a comparative study evaluating 60 patients, including 62 hips with OA undergoing THA. The following outcomes were compared: clinical (HHS), linear wear in mm/year, and volumetric wear rate in mm³ between individuals with a ceramic-on-polyethylene hip prosthesis and others with a metal-on-polyethylene hip prosthesis, all using a 26-mm femoral head. The follow-up time was 60 months.

Kim²²

This study comprised 52 patients aged up to 50 years, including 104 hips with OA. Participants underwent THA using either ceramic-on-polyethylene or metal-on-polyethylene prosthesis with a 28-mm femoral head. HHS outcomes, reviews performed, and linear and volumetric wear rates were evaluated. Follow-up time ranged from 60 to 96 months.

Kraay et al.²³

This was a clinical trial with 60 participants aged between 50 and 75 years and 104 hips with hip OA undergoing THA. The prostheses used had a femoral head of 28 mm and were made of ceramic-on-polyethylene or metal-on-polyethylene. The outcomes investigated were the number of reviews and the linear wear rate; clinical analysis was performed using the HHS tool. The follow-up time was 51 months.

Morison et al.²⁴

In total, 80 patients were selected, ranging in age from 18 to 65 years, with 91 hips with OA, submitted to a ceramic-on-polyethylene hip prosthesis, compared with a metal-on-polyethylene hip prosthesis, using a 28-mm femoral head. The following outcomes were evaluated: HHS, reviews, and linear wear rate in mm/year by radiographs. The follow-up time was 60 months.

Nakahara et al.²⁵

In total, 94 patients were compared, with a mean age of 58.5 years, with 102 hips with OA, submitted to a ceramic-on-polyethylene hip prosthesis, compared with a metal-on-polyethylene hip prosthesis, using a 26-mm femoral head, and the outcomes were evaluated: reviews and linear wear rate in mm/year by radiographs. Follow-up time was 72 months.

Zaoui et al.²⁶

In this study, 100 patients aged over 75 years with hip OA were included, submitted to a ceramic-on-polyethylene hip

prosthesis, compared with a metal-on-polyethylene hip prosthesis, using a 22.25-mm femoral head. The outcome evaluated include linear wear rate in mm/year by radiographs. The follow-up time was 48 months.

Bergvinsson et al.²⁷

A total of 50 patients with a mean age of 60 years and a mean body mass index (BMI) of 27 with primary hip OA were randomized, comparing ceramic-on-polyethylene hip prosthesis versus metal-on-polyethylene, using a 32-mm head. The outcomes evaluated were linear wear in mm/year, reviews, and hip disability and osteoarthritis outcome score. The follow-up time was 60 months.

Kayani et al.²⁸

This was a multicenter study including 401 patients aged over 18 years with degenerative/erosive disease due to hip OA, avascular necrosis, or rheumatoid arthritis undergoing THA. Prostheses with a 32-mm head, composed of metal-on-polyethylene versus ceramic-on-polyethylene, were compared. The outcomes of surgical review, linear wear in mm/year, and clinical outcome by the Western Ontario and McMaster Universities Arthritis index were analyzed. The follow-up time was 120 months.

Analysis of results by outcome

In the evaluation of linear wear (mm/year) between cross-linked ceramic polyethylene (XLPE) and metal XLPE, nine studies were included, with 448 patients in the ceramic XLPE group (intervention) and 443 patients in the metal XLPE group (control). Only one test had a lower wear result favorable to ceramic polyethylene³.

Ceramic XLPE reduces annual wear measured in mm/year compared to metal XLPE. However, this reduction is less than five thousandths of mm/year (Figure 2). The quality of available evidence is moderate (Table 2).

In the evaluation of linear wear (mm/year) between ultra-high-molecular weight ceramic polyethylene (UHMWPE) and metal UHMWPE, three studies were included, with 67 patients in the ceramic UHMWPE group and 71 patients in the metal UHMWPE group.

There is no difference in wear measured in mm/year when comparing ceramic UHMWPE and metal UHMWPE prostheses (Figure 3). The quality of available evidence is moderate (Table 2).

In the evaluation of surgical reviews of THA between ceramic XLPE and metal XLPE, seven studies were included, with 400 patients in the ceramic XLPE group and 400 patients in the metal XLPE group.

In surgical reviews of THA with a follow-up of more than 60 months, when comparing the use of ceramic XLPE and metal XLPE hip prostheses, there is no difference in the

number of procedures (Figure 4). The available evidence is of low quality (Table 2).

In the evaluation of the femoral head subgroup with a size ranging from 22 to 32 mm between ceramic XLPE and metal XLPE, in the linear wear outcome nine studies were included, with 518 patients in the ceramic XLPE group and 536 patients in the metal XLPE group.

Evaluating the subgroup of femoral stem heads with different measures 22, 26, 28, and 32 mm) in hip arthroplasty, there is no evidence of difference between ceramic XLPE and metal XLPE (Figure 5). The quality of available evidence is very low (Table 2).

Harris Hip Score

In the clinical evaluation of the HHS, there is a maximum score of 100 points, assessing pain, function, deformity, and range of motion, with pain and function having a greater weight. Five studies were included, with 147 patients in the ceramic XLPE group and 155 patients in the metal XLPE group.

In hip arthroplasty clinical reviews evaluated by the HHS, there is a difference in score favoring metal XLPE, when compared to ceramic XLPE (Figure 6). The available evidence is of very low quality (Table 2).

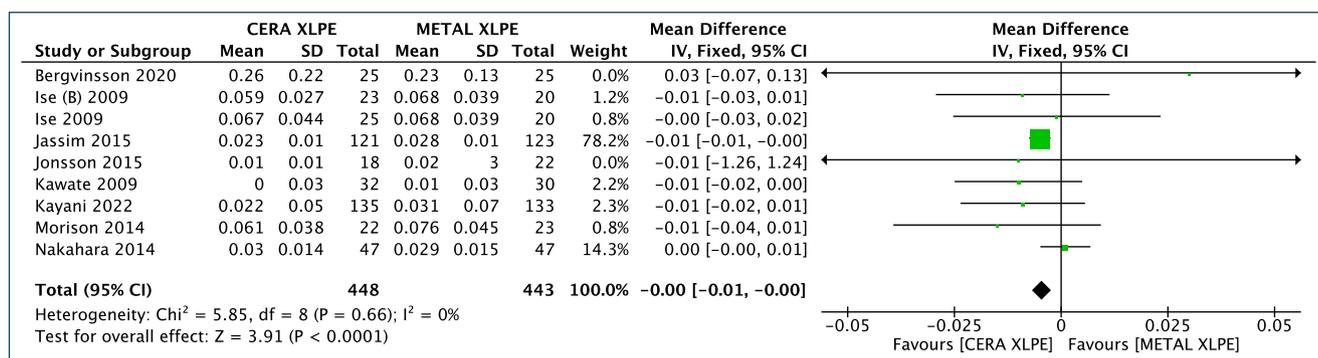


Figure 2. Forest plot of the ceramic XLPE vs. metal XLPE comparison analyzing wear outcome in mm/year.

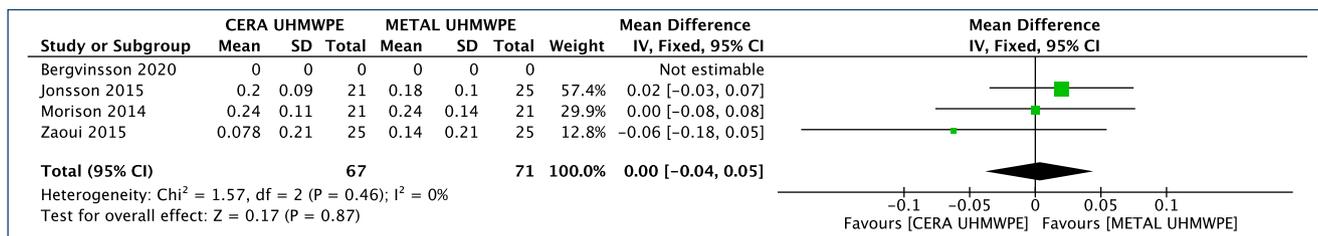


Figure 3. Forest plot of the ceramic UHMWPE vs. metal UHMWPE comparison analyzing wear outcome mm/year.

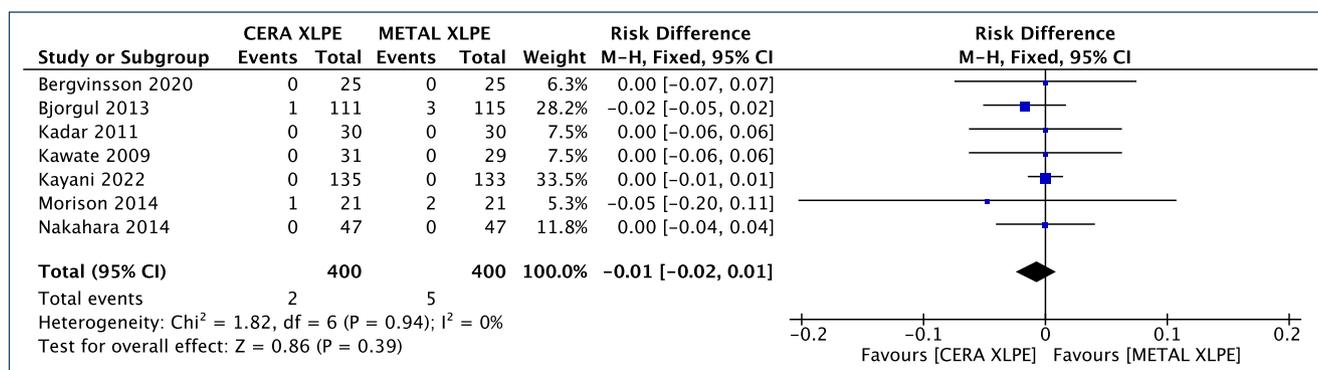


Figure 4. Forest plot of the ceramic XLPE vs. metal XLPE comparison analyzing the outcome reviews.

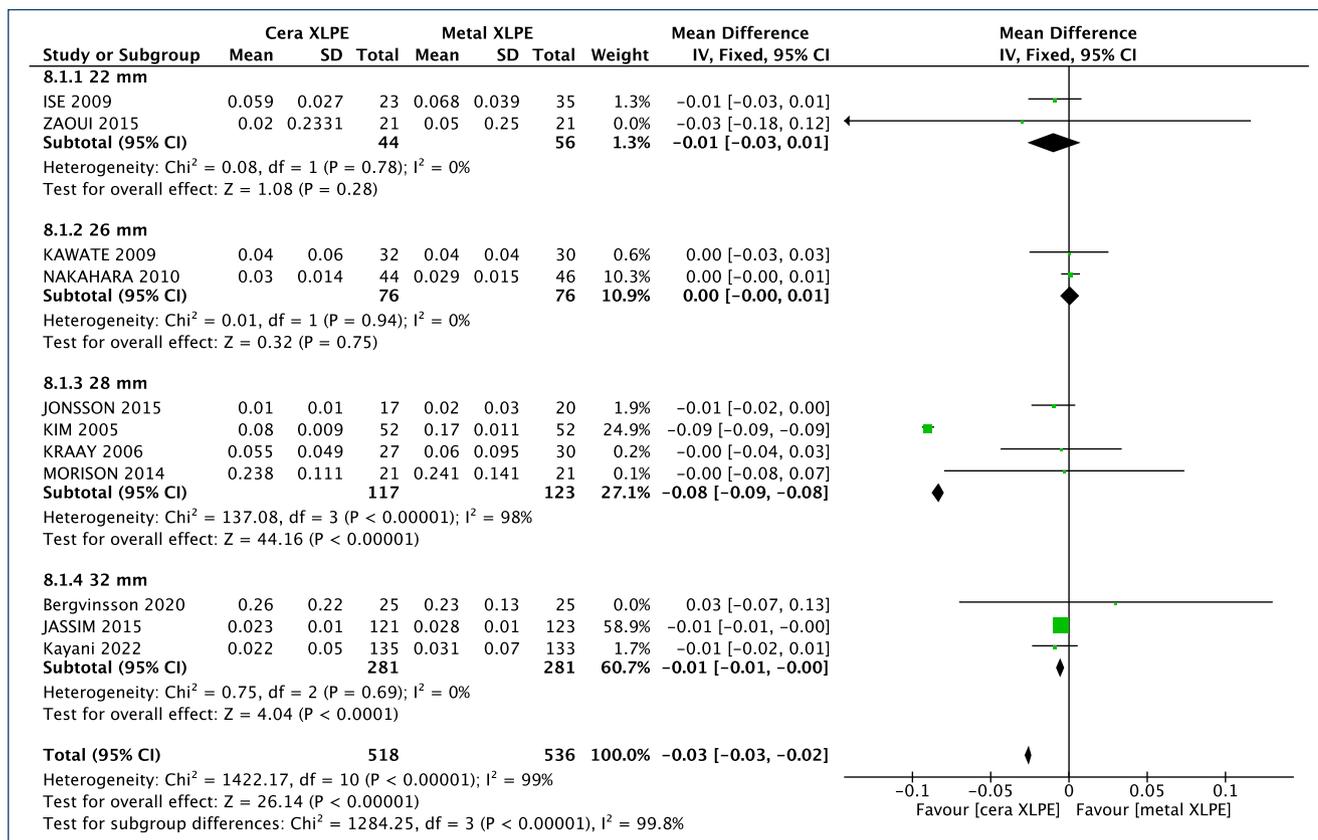


Figure 5. Forest plot of the ceramic XLPE vs. metal XLPE comparison analyzing a subgroup of femoral head size in the linear wear outcome.

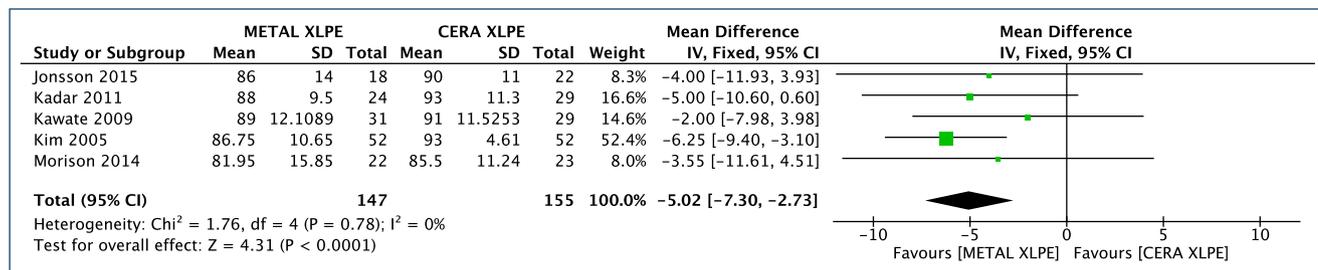


Figure 6. Forest plot of the ceramic XLPE vs. metal XLPE comparison analyzing hip arthroplasty clinical reviews evaluated by the HHS.

DISCUSSION

THA is an orthopedic procedure that has become increasingly common in Brazil, mainly due to the increase in life expectancy and the expansion in the performance of this surgery in younger and more active patients. In view of this, the search for data on durability has become increasingly frequent due to the emergence of new materials.

The main reason for THA failure is wear of the bearing surface and the resulting osteolysis induced by this wear, which can cause loosening and implant failure. Therefore, modern materials with better wear characteristics such as metal, ceramic, and high cross-linked polyethylene are now being used in THAs around the world.

Dumbleton et al.²⁹ reported that the incidence of osteolysis increases with a higher linear wear rate. *The literature suggests that osteolysis is infrequent when the wear rate is less than 0.1 mm/year and almost absent when the wear rate is less than 0.05 mm/year*²⁹. Jassim et al.¹⁸ found that the linear wear difference was 0.005 mm/year, while Kaiany et al.²⁸ found it to be 0.009 mm/year, favorable to ceramic XLPE components.

Similarly, the structural analysis performed in the literature also shows the absence of evidence of benefit of the ceramic XLPE prosthesis over the reference prosthesis (metal XLPE) for the surgical review rate and HHS outcomes. Although there is a proven

statistical difference in the radiological evaluation of wear, this is not demonstrated in numbers on the clinical reality of the patients analyzed, as in the follow-up period of 24–84 months, there was no difference found in the evaluation of the surgical reviews or in the postoperative clinical framework that this greater wear could provide.

Gosling et al.⁵ and López-López et al.⁷ showed results that are similar to those presented here.

The linear wear data analyzed in the RCTs of Jassim et al.¹⁸, Jonsson et al.¹⁹, Morrison et al.²⁴, and Zaoui et al.²⁶, comparing the acetabular components (XLPE vs. UHMWPE) on ceramic and metal surfaces did not demonstrate the superiority of ceramic to metal surfaces. However, when evaluating the wear within the same surface, whether ceramic or metal, we found a better performance of XLPE compared to UHMWPE.

The historical facts show that THA has increasingly prioritized the use of larger femoral heads, as they approximate the natural size of native femoral heads and provide the possibility of a lower rate of dislocations, to the detriment of the initial idea that smaller heads produce less wear.

Tsikandylakis et al.³⁰ concluded that the risk of surgical review due to dislocation is lower in femoral heads of 36 mm or more and volumetric wear together with frictional torque are greater in femoral heads greater than 32 mm. In addition, long-term survival is higher in femoral heads of 32 mm, especially when associated with the tribological pair of metal XLPE. In linear wear data in subgroups of femoral stem heads with different measures (22, 26, 28, and 32 mm) in hip arthroplasty, there is no evidence of difference between ceramic XLPE and metal XLPE.

The quality of evidence for the linear wear outcome is moderate (for both acetabular materials) due to the risk of severe bias (no blinding and no intention-to-treat analysis).

The outcome of surgical reviews is considered low due to the risk of bias (absence of blinding and analysis by intention to treat) and serious imprecision (long confidence interval).

In the HHS outcome, the evidence is of very low quality due to the risk of very severe bias (no blinding, intention-to-treat analysis, or sample size calculation) and severe imprecision (long confidence interval).

The quality of the femoral head outcome is also very low due to the risk of very severe bias (no blinding, no intention-to-treat analysis, and sample calculation), very severe inconsistency and indirect evidence (high heterogeneity), and high imprecision (long confidence interval).

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SUMMARY OF THE EVIDENCE

Comparing ceramic and metal surfaces in THAs for OA, we identified a statistical difference in the outcome of linear wear in patients who used the XLPE acetabular component, in favor of the ceramic surface. This evidence is of moderate quality. However, the amplitude of this effect is very small, almost negligible.

In the evaluation of linear wear, changing the acetabular component to the UHMWPE, there is no difference between the analyzed surfaces. This evidence is of moderate quality.

For the outcome of surgical reviews, no statistical differences were found and the quality of evidence is low.

In the HHS outcome, there was a difference in favor of the metal XLPE prosthesis in relation to the ceramic XLPE prosthesis, but with very low quality of evidence.

There is no evidence that less radiological wear of the ceramic XLPE surface can result in fewer surgical reviews and a better postoperative clinical evaluation.

CONCLUSION

Considering the linear wear, reviews, and clinical outcomes (HHS), there is no evidence in this evaluation demonstrating that ceramic XLPE prostheses are more effective than metal XLPE prostheses in THAs. Therefore, the higher cost of ceramics is not justified.

AUTHORS' CONTRIBUTIONS

IAZS: Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **AA:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **AU:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **HK:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **GT:** Writing – review & editing. **MMN:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **MA:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **OST:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **PO:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **WMB:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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Comment on “Obesity effects on sleep quality with anthropometric and metabolic changes”

André Pontes-Silva^{1*} 

Dear Editor,

I read with great appreciation the study by Tuna et al. entitled “Obesity effects on sleep quality with anthropometric and metabolic changes”¹. In this study, the authors aimed to determine the effect of obesity on sleep quality by using the Pittsburgh Quality Index and Berlin Question and evaluate the association of sleep with anthropometric and metabolic parameters. The study has an elegant rationale and is expected to generate new research in future. However, some methodological fragile reduces the clinical applicability of these results.

First of all, the authors used effect size for the sampling ($p=575$), but it is not described in the “Results” and/or “Discussion” sections (nor in tables). The results of this study are relevant; however, there is a need to look

beyond statistical significance (i.e., clinical relevance, not just p-value)².

Second, it is necessary to do Bonferroni’s correction for the outcome comparisons³. Why did the authors not do it? Besides, in the scientific context, technical terms should be used, e.g., “circumference” is wrong — measurements of body surfaces are called “perimeters.” In addition, “height” must be “stature” and “weight” should be “body mass” (note that the concept of BMI is body [mass] index, not body [weight] index)⁴.

Finally, the word “effects,” as described in the title, directs the reader’s reasoning to an experimental study (considering that only designs testing effects are clinical trials). In addition, this is an observational study, but the design is unclearly described. What checklist did the authors use to present these results?

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Comment on “Comparison of arterial stiffness and ultrasound indices in patients with and without chronic obstructive pulmonary disease”

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Dear Editor,

We have read with great interest the article entitled “Comparison of arterial stiffness and ultrasound indices in patients with and without chronic obstructive pulmonary disease” by Zhang et al.¹. In the study, the authors compared the differences in arterial stiffness and ultrasound indices between patients with chronic obstructive pulmonary disease (COPD) and non-COPD and found that COPD patients had higher intima-media thickness, total plaque area, number of plaques, arterial stiffness, and ankle-brachial blood pressure index compared with the control group. In addition, this study also found that arterial stiffness in COPD patients was negatively correlated with ankle-brachial blood pressure index and positively correlated with intima-media thickness. This study provides evidence to explain the underlying mechanisms behind the increased incidence of cardiovascular disease in COPD patients. However, the following concerns need to be addressed.

First, the subjects of this study were COPD patients. However, information on the duration of COPD and the frequency of exacerbations per month/year is lacking. Obviously, the longer the duration of COPD, the higher the level of arterial stiffness in COPD patients. This hypothesis has also been clarified by previous studies^{2,3}. In addition, information about drug therapy strategies and lung function in COPD patients is unknown. It is reasonable to assume that the treatment strategies in COPD patients, such as glucocorticoids, also have a potential influence on the level of arterial stiffness. Therefore, it

is highly recommended to provide information on COPD patients' disease duration and related drug treatment strategies to reduce confounding factors in conclusions.

Second, smoking, even household smoking or passive smoking, is one of the recognized risk factors for COPD. Passive smoking, both at home and at work, was associated with an increased incidence of COPD in a large population-based study⁴ (OR 3.80; 95%CI 1.29–11.2). In addition, a study involving 57,779 Chinese participants also demonstrated that cigarette smoking is one of the important risk factors for the development of COPD⁵. However, it is important to note that there was no significant difference in the percentage of smoking between COPD group and control group, as described in Table 1. This potentially leads to the misconception in clinical practice that smoking has no significant effect on the incidence of COPD and then resulting in a lack of necessary interventions and recommendations for smoking behaviors. Perhaps in the selection of patients, the authors have balanced for differences in smoking between the two groups. However, this process needs to be clarified in this study.

AUTHORS' CONTRIBUTIONS

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

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Comment on “Alemtuzumab improves cognitive processing speed in active multiple sclerosis – a longitudinal observational study”

Elnaz Asadollahzade¹ , Fereshteh Ghadiri¹ , Zahra Ebadi¹ , Abdorreza Naser Moghadasi^{1*} 

Dear Editor,

An interesting article was recently read about the effect of alemtuzumab on cognitive impairment (CI) in multiple sclerosis (MS)¹. This brings up the question whether other highly efficacious treatments can impact CI? If the answer is positive, is it permissible to select an escalation approach?

MS is a debilitating disease of central nervous system (CNS), which is common among young adults with noticeable economic consequences on the government². Due to widespread distribution of lesions, MS manifests a broad range of the symptoms. CI is one of the most critical symptoms, with prevalence rate ranging from 43 to 70%³, embracing all types of clinical courses and disease stages⁴. The evidence suggests that neuropsychological scores are better in relapsing-remitting (RR) patients compared to secondary-progressive (SP) and primary-progressive (PP) cases⁵. Cognitive impairment is more severe in SP patients than in PP patients⁶. Unfortunately, the effect of disease-modifying therapy (DMTs) on cognition is not well known. There is less evidence that DMTs are beneficial to improve the cognition¹. However, there is still no clear answer to this question: Do high potent DMTs significantly impact CI by slowing and stabilizing the course compared with low potent drugs? Clarification of this issue seems to make a significant change in treating MS patients.

Studies showed a link between CI and brain atrophy. Brain atrophy can be seen in the early stages of MS, which is associated with a decrease in brain volume and function. It was found that the higher the severity of cognition impairment in the patients, the higher the severity of brain atrophy^{7,8}. Previously, the prevention of relapses was an important goal for treating MS patients⁹. However, this approach has changed, and improving the patients' clinical condition and remission was considered a goal in advancements of the treatments and using new drugs. Therefore, in MS, as in many diseases, such as cancer and rheumatoid arthritis, the term “no evidence of disease activity” (NEDA) is used. NEDA is used as a criterion to

assess the clinical outcome of DMT¹⁰. Brain atrophy is known as NEDA-4 diagnostic benchmark, which can be used to diagnose better and understand the disease's activity and progression. Brain atrophy can manifest itself as CI¹¹. CI can affect patients' lifestyles and social activities¹². It seems that DMTs could alter the CI course¹³.

Two approaches (escalation and induction) were used to treat MS patients¹⁴. In recent years, it has been shown that the induction approach to patients with high performance drugs can reduce the survival of patients in terms of side effects of the drugs because drugs with a higher risk profile are used from the beginning¹⁵. In contrast, an escalation is an approach that starts treating patients with low-risk, moderately effective drugs. If the patient poorly responds to this treatment, more aggressive treatments are used to reduce the risk of complications¹⁵. Also, the advantage of escalation approaches is to allow many patients to have a satisfying control of the disease, while receiving relatively safe drugs and never escalating to more aggressive therapy¹⁶.

The diagnosis of MS with high accuracy along with the predictive feature is very useful because it can determine the initiation of early treatment. CI can be considered a prognostic factor for MS. Consequently, the CI value considered as a measure of exacerbation is a question that needs to be answered in future studies.

AUTHORS' CONTRIBUTIONS

EA: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, validation, visualization, writing – original draft, writing – review & editing. **FG:** Funding acquisition, methodology, writing – original draft, writing – review & editing. **ZE:** Conceptualization, data curation, formal analysis, investigation, project administration, resources, software, supervision, validation, visualization, writing – original draft. **ANM:** Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – review & editing.

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Comment on “Comparison of severe acute respiratory syndrome coronavirus 2 (COVID-19) vaccine side effects by age groups”

Qiongjiao Wang¹ , Yun Yang^{1*} 

Dear Editor,

In the article entitled “Comparison of severe acute respiratory syndrome coronavirus 2 (COVID-19) vaccine side effects by age groups” by Tosun et al¹, the authors compared differences in side effects of severe acute respiratory syndrome coronavirus 2 (COVID-19) vaccine across age groups. The online questionnaire was received by 411 participants who received the 2019 inactivated coronavirus disease vaccine. All patients were categorized into four groups according to their age (20–35, 36–50, 51–65, and >65 years), and their results found that vaccine-related side effects are most common in patients aged 20–35 years (68.2%) and least in those aged over 60 (38.8%) years. In addition, they also found that female and young age were important factors in determining the development of vaccine-related side effects. This study provides an important basis for the detection and tracking of vaccine-related side effects in different age groups. However, we note some issues that require further clarification.

First, the brand of COVID-19 vaccine that the participants received was unclear. Currently, the widely used COVID-19 vaccines include JNJ-78436735 (Johnson & Johnson), BNT162b2 (Pfizer/BioNTech), and mRNA-1273 (Moderna). The results of a previous study² had shown that there are significant differences in adverse events caused by different brands of COVID-19 vaccines. mRNA-1273-related adverse events

were the most (mRNA-1273 vs. BNT162b2; OR 2.00; 95%CI 1.86–2.15; $p < 0.001$), while JNJ-78436735-related vaccine adverse events were the least (JNJ-78436735 vs. BNT162b2; OR 0.64; 95%CI 0.52–0.79; $p < 0.001$). Therefore, it is necessary to clearly describe the vaccine brands received by the participants involved in the study by Tosun et al¹.

Second, the study did not clarify whether participants received the first or second dose of the COVID-19 vaccine. Vaccine-related side effects after the first and second doses were significantly different. Findings based on a large study showed that the allergic reaction or anaphylaxis after the first dose of the vaccine was higher than that of the second dose (0.3% vs. 0.2%)². Similarly, another study indicated that no side effects were reported by 18% of participants after the first dose and 31% of participants after the second dose³. Based on the evidence mentioned above, the vaccine-related side effects of the first dose appear to be higher than those of the second dose. Therefore, it is essential to clearly describe whether the vaccine-related side effects appear after the first dose or the second dose.

AUTHORS' CONTRIBUTIONS

QW, YY: Conceptualization, writing – original draft, writing – review & editing.

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Comment on “The role of Epstein-Barr virus in multiple sclerosis: from molecular pathophysiology to *in vivo* imaging”

Elnaz Asadollahzade¹ , Fereshteh Ghadiri¹ , Zahra Ebadi¹ , Abdorreza Naser Moghadasi^{1*} 

Dear Editor,

Vaccination to reduce the incidence of demyelination diseases is becoming an ever-increasing global health priority. This is largely due to neurological manifestations and sequelae from the existing and emerging central nervous system infections that account for significant morbidity and mortality worldwide. Some existing studies support the direct and indirect effects of viral infections on the nervous system. The link between Epstein-Barr virus (EBV) and multiple sclerosis (MS) is supported by the elevated EBV-specific antibody levels in MS patients when compared to healthy controls¹. In contrast, we also observed the association of demyelinating diseases with COVID-19 during the pandemic of this virus². Demyelinating disorders of the central nervous system (CNS) can appear after vaccination. Some case report studies have reported that vaccination against COVID-19 in MS patients who are in remission can cause MS relapse³. A 68-year-old woman with MS was diagnosed with neuromyelitis optica spectrum disorder (NMOSD) after immunization against COVID-19⁴. In this narrative review, the potential association between vaccination and the prevention of demyelination processes caused by viruses is discussed.

In the long term, the COVID-19 epidemic may be the cause of neurological diseases in the future⁵. The exact effects of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) on neurological diseases are currently unknown. But different pathophysiological theories support neurodegeneration with SARS-CoV-2. It is possible that COVID-19, as an aggravating factor, is the cause of neurological symptoms or the acceleration of neurological conditions in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD)⁶. The importance of other viral infections in the exacerbation of neurological diseases has been investigated in recent years. However,

the possible consequences of infection with SARS-CoV-2 for triggering neurodegeneration and causing neurodegenerative diseases are not precisely known⁷. We know that vaccination against SARS-CoV-2 prevents a severe course of disease⁵ and can prevent the onset of neurodegenerative diseases.

There are less data regarding the safety or efficacy of the vaccines in patients with preexisting neurological conditions. Given the widespread effect of the COVID-19 pandemic on adults with neurological disease, the risks and benefits of vaccination must be considered for each patient. Based on COVID-19 vaccine data from the general population and extrapolations from other vaccines studies in patients with neurological diseases, statements from the American Academy of Neuromuscular and Electrodiagnostic Medicine and the National Multiple Sclerosis Society support vaccination. The risk of onset or relapse of CNS demyelination following infections against which the vaccines are aimed to protect is substantially higher and the benefits of vaccinations surpass the potential risks of CNS inflammation⁸. As a link has been found between EBV and MS, other interventions that prevent EBV infection or treat EBV could also reduce the incidence of MS, for example, preventing EBV infection by vaccination at a very young age. However, EBV vaccine may have two complications. First, the vaccine itself can cause demyelinating disease after vaccination against viral diseases. Second, by suppressing EBV, the role of other viruses in the pathogenesis of demyelinating disease may increase⁹. Based on the existing evidence, neurologists should recommend COVID-19 vaccination to their patients. For those patients being treated with immunotherapies, attention should be paid to the timing of vaccination, concerning the treatment and the potential for an attenuated immune response.

Therefore, the point to keep in mind is that sometimes vaccination itself can cause demyelinating disease.

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Optical coherence tomography changes in ankylosing spondylitis patients on long-term adalimumab treatment

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SUMMARY

OBJECTIVE: Long-term ocular effects of tumor necrosis factor-alpha inhibitors remain to be elucidated. This study aimed to examine the long-term effects of adalimumab use on neural tissue of the anterior visual pathways using optical coherence tomography in patients with ankylosing spondylitis.

METHODS: This was a single-center, open-label, cross-sectional study conducted at the Giresun University Faculty of Medicine, Physical Medicine and Rehabilitation Department, between November 2019 and August 2020. This study included 26 ankylosing spondylitis patients receiving adalimumab for at least 1 year and 21 healthy controls. All subjects underwent a full ophthalmological examination and optical coherence tomography examination with the following measurements: peripapillary retinal nerve fiber layer thickness, peripapillary retinal thickness, peripapillary choroidal thickness, ganglion cell complex thickness, and the optic head properties.

RESULTS: Peripapillary retinal nerve fiber layer thickness and retinal thickness measurements were lower in the adalimumab group. In addition, ganglion cell complex thickness was significantly lower and the cup-to-disc ratio was significantly higher in the adalimumab group ($p < 0.05$). However, the two groups did not differ in terms of peripapillary choroidal thickness and disc area ($p > 0.05$).

CONCLUSION: Although tumor necrosis factor-alpha inhibitors have some favorable effects on the ocular involvement of patients with ankylosing spondylitis, they may also have paradoxical detrimental effects as evidenced by structural changes observed by optical coherence tomography. Future studies with better design, probably including a large number of patients with a range of rheumatological diseases and tumor necrosis factor-alpha inhibitors, are warranted.

KEYWORDS: Tumor necrosis factor alpha. Adalimumab. Optical coherence tomography.

INTRODUCTION

Ankylosing spondylitis, a form of axial spondyloarthritis (SpA), is a chronic inflammatory rheumatic disease and manifests itself by inflammatory back pain, radiographic sacroiliitis, and excess spinal bone formation, as well as non-skeletal manifestations such as uveitis, inflammatory bowel disease (IBD), or psoriasis¹. Reported prevalence rates range between 0.1 and 0.5%^{1,2}.

Tumor necrosis factor-alpha (TNF-alpha) is a cytokine with a key regulatory role in the inflammatory response, and impaired regulation of TNF-alpha has been suggested to have a role in the pathogenesis of inflammatory conditions. Blocking the action of TNF-alpha has been used in the treatment of chronic inflammatory conditions, including ankylosing spondylitis. Adalimumab is a recombinant human IgG1 monoclonal antibody, specific for human TNF-alpha.

Despite its revolutionary benefits in rheumatologic disease, adverse effects such as ocular complications, severe infection,

demyelinating conditions, malignancies, a lupus-like syndrome, induction of autoantibodies, injection site reactions, and heart failure have been reported with the use of TNF-alpha inhibitors³. Reported ocular side effects include periorbital infection, oculomotor nerve palsy, optic neuritis, central vein occlusion, as well as a paradoxical adverse event uveitis⁴⁻⁸. Most data on ocular side effects come from anecdotal reports, and pathophysiological processes are mostly unknown³; thus, ocular structural changes during long-term TNF-alpha inhibitor use may shed light on the pathogenesis of these conditions.

Optical coherence tomography (OCT) is a practical technique widely used in clinical practice, which enables detailed examination of the eye and allows the thicknesses of the choroid, retina, and peripapillary retinal nerve fiber layer (RNFL) to be measured.

This study aimed to examine the potential long-term adverse effects of adalimumab use on neural tissue of the anterior visual

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pathways through the measurements of peripapillary RNFL thickness, peripapillary retinal and choroidal thicknesses, optic nerve parameters, and ganglion cell complex (GCC), using OCT.

METHODS

The study protocol was approved by the Institutional Review Board of Hatay Mustafa Kemal University Medical Faculty (date June 3, 2021, number 07), and the study followed the tenets of the Declaration of Helsinki.

A total of 26 eyes of 26 ankylosing spondylitis patients on adalimumab attending the outpatient clinics of the Physical Therapy and Rehabilitation department (adalimumab group) and 21 eyes of 21 healthy controls were included in this single-center, open-label, cross-sectional study. All eyes underwent a full ophthalmological examination including refractive measurements with an autorefractor (Topcon Auto Ref-Keratometer, Tokyo, Japan), best corrected visual acuity (BCVA) evaluation, intraocular pressure measurements, biomicroscopic evaluation with slit-lamp examination, and OCT examination. Considering that outcome measurements from both eyes of the same subject tend to be positively correlated⁹, the right eye was selected for study measurements if both eyes were suitable for inclusion.

According to inclusion criteria, patients had to fulfill modified New York diagnostic criteria for ankylosing spondylitis, had to be >18 years of age, and should be receiving adalimumab for at least 1 year and provide informed consent. The patients attending the outpatient clinics of Giresun University Faculty of Medicine, Physical Medicine and Rehabilitation Department between November 2019 and August 2020 were included. Exclusion criteria were as follows: additional systemic disease, previous intraocular surgery or intracranial surgery, previous history of optic disc edema, and the presence of glaucoma and/or ocular hypertension, papillitis, or any other retinal disease.

In all, 21 patients without any systemic disease or ocular pathology who applied to the Physical Therapy and Rehabilitation outpatient department for other reasons were served as controls. Written informed consent was obtained from all subjects prior to the study.

Patients were receiving 40 mg of subcutaneous adalimumab every other week for at least 1 year and all were under remission (BASDAI<4) for at least 6 months. None of them were receiving concomitant glucocorticoids.

All eyes were examined by a spectral domain OCT device (Retinascan Advanced RS-3000; NIDEK, Gamagori, Japan). All OCT examinations were done by an investigator blinded to the patient groups, analyzed, and interpreted by using a software (NAVIS-EX, NIDEK, Tokyo, Japan). The following measurements were taken:

- **Peripapillary RNFL thickness** In the peripapillary region, a circular 3.45-mm diameter scan centered over the optic disc was used to measure RNFL thickness (using “disc circle” option). Average peripapillary RNFL thickness (360°) as well as peripapillary RNFL thicknesses at superior (46°–135°), inferior (226°–315°), nasal (136°–225°), and temporal (316°–45°) quadrants were measured.
- **Peripapillary retinal thickness** Using the same image and manually changing the lower border as the retina pigment epithelium, the average peripapillary retinal thickness (RT) and RTs at the superior, nasal, inferior, and temporal quadrants were measured. The temporal-superior-nasal-inferior thickness (TSNIT) graph (ILM-RPE/BM) was automatically displayed after this manual marking.
- **Peripapillary choroidal thickness** The average peripapillary choroidal thickness and peripapillary choroidal thicknesses of the four quadrants were estimated using the image obtained by the inversion of the peripapillary region image. Again, a circular 3.45-mm diameter scan centered over the optic disc was used with the “disc circle” option. The scans consisted of 1,024 “A scans” of high-definition (50 HD) frame enhancement software. To improve the choroidal image, the OCT device located the inversed image in closer proximity to the zero-delay line and displayed it on the upper part of the monitor. The outer border of the hyper-reflective retinal pigment epithelium (RPE/BM) was displayed automatically, and the sclera-choroidal vertical distance was manually drawn. The TSNIT graph (RPE/BM-manual [choroid]) was created automatically using the software.
- **GCC thickness and the optic head** Macula map X-Y (6.0–6.0 mm [256–256]) of the OCT device was used for GCC thickness map (ILMIPL/INL), and disc map X-Y (6.0–6.0 mm [256–256]) setting was used for optic nerve head properties.
- **Statistical analysis** The SPSS (Statistical Package for Social Sciences) software version 27.0 was used for statistical analyses. Descriptive data were presented as mean±standard deviation, median (range), and frequency (percentage), where appropriate. The normality of the data was tested using the Kolmogorov-Smirnov test. Independent samples t-test and Mann-Whitney U test were used for the comparison of quantitative data, depending on the distribution. A chi-square test was used for the comparison of qualitative data.

RESULTS

Patient characteristics

Table 1 shows the comparisons of the demographic and clinical characteristics. The groups did not differ in terms of age, gender distribution, distribution of the included eye (right or left), visual acuity, intraocular pressure, or spherical equivalent ($p > 0.05$ for all comparisons).

OCT findings

Table 2 summarizes the OCT findings of the two groups. The average peripapillary RNFL thickness was significantly lower in the adalimumab group compared to that of the controls ($p < 0.001$). In addition, the former group had lower RNFL measurements in all four quadrants ($p < 0.05$ for all). However, the two groups did not differ in terms of peripapillary choroidal thickness ($p > 0.05$ for all). The adalimumab group had significantly lower average peripapillary RT measurements ($p < 0.05$) than controls, and this relationship was true for all ($p < 0.05$) but the nasal quadrant ($p = 0.115$).

The GCC thickness was significantly lower (in both superior and inferior) and the cup-to-disc ratio (both horizontal and vertical) was significantly higher in the adalimumab group ($p < 0.05$ for all). Although the average cup area was higher in the adalimumab group ($p < 0.05$), the two groups did not differ in terms of disc area ($p = 0.358$).

DISCUSSION

In our study examining the potential effects of adalimumab use for a relatively long duration, we identified negative effects on several OCT parameters; however, such an effect does not seem to translate into significant ophthalmological problems, at least within 1 year, as evidenced by no change in visual acuity, spherical equivalents, or intraocular pressure. To the best of our knowledge, this is the first study to examine the effects

of longer adalimumab use on OCT parameters in comparison to healthy controls.

There is only one study in the literature examining the effect of TNF-alpha inhibitors on OCT findings in patients with ankylosing spondylitis¹⁰. In that 2015 study, 21 nonresponsive patients to non-steroidal anti-inflammatory drugs (NSAIDs) and sulfasalazine received one of the three TNF-alpha inhibitors (etanercept, adalimumab, or infliximab) and were followed with OCT measurements for peripapillary RNFL, GCIPL, and macular thickness. During the 6-month follow-up, no significant difference was detected in OCT parameters or intraocular pressure, although the treatment was effective in disease control. The authors concluded that TNF-alpha blockage does not influence the measured OCT parameters in patients with ankylosing spondylitis, at least in the short term. Those findings are in contrast with the findings of the present study, which found significant changes in OCT findings of ankylosing spondylitis patients receiving adalimumab for at least 1 year. There may be several explanations for such a difference. First, in that study, 21 patients received three different TNF-alpha inhibitors and only 7 patients received adalimumab. Less number of patients in the whole study group might have prevented to reach adequate statistical power. In addition, such a design with less number of patients receiving adalimumab may not be adequate for the determination of drug-specific effects on OCT findings. Second, 6-month follow-up may not be sufficient to detect any effect. On the contrary, our design may not be able to differentiate between disease- and drug-specific effects, since it did not include ankylosing spondylitis patients not receiving adalimumab or a TNF-alpha inhibitor; rather, our controls were healthy individuals. Thus, studies with a more detailed design and longer follow-up are required to examine any potential effects of TNF-alpha inhibitors in general, particularly adalimumab.

TNF-alpha is a cytokine with roles in the inflammatory processes. Understanding its key role in the pathogenesis of autoinflammatory diseases paved the way to the development

Table 1. Demographic and clinical features of the groups.

	Adalimumab group (n=26)	Control group (n=21)	p-value
Age, years	45.3 (9.6)	40.5 (12.1)	0.141 ^t
Male gender, n (%)	17 (65.4)	13 (61.9)	0.805 ^x
Right eye, n (%)	20 (76.9)	16 (76.2)	0.953 ^x
BCVA	1.00 (0.10)	1.00 (0.00)	0.122 ^m
SE, diopter	0.00 (1.00)	0.00 (1.13)	0.786 ^m
IOP, mmHg	13.50 (5.25)	13.00 (5.00)	0.620 ^m

BCVA: best corrected visual acuity; SE: spherical equivalent; IOP: intraocular pressure; M/F: male/female; R/L: right/left. Unless otherwise stated, data presented as mean (standard deviation) for parametric test and median (interquartile range) for nonparametric test. ^tt-test; ^mMann-Whitney U test; ^x χ^2 test.

Table 2. Comparison of the groups for optical coherence tomography parameters.

	Adalimumab group (n=26)	Control group (n=21)	p-value
RNFL thickness (µm)			
Average	89.9 (10.1)	107.2 (7.8)	<0.001^t
Superior	121.1 (17.9)	136.4 (13.0)	<0.05^t
Nasal	61.7 (9.8)	77.5 (8.2)	<0.001^t
Inferior	119.5 (13.2)	139.3 (11.8)	<0.001^t
Temporal	66.0 (10.8)	75.1 (7.6)	<0.05^t
Choroidal thickness (µm)			
Average	191.2 (9.2)	193.6 (12.4)	0.437 ^t
Superior	189.0 (7.8)	190.5 (10.7)	0.341 ^t
Nasal	186.8 (8.5)	188.4 (13.1)	0.655 ^t
Inferior	187.2 (8.7)	188.8 (9.0)	0.251 ^t
Temporal	193.9 (11.2)	191.3 (10.1)	0.399 ^t
Retinal thickness (µm)			
Average	310.7 (14.8)	336.3 (11.7)	<0.05^t
Superior	324.1 (10.8)	330.3 (10.1)	<0.05^t
Nasal	315.5 (11.5)	321.3 (11.7)	0.115 ^t
Inferior	314.1 (14.8)	344.7 (13.1)	<0.05^t
Temporal	305.0 (16.1)	335.7 (10.8)	<0.05^t
GCC thickness (µm)			
Superior	85.9 (14.3)	104.7 (7.9)	<0.001^t
Inferior	87.5 (13.9)	107.7 (8.8)	<0.001^t
Cup-to-disc ratio			
Horizontal	0.491 (0.182)	0.385 (0.116)	<0.05^t
Vertical	0.513 (0.221)	0.397 (0.137)	<0.05^t
Average cup area (mm ²)	0.837 (0.49)	0.638 (0.291)	<0.05^t
Disc area (mm ²)	2.396 (0.604)	2.481 (0.454)	0.358 ^t

OCT: optical coherence tomography; RNFL: retinal nerve fiber layer; GCC: ganglion cell complex. Data presented as mean (standard deviation) for parametric test and median (interquartile range) for nonparametric test. ^tt-test. Bold values indicate statistical significance.

of biological treatments for the blockade of TNF-alpha with good treatment responses. They have been found effective for the extra-articular involvement of rheumatological disease including the eye as well as for musculoskeletal manifestation^{1,11,12}. For example, noninfectious uveitis attacks can develop during autoinflammatory diseases such as ankylosing spondylitis or Behçet's disease, where disease activity seems to be correlated with TNF-alpha. Adalimumab, a recombinant human immunoglobulin G1 monoclonal antibody specifically binding TNF-alpha, has been shown to be effective in the treatment of intermediate, noninfectious posterior uveitis and panuveitis cases. In the VISUAL III study, intraocular inflammation was better controlled and systemic steroid use was reduced in patients with active and inactive noninfective uveitis who received adalimumab for more than 78 weeks¹².

TNF-alpha antagonist may be associated with paradoxical adverse events, including uveitis⁸. However, in such cases, infectious conditions such as tuberculosis should be ruled out. Several other ocular conditions such as severe inflammation of the orbit have been reported in association with adalimumab⁴. Optical neuritis leading to irreversible vision loss may also develop in association with anti-TNF-alpha treatments. In a study with 68 multiple sclerosis patients receiving anti-TNF-alpha treatment, more recurrences were observed when compared to the controls¹³.

Changes in OCT findings in association with adalimumab use found in this study potentially suggest a neurotoxic side effect. Although rare, TNF-alpha inhibition-related neurotoxicities/neuropathies, such as multiple sclerosis, Guillain-Barre and Miller-Fischer syndrome, chronic inflammatory demyelinating

peripheral neuropathy, mononeuropathy simplex, mononeuropathy multiplex, and multifocal motor neuropathy, have been reported, mostly in case reports¹⁴. In a large safety analysis of adalimumab, it is estimated that the incidence of demyelinating disorders is less than 0.1 per 100 patient-years¹⁵. The fact that functional impairment in vision was not evaluated in this study highlights the need for a more detailed investigation of such a potential effect. The decrease in apoptosis of retinal ganglion cells with neutralizing antibodies against TNF- α ¹⁶ also weakens the claim that inhibition of TNF- α may have a direct detrimental effect on retinal nerves.

This study has several limitations. First, a control group consisting of ankylosing spondylitis patients who did not receive adalimumab would explain whether any change is associated with the disease itself. Second, a prospective design would elucidate the relation of any changes with time. Finally, a design with a larger number of participants would improve study power.

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CONCLUSION

Although TNF- α inhibitors have some favorable effects on the ocular involvement of patients with ankylosing spondylitis, they may also have detrimental effects, some of which may be paradoxical. The findings from this study suggest that at least some structural changes detectable with OCT may occur in the long-term use of adalimumab in patients with ankylosing spondylitis. However, whether such changes translate into functional impairment remains to be elucidated in future studies with better controlled designs, probably including a large number of patients with a range of rheumatological diseases.

AUTHORS' CONTRIBUTIONS

NCY: Conceptualization, Writing – original draft, Writing – review & editing. **MAO, IFS:** Data curation, Formal analysis, Writing – review & editing. **SK:** Data curation, Writing – review & editing. **ST, HO:** Writing – review & editing.

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The willingness and attitudes of medical students regarding organ donation and transplantation: a cross-sectional study from Turkey

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SUMMARY

OBJECTIVE: Positive attitudes and motivation on the part of medical students concerning organ donation and transplantation are very important in terms of the growing need for these. This study aimed to evaluate the willingness and attitudes of medical students toward organ donation and transplantation.

METHODS: This cross-sectional study was performed at a state university in Turkey in February–March 2020. The questionnaire investigated sociodemographic characteristics and willingness toward organ donation and transplantation and contained the Organ Donation Attitude Scale.

RESULTS: A total of 309 medical students participated, of which 71.2% were willing to donate their organs. Medical students' willingness to donate organs increased depending on gender, academic year, receipt of education on the subject, discussing donation with family and friends, possession of an organ donation card, knowledge of the organ donation system, and willingness to receive organ donation if necessary. Positive attitudes toward organ donation increased after discussing the subject with family and friends, possessing an organ donation card, and knowing the path to be followed for organ donation.

CONCLUSION: Medical students exhibited high willingness and positive attitudes regarding organ donation and transplantation. However, education on the subject of organ donation and transplantation is needed.

KEYWORDS: Attitude. Students, medical. Organ transplantation. Tissue transplantation. Tissue and organ procurement.

INTRODUCTION

More than 15 million people aged between 30 and 69 years die from noninfectious diseases every year. In total, 85% of these “early” deaths occur in low- and moderate-income countries¹. Changing population demographics and an increasing prevalence of risk factors have contributed to the growing demand for organ replacement therapies. Transplantation is, therefore, the only option for the restoration of organ function and preventing early death for many patients². Transplantation restores not only organ function but also the quality of life. Although there are differences between countries, an estimated 78,627 kidney, 31,044 liver, 7,928 heart, and 5,788 lung transplantations were performed worldwide in 2020³.

Nonmedical obstacles to transplantation include negative attitudes, inadequate role perception, poor motivation, knowledge, expertise, and deficient communication skills at the level of the health worker². Information concerning the benefits and practical aspects of transplantation must therefore be included in the curricula of all health practitioners, from undergraduate

medical students (MS) to postgraduate training for specialists and practicing physicians. The presence of health professionals trained in patient education as part of the treatment team will positively impact the donation process. Positive developments in terms of knowledge and attitudes concerning organ donation (OD) may be a useful strategy for expanding the limited donor pool⁴. Understanding the factors affecting OD and attitudes toward the subject is, therefore, very important in terms of the process involved. The purpose of this study was to evaluate the willingness and attitudes of MS toward OD and transplantation.

METHODS

This cross-sectional survey study was performed between February 14, 2020, and March 10, 2020. A total of 1,569 MS from all academic years were enrolled in the faculty at that time. Assuming an OD willingness level of 50%, a total study sample size of 309 MS was calculated at a 95% confidence interval

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($\alpha=0.05$) and a sample error of $d=0.05$. The layered sampling by years method was employed. The study included people with MS over the age of 18 years. Informed consent was obtained from the MS, and approval for the study was granted by the Ethical Committee. Data were collected with a questionnaire at face-to-face interviews. The questionnaire consisted of two sections. The first section contained questions eliciting sociodemographic information. The second section contained questions concerning the receipt of education concerning OD (yes/no) and the organs the MS would be willing to OD. The Organ Donation Attitude Scale (ODAS) was employed in the second part. The ODAS was developed by Parisi and Katz⁵ and subsequently revised by Kent and Owens⁶. The reliability and validity of the Turkish-language version were established by Yazici Sayin et al.⁷ The ODAS is a 40-item 6-point Likert-type scale. A total of 20 items measure positive attitudes and 20 measure negative attitudes. High positive attitude scores and low negative attitude scores indicate a positive attitude toward OD.

The data were analyzed using the IBM SPSS version 21.00 software and were expressed as number, percentage, and mean \pm standard deviation. The chi-square test was used to compare categorical variables. The normality of distribution was assessed using the Kolmogorov-Smirnov test and graphs. The independent-sample t-test and ANOVA were applied to compare continuous variables. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 309 MS participated in the study. The mean age of MS was 21.6 ± 2.3 years, 59.9% were female, 23.6% were in the first year, and 8.1% had a chronic disease. In total, 46% of MS had received education concerning OD, and 69.9% wished to see education about OD being given every year. In addition, 46.9% reported having discussed OD with their families and 70.6% with their friends. A total of 37.2% of MS understood the path followed for OD in Turkey, while 73.8% reported knowing about the “record system” in Turkey, and 71.2% considered donating organs. In addition, 9.7% of MS had OD cards. Notably, 57.6% of MS reported that organ transplantation from live donors was most frequently performed in Turkey, and 95.8% stated that they would accept ODs if necessary (Table 1). The organs that MS were most willing to donate, in descending order, were blood, kidneys, and liver, while the organs they were least willing to donate were upper airway organs, the extremities, the face, and hairy skin (Figure 1).

MS' willingness regarding OD varied significantly depending on gender ($p=0.034$), their academic year ($p=0.031$), receipt of education on the subject of OD ($p<0.001$), discussion of

OD with the family ($p<0.001$), discussion of OD with friends ($p<0.001$), possession of an OD card ($p=0.001$), knowledge of the OD system in Turkey ($p<0.001$), and their own willingness to accept OD if necessary ($p=0.042$) (Table 1).

ODAS positive attitude subscale scores were higher among women ($p=0.012$), MS who had discussed OD with their families ($p=0.008$), those who were willing to donate organs ($p<0.001$), and those with OD cards ($p=0.022$) (Table 2). ODAS negative attitude subscale scores were higher among men ($p=0.006$), MS with no education concerning OD ($p<0.001$), those who had not discussed OD with their families ($p<0.001$), who had not discussed OD with friends ($p<0.001$), who were unaware of the OD process ($p<0.001$), who were reluctant to donate organs ($p<0.001$), who did not possess OD cards ($p<0.001$), who were unaware of the OD system in Turkey ($p<0.001$), and who stated they would not accept OD if they required it ($p=0.001$). The first-year MS had the highest negative attitude scores, while the lowest scores were observed in the fifth-year MS. Negative attitude scores differed significantly between the academic years ($p<0.001$) (Table 2).

DISCUSSION

A positive attitude toward OD among physicians and MS is an important factor in raising OD rates in society as a whole. The rate in the present study (71.2%) was slightly lower than that in other countries. The reported rates of OD willingness among MS patients range from 80 to 93%^{4,8,9}. OD willingness among MS indicated a positive attitude. A study reported a significant association between positive attitudes toward OD and willingness to donate¹⁰. Deficiencies regarding education have been observed to underlie MS' prejudices toward OD. Misunderstandings and myths on the subject break down as the levels of education rise. A study showed that willingness to donate could be increased through interviews with MS of an educational nature⁸. A study reported an increase in participants' willingness to donate and the disappearance of myths and false beliefs after education¹¹. MS reported feeling a need for education on the subject of OD¹². In the present study, education on the subject of OD was found to increase the willingness to donate. Individuals who had received education on the subject were more willing to donate organs. In addition, most MS stated that they wished to receive such education every year. Including this subject in the curriculum, every year can help keep OD a dynamic topic and encourage and increase willingness to donate.

Consistent with the previous literature, the MS in the present study looked favorably on OD^{12,13}. In terms of the relationship

between gender and OD, studies have reported that women hold more positive attitudes than men^{4,14}. Women also regarded OD more positively than men in the present study. However, another study reported no gender difference regarding attitudes toward OD¹⁵. In contrast to the present research findings, a study from India reported that men exhibited more positive

attitudes than women¹⁶. These differences may be derived from sociocultural variations among the study communities. Increase in positive attitudes to OD was in line with academic years^{4,17}, as MS became more familiar with death and the donation process and were in closer contact with clinical specialties⁴. In one study, the first- and third-year MS had more positive attitudes

Table 1. Characteristics of medical students and their willingness to donate organs.

Variable	Category	Total 309 (100)	Yes 220 (77.2)	No 89 (22.8)	p-value
		n (%)	n (%)	n (%)	
Genderw	Female	185 (59.9)	140 (75.7)	45 (24.3)	0.034
	Male	124 (40.1)	80 (64.5)	44 (35.5)	
Academic year	First	73 (23.6)	45 (61.6)	28 (38.4)	0.031
	Second	57 (18.4)	37 (64.9)	20 (35.1)	
	Third	45 (14.6)	30 (66.7)	15 (33.3)	
	Fourth	46 (14.9)	34 (73.9)	12 (26.1)	
	Fifth	44 (14.2)	38 (86.4)	6 (13.6)	
	Sixth	44 (14.2)	36 (81.8)	8 (18.2)	
Chronic disease	Yes	25 (8.1)	18 (72.0)	7 (28.0)	0.926
	No	284 (91.9)	202 (71.1)	82 (28.9)	
Receipt of education concerning organ donation	Yes	142 (46.0)	114 (82)	25 (18)	<0.001
	No	167 (54.0)	106 (62.4)	64 (37.6)	
Wishing to receive annual education concerning organ donation	Yes	216 (69.9)	156 (72.2)	60 (27.8)	0.544
	No	93 (30.1)	64 (68.8)	29 (31.2)	
Discussion of organ donation with the family	Yes	145 (46.9)	123 (84.8)	22 (15.2)	<0.001
	No	164 (53.1)	97 (59.1)	67 (40.9)	
Discussion of organ donation with friends	Yes	218 (70.6)	167 (76.6)	51 (23.4)	0.001
	No	91 (29.4)	53 (58.2)	38 (41.8)	
Organ transplantation in a relative	Yes	39 (12.6)	25 (64.1)	14 (35.9)	0.295
	No	270 (87.4)	195 (72.2)	75 (27.8)	
A relative on the organ transplantation waiting list	Yes	19 (6.1)	14 (73.7)	5 (26.3)	0.805
	No	290 (93.9)	206 (71.0)	84 (29.0)	
Knowledge of the organ donation process	Yes	115 (37.2)	89 (77.4)	26 (22.6)	0.064
	No	194 (62.8)	131 (67.5)	63 (32.5)	
Possession of an organ donation card	Yes	30 (9.7)	29 (96.7)	1 (3.3)	0.001
	No	279 (90.3)	191 (68.5)	88 (31.5)	
The most common type of organ transplantation in Turkey	Living donor	178 (57.6)	131 (73.6)	47 (26.4)	0.278
	Cadaveric	131 (42.4)	89 (67.9)	42 (32.1)	
Knowledge of the organ donation record system in Turkey	Yes	228 (73.8)	175 (76.8)	53 (23.2)	<0.001
	No	81 (26.2)	45 (55.6)	36 (44.4)	
Willingness to receive organ donation if necessary	Yes	296 (95.8)	214 (72.3)	82 (27.7)	0.042
	No	13 (4.2)	6 (46.2)	7 (53.8)	

Bold values indicate statistical significance.

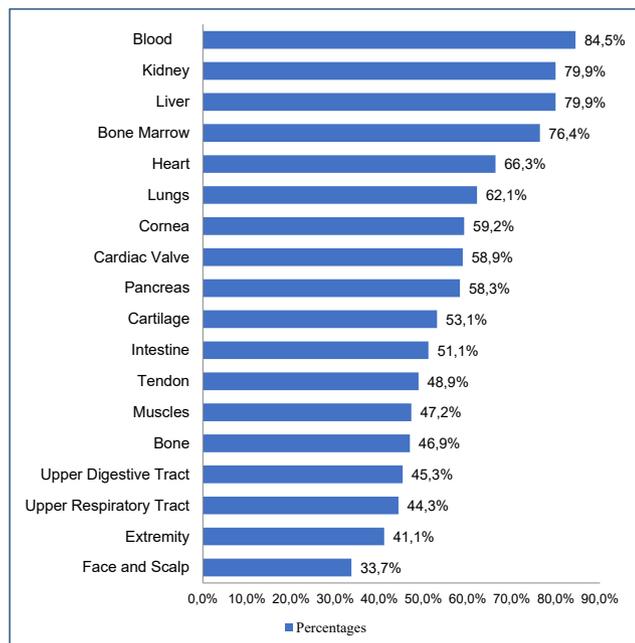


Figure 1. Percentage of organs that medical students were willing to donate.

toward OD¹⁶. However, a study from Pakistan reported no change in attitudes toward OD as education progressed¹⁸. Another study reported that MS' positive attitudes toward OD increased in line with their academic years, while no such changes occurred in nursing or health sciences students¹³. A study from China also reported no association between attitudes toward OD and academic years¹⁵. Although there was no regular increase in positive attitudes by years in the present study, MS' highest negative attitudes toward OD scores were seen in the first year, while the lowest scores were seen in the fifth year. Therefore, it may be concluded that education has a generally positive effect on OD. It will also be helpful for OD and transplantation to be included in the medical curriculum. The findings of this study showed that medical knowledge about OD, the sufficiency of that knowledge, knowing the relevant processes, and discussing the issue with family and friends positively affect attitudes toward OD. However, only 47% of MS had discussed OD with their families. Reported rates of discussion with families are 54–68%^{9,19,20}. Studies reported that individuals who discussed OD with their families exhibited more positive attitudes toward it^{4,15}. A study from Spain also described discussing OD with friends and family as one of the reasons underlying the willingness to donate organs²¹. Finally, a study from Turkey concluded that negative attitudes toward OD on the part of the family adversely affected students' attitudes toward donation²². An environment and family favorably disposed to OD can increase the willingness to donate organs. Observing family approval and willingness may make

it easier for students to make positive decisions on the subject. Therefore, it is very important to explain the issue to all family members by using plans and brochures for all family members in community educational activities. It may be beneficial to plan interactive training sessions with the family from primary school onward for children to raise familial awareness of OD and transplantation.

A study reported that the presence of end-stage renal disease or a friend waiting for a renal transplant did not significantly affect attitudes toward OD¹⁷. Similarly, in the present study, the attitudes of MS acquainted with individuals who had undergone organ transplantation or who were on the waiting list were slightly higher, although the difference was not statistically significant. In another study, being acquainted with a transplantation patient or donor significantly enhanced positive attitudes toward OD among MS⁴. This may be due to low societal awareness of OD. The importance of this subject now needs to be emphasized by means of interventions aimed at raising such awareness. In the present study, 37% of MS reported knowing how OD is performed. A study reported that 34% of participants knew where to register for OD¹¹. The fact that even MS were aware of the path involved in OD suggests that there is a deficiency in society in this area. Education on the subject should be brought down as far as middle and high schools, and it should be explained that OD is a major need for all. However, most MS described themselves as willing to donate organs, and only 1 in 10 possessed OD cards. Another study reported OD card possession rates of 8% among the first-year MS and 10% among the sixth-year MS²³. A study reported that 59% of MS would record their wish to donate organs onto electronic health cards¹³. OD card possession rates have been reported at 21–43%^{9,10,20,24}. Interestingly, a study from China reported that no MS taking part held OD cards¹⁵. Lack of knowledge about OD cards can result in a failure to fully understand the procedure involved in obtaining one and the postponement of action on the subject of OD. Making it easy to get OD cards or record the wish to donate organs onto electronic health cards within the health system will significantly facilitate donation.

In the studies, 92–98% of MS reported that they would accept donated organs if required^{10,25}. Similarly, in the present study, 96% of MS stated they would accept ODs if necessary. There are differences in studies between willingness to receive organs and willingness to donate them^{10,25}. When MS were shown a list of organs capable of being donated, the organs and tissues they were most willing to donate were blood, kidneys, and liver, mentioned in descending order. A study from

Brazil found that the organs they were most willing to donate were the kidneys, liver, and bone marrow¹⁹. In a study from Croatia, the most desirable organs were the kidneys, and the least popular was the skin²⁴. This may be due to their appearing

less frequently in the media. The least popular organs and tissues for donation were the faces and hairy skin, respectively. This may be due to concerns about individuals' external appearance being affected.

Table 2. Comparison of medical students' characteristics and ODAS scores.

Variable	Category	Positive attitudes		Negative attitudes	
		Mean±SD	p	Mean±SD	p
Gender	Woman	102.5±13.3	0.012	43.1±16.5	0.006
	Man	98.3±15.5		50.1±22.1	
Academic year	First	100.9±14.4	0.207	53.7±17.2 ^a	<0.001
	Second	104.1±13.3		45.8±17.6 ^{ac}	
	Third	101.1±15.8		52.8±28.3 ^{ac}	
	Fourth	97.1±15.5		44.4±16.1 ^{abc}	
	Fifth	100.3±13.3		35.3±14.1 ^b	
	Sixth	100.6±13.9		38.1±11.3 ^{bc}	
Chronic disease	Yes	101±13.1	0.907	45.4±17.8	0.968
	No	100.8±14.5		46±19.4	
Receipt of education concerning organ education	Yes	102.6±12	0.156	39.7±17.5	<0.001
	No	99.4±16		51±19.2	
Wishing to receive annual education concerning organ donation	Yes	101.4±14.3	0.223	45.8±17.8	0.648
	No	99.4±14.6		46.1±22.3	
Discussion of organ donation with the family	Yes	103.5±12	0.008	41.9±17.6	<0.001
	No	98.5±15.9		49.5±20	
Discussion of organ donation with friends	Yes	101.1±14	0.566	43.5±17.8	<0.001
	No	100.1±15.3		51.6±21.4	
Organ transplantation in a relative	Yes	101.9±16	0.349	45.3±21.8	0.413
	No	100.7±14.2		46±18.9	
A relative on the organ transplantation waiting list	Yes	105.2±13	0.123	48.8±18.4	0.464
	No	100.5±14.5		45.7±19.3	
Knowledge of the organ donation process	Yes	102.5±12.9	0.161	42±18.4	0.002
	No	99.8±15.1		48.2±19.4	
Willing to donate organs	Yes	103.4±13.1	<0.001	41.7±18.1	<0.001
	No	94.4±15.5		56.3±18.1	
Possession of an organ donation card	Yes	105.9±12.5	0.022	35.6±16.1	<0.001
	No	100.3±14.5		47±19.2	
The most common type of organ transplantation in Turkey	Living donor	100.4±14.5	0.604	45.9±19.7	0.989
	Cadaveric	101.4±14.3		45.9±18.7	
Knowledge of the organ donation record system in Turkey	Yes	100.8±14.9	0.774	43.1±19.1	<0.001
	No	101±13.1		53.7±17.7	
Willingness to receive organ donation if necessary	Yes	101±14.6	0.126	45.1±18.9	0.001
	No	97.3±9.5		63.9±18.3	

There is no difference between the same letters in a column. Bold values indicate statistical significance.

There are a number of limitations to this study. First, it is a single-center study, and hence the findings cannot be generalized. Second, since it involved the application of a questionnaire, it may not reflect actual behaviors, which may be lower than the reported willingness rates. Finally, few factors were evaluated in terms of willingness and attitudes toward donation and transplantation; future research should increase these factors.

CONCLUSION

Medical students appear to look favorably on OD. Some had received education on the subject but wished to be given more. We identified a need for greater discussion of the issue with family and friends in the community. Conversation with family and friends was found to have a positive impact on attitudes. Women exhibited more positive attitudes than men. More students reported a willingness to receive donated organs if

necessary than reported a willingness to donate organs. It will be useful to improve societal understanding of OD and encourage and explain the OD system more in detail.

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AUTHORS' CONTRIBUTIONS

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

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The prognostic importance of PD-L1, PTEN, PHH3, and Ki-67 expressions in invasive breast carcinoma

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the relationship of PD-L1, PTEN, PHH3, and Ki-67 immunohistochemical stain expressions with prognostic clinicopathological parameters in breast cancer.

METHODS: Lumpectomy and mastectomy materials from 85 patients operated at the Department of Pathology, Bolu Abant İzzet Baysal University, Faculty of Medicine between 2014 and 2019 were retrospectively reviewed. PD-L1, PTEN, PHH3, and Ki-67 expressions were examined. Immunohistochemical staining results were compared with clinicopathological parameters and found to be associated with prognosis.

RESULTS: A statistically significant correlation was found between PD-L1 and large tumor size, high histological grade, multifocality, and lymphovascular invasion. A statistically significant correlation was found between the loss of PTEN and large tumor size and histological grade. There was a statistically significant correlation between PHH3 and advanced age, large tumor size, and high histological grade. A statistically significant correlation was found between Ki-67 and large tumor size, high histological grade, and lymphovascular invasion.

CONCLUSION: PD-L1, PTEN, PHH3, and Ki-67 are regarded as potential biomarkers that can be used to predict the prognosis of breast cancer and to develop targeted therapies.

KEYWORDS: Carcinoma, ductal. Breast. Immunohistochemistry. Immune checkpoint inhibitors. Ki-67 antigen.

INTRODUCTION

Breast carcinoma is the most common cancer in women¹. The presence of tumor-infiltrating lymphocytes and immune response markers are good prognostic features in breast tumors². Programmed cell death 1 (PD-1) is a surface membrane antigen expressed by various cells of the immune system, including T lymphocytes³. Programmed cell death ligand 1 (PD-L1) is expressed in breast tumors and tumor-infiltrating lymphocytes, but not in healthy breast tissue⁴. PTEN is a novel tumor suppressor gene located on chromosome band 10q23. The main function of PTEN is to inhibit the PI3K/AKT pathway, which plays a role in cell proliferation and cell survival⁵. Loss of PTEN function plays a role in the progression of several cancers⁶.

Many prognostic factors in breast cancer are directly or indirectly related to proliferation. One of the best known methods for immunohistochemical measurement of proliferation is Ki-67⁷. Studies have revealed that Ki-67 correlates strongly with biomarkers such as hormone receptor status, HER2 status, tumor staging, and axillary lymph node status⁸. Phosphohistone H3 (PHH3) has long been known as a marker of cellular proliferation in various cancers⁹.

The aim of this study was to investigate the correlation of the expressions in PD-L1, PTEN, PHH3, and Ki-67 immunohistochemical stains with prognostic clinicopathological parameters in breast cancer.

METHODS

Case selection

Lumpectomy and mastectomy materials from 85 patients (with invasive ductal carcinoma and lobular carcinoma) treated and operated at the Department of Pathology, Bolu Abant İzzet Baysal University, Faculty of Medicine between 2014 and 2019 were included in the study. The blocks that best reflected the tumor and were most suitable for immunohistochemical examination were selected.

Immunohistochemical staining

Thin sections at a thickness of 4 microns were taken from the formalin-fixed, paraffin-embedded blocks. The sections were stained with primary antibodies PD-L1, PTEN, PHH3, and Ki-67 according to the manufacturers' instructions using a Leica

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Bond-Max fully automated immunohistochemistry machine. Staining was performed using a standard compact polymer technology kit for immunohistochemistry.

Sections were stained with PD-L1 antibody (Cell Signaling Technology, clone: E1L3N; diluted 1:200). Placental sections were used as positive controls for PD-L1. Membranous and cytoplasmic staining were considered positive³. The total percentage of positive cells and their staining intensities (0–3) were determined. The modified Histo-score (H-score) was used [PD-L1 staining intensity (0–3) × percentage of positive cells, between 0 and 300]. PD-L1 expression was dichotomized into two groups, using a cutoff score of 100 (H-score 0–99: negative/low expression, and 100–300: positive expression)⁴.

Sections were stained with anti-PTEN antibody (Dako, Monoclonal Mouse anti-PTEN, clone 6H2.1; diluted 1:40). Colon tumor sections were used as positive controls for PTEN. Both cytoplasmic and nuclear staining were observed with PTEN. The staining intensity for PTEN was scored 0–3¹⁰. The percentage of positively stained tumor cells was calculated. The total PTEN index was calculated using the following formula: H score = (Percentage of positive cells) × (Immunostaining intensity). The H-score ranged from 0 to 300, and case with a score of less than 90 was considered PTEN loss¹¹.

Sections were stained with PHH3 (Cell Marque Corp. Rocklin, rabbit polyclonal antibody; diluted 1:200). Tonsil sections were used as positive controls. The percentage of positively stained tumor cells in the region with the highest PHH3 concentration was determined¹².

Ki-67 (Dako Autostainer/Autostainer Plus, monoclonal Mouse Anti-Human Ki-67 antigen clone MIB-1) was applied to formalin-fixed tissues. In determining the score, the entire section taken from the invasive border of the tumor was examined, and at least 1000 cells with the highest staining in the 40x objective were evaluated and averaged¹³. The percentage of tumor cells that showed only nuclear staining with Ki-67 determined the Ki-67 score¹³.

Statistical analysis

To analyze the data, descriptive statistics were given with frequency, percentage, mean, and standard deviation. The Mann-Whitney U test was performed to investigate whether prognostic factor measures in the study differed according to immunohistochemical staining positivity. When evaluating PD-L1, PHH3, Ki-67, and PTEN positivity, the Mann-Whitney U test was used because the number in the positive groups was small and the measurements did not have a parametric distribution. Chi-square analysis and Fisher's exact test were applied to examine the relationships between the proportional values

of prognostic factors according to PD-L1, PHH3, Ki-67, and PTEN positivity status. The SPSS 25.0 program was used for statistical analysis ($p < 0.05$).

RESULTS

Clinicopathological features

The study covered 85 cases of invasive breast carcinoma. Of the patients aged between 35 and 88 years (mean 58.33 ± 12.50 years), 82 (96.4%) were female and 3 (3.6%) were male. Of the cases diagnosed with breast carcinoma, 77 (90.5%) had invasive ductal carcinoma and 8 (9.5%) had invasive lobular carcinoma. According to tumor size, patients were divided into two groups: <2 cm in 34 (40%) patients and ≥ 2 cm in 51 (60%) patients.

Nottingham histological grade was found: 12.9% in grade 1, 56.5% in grade 2, and 30.6% in grade 3. Tumor metastasis to the axillary lymph node was detected: 1–3 lymph nodes in 17 (20.0%), 4–9 lymph nodes in 15 (17.6%), and 10 or more lymph nodes in 13 (15.3%) patients. Of the patients, 30% were found with stage 1, 31.8% with stage 2, 20.2% with stage 3, and 17.9% with stage 4.

Immunohistochemical staining with clinicopathological parameters

PD-L1 expression and its relationship to clinicopathological factors

Table 1 shows the relationship between PD-L1 and prognostic factors. In sections prepared from invasive breast cancer blocks, 21 (24.7%) of 85 patients expressed PD-L1. Tumor cells and/or lymphocytes showed membranous staining in 13 (15.3%) cases, cytoplasmic staining in 3 (3.5%) cases, and cytoplasmic and membranous staining in 21 (24.7%) cases (Figures 1A–D); 48 (56.5%) cases were scored as skor 0, 20 (23.5%) as skor 1, 12 (14.1%) as skor 2, and 5 (5.9%) as skor 3. No significant association was found between PD-L1 expression and age, gender, tumor location, surgical procedure of patients, lymph node metastasis, perineural invasion, stage, and HER2 score.

PTEN expression and its relationship with clinicopathological factors

The relationship between PTEN-1 and prognostic factors is given in Table 1. Loss of PTEN expression was observed in 42 (49.4%) of 85 cases with invasive breast carcinoma. It was found that there was no staining in 14 (16.4%) cases, weak staining in 24 (28.2%) cases, moderate staining in 30 (35.2%) cases, and strong staining in 17 (20%) cases (Figures 1E–H).

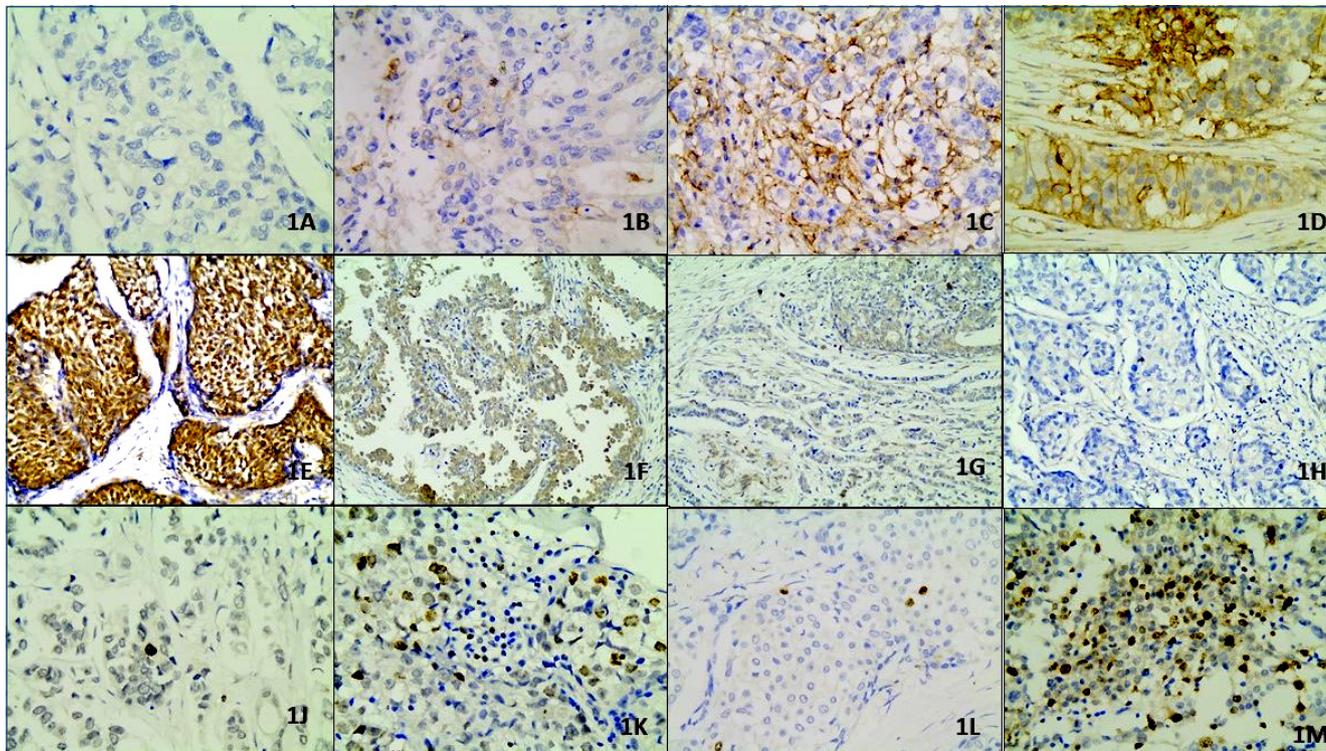


Figure 1. (A) Negative expression of PD-L1 in tumor cells, immune peroxidase, 400x. (B) Mild expression of PD-L1 in tumor cells, immune peroxidase, 400x. (C) Moderate expression of PD-L1 in tumor cells, immune peroxidase, 400x. (D) Severe expression of PD-L1 in tumor cells, immune peroxidase, 400x. (E) Intense expression of PTEN in tumor cells, immune peroxidase, 400x. (F) Moderate-intense expression of PTEN in tumor cells, immune peroxidase, 400x. (G) Mild-intense expression of PTEN in tumor cells, immune peroxidase, 400x. (H) Negative expression of PTEN in tumor cells, immune peroxidase, 400x. (J) Low nuclear PHH3 expression in tumor cells, immune peroxidase, 400x. (K) High nuclear PHH3 expression in tumor cells, immune peroxidase, 400x. (L) Low nuclear Ki-67 expression in tumor cells, immune peroxidase, 400x. (M) High nuclear Ki-67 expression in tumor cells, immune peroxidase, 400x.

No significant correlation was found between loss of PTEN expression and age, gender, histological type, lymphovascular invasion, perineural invasion, histological grade, stage, ER, PR, HER2 score, and Ki-67 expression.

PHH3 expression and its relationship with clinicopathological factors

Table 2 shows the relationship between PHH-3 and prognostic factors. PHH3 stained positive in 36 (42.3%) cases and negative in 49 (47.7%) cases (Figures 1J and K). It was observed that the PHH3 group did not differ by pathological T, N, M grading and staging ($p=0.06$; $p=0.12$; $p=0.51$; $p=0.35$, respectively). No significant correlation was found between loss of PHH3 expression and gender, tumor localization, lymphovascular invasion, HER2 score, and stage.

Ki-67 expression and its relationship with clinicopathological factors

The relationship between PHH-3 and prognostic factors is shown in Table 2. Ki-67 expression was $<20\%$ in 57 (67%) cases and

$\geq 20\%$ in 28 (33%) cases (Figures 1L and M). The values of the Ki-67 group differed by tumor size, which was larger than 2 cm ($p=0.01$). Patient's age, gender, tumor localization, histological type of tumor, and the number of metastatic lymph nodes did not differ in Ki-67 groups below or above 20%.

DISCUSSION

The increase in the incidence of breast cancer in recent years has led to studies on the development of new methods of diagnosis and treatment¹⁴. Many studies have shown the expression of PD-L1 in both tumor cells and TIL in breast carcinoma. In studies, PD-L1 expression in all breast cancer subtypes varies from 0 to 83%, and most studies show PD-L1 expression below 50%¹⁵. In this study, we identified PD-L1 positivity in 21 (24%) of 85 breast cancer patients and found that it was associated with poor prognostic parameters such as high histological grade, large tumor size, ER and PR negativity, and high Ki-67 expression. Thus, we obtained similar results to recent studies^{14,16,17}.

Table 1. PD-L1 and PTEN by prognostic factors.

Prognostic factors		PD-L1				p	PTEN				p-value
		Negative		Positive			<90		≥90		
		n	%	N	%		n	%	n	%	
Age	<40	5	7.8%	2	9.5%	0.67	4	9.5%	3	7.0%	0.60
	≥40	59	92.2%	19	90.5%		38	90.5%	40	93.0%	
Gender	Female	62	96.9%	20	95.2%	0.73	42	100.0%	40	93.0%	0.08
	Male	2	3.1%	1	4.8%		0	0.0%	3	7.0%	
Tumor Localization	Right	31	48.4%	13	61.9%	0.01*	25	59.5%	19	44.2%	0.11
	Left	33	51.6%	8	38.1%		17	40.5%	24	55.8%	
Tumor size (cm)	<2	27	42.2%	7	33.3%	0.04*	14	33.3%	20	46.5%	0.03*
	≥2	37	57.8%	14	66.7%		28	66.7%	23	53.5%	
Histological grade	1	10	15.6%	1	4.8%	0.03*	7	16.7%	4	9.3%	0.03*
	2	37	57.8%	11	52.4%		25	59.5%	23	53.5%	
	3	17	26.6%	9	42.9%		10	23.8%	16	37.2%	
Lymphovascular invasion	None	38	59.4%	9	42.9%	0.01*	22	52.38%	25	58.1%	0.13
	Yes	26	40.6%	12	57.1%		20	47.62%	18	41.9%	
Perineural invasion	None	50	78.1%	16	76.2%	0.56	32	76.19%	34	79.07%	0.21
	Yes	14	21.9%	5	23.8%		10	23.81%	9	20.93%	
Pathologic T	T1	29	45.3%	7	33.3%	0.06	15	35.71%	21	48.84%	0.24
	T2	27	42.2%	13	61.9%		23	54.76%	17	39.53%	
	T3	3	4.7%	1	4.8%		2	4.76%	2	4.65%	
	T4	5	7.8%	0	0.0%		2	4.76%	3	6.98%	
Pathologic N	N0	3	9.4%	0	0.0%	0.12	1	4.55%	2	9.09%	0.13
	N1	5	15.6%	2	16.7%		1	4.55%	6	27.27%	
	N2	15	46.9%	8	66.7%		13	59.09%	10	45.45%	
	N3	9	28.1%	2	16.7%		7	31.82%	4	18.18%	
Pathologic M	M0	57	89.1%	21	100.0%	0.03*	38	90.48%	40	93.02%	0.11
	M1	7	10.9%	0	0.0%		4	9.52%	3	6.98%	
Stage	1A	20	31.3%	5	23.8%	0.14	12	28.57%	13	30.23%	0.25
	1B	1	1.6%	0	0.0%		0	0.00%	1	2.33%	
	2A	12	18.8%	5	23.8%		9	21.43%	8	18.60%	
	2B	9	14.1%	1	4.8%		4	9.52%	6	13.95%	
	3A	7	10.9%	7	33.3%		7	16.67%	7	16.28%	
	3B	2	3.1%	0	0.0%		0	0.00%	2	4.65%	
	3C	10	15.6%	3	14.3%		9	21.43%	4	9.30%	
	4	3	4.7%	0	0.0%		1	2.38%	2	4.65%	
ER	Negative	7	10.9%	5	23.8%	0.03*	9	15.00%	3	12.00%	0.08
	Positive	57	89.1%	16	76.2%		51	85.00%	22	88.00%	
PR	Negative	11	17.2%	9	42.9%	0.01*	14	23.30%	6	24.00%	0.21
	Positive	53	82.8%	12	57.1%		46	76.70%	19	76.00%	
HER2	0	32	50.0%	8	38.1%	0.16	17	40.48%	23	53.49%	0.13
	1	12	18.8%	5	23.8%		10	23.81%	7	16.28%	
	2	10	15.6%	4	19.0%		9	21.43%	5	11.63%	
	3	10	15.6%	4	19.0%		6	14.29%	8	18.60%	

*Statistical significance at the p<0.05 level.

In a study by Ming Li et al., PD-L1 was found to be positive in 38.6% of patients, and PD-L1 positivity was significantly associated with high histological grade, recurrence, and distant metastasis¹⁷. Hazem et al. identified PD-L1 positivity in 34% of cases and found that PD-L1 expression was associated with

large tumor size, histological grade, HER2 positivity, and ER and PR negativity¹⁶. In this study, similar to the literature, PD-L1 expression increased with the increasing histological grade of the tumor^{4,18}. Moreover, in agreement with the literature, PD-L1 expression was found to be higher in ER- and PR-negative cases¹⁸.

Table 2. PHH3 and Ki-67 by prognostic factors.

Prognostic factors		PHH3					Ki-67				
		<2		≥2		p	<20%		≥20%		p
		n	%	n	%		n	%	n	%	
Tumor size (cm)	<2	23	46.9%	11	30.6%	0.01*	28	49.1%	6	21.4%	0.01*
	≥2	26	53.1%	25	69.4%		29	50.9%	22	78.6%	
Histological grade	1	10	20.4%	1	2.8%	0.02*	1	1.8%	0	0.0%	0.01*
	2	26	53.1%	22	61.1%		11	19.3%	0	0.0%	
	3	13	26.5%	13	36.1%		35	61.4%	13	46.4%	
Lymphovascular invasion	None	29	59.2%	18	50.0%	0.58	37	64.9%	10	35.7%	0.01*
	Yes	20	40.8%	18	50.0%		20	35.1%	18	64.3%	
Perineural invasion	None	39	79.6%	27	75.0%	0.01*	43	75.4%	23	82.1%	0.01*
	Yes	10	20.4%	9	25.0%		14	24.6%	5	17.9%	
Pathologic T	T1	24	49.0%	12	33.3%	0.05	29	50.9%	7	25.0%	0.05
	T2	20	40.8%	20	55.6%		23	40.4%	17	60.7%	
	T3	2	4.1%	2	5.6%		3	5.3%	1	3.6%	
	T4	3	6.1%	2	5.6%		2	3.5%	3	10.7%	
Pathologic N	N0	2	8.0%	1	5.3%	0.36	1	3.7%	2	11.8%	0.28
	N1	3	12.0%	4	21.1%		4	14.8%	3	17.6%	
	N2	15	60.0%	8	42.1%		14	51.9%	9	52.9%	
	N3	5	20.0%	6	31.6%		8	29.6%	3	17.6%	
Pathologic M	M0	45	91.8%	33	91.7%	0.51	53	93.0%	25	89.3%	0.30
	M1	4	8.2%	3	8.3%		4	7.0%	3	10.7%	
Stage	1A	16	32.7%	9	25.0%	0.35	21	36.8%	4	14.3%	0.38
	1B	1	2.0%	0	0.0%		1	1.8%	0	0.0%	
	2A	9	18.4%	8	22.2%		10	17.5%	7	25.0%	
	2B	6	12.2%	4	11.1%		6	10.5%	4	14.3%	
	3A	6	12.2%	8	22.2%		7	12.3%	7	25.0%	
	3B	2	4.1%	0	0.0%		1	1.8%	1	3.6%	
	3C	7	14.3%	6	16.7%		9	15.8%	4	14.3%	
	4	2	4.1%	1	2.8%		2	3.5%	1	3.6%	
ER	Negative	4	8.2%	8	22.2%	0.02*	4	7.0%	8	28.6%	0.01*
	Positive	45	91.8%	28	77.8%		53	93.0%	20	71.4%	
PR	Negative	9	18.4%	11	30.6%	0.01*	8	14.0%	12	42.9%	0.01*
	Positive	40	81.6%	25	69.4%		49	86.0%	16	57.1%	
HER2	0	9	18.4%	8	22.2%	0.05	31	54.4%	9	32.1%	0.07
	1	7	14.3%	7	19.4%		12	21.1%	5	17.9%	
	2	8	16.3%	6	16.7%		10	17.5%	4	14.3%	
	3	9	18.4%	8	22.2%		4	7.0%	10	35.7%	

*Statistical significance at the p<0.05 level.

Recent studies have shown that PTEN has a regulatory role in breast carcinoma and may be a predictive and prognostic marker¹⁹. Shaham et al. found that the loss of PTEN was significantly associated with large tumor size, high grade, and triple-negative breast cancer¹¹. In our study of 85 patients, the loss of PTEN was observed in 49.4% of patients. This result from our study was evaluated in accordance with the loss of PTEN expression reported in the literature as 8–86%^{11,20}. In this study, the loss of PTEN expression was associated with large tumor size and low histological grade. The low histological grade was probably due to the fact that only a small number of cases were studied. Although a higher loss of PTEN expression was observed in the group with LN metastases in this study, this was statistically insignificant.

PHH3 expression has been shown to be stronger than classic prognostic factors such as axillary lymph node status, tumor size, histological grade, and ER and PR negativity^{12,21}. Ivar et al. showed that low PHH3 expression is associated with an excellent prognosis. PHH3 was associated with age, tumor size, grade, tubular formation, nuclear atypia, mitotic activity index, and ER and PR negativity¹². In this study, high PHH3 expression was associated with advanced age, large tumor size, high histological grade, ER and PR negativity, and high Ki-67 expression. This result suggests that nuclear PHH3 expression may be a poor prognostic factor for breast cancer patients.

The percentage of cells staining positive for Ki-67 has been used as a measure of proliferation and as a prognostic factor²². Wiesner et al. found that Ki-67 correlated strongly with other biomarkers such as negative ER and PR, HER2 status, tumor

staging, and histological grade⁸. In this study, high Ki-67 expression was found to be associated with large tumor size, high histological grade, lymphovascular invasion, and ER and PR negativity.

Our study has several limitations. Our cases were few in number, and some of the blocks had technical difficulties, such as failure to obtain optimal staining during immunohistochemical staining.

CONCLUSION

As a result of this study, expressions of PD-L1, PHH3, Ki-67, and loss of PTEN were found to be associated with poor prognostic factors. We believe that these biomarkers, in addition to being used for prognosis in breast cancer, can be used for therapeutic purposes and may contribute to the development of appropriate immunotherapies through a better understanding of the immunological basis of the tumor.

AUTHORS' CONTRIBUTIONS

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

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Evaluation of subclinical atherosclerosis by ultrasound radiofrequency data technology in patients with psoriatic arthritis

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SUMMARY

OBJECTIVE: This study aimed to assess the presence of subclinical atherosclerosis in patients with psoriatic arthritis using ultrasound radiofrequency data technology.

METHODS: In all, 29 psoriatic arthritis patients and 42 age- and sex-matched healthy controls were included in this cross-sectional study. Arterial stiffness and carotid intima-media thickness measurements were performed in bilateral common carotid arteries using ultrasound radiofrequency data technology in all participants.

RESULTS: In psoriatic arthritis patients, the mean carotid intima-media thickness, α and β stiffness indices, and pulsed wave velocity value were significantly higher than those in the control group (542.3 (81.3) vs. 487.9 (64.1), 9.3 (6.3) vs. 3.9 (0.1), 18.7 (17.7) vs. 8.04 (4.2), and 10.2 (3.8) vs. 6.4 (1.5), $p < 0.05$). The mean distensibility coefficient and compliance coefficient values of the patient group were significantly lower than those of the control group (0.014 (0.01) vs. 0.03 (0.01) and 0.57 (0.33) vs. 1.02 (0.4), $p < 0.05$). No significant correlation was found between carotid artery hemodynamic parameters and symptom duration, duration of diagnosis and treatment, disease activity index for psoriatic arthritis scores, erythrocyte sedimentation rate, and C-reactive protein levels ($p > 0.05$).

CONCLUSION: In the results of our study, evidence of subclinical atherosclerosis has been detected in psoriatic arthritis patients without clinically evident cardiovascular disease or traditional cardiovascular risk factors.

KEYWORDS: Arterial stiffness. Carotid intima-media thickness. Psoriatic arthritis. Atherosclerosis. Ultrasonography.

INTRODUCTION

Psoriatic arthritis (PsA) is a heterogeneous and inflammatory joint disease that occurs in patients who have psoriasis, will develop psoriasis, or have a family history of psoriasis. Patients with PsA have heterogeneous clinical manifestations, including synovitis, enthesitis, dactylitis, axial inflammation, and skin and nail involvement^{1,2}. Recently published data revealed that the pooled prevalence and incidence rates of PsA are 133 patients per 100,000 subjects and 83 per 100,000 person-years, respectively³.

Atherosclerosis is a chronic inflammatory vascular disease characterized by the accumulation of lipids, inflammatory cells, and fibrous elements in the walls of arteries, causing progressive luminal narrowing of these vessels^{4,5}. Vascular inflammation plays a critical role in pathophysiology of atherosclerosis, but the contribution of inflammation to this pathophysiology is complex and probably not fully understood^{5,6}. It is widely accepted that both innate and adaptive immune responses are important for initiation and progression of atherosclerosis⁵.

Carotid intima-media thickness (CIMT) is measured between the intima-lumen and the media-adventitia interfaces of the carotid artery⁷. The CIMT measurements are used as a surrogate marker for atherosclerosis and subclinical cardiovascular diseases (CVD), and as a variable predictor of cardiovascular events^{7,8}.

Arterial stiffness is a term used to qualitatively describe the reduction in the elastic vessel wall properties. It is one of the earliest markers of functional and structural changes in arterial walls^{4,9}. Measurement of arterial stiffness is important because it is an independent predictor of the risk of future fatal and nonfatal cardiovascular (CV) events^{4,10}. The pulse wave velocity (PWV) is the most widely used measure of arterial stiffness⁴.

Ultrasound (US) radiofrequency (RF) data technology is a novel sonographic method for the evaluation of vascular disease. The innovations in the US technology have allowed the automatic and accurate measurement of CIMT and arterial stiffness^{11,12}. During examinations with this technology, the pulsation of the arterial wall can be automatically monitored

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using RF energy, and it also gives feedback on the quality of the measurement. Thus, this measurement method is less dependent on the experience of the operator^{11,13}. The quality arterial stiffness software provides real-time measurement of the expansion of blood vessel walls caused by a moving blood pressure wave resulting from heart pumping. Distension, a fundamental parameter measured by quality arterial stiffness software, is the difference between systolic and diastolic diameters. The pressure and distension values are used to calculate the stiffness parameters using the software¹³. In addition, this technique presents CIMT measurements at the micrometer level, which facilitates the acquisition of more precise and clearer results compared to other methods with millimeter accuracy¹².

We aimed to evaluate subclinical atherosclerosis using the US RF data technology in patients with PsA without clinically evident CVD or traditional CV risk factors.

METHODS

Study design and population

This cross-sectional study was conducted at the Diskapi Yildirim Beyazit Training and Research Hospital, Rheumatology and Radiology outpatient clinics between May 2019 and July 2019. This study included 29 PsA patients and 42 age- and sex-matched healthy controls. PsA patients fulfilled the classification criteria for the diagnosis of PsA (CASPAR)¹⁴. The control group consisted of healthy volunteers who applied to the rheumatology outpatient clinic and were not diagnosed with any rheumatological disease as a result of the evaluations. The age of PsA patients and controls were 18–60 years. The exclusion criteria for both groups were as follows: diabetes mellitus, arterial hypertension, obesity, dyslipidemia, smoking, primary cardiovascular or cerebrovascular disease, acute or chronic renal insufficiency, chronic obstructive pulmonary disease, and chronic infectious or inflammatory disease.

The study protocol was approved by the Clinical Trials Ethics Committee of our hospital (29.04.2019 – 62/03). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all PsA patients and controls before enrollment.

Clinical assessment of psoriatic arthritis

The physical examination included recording the number of tender and swollen joints using the 68 tender/66 swollen joint counts. Laboratory markers of disease activity included erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Disease activity was assessed using the Disease Activity Index for

Psoriatic Arthritis (DAPSA) score¹⁵. The disease activity levels were defined, with disease remission as ≤ 4 , low disease activity (LDA) as >4 to ≤ 14 , moderate disease activity (MDA) as >14 to ≤ 28 , and high disease activity (HDA) as >28 .

Ultrasonographic assessment

Participants' details were recorded in a US device, which functioned on a MyLab 60 platform (Esaote SpA, Genoa, Italy) with a high-resolution 12-MHz linear sequence transducer (LA523). The device was equipped with the RF Quality Intima-Media Thickness Analysis and RF Quality Arterial Stiffness Analysis software, which worked using the RF method. All participants lied in the supine position with the head 45° up and horizontally 30° turned contralateral to the side being examined. The CIMT measurements were obtained from the B-mode examinations using the US RF monitoring technology from the distant common carotid arteries (CCAs) wall at a 10-mm distal segment, where appropriate images were obtained from the longitudinal plane and no plaque was visualized. After six cardiac cycles, the software calculated a real-time mean CIMT value and its standard deviation (Figure 1).

Using the RF Quality Arterial Stiffness Analysis software, arterial wall movements were monitored by RF signals at systolic and diastolic phases during six cardiac cycles in B-mode examinations. The RF Quality Arterial Stiffness Analysis technology provides a list of stiffness parameters calculated by measuring the arterial distension waveform combined with the brachial artery BP. These parameters are distensibility coefficient (DC), compliance coefficient (CC), α , β , and PWV. If the artery is stiffer, DC and CC will be lower, and α , β , and PWV will be higher.

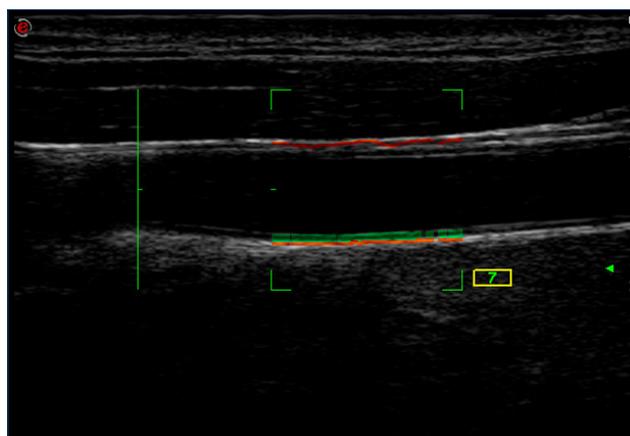


Figure 1. Left CCA quality intima-media thickness analysis. The orange line represents the RF signal following the anterior edge of the media-adventitia interface, and the green line represents the RF signal following the anterior edge of the lumen-intima interface.

Arterial stiffness parameters and CIMT measurements were made from the right and left carotid arteries of each participant, and their mean values were recorded.

Statistical analysis

The data were evaluated using the SPSS (Statistical Package for Social Sciences) program for Windows 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed as mean (standard deviation), frequency, and percentage. χ^2 test was used to evaluate categorical variables. Visual (histograms and probability diagrams) and analytical methods (Shapiro-Wilk test) were used to check whether or not the variables were normally distributed. For normally distributed variables, Student's t-test was used to measure any statistically significant differences between two independent groups. As a statistical method for variables without a normal distribution, Mann-Whitney U test was used to measure the statistical significance between two independent groups. The relationships between variables were analyzed using Spearman's correlation coefficients. A p-value of <0.05 was considered statistically significant.

RESULTS

There was no significant difference between the groups in terms of demographic, clinical, and laboratory characteristics ($p>0.05$), except for ESR and CRP levels ($p<0.05$) (Table 1).

The mean symptom duration of the patient group was 4.5 ± 2.9 (1–15) years, and the mean duration of diagnosis and treatment was 3.3 ± 2.4 (1–12) years. All of the patients were using disease-modifying antirheumatic drug (DMARD) therapy and 4 (13.8%) patients were using anti-tumor necrosis factor (TNF)-alpha treatment in addition to the DMARD therapy. The mean DAPSA score of patients was 9.3 ± 6.5 (2.3–22.2). In terms of DAPSA scores, 12 patients (41.4%) were in remission, 10 patients (34.5%) were in LDA, and 7 patients (24.1%) were in MDA periods.

In PsA patients, the mean CIMT, α and β stiffness indices, and PWV values were significantly higher than those in the controls ($p<0.05$). The mean DC and CC values of the patient group were significantly lower than those of the control group ($p<0.05$) (Table 2).

There was no statistically significant difference between DAPSA groups in terms of carotid artery hemodynamic parameters (CIMT, DC, CC, PWV, and α and β stiffness indices) ($p>0.05$).

Table 1. Demographic, clinical, and laboratory characteristics of PsA patients and healthy controls.

	PsA Patients (n=29)	Controls (n=42)	p-value
	Mean (SD)	Mean (SD)	
Age (years)	40.3 (9.6)	38.2 (7.9)	0.325 ^a
Gender (Female:male)	18:11	26:16	0.989 ^b
Glucose (mg/dL)	84.3 (7.9)	83.8 (6.2)	0.782 ^a
Total Cholesterol (mg/dL)	179.5 (16.2)	172.4 (18.7)	0.109 ^c
LDL (mg/dL)	123.4 (17.6)	114.7 (22.5)	0.112 ^c
HDL (mg/dL)	50.6 (12.8)	47.9 (12.7)	0.383 ^a
Triglycerides (mg/dL)	117.4 (41.4)	108.9 (33.7)	0.348 ^a
ESR (mm/h)	17.5 (14.6)	10.2 (6.1)	0.03 ^c
CRP (mg/L)	6.3 (5.8)	3.3 (2.1)	0.006 ^c
BMI (kg/m ²)	26 (2.2)	25.4 (2.1)	0.082 ^c
BMI categories (n, %)			
<18.5 (underweight)	0 (0)	0 (0)	0.075 ^b
18.5–24.9 (normal weight)	9 (31)	22 (52.4)	
25–29.9 (overweight)	20 (69)	20 (47.6)	
≥30 (obesity)	0 (0)	0 (0)	
SBP (mm Hg)	123.6 (3.8)	122.4 (3.3)	0.206 ^c
DBP (mm Hg)	81.6 (3.2)	80.2 (3.9)	0.252 ^c
Pulse (beats/min)	77.7 (6.8)	77.5 (6.4)	0.876 ^a

PsA: psoriatic arthritis; SD: standard deviation; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. ^aStudent's t-test, ^b χ^2 test, ^cMann-Whitney U test.

Table 2. Distribution of carotid artery hemodynamic parameters between PsA patients and healthy controls.

	PsA Patients (n=29)	Controls (n=42)	p-value
	Mean (SD)	Mean (SD)	
*Mean-CIMT (μm)	542.3 (81.3)	487.9 (64.1)	0.002 ^a
*Mean-DC (1/kPa)	0.014 (0.01)	0.03 (0.01)	<0.001 ^b
*Mean-CC (mm^2/kPa)	0.57 (0.33)	1.02 (0.4)	<0.001 ^b
*Mean- α Stiffness Index	9.3 (6.3)	3.9 (0.1)	<0.001 ^b
*Mean- β Stiffness Index	18.7 (17.7)	8.04 (4.2)	<0.001 ^b
*Mean-PWV (m/s)	10.2 (3.8)	6.4 (1.5)	<0.001 ^b

PsA: psoriatic arthritis; SD: standard deviation; CIMT: carotid intima-media thickness; DC: distensibility coefficient; CC: compliance coefficient; PWV: pulsed wave velocity. ^aStudent's t-test, ^bMann-Whitney U test. *Values were given as the mean of right and left carotid artery hemodynamic parameters.

No significant correlation was found between carotid artery hemodynamic parameters and symptom duration, duration of diagnosis and treatment, DAPSA scores, ESR, and CRP levels ($p>0.05$).

DISCUSSION

The evidence for increased CVD burden and CV risk in patients with inflammatory rheumatic diseases is well recognized¹⁶. Inflammation is associated with endothelial dysfunction and atherosclerosis. Endothelial dysfunction, characterized by reduced nitric oxide (NO) bioavailability, is an early stage in the pathogenesis of atherosclerosis. The circulating inflammation mediators such as CRP can directly alter endothelial NO bioavailability¹⁷. PsA is associated with reduced levels of endothelial progenitor cells (EPCs) and impaired EPC function, leading to decreased release of NO¹⁸. Inflammatory cytokines such as TNF, interleukin (IL)-6, and IL-17 are implicated in the pathogenesis of endothelial dysfunction and atherogenesis. Inflammation leads to alterations in coagulation, increased vasoconstriction and impaired vasodilatation, and the formation of reactive oxygen species¹⁶. Inflammatory cells such as macrophages and polymorphonuclear neutrophils produce a variety of matrix metalloproteinases, which can alter the balance of elastin/collagen¹⁸. All these changes lead to subclinical and clinical atherosclerosis and adverse CV outcomes.

Early detection of subclinical atherosclerosis is important to reduce patients' CV risk⁴. Detection of increased arterial stiffness and CIMT measurement are the most commonly used methods for the diagnosis of subclinical atherosclerosis^{4,7}.

CIMT is a noninvasive measurement of the artery wall thickness and a surrogate marker for the presence and progression of atherosclerosis^{7,8}. CIMT is used worldwide to evaluate the

risk and incidence of CVD, because it can be simply, reproducibly, and noninvasively measured⁷. Similar to the results of our research, there are studies in which CIMT measurements were significantly higher in the PsA group than in the control group¹⁹⁻²⁴. In a study by Bilgen et al., no significant difference was found between the PsA patients and control group in terms of CIMT values²⁵. In our study, there was no significant correlation between CIMT values and symptom duration, duration of diagnosis and treatment, DAPSA score, CRP, and ESR levels. This result is thought to be due to the relatively small number of our patients. Similarly, Garg et al. reported that there was no correlation between CIMT and disease duration, ESR, CRP, disease activity score of 28 joints, and DAPSA scores²⁰. In the other two studies, a significant correlation was found between CIMT and disease duration, ESR, and CRP levels^{19,23}.

There is a close relationship between arterial stiffness and atherosclerosis. Increased luminal pressure and shear stress due to arterial stiffening cause endothelial dysfunction, accelerate the formation of atheroma, and stimulate excessive collagen production and deposition in the arterial wall, leading to the progression of atherosclerosis⁴. PWV is a noninvasive method to measure arterial stiffness and is a strong predictor of future CV events and CV mortality¹⁶. Similar to our results, in a previous study, PWV measurements were significantly higher in PsA patients than in the control group¹⁸. In our study, no significant correlation was found between PWV measurements and symptom duration, duration of diagnosis and treatment, DAPSA scores, ESR, and CRP levels. Shen et al. showed that there was a significant correlation between PWV and cumulative-ESR but not cumulative CRP levels¹⁸.

In our results, there was no statistically significant difference between DAPSA groups (remission, LDA, and MDA) in terms of carotid artery hemodynamic parameters, which might be due to the low number of patients in DAPSA groups.

The obvious limitation of this study was the relatively small number of patients in the sample. The exclusion criteria of our study were the most important reason for this limitation. On the contrary, the strongest aspect of our study was our exclusion criteria. Thus, we evaluated the relationship of our main variables with PsA.

CONCLUSION

In the results of our study, evidence of subclinical atherosclerosis has been detected in PsA patients without clinically evident CVD or traditional CV risk factors. To the best of our knowledge, this is the first study that detected subclinical atherosclerosis in PsA patients using US RF data technology. This

ultrasonographic method is an easy, reproducible, noninvasive, sonographic technique that provides more precise and clear findings of subclinical atherosclerosis.

AUTHORS' CONTRIBUTIONS

CO: Conceptualization, data curation, formal analysis, investigation, methodology, supervision, writing – original draft, writing – review and editing. **HK:** Conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review and editing. **SCS:** Data curation, formal analysis, writing – original draft, writing – review and editing. **ZO:** Data curation, formal analysis, software, writing – original draft, writing – review and editing.

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Clinical analysis on the influencing factors related to preterm twin pregnancy

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SUMMARY

OBJECTIVE: The aim of this study was to explore the influencing factors of preterm twin pregnancy.

METHODS: In total, 602 twin-pregnant women delivered from February 2016 to February 2020 were analyzed retrospectively. According to whether the pregnant women were preterm or not, they were divided into preterm group (n=363) and term group (n=239). Baseline information, such as maternal age, address, and education level of the pregnant women, were collected. The clinical information of pregnant women, such as chorionic, preeclampsia, gestational diabetes, premature rupture of membranes, abnormal fetal position, and fetal weight, were analyzed. Logistic regression analysis was used to analyze the risk factors. p-value <0.05 was considered statistically significant.

RESULTS: In the preterm group, monochorionic diamniotic comorbidities were significantly higher compared with the control group (p<0.05). Higher risks of preterm group have lower education (p<0.05). Multiple logistic regression analysis demonstrated that education, preeclampsia, and premature rupture of membranes were risk factors for preterm twin pregnancy.

CONCLUSIONS: Preterm birth in twin pregnancy is associated with many risk factors, such as education, preeclampsia, and premature rupture of membranes. Pregnancy supervision and prenatal guidance for twin pregnancy should be strengthened. Furthermore, early detection and diagnosis of comorbidities can improve maternal and neonatal outcomes.

KEYWORDS: Twin pregnancy. Preterm birth. Incidence rate.

INTRODUCTION

Twin pregnancy is the most common form of multiple pregnancy, and it has been reported that the incidence has increased dramatically over the past few decades. Since 1987, with the rapid development of related assisted reproductive technology (ART), the incidence of twin pregnancy has been increasing year by year¹. The twin pregnancy rate in the United States has increased from 1.89–3.33% in 30 years².

The incidence of twin pregnancy is increasing year by year, followed by a variety of complications during pregnancy, child-birth, and puerperium, such as anemia, hypertensive disorders, gestational diabetes mellitus, and postpartum hemorrhage³. Preterm birth is one of the most important problems in twin pregnancy and it seriously increases the perinatal morbidity and mortality^{4,5}. A previous study demonstrated that for twin gestations, the rates of preterm birth are higher than that for singleton gestations⁶. A study reported that the rate of preterm birth in twin gestation ranges from 31–63%⁷. The incidence of preterm birth in twin gestation has also shown an increasing trend as the rate of twin pregnancy accelerates, thereby

leading to more public health challenges⁸. It is urgent to pay attention to the research and treatment of premature birth in twin pregnancy and to actively summarize the experience from clinical data.

A better understanding of patients at higher risk of preterm birth would enable optimization of the available interventions to reduce the adverse perinatal outcomes associated with preterm birth. Methods for predicting preterm birth and risk factors for preterm birth in twin pregnancy have long been studied. History of preterm birth⁹, maternal clinical characteristics (age and height)¹⁰, gestational weight gain^{11,12}, and maternal complications (preeclampsia)¹³ are all proposed to aid in identifying and optimizing the management of preterm birth in twin pregnancy. Another retrospective study showed that self-reported pain scores are a predictor of preterm birth in symptomatic twin pregnancy¹⁴.

Studies on twin pregnancy are more frequent in Western countries, while there are fewer studies analyzing the factors of preterm birth in twin pregnancy in Chinese pregnant women. The objective of this study was to investigate

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the factors influencing preterm birth in twin pregnancy in China by retrospectively analyzing the prenatal information and clinical data of pregnant women with twin pregnancy.

METHODS

Research objects

A total of 602 women with twin pregnancy who delivered in Shanxi Maternal and Child Health Hospital from February 2018 to February 2020 were selected for retrospective analysis. Initially, 630 women with twin pregnancy were selected for information and clinical data collection. A total of 28 (4.4%) cases were excluded due to incomplete data or pregnant women with complications of important organs such as the heart, brain, liver, and kidney. A total of 602 women with twin pregnancy were finally included in the study, including 363 in the preterm delivery group and 239 in the term delivery group (Figure 1). This study was approved by the Ethics Committee of our hospital. Inclusion criteria were as follows:

1. 18–55 years old;
2. gestational age <34 weeks at the time of visit;
3. without evidence of ruptured membranes; and
4. both alive fetuses.

Exclusion criteria were as follows:

1. women without data on the exact age and gestational age at delivery and
2. incomplete medical records.

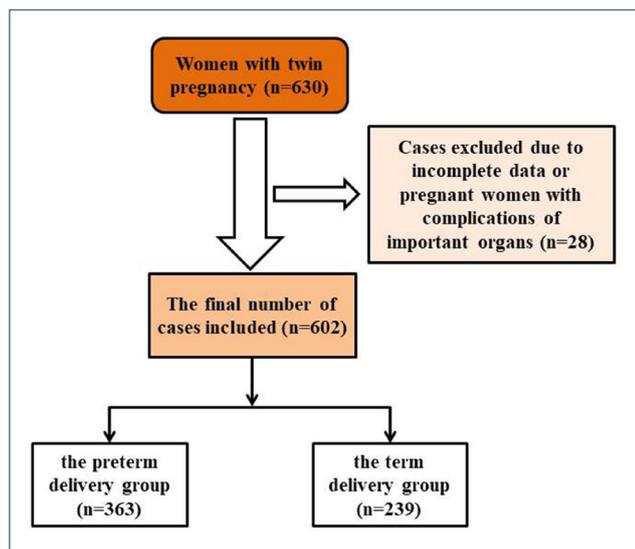


Figure 1. Flowchart for subject selection.

General information

Retrospective analysis of the general conditions of the patients in the preterm group and the term group, including age, education, place of residence, way of conception including natural conception and assisted reproductive assisted pregnancy, chorionic including monochorionic diamniotic (MCDA) and dichorionic diamniotic (DCDA), preeclampsia, gestational diabetes, premature rupture of membranes, abnormal fetal position, and fetal weight. The diagnostic criteria for preterm labor, chorionicity, preeclampsia, and other symptoms refer to the 9th edition of Obstetrics and Gynecology published by the People's Health Publishing House¹⁵.

Statistical analysis

The SPSS 22.0 statistical software was used to process the data. Continuous variables were described by means±standard deviation (range) if they were normally distributed. Categorical variables were expressed as counts (percentage). Variables were compared between the two outcomes (preterm birth or full-term birth) using univariate analysis. Continuous variables were compared using the Student's t-test or the Mann-Whitney U-test, depending on their distribution. Categorical variables were compared using the chi-squared test or Fisher's exact test. Variables in the univariate analysis with p-values <0.5 were selected for potential inclusion in the multivariate logistic regression. Then, stepwise logistic regression was performed to determine potential risk factors for preterm birth. Odds ratios (ORs) with 95% confidence intervals (CIs) and p-values were calculated. A two-tailed p-value <0.05 was considered significant.

RESULTS

Univariate analysis of two groups

Univariate analysis showed that there was no significant difference between the preterm group and the control group in terms of age, place of residence, and method of conception among pregnant women with twin pregnancy ($p>0.05$). However, educational background, chorionicity, and comorbidities significantly affected the incidence of preterm birth in twin pregnancy ($p<0.05$), and the difference was statistically significant ($p<0.05$), as shown in Table 1. The preterm birth rate of people with an associate degree and below was 73.1%, which was significantly higher than that of people with a university degree and

above (53.8%) ($p < 0.05$), which implied that the higher the preterm birth rate was at the end of the education background. In addition, the preterm birth rate of MCDA pregnant women (76.5%) was significantly higher than that of DCDA pregnant women (59.3%) ($p < 0.05$). It can be seen that the preterm birth rate of pregnant women with MCDA is higher. As for the impact of comorbidities on preterm birth, preeclampsia (75.7%) is more likely to cause preterm birth than gestational diabetes (56.4%) and premature rupture of membranes (69.9%).

Logistic regression analysis of the influencing factors of preterm birth

Logistic regression analysis showed that education (OR 0.651; 95%CI 0.525–0.807; $p < 0.001$), preeclampsia (OR 2.456; 95%CI 1.391–4.336; $p = 0.002$), and premature rupture of membranes (OR 1.770; 95%CI 1.057–2.964; $p = 0.030$) were high risk factors for premature twin birth, and the difference was statistically significant ($p < 0.05$), as shown in Table 2. In addition, preeclampsia has the greatest impact on preterm birth (OR 2.456; 95%CI 1.391–4.336; $p = 0.002$).

Table 1. Univariate analysis of twin pregnancy in preterm group and term group.

	Preterm group (n=363)			Term group (n=239)		χ^2	p-value
	Case	Proportion (%)	Preterm birth rate	Case	Proportion (%)		
Age							
<35 years	319	87.9	60.9	205	85.8	0.56617.6	0.452
≥ 35 years	44	12.1	56.4	34	14.2		
Education							
University and above	214	58.9	53.8	184	76.9	21.741	<0.001
Associate degree and below	149	41.1	73.1	55	23.1		
Place of residence							
City	278	76.6	58.5	197	82.4	2.956	0.086
Rural	85	23.4	66.9	42	17.6		
Way of conception							
Natural conception	97	26.7	62.6	58	24.3	0.454	0.500
Embryo transfer	266	73.3	59.5	181	75.7		
Chorionic							
DCDA	337	92.8	59.3	231	97.1	3.937	0.047
MCDA	26	7.2	76.5	8	2.9		
Comorbidities							
Preeclampsia	56	15.4	75.7	18	7.5	6.805	0.033
Gestational diabetes	44	12.1	56.4	34	14.2		
Premature rupture of membranes	58	16	69.9	25	10.5		

Table 2. A logistic regression analysis to identify independent factors associated with preterm birth in twin pregnancy in China from February 2018 to February 2020.

Risk factors	OR	95%CI	p-value
Education	0.651	(0.525–0.807)	<0.001
Monochorionic	0.167	(0.813–3.416)	0.163
Comorbidities			
Preeclampsia	2.456	(1.391–4.336)	0.002
Gestational diabetes	0.972	(0.617–1.531)	0.903
Premature rupture of membranes	1.770	(1.057–2.964)	0.030

DISCUSSION

With the promotion of ART, the incidence of twin pregnancy is on the rise [1], and subsequently, the risk of pregnancy complications in women with twin pregnancy is significantly higher than that in women with single pregnancy, such as preterm birth, high gestation period blood pressure disease, gestational diabetes, and premature rupture of membranes.

This study retrospectively analyzed the clinical data of 363 pregnant women with preterm twin pregnancy and 239 pregnant women with full-term twin pregnancy in our hospital. The incidence of preterm birth in twin pregnancy was 60.29%. Preterm birth is a common complication of twin pregnancy and the leading cause of perinatal morbidity and mortality. Preterm births account for 5–15% of all deliveries, and the incidence of preterm birth in twin pregnancy is approximately nine times higher than in singleton pregnancy⁶. The causes of premature twin birth are premature rupture of membranes, chorionicity, and iatrogenic premature birth caused by other complications of twins¹⁶. This analysis showed that education, chorionicity, and comorbidities were high-risk factors for twin preterm birth.

The results showed that the incidence of premature rupture of membranes in the preterm group was significantly higher than that in the term group, which was one of the risk factors for preterm birth in twin pregnancy. Studies have shown that the risk of preterm birth within 72 h after premature rupture of membranes in twin pregnancy is greater than that in singleton pregnancy¹⁷, which is related to factors such as reproductive tract infection, increased amniotic pressure, and uneven force on fetal membranes¹⁸. Therefore, for the prevention of premature rupture of membranes and active treatment of premature rupture of membranes, delaying the gestational age as much as possible is suggested in order to reduce the occurrence of premature birth in twin pregnancy, strict monitoring of reproductive tract infection during pregnancy, avoid strenuous activities in the late stage¹⁹, prohibit sexual life²⁰, and develop a healthy lifestyle habits²¹.

The proportion of premature birth among women with a college education and above is 53.8%, and the proportion of premature birth among women with an associate degree and below is 73.1%, indicating that the risk of premature birth is higher with low education level ($p < 0.05$). Most pregnant women generally live in rural areas, where health awareness and living standards are poor and the local medical level is limited²². We should popularize reproductive health knowledge during pregnancy, vigorously publicize and advocate regular prenatal examinations for pregnant and lying-in women, and strengthen supervision during pregnancy.

The incidence of MCDA in the preterm group was significantly higher than that in the control group. Due to the extensive vascular communication on the surface of the placenta in twin pregnancy, complications such as twin-to-twin transfusion syndrome and selective fetal growth restriction were prone to occur clinically. The gestational age of termination of pregnancy in the preterm group was also relatively earlier than that of double chorionic twins, and preterm birth was more likely to occur. Furthermore, in monochorionic twin pregnancy, the generally accepted approach is to terminate the pregnancy before term²³, which apparently contributes to a higher incidence of preterm birth in this group of pregnant women.

In a retrospective study involving 193 pairs of monoamniotic twins, it was found that the mortality rate of the fetus and neonate was lower when the pregnancy was closely monitored at 26–28 weeks of gestation and terminated at 33 weeks of gestation²⁴. Liu et al.²⁵ reported that the incidence of monochorionic twins in the preterm group was 32.94%, which was significantly higher than the 10.98% in the full-term group, which was consistent with the results of this study. Interestingly, although univariate analysis suggested that chorionicity was associated with preterm birth in twin pregnancy, a logistic regression analysis showed that chorionicity was not a risk factor for preterm birth in twin pregnancy ($p = 0.163$). We speculate that preterm birth in twin pregnancy may be caused by a combination of factors. In this study, univariate analysis was used to explore the association between chorionicity and preterm birth in twin pregnancy. In the next step, we will analyze the relationship between these risk factors and preterm delivery in twin pregnancy through correlation analysis and will also conduct a large sample multicenter prospective study to further confirm the impact of chorionicity on preterm birth in twin pregnancy.

Preeclampsia is a pregnancy-specific disease and one of the important causes of maternal and perinatal death, with an incidence of about 9.4% in China²⁶. Twin pregnancy is more likely to cause preeclampsia than singleton pregnancy^{27,28}. In this study, the incidence of preeclampsia in the preterm group was 75.7%, which was significantly higher than that in the full-term group, which was consistent with the findings of Butali et al.²⁹.

Studies have shown that the rate of preterm birth in the twin pregnancy group is higher than that in the singleton pregnancy group in ART patients ($p < 0.05$).

However, the results of this study showed that different methods of conception had no effect on the occurrence of preterm birth in twin pregnancy, which may have been inconsistent due to differences in the selected samples. Chambers et al.³⁰ suggested that blastocyst-stage transfer may be associated

with preterm birth in twin pregnancy compared with cleavage-stage embryo transfer (ET) in pregnant women undergoing ART. Therefore, potential confounding factors such as the ET method were not considered in the sample selection in this study, resulting in differences between samples. In the future, ET methods will be included in the evaluation for more in-depth exploration.

This study also has some limitations. First, this study is a single-center study, and the conclusions are not generalized. The next step will be a multicenter in-depth study. Second, there is a lack of consideration of potential variables. For example, the income of the families of the women studied was not included in the assessment, which in fact must be the main factor to explain the higher incidence of prematurity in pregnant women with lower levels of education.

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CONCLUSIONS

Twin pregnancy has a high rate of premature birth with many complications. Educational background, preeclampsia, and premature rupture of membranes are high risk factors for twin pregnancy. Pregnancy supervision and prenatal guidance for twin pregnancy should be strengthened to detect early. It is of great significance to reduce the incidence of preterm birth in twin pregnancy.

AUTHORS' CONTRIBUTIONS

LJD: Conceptualization, Formal Analysis, Supervision, Writing – original draft. **XPD:** Data curation, Formal Analysis. **WJW:** Data curation, Formal Analysis. **LYH:** Data curation. **CFQ:** Formal Analysis. **RS:** Formal Analysis.

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Varicose Vein Stripping in 66,577 patients in 11 years in public hospitals in São Paulo

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SUMMARY

OBJECTIVES: The aim of this study was to evaluate the epidemiology of varicose vein stripping in Brazil's largest city, São Paulo.

METHODS: Open and anonymous data regarding varicose vein surgeries between 2008 and 2018 were evaluated from the TabNet platform of the Municipal Health Secretary of São Paulo, Brazil.

RESULTS: Most patients were female and adults. A total of 66,577 varicose vein surgeries were performed in public hospitals and outpatient clinics in São Paulo, with a statistically significant increase for both unilateral ($p=0.003$) and bilateral ($p<0.001$) procedures. Since 2016, unilateral procedures have been performed more frequently than bilateral procedures. Most procedures were associated with same-day (54.8%) or next-day (32%) discharge. The in-hospital mortality rate was 0.0045%. The total amount reimbursed was \$20,693,437.94, corresponding to a mean value of \$310.82 per procedure.

CONCLUSION: Surgeries to treat chronic vein disease totaled 66,577 in 11 years, demanding \$20,693,437.94 from the public health system. The majority of treated patients were female, over 40 years of age, and local residents. Procedure rates have increased over the years. The in-hospital mortality rate was very low (0.0045%).

KEYWORDS: Big data. Varicose veins. Database. Epidemiology. Vascular diseases.

INTRODUCTION

Chronic venous disease (CVD) is a spectral and progressive disease, ranging from mild presentations, such as telangiectasia, reticular, and varicose veins, to severe complications, including venous thrombosis, varicorrhage, and skin alterations such as ochre dermatitis and venous ulcers¹⁻³.

In the United States, the estimated prevalence of CVD is approximately 23% of the adult population¹, while in Brazil, it is estimated at 47.6%⁴, and these numbers are expected to increase as the population ages⁵.

Therapeutic options for CVD range from conservative measures to invasive procedures. Conservative measures include lifestyle changes, encompassing weight loss, physical activity, and compressive therapy, whereas invasive procedures include sclerotherapy, endovenous ablation, and surgery.

Varicose vein stripping (VVS) refers to collateral phlebectomies through staggered incisions combined with eventual high ligation and removal of the saphenous vein. Even though

endovenous ablation of saphenous vein and hybrid procedures are becoming more popular^{6,7}, VVS is still the most performed treatment for CVD in Brazilian public hospitals and outpatient clinics⁸ as it is in other countries, such as China⁹.

Despite being the most commonly performed surgical treatment for the most common vascular disease¹⁰, whose burden is estimated to progressively increase in the coming years⁵, there are only a few population-based studies on statistics referring to VVS for CVD treatment. One nationwide study observed a slight downward trend in VVS rates, a very low mortality rate, and low reimbursement. However, demographics of the patients, which may impact the surgery and mortality rates, and length of intensive care unit (ICU) and hospital stay, which may affect hospitals' revenue, were not evaluated⁸.

Therefore, we designed the present study to evaluate the epidemiology of VVS performed between 2008 and 2018 in public hospitals in Brazil's largest city, São Paulo, which has an estimated population of more than 12 million¹¹, of which

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5 million depend exclusively on the public health system¹². In addition to being the largest and most populous city, the Municipal Health Secretary of São Paulo database provides the most detailed health data¹³, yielding more information than the national database, including demographics and hospital and ICU length of stay.

METHODS

Data were retrieved from the TabNet platform, a public health information application developed by DATASUS, the Health Informatics Department of the Brazilian Ministry of Health¹⁴. The TabNet system provides open and anonymous data regarding procedures performed within the Brazilian public health system, by hospitals adequately accredited as vascular surgery centers. Such accreditation is a prerequisite for government reimbursement.

This study was approved by the Ethics Committee of the institution where it was conducted. Since data are anonymous, the Institutional Revision Board (IRB) waived the application of informed consent forms.

Statistics referring to varicose vein surgeries between 2008 and 2018 were selected from the TabNet platform of the Municipal Health Secretary of São Paulo, Brazil. Among the selections, analysis included gender, age, municipality of residence, number and types of procedures performed, in-hospital mortality, length of hospital stay, ICU stay, and reimbursement by the public health system.

Two procedures were evaluated for the treatment of varicose veins, according to coding as established by the management system for procedures and medications of the Brazilian public health system – SIGTAP (Sistema de Gerenciamento da Tabela de Procedimentos, Medicamentos e OPM): *bilateral surgical treatment of varicose veins* (04.06.02.056-6) and *unilateral surgical treatment of varicose veins* (04.06.02.057-4).

All data were collected from public access sites through computer programs of automated content access (*web scraping*). These automated navigation codes were programmed in the Python language (v. 2.7.13, Beaverton, OR, USA) using the Windows 10 Single Language operational system.

The data collection, platform field selection, and table adjustment steps were performed using the selenium-webdriver packages (v. 3.1.8, Selenium HQ, several collaborators worldwide) and pandas (v. 2.7.13, Lambda Foundry, Inc. and PyData Development Team, New York, NY, USA). The Mozilla Firefox browser (v. 59.0.2, Mountain, CA, USA) and geckodriver webdriver (v. 0.18.0, Mozilla Corp., Bournemouth, England) were used.

Following collection and treatment, data were organized and grouped in spreadsheets using the Microsoft Office Excel 2016® (v. 16.0.4456.1003, Redmond, WA, USA) software and analyzed.

Reimbursement values in Brazilian Real (R\$, the Brazilian official currency) were converted into US dollars (U\$), using the exchange rate of December 31, 2012, the median date between the first and last cases evaluated.

STATISTICAL ANALYSIS

Linear regression was performed to evaluate the trends in the distribution of VVS procedures throughout the years, using the Stata SE® (StataCorp LLC, College Station, TX, USA). The level of statistical significance was set at <0.05.

RESULTS

Most patients were female (79.31%). The distribution of age group of patients treated with VVS is shown in Figure 1. Age group distribution was bell-shaped, with peaks between the ages of 35 and 60 years, corresponding to approximately three-quarters of all patients who underwent VVS. Only 5% were elderly, aged 65 years or more; 90.78% of the procedures were performed in patients with a registered residence address in São Paulo.

A total of 66,577 VVS to treat CVD were performed in São Paulo from 2008 to 2018, either in public hospitals or outpatient clinics, the proportion of which is unknown. The distribution of unilateral and bilateral VVS to treat CVD over time is depicted in Figure 2. There was a statistically significant upward trend for both unilateral ($p=0.003$) and bilateral ($p<0.001$) procedures over time, with a more expressive increase observed for unilateral surgeries (861% increase for unilateral VVS vs. 254% increase for bilateral VVS), which became the most performed procedure from 2016 onward.

Most procedures were associated with discharge on the same day (54.8%), 32% of procedures were associated with 1 day of hospitalization, and 11.6% with 2 days. Hospital stay longer than 3 days was rare (1.6%).

Regarding the need for ICU stay, only 29 overnight stay was charged throughout the study years.

Three in-hospital deaths were observed throughout the evaluated years. Considering the total of 66,577 procedures, the in-hospital mortality rate was 0.0045%.

The total amount reimbursed by the government for the surgeries during the 11 years evaluated was \$20,693,437.94, corresponding to an average amount of \$310.82 per procedure.

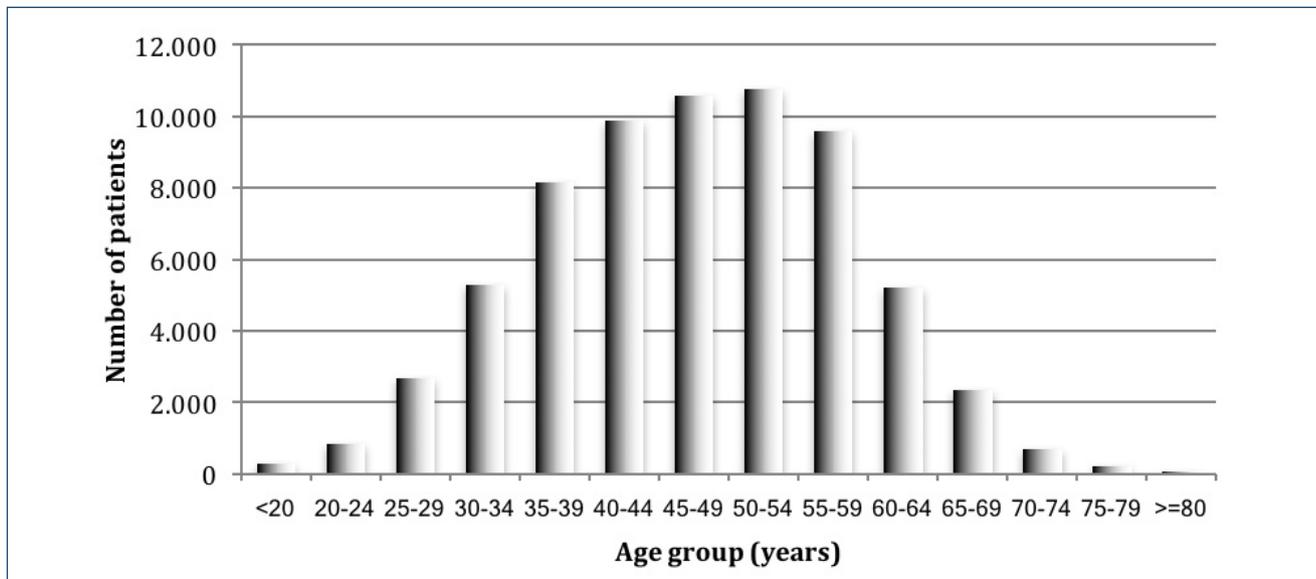


Figure 1. Distribution of age groups of the patients who underwent varicose vein surgery.

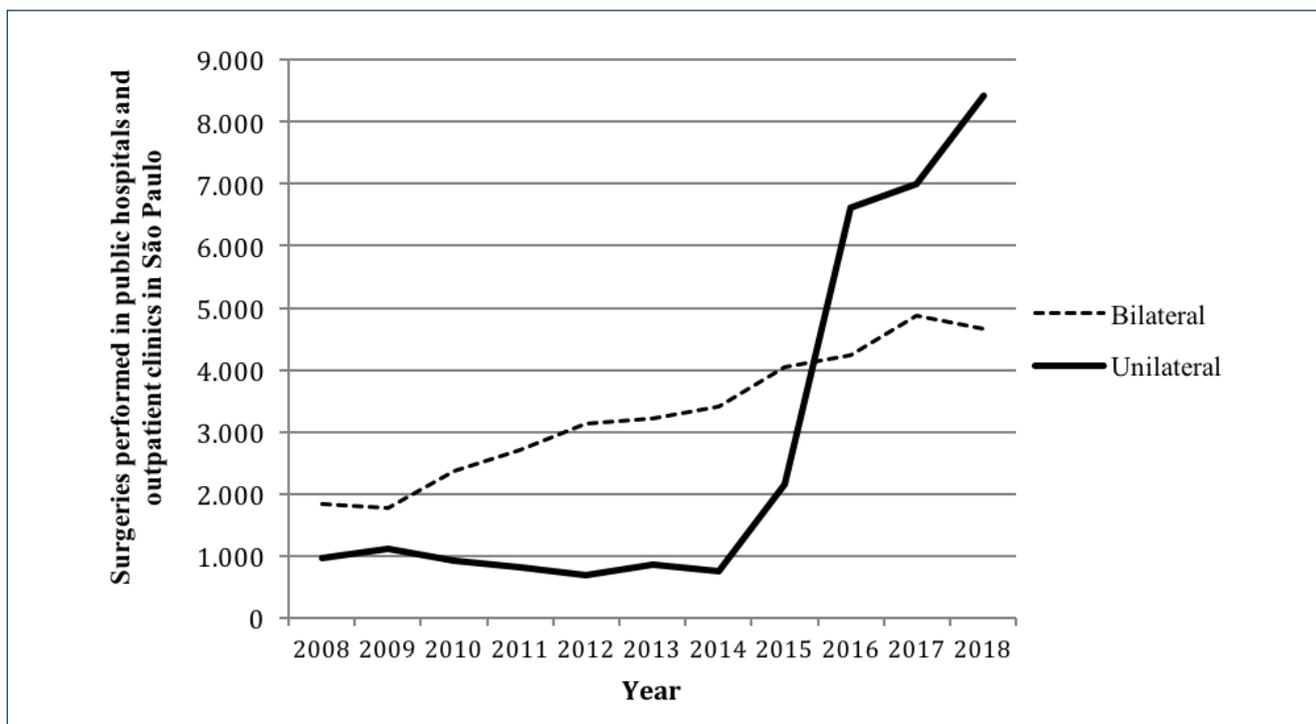


Figure 2. Distribution of unilateral and bilateral varicose vein surgeries between 2008 and 2018.

DISCUSSION

Our demographic data are in agreement with those of previous studies.

Along with a higher prevalence of CVD in women^{4,15}, this population possibly seeks treatment more often and/or earlier

than men, as female patients comprise the majority of the patients treated for CVD in our population and other studies¹⁶. Possible explanations for female predominance may involve sociocultural factors, such as a greater concern with health in general and with aesthetics in particular.

Since CVD is progressive, its prevalence is expected to increase with age. We observed a progressive increase in patients' age of up to 54 years and a decline thereafter, probably due to an increase in the prevalence of comorbidities likewise, elevating surgical risks. Only 5% of the patients were elderly, suggesting that perhaps nonsurgical CVD treatments are being preferentially offered to this group, such as foam sclerotherapy or conservative measures.

As for primary residence address, more than 90% of the patients were domiciled in São Paulo, which is a higher proportion than that observed in a previous report on surgical treatment for cerebrovascular disease in São Paulo¹⁷. As VVS is a less complex vascular intervention, it does not require referral to highly specialized centers and can be addressed in the city of the primary residence.

The trends for VVS that we observed were upward, consistent with other reports⁷, which is probably a reflection of increasing demand. Although the population of the city of São Paulo increased from 2008 to 2018, this may not fully explain the increase in VVS, as the population increased by 10.8%¹¹ in the period, while unilateral procedures increased by 861% and bilateral procedures increased by 254%. Thus, we hypothesize that the rise in demand was also influenced by the fact that the growing population of the city of São Paulo is also getting older¹¹ and more obese¹⁸, which are important risk factors for CVD^{19,20}. This finding contrasts with the national data, as the standardized procedure rates of VVS are not increasing at national level⁸. This difference is unlikely to be fully explained by a higher prevalence of CVD at the municipal level compared to the national level, even on account that the obesity prevalence in São Paulo is one of the lowest for men among other Brazilian capitals and intermediate for women¹⁸. One possible explanation is that at the municipal level, the public health system is able to meet the increased demand, which may not be happening at the national level. Another possible explanation is that outside São Paulo, foam sclerotherapy to treat CVD is being performed more relatively than VVS.

Unilateral procedures increased faster than bilateral ones, becoming the most frequently performed VVS since 2016, which was also observed in other studies^{16,21}. One possible explanation for this change may be the fact that, as VVS has been performed more frequently, the waiting lines may have shortened; hence, more patients may have been treated for less advanced and still unilateral disease. In addition, the population is aging, and unilateral procedures may be safer for older patients with more comorbidities and large varicose veins, as they are expected to feel a greater impact from bleeding.

The low in-hospital mortality rate that we observed (0.0045%) is similar to that observed in the national study (0.0056%). This is likely the result of a low-risk procedure performed on a low-risk patient, as we observed that 95% of the patients were under 65 years of age.

The high proportion of same-day discharges corroborates the safety of the procedure. Early complications that could explain 1.6% of procedures associated with hospital stay for 3 days or more and the three deaths we observed are mainly due to anesthetic complications and bleeding, which have been reported by other authors²¹. Another population-based study that evaluated mortality after surgery to treat CVD was the Japanese Society for Vascular Surgery Report, which did not observe any 30-day death⁶. However, their sample was almost half of ours.

Even though endovenous thermoablations are getting more popular and have shown good results^{22,23}, they are not superior to the VVS in all aspects^{22,23}, and their initial expenditures may be prohibitively high²⁴, given that the use of the endovenous thermoablation adds at least \$735 to the procedure while a standard disposable phleboextractor costs \$39. Moreover, the Brazilian public health system does not ordinarily reimburse them. It can be argued that endovenous thermoablations have the advantage of earlier postoperative recovery when compared to VVS, thus decreasing the economic burden due to lost work-days²⁴. However, so does the treatment with foam sclerotherapy, which is cheaper, with a vial cost of \$2, and is a standard treatment reimbursed by the government.

We observed an average amount of \$310.82 per VVS for the treatment of CVD, which is a relatively low amount. Considering that the health care burden of CVD is estimated to be 1–2% of the U.S. national budget, with more than \$1 billion being spent on wound care alone²⁵, public health care strategies should focus on treating CVD earlier by stimulating VVS, especially in outpatient clinics.

Limitations

As inherent in a retrospective analysis with automated data collection, our study is limited by the loss of patient information and eventual miscoding, which should not be significant given the large size of our population.

Another important limitation is that data are anonymous; therefore, we were not able to assess patients' clinical, etiological, anatomical, and pathological classifications or quality-of-life scores. In addition, we were also not able to perform adjusted analysis, and follow-up was impossible; hence, we do not know the complication rates, the causes of death of the three individuals, and whether there were any VVS-related deaths after discharge, due to pulmonary embolism, for example.

In addition, we were unable to distinguish how many patients were treated, because we only had access to the total number of procedures and some patients may have undergone more than one VVS – two unilateral procedures or even two bilateral procedures, given the long study period. We also do not know how many of the procedures involved saphenous treatment and how much was paid for each type of procedure. Moreover, governmental reimbursement is based on a fixed compensation table and may not reflect actual hospital expenses.

Despite these limitations, this is a comprehensive and detailed epidemiological analysis of VVS for the treatment of CVD, analyzing objective and compulsorily recorded data in the public data. Our findings show patients' demographics and procedure trends and highlight the safety of the treatment.

CONCLUSION

In a city whose population surpasses that of some European countries, surgeries to treat CVD were frequent and totaled 66,577 in 11 years, demanding \$20,693,437.94 from the public health system, which is a relatively low cost.

The majority of treated patients were female, over 40 years of age, and local residents. An increase in the number of procedures was observed over the years, for both unilateral and bilateral procedures. The in-hospital mortality rate was very low (0.0045%).

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AUTHORS' CONTRIBUTIONS

MFAS, ACSL: Data curation, formal analysis, project administration, writing – original draft, and writing – review & editing. **MPT, EAJ:** Conceptualization, formal analysis, and writing – review & editing. **DBDL:** Formal analysis, project administration, and writing – review & editing. **MFCP:** Data curation, formal analysis, project administration, and writing – review & editing. **NS:** Data curation, formal analysis, and writing – review & editing. **NW:** Conceptualization, formal analysis, project administration, and writing – review & editing.

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The value of next-generation sequencing for the diagnosis of *Streptococcus suis* meningitis

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the value of next-generation sequencing for the diagnosis of *Streptococcus suis* meningitis.

METHODS: Patients with meningitis in the Department of Neurology of the Hainan General Hospital were recruited and divided into a next-generation sequencing group and a control group. In the next-generation sequencing group, we used the next-generation sequencing method to detect the specific pathogenic bacteria in the patients. In the control group, we used the cerebrospinal fluid bacterial culture method to detect the specific pathogenic bacteria in the patients.

RESULTS: A total of 28 participants were recruited for this study, with 14 participants in each group. The results showed similarities in both the average age and average course of the disease between the two groups ($p > 0.05$). The white blood cell count, percentage of neutrophils, and level of C-reactive protein in the next-generation sequencing group were significantly higher than those in the control group ($p < 0.05$). There were similarities in both the temperature and intracranial pressure between the two groups ($p > 0.05$). In the next-generation sequencing group, all patients (100%) were detected as having had the *S. suis* meningitis infection by next-generation sequencing, while only 6 (43%) patients in the control group had been detected as having the *S. suis* meningitis infection by cerebrospinal fluid bacterial culture.

CONCLUSIONS: The positive detection rate of *S. suis* by the next-generation sequencing method was significantly higher compared with using a cerebrospinal fluid bacterial culture. Therefore, the next-generation sequencing method is valuable for the diagnosis of *S. suis* meningitis and is worthy of clinical application.

KEYWORDS: *Streptococcus suis*. Cerebrospinal fluid. Next-generation sequencing. Meningitis. Bacterial culture.

INTRODUCTION

Streptococcus suis (*Ss*) meningitis is an acute infectious disease caused by *Ss*, invading the central nervous system. The main form of transmission is through close contact between people and pigs; the pathogen invades the body through damaged skin or mucous membranes and infects the central nervous system via the blood. It is the primary type of *Ss* infection among humans, and its main clinical symptoms include fever, headache, and hearing loss, and in severe cases, consciousness disturbances may occur¹. Most of the cerebrospinal fluid of patients with *Ss* meningitis showed purulent meningitis², and some patients reflected similar viral meningitis³ or tuberculous meningitis⁴.

Next-generation sequencing (NGS) is a large-scale parallel sequencing technology that allows for thousands to billions of deoxyribonucleic acid (DNA) fragments to be simultaneously and independently sequenced⁵. Since most infectious agents include DNA or RNA genomes, existing research findings

on NGS technology have been widely applied to infectious diseases. Studies have shown that the application of NGS to infectious disease outbreaks of unknown causes and patients with suspected infections has positive results⁶. The applications of NGS included whole-genome sequencing of microbial isolates, microbiome studies, drug resistance testing of viruses or culture isolates, and lineage tracing⁷. Additionally, NGS technology exhibited efficient potential through unbiased pathogen detection. It has been demonstrated in the field of microbiology and in clinical contexts, and its application is rapidly becoming routine⁸.

Traditional blood culture and cerebrospinal fluid culture detection of pathogens have the disadvantages of long detection times and low positive rates. This can make it easy to misdiagnose and affect patients' prognoses. Cerebrospinal fluid pathogen NGS technology is an emerging molecular diagnostic method that can quickly detect intracranial pathogens. The literature

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evidenced its use in the diagnosis of encephalitis; however, its applicability for routine diagnosis has not been evaluated⁹. Accordingly, this study investigated the value of NGS for the diagnosis of *Ss* meningitis.

METHODS

Participants

From August 2018 to January 2021, patients with meningitis in the Department of Neurology of the Hainan General Hospital were recruited and divided into an NGS group and a control group. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of our hospital. All participants signed informed consent forms for inclusion in the study.

The diagnostic criteria of *Ss* meningitis are as follows:

1. Coma may occur in patients with acute onset, fever, chills, general discomfort, fatigue, headache, dizziness, nausea, and vomiting (possibly jet vomiting). There are no bleeding spots, ecchymosis spots, and ecchymosis spots on the skin. No shock.
2. The meningeal stimulation sign was positive, and the cerebrospinal fluid showed suppurative changes.
3. The NGS or cerebrospinal fluid bacterial culture method was used to detect specific pathogens in patients.

Inclusion and exclusion criteria

The inclusion criteria were as follows:

1. Patients who had been diagnosed with meningitis;
2. Those older than 18 years;
3. Patients suspected of suffering from *Ss* meningitis:
 - a. recent history of close contact with sick pigs and their products, especially those with damaged skin and mucosa;
 - b. acute onset, chills, fever, and other acute infectious poisoning symptoms; and
4. Patients who provided signed informed consent for inclusion in the study.

The exclusion criteria were as follows:

1. Patients who had an advanced malignant tumor and
2. Patients whose data were incomplete.

METHODS

In the NGS group, we used the NGS method to detect specific pathogenic bacteria in the patients. In the control group, we

used the cerebrospinal fluid bacterial culture method to detect the specific pathogenic bacteria in the patients.

Cerebrospinal fluid detection

For the lumbar puncture, approximately 2–3 mL of cerebrospinal fluid was collected, sterilely sealed, and stored at -20°C or transported on dry ice to Yuguo Biotechnology (Beijing) Co., Ltd., for metagenomics NGS detection. Following the TIANGEN DNA Mini kit DP316 (TIANGEN Biotechnology Company, Beijing, TIANGEN DNA) instructions, 200 µL of cerebrospinal fluid was collected to extract DNA and purify it. Nucleic acid concentration and quality were detected and confirmed using the Qubit dsDNA HS kit (Thermo Fisher Scientific Co., Ltd., Shanghai) and agarose gel electrophoresis.

The Qiagen library construction kit (QIAseq™ Ultralow Input Library Kit) operation manual was followed to complete the DNA library construction. The Qubit dsDNA HS kit (Thermo Fisher Scientific Co., Ltd.) and agarose gel electrophoresis were used to detect DNA concentration and quality. Qualified DNA libraries with different barcode tags were collected and sequenced by the Illumina NextSeq sequencing platform.

After obtaining the sequencing data, high-quality data were generated by filtering out joints, low-quality, low-complexity, and short sequences. Then, the human sequence that matched the human reference genome was removed using the SNAP software. Burrow-Wheeler alignment was used to compare the remaining data with the microbial genome database. The database collects a large number of microbial genomes from NCBI, including more than 20,000 microorganisms, 11,910 bacteria species, 7,103 virus species, 1,046 fungi species, and 305 parasite species. Finally, the microbiological composition of the sample is obtained. It took approximately 2–3 days to obtain the NGS microbial identification results from the cerebrospinal fluid.

Main observation indexes

In this study, the main observation indexes included sex, age, the course of the disease, temperature, white blood cell count, the percentage of neutrophils, the level of C-reactive protein, intracranial pressure, and cerebrospinal fluid detection.

Statistical analysis

This study used the SPSS Statistics version 20.0 (IBM, Chicago, IL, USA) software to conduct the statistical analysis. The continuous variables of normal distribution were expressed as mean±standard deviation, the continuous variables of non-normal distribution were expressed as a median

(interquartile range), and the categorical variables were expressed as a frequency (percentage [%]). For the two comparisons, each value was compared using a t-test when each datum conformed to a normal distribution, while non-normally distributed continuous data were compared using non-parametric tests. The counting data were tested by the chi-square test; $p < 0.05$ was considered statistically significant.

RESULTS

General characteristics

A total of 28 participants were recruited for this study, including 4 females and 24 males. There were 14 patients in the NGS group; the average age was 54.79 ± 10.34 years and the average course of the disease was 5.93 ± 3.52 days. There were 14 patients in the control group; the average age was 46.71 ± 18.96 years and the average course of the disease was 8.07 ± 7.49 days. The results showed similarities in both the average age ($p = 0.151$) and the average course of the disease ($p = 0.350$) between the two groups.

Comparison of routine and biochemical indexes between the two groups

In the NGS group, the temperature was $39.10 \pm 0.44^\circ\text{C}$, the white blood cell count was $12.45 \pm 4.21 \times 10^9$, the percentage of neutrophils was $82.48 \pm 8.50\%$, and the level of C-reactive protein was 134.07 ± 88.86 mg/L. In the control group, the temperature was $38.46 \pm 0.98^\circ\text{C}$, the white blood cell count was $7.95 \pm 2.91 \times 10^9$, the percentage of neutrophils was $71.99 \pm 9.23\%$, and the level of C-reactive protein was 4.70 ± 6.03 mg/L. The

results showed that the white blood cell count ($p = 0.005$), the percentage of neutrophils ($p = 0.004$), and the level of C-reactive protein ($p < 0.001$) in the NGS group were significantly higher than in the control group. There were also similarities in temperature ($p = 0.082$) between the two groups. The details are shown in Figure 1.

Cerebrospinal fluid test results between the two groups

In the NGS group, the intracranial pressure was 214.29 ± 67.14 mmH₂O. In the control group, the intracranial pressure was 190.86 ± 55.99 mmH₂O. The results showed similar intracranial pressure between the two groups ($p = 0.350$).

In the NGS group, all patients (100%) had been detected as having the *Ss* meningitis infection by NGS, while only 6 (43%) patients in the control group had been detected as having the *Ss* meningitis infection by cerebrospinal fluid bacterial culture.

DISCUSSION

S. suis is a Gram-positive and anaerobic zoonotic pathogen that can be divided into 35 serotypes, the most common of which is serotype II. Anyone is susceptible to the *Ss* pathogen and, following infection, it can cause meningitis (the most common manifestation), sepsis, endocarditis, and arthritis. Most of these conditions can lead to serious cochlear nerve and vestibular nerve damage sequelae¹⁰. The pathogen enters the central nervous system through the brain microvascular epithelial cells or choroidal epithelial cells via the blood-brain barrier or blood-cerebrospinal fluid barrier and causes inflammation of the meninges and brain parenchyma¹¹. The main pathogenic

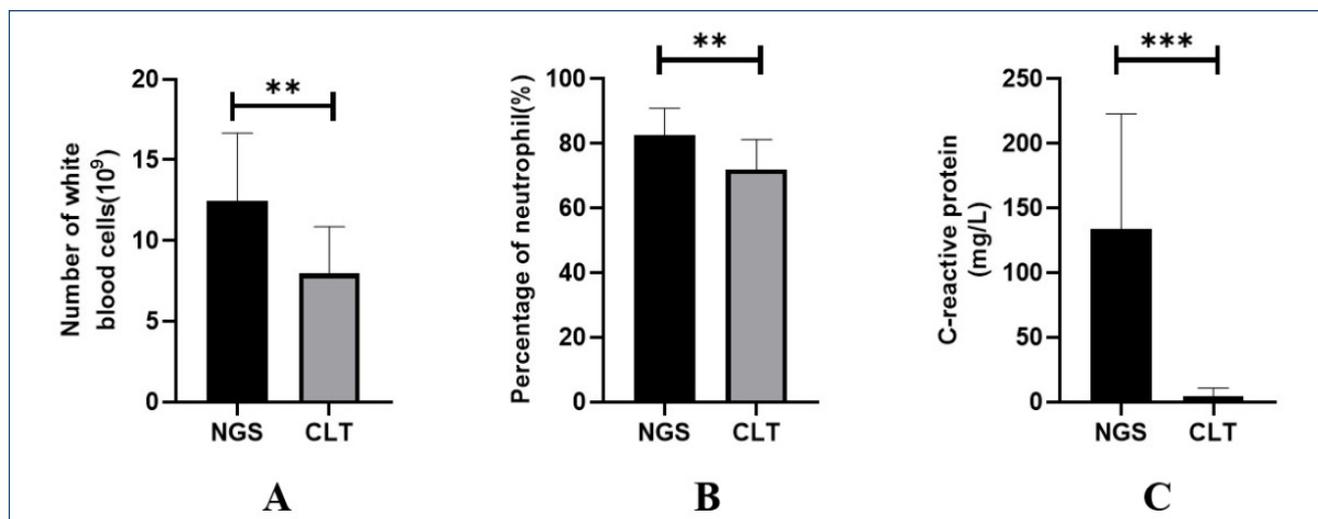


Figure 1. (A) The comparison of white blood cell between the two groups. (B) The comparison of the percentage of neutrophils between the two groups. (C) The comparison of the level of C-reactive protein between the two groups. ** $p < 0.01$. *** $p < 0.001$.

risk factors include occupational exposure to pigs and raw pork or eating raw pork products¹². The diagnosis of this disease depends on the positive culture of *Ss* in the blood-cerebrospinal fluid¹³. However, due to factors such as low blood-cerebrospinal fluid, insufficient sampling, or early antibiotic treatment, the positive rate of blood-cerebrospinal fluid cultures is low, and the positive rate of bacterial blood cultures is approximately 32%. The positive rate of cerebrospinal fluid bacterial cultures is 18.5%, leading to a higher incidence of poor prognosis¹⁴. Early diagnosis and treatment can help improve the clinical outcome of patients. Therefore, a rapid and accurate detection method is needed to assist with diagnosis in clinical practice. The NGS of cerebrospinal fluid is used as a molecular diagnosis of infectious diseases of unknown pathogens. This new technology has attracted significant attention⁵.

The common symptoms and signs of *Ss* meningitis include fever, headache, and neck stiffness¹⁵. Bilateral hearing loss can occur during early onset with an incidence as high as 66.4%¹⁶. All five patients had a fever, headache, and bilateral deafness. The incidence was 100%, which was higher than noted in existing literature reports^{15,16}. Reports in the literature showed that the classification of cerebrospinal fluid white blood cells in this disease was dominated by multiple nuclear cells, which were transformed into mononuclear cells or lymphocytes quickly after antibiotic treatment². If the epidemiological data of patients are not clear, the condition can be easily confused with tuberculous meningitis, resulting in a misdiagnosis.

The NGS of cerebrospinal fluid pathogens as a molecular technology to assist in the diagnosis of central nervous system infections can quickly and sensitively identify pathogens¹⁷. This can enable clinicians to make reasonable interpretations of test results, based on a patient's clinical manifestations, and select an appropriate antibiotic treatment plan¹⁸.

The results showed that all patients (100%) had been detected as having the *Ss* meningitis infection by NGS in the NGS group, while only 6 (43%) patients in the control group had been detected as having the *Ss* meningitis infection by cerebrospinal fluid bacterial culture. Therefore, the positive detection rate of *Ss* using the NGS method was significantly higher compared with detection via cerebrospinal fluid bacterial culture.

This study had multiple limitations. First, it was not a randomized controlled trial. Second, the sample size was limited and, as such, a larger trial with more participants is necessary for further study.

CONCLUSIONS

The positive detection rate of *Ss* by the NGS method was significantly higher compared with detection via cerebrospinal fluid bacterial culture. Therefore, the NGS method represents value for the diagnosis of *Ss* meningitis and is worthy of clinical application.

AUTHORS' CONTRIBUTIONS

EZ: Conceptualization, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **DW:** Formal analysis, Writing – original draft, Writing – review & editing. **ZZ:** Data curation, Writing – original draft, Writing – review & editing. **LX:** Data curation, Software, Writing – original draft, Writing – review & editing. **XH:** Software, Writing – original draft, Writing – review & editing. **PH:** Validation, Writing – original draft, Writing – review & editing. **FO:** Investigation, Writing – original draft, Writing – review & editing. **GW:** Resources, Writing – original draft, Writing – review & editing. **SH:** Supervision, Writing – original draft, Writing – review & editing. **YG:** Methodology, Writing – original draft, Writing – review & editing.

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Serum Calcium/Phosphorus Ratio in Biochemical Screening of Primary Hyperparathyroidism

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SUMMARY

OBJECTIVE: Primary hyperparathyroidism is a common endocrine disease and most cases are asymptomatic. Currently, in a hypercalcemic patient, the first laboratory investigation is serum primary hyperparathyroidism measurement. However, the primary hyperparathyroidism level cannot be measured in many primary healthcare centers in our country. In addition, serum calcium levels are normal in normocalcemic primary hyperparathyroidism patients, even if most centers have serum calcium levels measured. Therefore, a simple and inexpensive laboratory biochemical marker is required for the diagnosis of primary hyperparathyroidism. Recently, the calcium/phosphorus ratio has been proposed as a suitable tool for diagnosing primary hyperparathyroidism. This study aimed to investigate the diagnostic value of serum calcium/phosphorus ratio in primary hyperparathyroidism screening.

METHODS: A total of 462 patients followed in our clinic with a diagnosis of primary hyperparathyroidism were reviewed in this retrospective study. Out of these patients, 148 with normal levels of serum parathyroid hormone, calcium, and phosphorus were selected as the control group. Serum calcium, corrected calcium, phosphorus, albumin, parathyroid hormone, 25-hydroxyvitamin D, and creatinine were evaluated. The diagnostic accuracy of the calcium/phosphorus ratio was investigated using receiver operating characteristic curve analysis.

RESULTS: There were 404 (87.4%) females and 58 (12.6%) males in the primary hyperparathyroidism group. Calcium, parathyroid hormone, and calcium/phosphorus ratio were significantly higher in primary hyperparathyroidism than in controls ($p < 0.001$ for each). Receiver operating characteristic curve analyses identified a cutoff value of 2.59 (3.35 if calcium and phosphorus are measured in mg/dL) for the calcium/phosphorus ratio, with a sensitivity of 90.5% and specificity of 93.2% ($p < 0.001$).

CONCLUSION: The calcium/phosphorus ratio is a simple and inexpensive method for primary hyperparathyroidism screening when a cutoff value of 2.59 is used.

KEYWORDS: Calcium. Hyperparathyroidism. Hypercalcemia. Hypophosphatemia. Parathyroid hormone.

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a relatively common endocrine disease caused by overactive parathyroid glands and it is the most common cause of outpatient hypercalcemia^{1,2}. It is generally characterized by elevated serum calcium (Ca) levels along with parathyroid hormone (PTH) that are either elevated or inappropriately normal. Prevalence is 0.1–0.4%, being nearly three times more frequent in females than males³. In most patients with mild forms of PHPT, serum levels of Ca are mildly elevated, often within 1 mg/dL of the upper limit of normal, while phosphorus (P) levels are in the lower half of the normal range. Only in severe forms of the disease, P might be significantly lower because of increased P excretion⁴⁻⁶. Currently, when PHPT is suspected in a patient, the first laboratory investigation is the measurement of serum PTH

and 25-hydroxyvitamin D. In many cases, advanced investigations such as 24-h urinary Ca, parathyroid ultrasonography, and sestamibi scanning are required to exclude other causes of hypercalcemia and make the diagnosis clear.

PHPT is often recognized during biochemical screening. Historically, most patients presented with overt symptoms and signs of PHPT. However, with the advent and widespread use of automated blood analyzers, the majority of patients are diagnosed through routine biochemical laboratory testing done for other reasons⁷. Thus, in recent years, clinicians have more frequently come across asymptomatic PHPT and normocalcemic PHPT (NPHPT) cases^{8,9}. Accurate screening and early diagnosis are important to prevent untreated clinical progression in this group. In addition, PTH measurement may not be possible in primary healthcare institutions with limited resources.

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Therefore, more practical and inexpensive biochemical markers are needed for the early diagnosis and screening of PHPT.

Recent studies have focused on the use of serum calcium/phosphorus (Ca/P) ratio for the diagnosis of PHPT, considering the inverse relationship between serum Ca and P in pathogenesis. Madeo et al.¹⁰ examined the Ca/P ratio in the diagnosis of PHPT. They reported a cutoff value of 2.71 (3.5 if Ca and P are measured in mg/dL) for the Ca/P ratio in the diagnosis of PHPT, with a sensitivity of 86% and specificity of 87%. When this ratio was evaluated in 35 patients with NPHPT, the sensitivity decreased to 71%, while the specificity of 88% was maintained. A recent study in the Chinese population confirmed that a Ca/P ratio of over 2.94 can distinguish PHPT patients from healthy controls with a sensitivity of 95.5% and a specificity of 98.7%¹¹. In this study, we aimed to investigate the diagnostic value of serum Ca/P ratio in PHPT screening in our patient population with PHPT.

METHODS

In this retrospective, single-center, case-control study, patients diagnosed with PHPT in our clinic between January 2016 and January 2019 were examined. In all, 462 patients with PHPT were included in the study, and 148 healthy control groups who applied to our center in the same period were selected retrospectively. In our series, 396 of 462 patients with PHPT were operated on and the diagnosis was confirmed histopathologically. There was no clinical indication for surgery in 66 patients who did not undergo surgery. All patients operated on had clinical indications for surgery according to criteria established by PHPT guidelines¹². Exclusion criteria for both cases and controls were less than 18 years of age, severe renal (GFR <30 mL/min) or hepatic failure, secondary (including vitamin D deficiency or chronic kidney disease) or tertiary causes of hyperparathyroidism, metabolic bone disease (such as Paget's disease and osteomalacia), known malignancy of any kind, familial hypocalciuric hypercalcemia (FHH), non-PHPT-related hypophosphatemia, and drugs that interfere with Ca or bone metabolism (steroids, calcitriol, thiazides, phosphate binders, lithium, cinacalcet, bisphosphonates, and denosumab). Biochemical data, including serum Ca (mmol/L), P (mmol/L), albumin (g/dL), PTH (pg/mL), 25-hydroxyvitamin D (μ g/L), and creatinine (Cr) (mg/dL) were obtained from medical records. Local ethical committee approval was obtained in accordance with the ethical standards of the Declaration of Helsinki.

Serum total Ca was determined using a reference clinical chemistry laboratory (Roche Diagnostics), with a normal reference range of 2.12–2.62 mmol/L (8.5–10.5 mg/dL). The normal

reference range for serum P was 0.81–1.45 mmol/L (2.5–4.5 mg/dL). The reference range for albumin and Cr was 3.5–5.2 and 0.5–1.1 mg/dL, respectively. Plasma intact PTH was measured using the Allegro IRMA (Roche Diagnostics) with a detection limit of 1 pg/mL (normal range, 15–60 pg/mL) and a 2 and 10% intra- and inter-assay coefficient of variation, respectively. 25-Hydroxyvitamin D was measured using liquid chromatography coupled with tandem mass spectrometry (Schimadzu-API LC-MS-MS API 3200, Canada) with lower and upper detection limits of 4 and 150 μ g/L (normal range, 20–80 μ g/L), respectively. Serum Ca was corrected according to the following formula:

$$\text{Corrected calcium} = \text{Total calcium} + [0.8 \times (4.0 - \text{albumin})]$$

Patients with PHPT were subgrouped as hypercalcemic and NPHPT. The diagnosis of hypercalcemic PHPT was based on elevated serum albumin-corrected calcium (>2.62 mmol/L) with high serum PTH concentrations (>60 pg/mL). NPHPT was diagnosed in the presence of elevated serum PTH concentrations (>60 pg/mL) with normal serum albumin-corrected calcium (\leq 2.62 mmol/L) on two separate occasions. Patients with NPHPT with 25-hydroxyvitamin D3 levels below 30 μ g/L received maintenance cholecalciferol (vitamin D3) replacement of 1500–2000 IU/day for at least 8 weeks for accurate diagnosis¹³. After vitamin D3 replacement, serum-corrected Ca levels were within normal limits, but the elevation in PTH level persisted.

The SPSS 24.0 software package (IBM Corp., Armonk, NY, USA) was used for statistical analysis. We presented the descriptive statistics as mean \pm standard deviation for normally distributed variables, median (minimum-maximum) for non-normally distributed variables, and the number of cases and percentages for nominal variables. A comparison between categorical variables was made using the chi-square test. The Student's t-test was used for parametric variables and the Mann-Whitney U test was used for nonparametric variables to investigate the difference between groups. For all comparisons, $p < 0.05$ was considered statistically significant. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated. The diagnostic accuracies of Ca/P and corrected Ca/P were investigated using receiver operating characteristic (ROC) curves to identify cutoff points that better define affected patients based on their biochemical profiles.

RESULTS

There were 404 (87.4%) females and 58 (12.6%) males in the PHPT group, and the median age of the cohort was 55 (20–82) years. There were 96 (64.9%) females and 52 (35.1%) males in the control group, and the median age was 51 (19–76) years.

The median age was similar in both groups ($p=0.059$). Serum total Ca, corrected Ca, and PTH were significantly higher, and serum P was significantly lower in PHPT than in controls ($p<0.001$ for each). 25-Hydroxyvitamin D levels were similar in the two groups ($p=0.058$). The median serum total Ca/P ratio was 3.32 (1.84–8.61) in the PHPT and 2.10 (1.53–2.91) in the control group ($p<0.001$). The median-corrected Ca/P ratio was also significantly higher in the PHPT group than in the control group [3.20 (1.71–7.85) vs. 1.94 (1.47–2.77), $p<0.001$] (Table 1).

Of all the patients, 328 (71%) had hypercalcemic PHPT and 134 (29%) had NPHPT. The mean age was similar in both the hypercalcemic PHPT and NPHPT groups compared to the control group. Serum Ca, corrected Ca, and PTH were significantly higher in the hypercalcemic PHPT group than in the control group, while serum P and albumin levels were significantly lower ($p<0.001$ for each). Hypercalcemic PHPT patients had a significantly higher serum Ca/P ratio and corrected Ca/P ratio than the controls ($p<0.001$ for each). 25-Hydroxyvitamin D levels were similar to controls in both groups ($p=0.061$ and $p=0.053$, respectively). Similarly, serum Ca, corrected Ca, and PTH were significantly higher in the NPHPT group compared to that in the control group, while serum P was significantly lower ($p<0.001$ for each). NPHPT had a significantly higher serum Ca/P ratio and corrected Ca/P ratio compared to controls ($p<0.001$ for each) (Table 1).

The Ca/P threshold of 2.59 (3.35 if Ca and P are measured in mg/dL) obtained by the ROC curve analysis had the highest sensitivity and specificity for the diagnosis of PHPT (90.5%

sensitivity and 93.2% specificity, $p<0.001$) (Figure 1A). In addition, the optimal corrected Ca/P threshold was 2.46 (3.18 if Ca and P are measured in mg/dL) with a sensitivity of 91.8% and specificity of 92.6% for the PHPT diagnosis (Figure 1B).

The cutoff value of 2.59 for the Ca/P ratio was able to accurately identify 418 out of 462 PHPT patients, while only 10 out of 148 controls were incorrectly diagnosed with PHPT. The cutoff value of 2.59 for the Ca/P ratio correctly identified 312 out of 328 hypercalcemic PHPT patients and 106 out of 134 NPHPT patients.

The diagnostic values of serum Ca/P ratio, corrected Ca/P ratio, and PTH for the diagnosis of PHPT are given in Table 2. Accordingly, the accuracy of Ca/P ratio of 2.59 was 94.5% in hypercalcemic and 86.5% in NPHPT patients. Considering the corrected Ca/P of 2.46, the diagnostic accuracy was 95.6% in the hypercalcemic and 86.1% in the NPHPT patients.

Bone mineral densitometry (BMD) was evaluated with dual-energy x-ray absorptiometry (DEXA) in all patients with PHPT. Of the 462 patients, 206 (44.6%) had osteoporosis (T score ≤ 2.5 in one of three areas), 149 (32.2%) had osteopenia (T score between -1 and -2.5), and 107 (23.2%) had normal scores. Nephrolithiasis was detected in 117 (25.3%) patients on renal ultrasound. Of the 462 patients with PHPT, 396 were operated on for clinical indications and the diagnosis was confirmed histopathologically. In the operated patients, parathyroid adenoma was found in 348 (87.9%), parathyroid hyperplasia in 16 (4%), atypical parathyroid adenoma in 30 (7.6%), and 2 (0.5%) parathyroid carcinoma was detected.

Table 1. Comparison of demographic, biochemical, and hormonal data in patients with hypercalcemic primary hyperparathyroidism, normocalcemic primary hyperparathyroidism, and controls.

	All patients PHPT (n=462)	Hypercalcemic PHPT (n=328)	Normocalcemic PHPT (n=134)	Control (n=148)	p ¹	p ²	p ³
Age (years)	55 (20–82)	55 (20–82)	54 (27–77)	51 (19–76)	0.059	0.062	0.079
Sex, n (%)							
Female	404 (87.4)	283 (86.3)	121 (90.3)	96 (64.9)	<0.001	<0.001	<0.001
Male	58 (12.6)	45 (13.7)	13 (9.7)	52 (35.1)			
Ca (2.12–2.62 mmol/L)	2.80 (2.30–4.02)	2.85 (2.6–4.02)	2.67 (2.30–2.82)	2.34 (2.17–2.62)	<0.001	<0.001	<0.001
P (0.81–1.45 mmol/L)	0.84 (0.36–1.42)	0.82 (0.36–1.26)	0.91 (0.55–1.42)	1.13 (0.81–1.45)	<0.001	<0.001	<0.001
Ca/P ratio	3.32 (1.84–8.61)	3.51 (2.26–8.61)	2.94 (1.84–4.55)	2.10 (1.53–2.91)	<0.001	<0.001	<0.001
Corrected Ca (2.12–2.62 mmol/L)	2.68 (2.19–4.12)	2.75 (2.62–4.12)	2.56 (2.19–2.62)	2.24 (2.10–2.43)	<0.001	<0.001	<0.001
Corrected Ca/P ratio	3.20 (1.71–7.85)	3.39 (2.10–7.85)	2.79 (1.71–4.65)	1.94 (1.47–2.77)	<0.001	<0.001	<0.001
PTH (15–60 pg/mL)	132 (7.60–1210)	144 (7.60–1210)	97 (30.17–494)	54 (21–60)	<0.001	<0.001	<0.001
Creatinine (0.5–1.1 mg/dL)	0.69 (0.30–1.1)	0.69 (0.30–1.1)	0.68 (0.33–0.99)	0.67 (0.41–1.11)	0.007	0.031	0.007
Albumin (3.5–5.2g/dL)	4.56 (3.49–5.78)	4.52 (3.49–5.78)	4.62 (3.70–5.10)	4.50 (4.0–5.20)	0.035	0.001	0.459
25-Hydroxyvitamin D (20–80 µg/L)	16.10 (2–121)	14.70 (2–121)	18.79 (3–67)	23 (8–53)	0.058	0.061	0.053

PHPT: primary hyperparathyroidism; Ca: serum calcium; P: serum phosphorous; PTH: parathyroid hormone. p¹ indicates statistical difference between all primary hyperparathyroidism patients and control group. p² indicates statistical difference between hypercalcemic primary hyperparathyroidism patients and control group. p³ indicates statistical difference between normocalcemic primary hyperparathyroidism patients and control group.

Bold values indicate statistical significance at the $p<0.05$ level.

DISCUSSION

This study showed that a serum Ca/P value above 2.59 (3.35 if Ca and P are measured in mg/dL) is an ideal indicator for PHPT screening. Our results show that the Ca/P ratio is a simple, inexpensive, and valuable tool in the diagnosis of PHPT and can be used in both normocalcemic and hypercalcemic patients.

PTH is one of the most important hormones that maintain Ca and P homeostasis. It regulates serum Ca and P concentrations through its receptor-mediated, combined actions on the bone, intestine, and kidney. In the kidney, PTH increases the reabsorption of Ca, predominantly in the distal convoluted tubule, and inhibits the reabsorption of P in the renal proximal

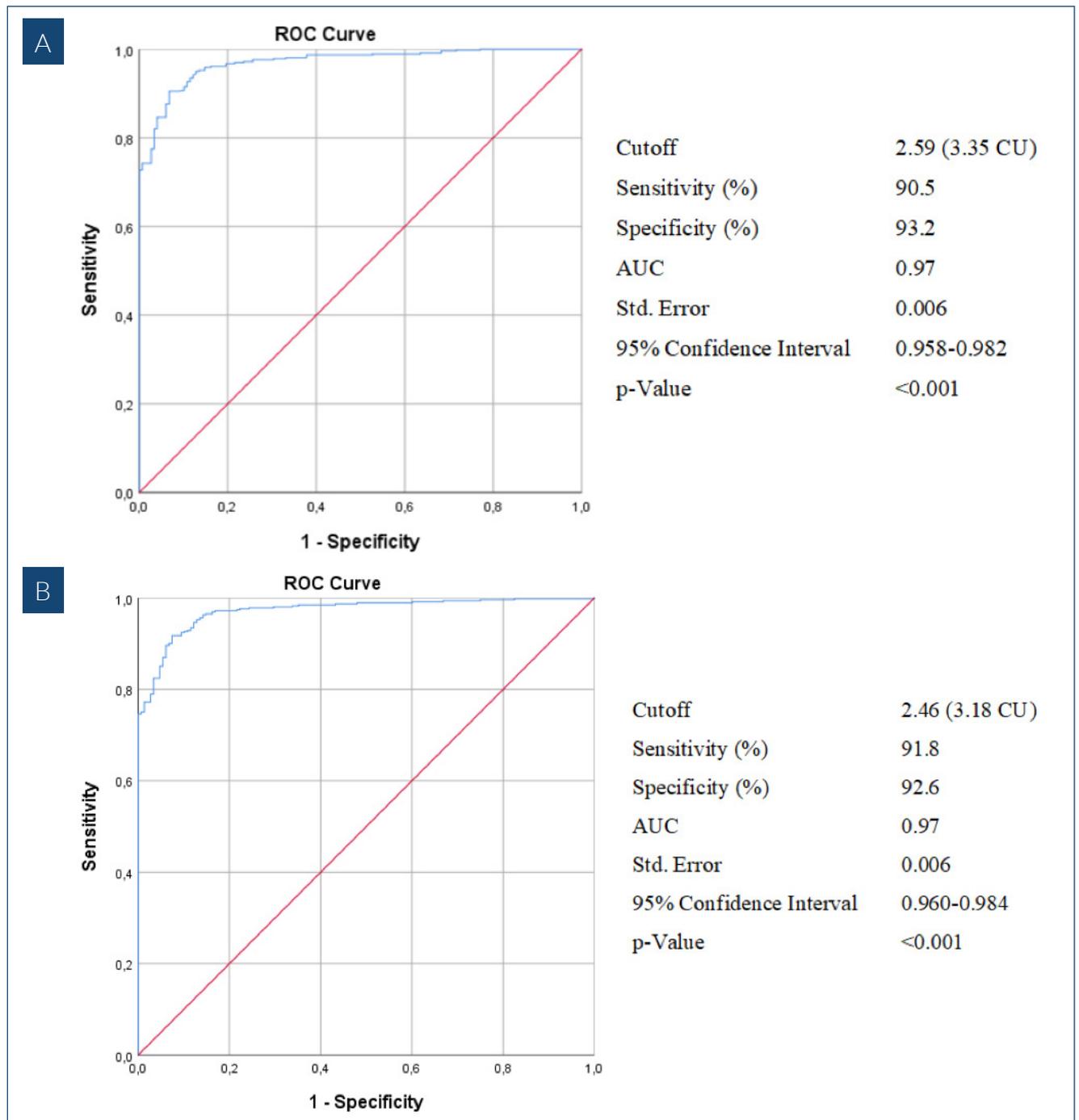


Figure 1. (A) Receiver operating characteristic (ROC) curve analysis for calcium/phosphorus with cutoff, sensitivity, specificity, AUC, standard error, and 95% confidence interval values. (B) ROC curve analysis for corrected calcium/phosphorus with cutoff, sensitivity, specificity, AUC, standard error, and 95% confidence interval values. CU: conventional units (calcium and phosphorus measured in mg/dL); AUC: area under the curve.

Table 2. Diagnostic value of Ca/P ratio, corrected Ca/P ratio, and serum parathyroid hormone level for primary hyperparathyroidism diagnosis in the entire cohort, hypercalcemic primary hyperparathyroidism, and normocalcemic primary hyperparathyroidism.

	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
All patients						
Ca/P ratio	2.59 (3.35 CU)	90.5	93.2	97.7	75.8	91.2
Corrected Ca/P	2.46 (3.18 CU)	91.8	92.6	97.5	78.3	92.0
Serum PTH	60	89.0	100	100	74.4	91.6
Hypercalcemic PHPT						
Ca/P ratio	2.59 (3.35 CU)	95.1	93.2	96.9	89.6	94.5
Corrected Ca/P	2.46 (3.18 CU)	97.0	92.6	96.7	93.2	95.6
Serum PTH	60	93.0	100	100	86.6	95.2
Normocalcemic PHPT						
Ca/P ratio	2.59 (3.35 CU)	79.1	93.2	91.4	83.1	86.5
Corrected Ca/P	2.46 (3.18 CU)	79.0	92.6	90.5	83.0	86.1
Serum PTH	60	79.1	100	100	84.1	90.1

PTH: parathyroid hormone; PHPT: primary hyperparathyroidism; Ca: serum calcium; P: serum phosphorus; PPV: positive predictive value; NPV: negative predictive value; CU: conventional units (Ca and P measured in mg/dL).

tubule, with the net result of hypercalcemia and hypophosphatemia¹⁴. Due to this inverse relationship in Ca and P homeostasis, it was predicted that the Ca/P ratio could be used in the diagnosis of PHPT, and recent studies have focused on this ratio. Madeo et al.¹⁰ published the first study examining the Ca/P ratio in the diagnosis of PHPT. They retrospectively evaluated 97 patients with PHPT and 96 control subjects with normal PTH and Ca. They reported a cutoff value of 2.71 (3.5 mg/dL) for the Ca/P ratio in the diagnosis of PHPT with a sensitivity of 86% and specificity of 87%, and it was confirmed by the independent big database. When this ratio was evaluated in 35 patients with NPHPT, the sensitivity decreased to 71%, while the specificity of 88% was maintained. This study highlights the greater diagnostic power of the Ca/P ratio compared to total or corrected calcium in the diagnosis of PHPT, including the NPHPT form. The authors concluded that the Ca/P ratio could be a valuable and inexpensive method for detecting PHPT in healthcare institutions where PTH measurement cannot be performed. In another study by Madeo et al.¹⁵ involving 142 patients diagnosed with NPHPT, the Ca/P ratio cutoff value of 2.55 showed high sensitivity (71.1%) and specificity (87.9%). Yin et al.¹¹ recently reported a sensitivity of 95.5% and specificity of 98.7% with a Ca/P ratio cutoff value of 2.94, and this index was positively correlated with the PTH level ($r=0.875$, $p<0.001$). In another study, the cutoff value of 2.55 for the Ca/P ratio served as a reliable predictor for the diagnosis of PHPT with 95.6% sensitivity and 63.6% specificity¹⁶. These results show that this ratio can be a promising screening method for PHPT diagnosis.

In our study, Ca/P ratio above 2.59 was 90.5% sensitive and 93.2% specific for the diagnosis of PHPT. The cutoff value of 2.59 was able to correctly identify 418 out of 462 PHPT patients, and only 10 out of 148 controls were diagnosed with false PHPT. The sensitivity of the Ca/P ratio was 95.1% and the specificity was 93.2% in the hypercalcemic PHPT group, and as expected, the diagnostic sensitivity of the Ca/P ratio was highest in the hypercalcemic group. Our results were similar to the results of Madeo et al.'s¹⁰ first multicenter study that evaluated the diagnostic value of Ca/P in the diagnosis of PHPT. Our findings support the notion that the Ca/P ratio can be a simple and inexpensive tool for early detection and screening of PHPT. Especially in primary healthcare, it can provide great convenience to general practitioners in diagnosing and guiding patients correctly.

NPHPT is characterized by persistently increased serum PTH levels in the setting of normal albumin-adjusted and ionized serum Ca. Secondary causes of hyperparathyroidism such as renal failure, vitamin D deficiency, and the use of thiazide diuretics should be excluded from the diagnosis¹⁷⁻¹⁹. In recent years, the diagnosis of NPHPT has increased due to the widespread use of PTH tests. PTH levels are measured in some patients, even when serum Ca levels are normal. Especially in the comprehensive approach to biochemical evaluation of osteoporosis and metabolic bone diseases, PTH levels are measured despite normal serum Ca levels. In this regard, more individuals are diagnosed with NPHPT. Some patients with NPHPT may progress to a hypercalcemic state or classic conditions that require surgical intervention, such as fractures and kidney

stones. Recent reports showed high rates of osteoporosis (57%), fractures (11%), and nephrolithiasis (14%) in these patients²⁰. There are also studies showing an increase in comorbidities in NPHPT⁹. Therefore, it is important to diagnose these patients early. Evaluating the Ca/P ratio in patients with normal serum Ca levels but suspicious of NPHPT can make a significant contribution in terms of early diagnosis. Thus, delays in the diagnosis and treatment of the disease can be prevented and PHPT-related comorbidities can be reduced. In our study, the diagnostic sensitivity of the Ca/P ratio of 2.59 was 79.1% and the specificity was 93.2% in the NPHPT group. Although the diagnostic performance of the Ca/P ratio was better in the hypercalcemic group, our findings suggest that this ratio is a simple but effective tool that can be used for screening and early diagnosis of both classical PHPT and NPHPT.

The serum Ca/P ratio can be used as an accurate tool to differentiate patients with PHPT from healthy subjects. However, although PHPT is the most common cause of hypercalcemia, other disorders of Ca-P metabolism can impair the Ca/P ratio^{1,8}. For example, FHH, malignancy hypercalcemia due to parathyroid hormone-related peptide (PTHrP), and hereditary hypophosphatemic rickets with hypercalciuria (HHRH) can all be present with hypercalcemia and hypophosphatemia, and therefore a high Ca/P ratio²¹⁻²³. In addition, hypophosphatemia is frequently observed in patients with human immunodeficiency virus (HIV) infection and receiving highly active antiretroviral therapy (HAART)^{24,25}. For all these reasons, it does not seem appropriate to use it alone for a definitive diagnosis without PTH measurement, because there may be other causes of hypercalcemia and/or hypophosphatemia that can be overlooked by using the Ca/P ratio alone. All these conditions should be considered, and the Ca/P ratio should be used in an appropriate clinical context.

The most important limitation of our study is its retrospective design. This also kept us from evaluating the ionized

Ca level in the PHPT patient group. Including ionized calcium as a mandatory parameter may change patient categories, but ionized Ca levels are not routinely checked in our center or in many centers. Therefore, we chose our criteria based on serum total Ca because patients would initially be confronted with serum Ca and P levels. In addition, we corrected serum Ca levels according to albumin levels and also did statistical analysis including this parameter. Another limitation of this study was that patients and controls did not match very well by gender.

CONCLUSION

Evaluating serum Ca/P ratio in addition to serum Ca and P level is a very simple, inexpensive, and valuable tool for early diagnosis of PHPT. Moreover, the diagnostic value of this ratio is high in patients with hypercalcemic PHPT as well as with NPHPT. When evaluating a patient for PHPT, the serum Ca/P ratio should also be evaluated instead of just the serum Ca and P level, but the PTH measurement is still required for a definitive diagnosis. The serum Ca/P ratio can be useful in PHPT screening and early diagnosis and can guide clinicians to refer patients for further examination in primary healthcare institutions where PTH measurement cannot be performed.

AUTHORS' CONTRIBUTIONS

NB: Conceptualization, data curation, formal analysis, methodology, writing – original draft, writing – review & editing. **FNC, BP, BEO:** Data curation, formal analysis. **DO:** Conceptualization, formal analysis, methodology, writing – original draft, writing – review & editing. **RE:** Conceptualization. **BC:** conceptualization, writing – original draft, writing – review & editing.

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Perception of Brazilian medical students toward organ donation

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SUMMARY

OBJECTIVE: Brazil ranks second in gross numbers of kidney and liver transplantations, but deceased organ donation is still far below the national demand for organs. Apart from a high family refusal rate, another significant barrier is healthcare workers' inappropriate knowledge concerning organ donation and transplantation. Since most of them have recently graduated from university, this study aimed to evaluate the awareness and attitudes of medical students concerning organ donation.

METHODS: Between August and September 2021, a web-based survey with 10 overlapping questions was sent to medical students from several Brazilian universities after advertisements on social media.

RESULTS: A total of 391 (60% female, mean age: 23 [17–41] years) students answered the survey. Most of them (91%) would like to donate organs in case of death, and 75% have already notified their family. The main reasons for not becoming a donor were fear of body manipulation (n=3) or family reaction (n=2) and religious reasons (n=2). When inquired, 54% were unaware of brain death criteria, and half of them claimed to have never discussed the topic in medical classes. Except for Glasgow coma scale (97%), pupillary (72%), and corneal (53%) reflexes, less than 25% knew other reflexes required for brain death diagnosis.

CONCLUSION: Most medical students declared themselves as organ donors, and most informed their families. However, many students have never learned about crucial steps toward organ donation, which may impact their adequate recognition of potential organ donors later in life.

KEYWORDS: Organ transplantation. Medical Students. Knowledge. Attitudes.

INTRODUCTION

Brazil ranks second in gross numbers of kidney and liver transplantations worldwide after the United States (US), but performs far lower when compared to the US and Western Europe in terms of organ donation and transplantation per million people (pmp)¹. According to data from the Brazilian Association of Organ Transplantation (ABTO), from January to September 2021, 25,912 subjects were placed on a waiting list for organ transplantation in Brazil. Likewise, 1,772 of them died in the same period waiting for an appropriate donor. Reports from the Brazilian Ministry of Health estimate that around 35,000 subjects are still waiting to be submitted, mainly for kidney and liver transplantation, with a long average waiting time due to organ shortage. Transplantation

activity in Brazil is funded by the United Public Health System (SUS) and is mainly dependent on deceased organ donation. The country's donation rate from 2018 to 2019 varied from 15.8 to 18.1 effective donors pmp, with a considerable gap between the supply and demand for organs¹.

Brazil has opted for an opt-out donation system due to the requirement of family consent for donation¹. There is a striking variability in donation and family refusal rates across the country, with frequencies approaching those seen in Spain and Croatia in the South and Southern regions, with a high human development index (HDI) compared to others with low HDI. Suboptimal identification of potential donors by healthcare personnel unaware of the brain death criteria adopted by the Brazilian legislation or without proper

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knowledge about notification and management of potential donors is also well recognized as a significant barrier to organ donation in Brazil and several other countries. Since most healthcare workers currently employed as physicians in Brazilian emergency departments or intensive care units are physicians who recently graduated from medical universities, the authors performed a web-based survey among medical students regarding knowledge and attitudes concerning post-mortem organ donation (PMOD). This manuscript summarizes its major findings.

METHODS

This study was conducted between August and September 2021 using a web-based questionnaire sent to medical students by 15 leaders of scientific leagues through social media platforms.

The questions were divided into two blocks, comprising demographic variables and specific questions regarding the research subject.

Demographic variables included age, gender, type of medical school (MS), years of studying medicine, and affiliation in a medical student league.

The specific questions (Q) concerning attitudes toward organ donation and knowledge about brain death criteria and their possible answers (under parentheses) were as follows:

- Q1: In case of death, would you like to donate your organs (yes or no)?
- Q2: If your decision is not to become an organ donor in case of death, for which reasons below would you not want to donate your organs (religious beliefs; age; no interest in organ donation or lack of interest to talk about issues related to death; fear of physical mutilation after death; presence of comorbidities that could exclude organ donation; fear of body handling after death; fear of pre-mortem organ retrieval; concerns about human organ trafficking; concerns about family disapproval; no trust in brain death criteria; no trust in the organ donation and transplantation system; other reasons)?
- Q3: If your decision is to become an organ donor in case of death, do your relatives or offspring know about your will to donate your organs (yes or no)?
- Q4: Do you have any relatives who benefited from organ transplantation (yes or no)?
- Q5: Would you consent to an organ donation of a relative without knowledge concerning his or her previous will (yes or no)?
- Q6: Have you ever had any subject or class about brain death criteria in your MS (yes or no)?

- Q7: Are you aware of the brain death criteria adopted in your country (yes or no)?
- Q8: Do you know the steps required to consider the diagnosis of brain death (Glasgow coma scale; absence of pupillary response to bright light; absence of ocular movements using oculocephalic testing; absence of oculovestibular reflexes; absence of corneal reflexes; and absence of pharyngeal “gag” and tracheal “cough” reflexes)?

All participants gathered online informed consent prior to taking part in the study. All data were de-identified before further analysis.

Statistical analysis

Data in text and tables are expressed as percentages or means and standard error. Demographics were compared to those answers to specific questions using the χ^2 test or Fisher's exact probability test when appropriate. Continuous variables were compared using the t-test. p-values ≤ 0.05 were considered significant.

RESULTS

A total of 382 medical students (235 females, 23 ± 4 years) living in the southeast (n=119), northeast (n=112), north (n=102), midwest (n=38), and south (n=21) regions of Brazil answered the questionnaire. They were studying in 72 different MS. Most of them (n=219) were public MS funded by federal and state governments. Most of the participants were in the fourth year of MS (n=108). The remaining students were in the first (n=83), second (n=51), third (n=78), fifth (n=42), and sixth (n=30) years at their MS. Only 153 (39%) students declared to be studying in an institution linked to an organ transplantation program. The remaining students were either unaware (n=200) or enrolled in an institution without any transplantation activity (n=39). As expected, most students took part in one scientific academic league (n=239). The answers to Q1 and Q3 to Q7 are depicted in Figure 1 and Table 1. Briefly, 356 (91%) students were willing to donate their organs in case of death. Notably, 27 students did not know yet, and only 6 were not in favor of PMOD due to religious beliefs (n=1), religious beliefs and concerns about human organ trafficking (n=1), fear of body handling after death (n=3), no interest to talk about issues related to death (n=1), and unknown reasons (n=1). Most of the students (68%) have already informed their families about their will for PMOD. Few (21.5%) have one or more relatives who have benefited from organ transplantation, and half would consent to organ donation from a relative

without knowledge concerning his or her previous will. Almost half of the students claimed to have heard about organ donation in their MS or disclosed to be aware of the brain death

criteria adopted in Brazil (Table 1). No differences in organ donation attitudes were observed according to gender, except for communication of donation willingness to a relative (Q3),

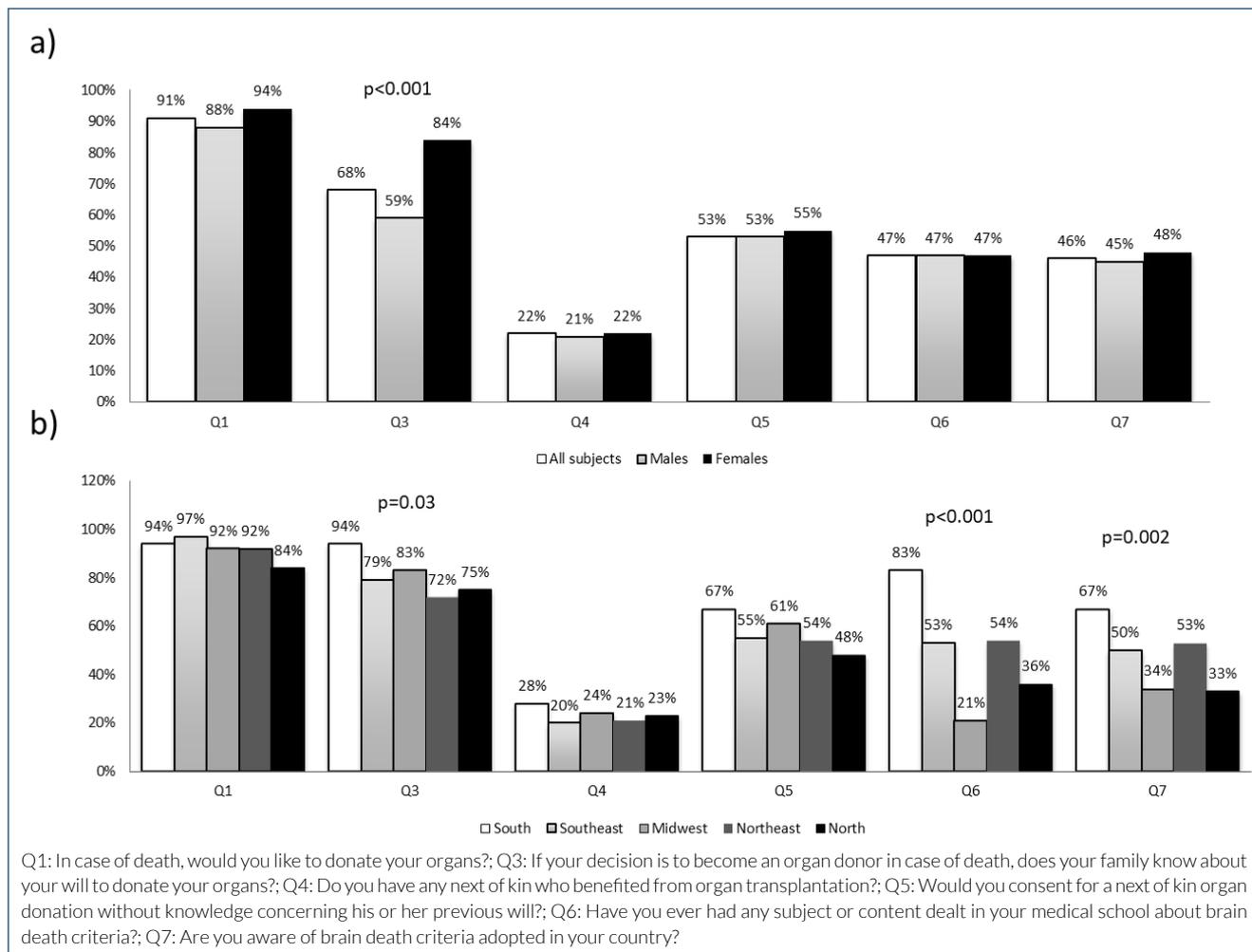


Figure 1: Frequency (%) of positive answers to Q1 and Q3 to Q7 according to demographics: a) Gender and b) geographical region.

Table 1. Percentages of positive answers to questions concerning organ donation according to MS features.

Question	Total	Public MS	Private MS	Partnership with transplantation center	No partnership with transplantation center
Q1 (%)	356 (90.8)	196 (90.7)	160 (92.5)	141 (92)	215 (91)
Q3 (%)	256 (67.9)	141 (71.9)	125 (78.1)	109 (77)	157 (73)
Q4 (%)	84 (21.5)	44 (20.4)	40 (20.3)	39 (25)	45 (19)
Q5 (%)	209 (53.3)	109 (50.5)	100 (57.8)	88 (57)	121 (51)
Q6 (%)	183 (46.7)	83 (38.4) ^a	100 (57.8) ^b	77 (50)	106 (45)
Q7 (%)	181 (46.2)	87 (40.3) ^c	94 (54.3) ^d	68 (44)	113 (47)

Q1: In case of death, would you like to donate your organs? Q3: If your decision is to become an organ donor in case of death, does your family know about your will to donate your organs? Q4: Do you have any relatives who benefited from organ transplantation? Q5: Would you consent to an organ donation of a relative without knowledge concerning his or her previous will? Q6: Have you ever had any subject or class in your medical school about brain death criteria? Q7: Are you aware of the brain death criteria adopted in your country? ^{a,b}p<0.0001; ^{c,d}p=0.004.

which was more frequently observed in women (84% vs. 59% in men, $p < 0.001$). Heterogeneity in the positive answers to Q3 as well as to Q6 and Q7 (Table 1) was noted according to the place of living. In general, students from the south and midwest regions talked more to their relatives about their willingness to donate organs after death. Participants from the south, southeast, and northeast regions answered more frequently about learning brain death criteria in MS and about knowledge concerning brain death criteria (Figure 1). No differences regarding responses to Q1, Q3, Q4, and Q5 were disclosed according to years of medical studies. However, positive answers to Q6 and Q7 were more frequently observed in students from private MS when compared to their counterparts from public MS (Table 1). As expected, positive answers to questions Q6 and Q7 were directly related to years of schooling. Likewise, the ability to perform all clinical steps involved in brain death diagnosis was shown to increase over time significantly (Figure 2). All students with six or more years of school claimed to be used to at least one of those critical steps for brain death diagnosis, but 30–67% of them who were at

the end of their medical studies were unaware of how to perform at least one of those steps (Figure 2).

DISCUSSION

Our results demonstrated that more than 90% of the Brazilian medical students would consent to PMOD. Approximately two-thirds of them have talked to their relatives about their decision, and more than half would also consent to donate organs of a relative even without knowledge about their will. Those attitudes were reported even though few declared to be acquainted with someone who benefited from an organ transplant and were not shown to vary according to the source of MS funding or partnership with a transplantation center. Those findings are different from those recently reported in a survey conducted on a representative sample of the Brazilian population (personal communication), which revealed that 67% of the students would consent to PMOD, and only half have talked about this issue with a relative. This is in accordance with some^{2,3}, but not all, reports⁴ from Brazil and elsewhere^{5,6}

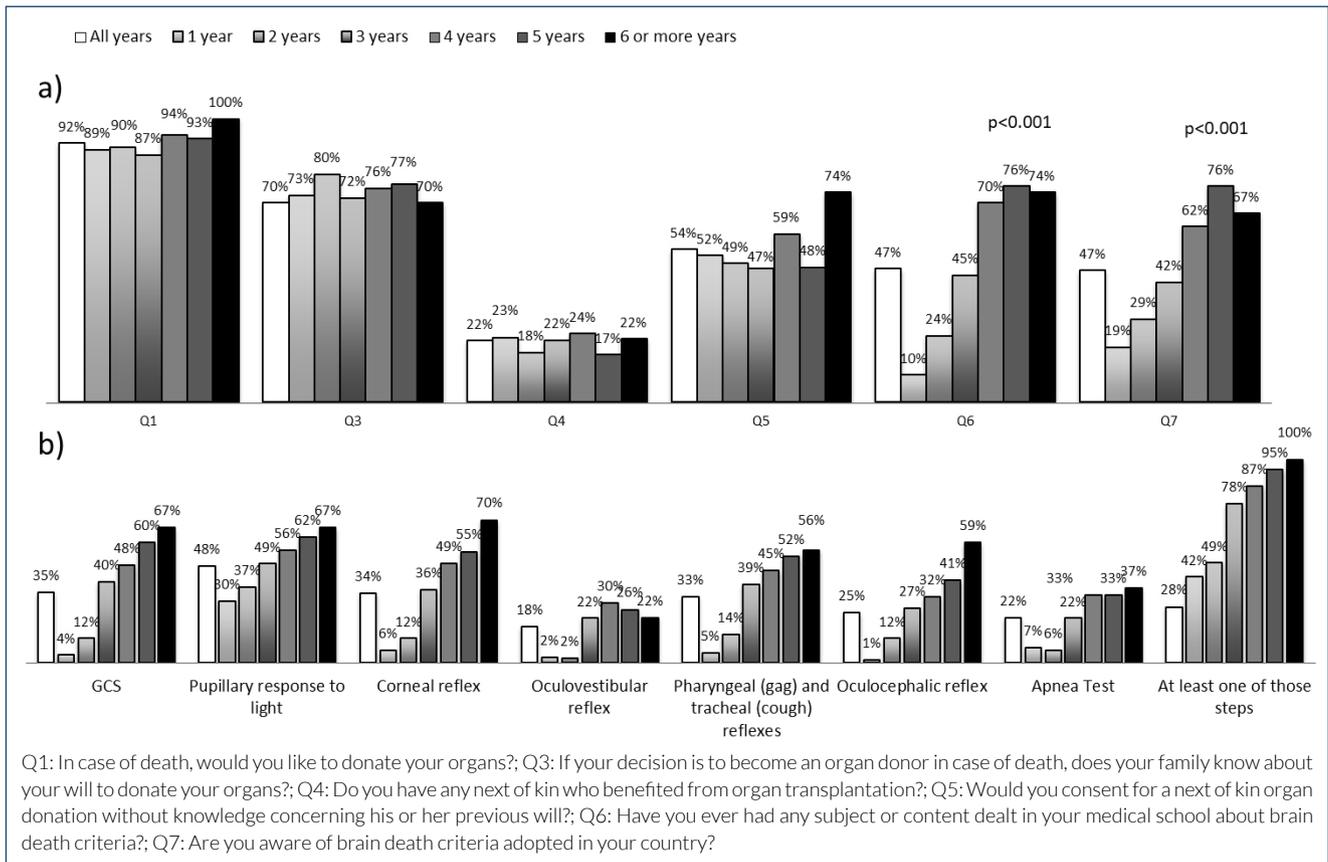


Figure 2. (a) Frequency (%) of positive answers to Q1 and Q3 to Q7 and (b) knowledge (%) about performance of different steps required for the diagnosis of brain death according to years of studying medicine.

indicating that knowledge of medical students is far better when compared to laypeople. Unlike other reports^{7,8}, years of school were not shown to influence attitudes toward organ donation, indicating positive attitudes toward donation from the beginning of MS studies in Brazil. Several previous studies have evaluated knowledge about organ transplantation, including potential deceased donors' identification and brain death criteria understanding⁷⁻⁹. Some have reported a lack of adequate information about donation and transplantation in MS curricula^{10,11}. In the present study, less than half of the medical students claimed to have had classes regarding donation and transplantation or to know the brain death criteria adopted in Brazil. As expected¹², awareness was shown to increase according to school years, but it is troublesome to observe that one-quarter of the medical students are unaware of organ donation issues after 6 years of schooling in Brazil. Some steps involved in brain death diagnosis were more challenging to learn, particularly oculovestibular and oculocephalic reflexes and the apnea test. Lower awareness about transplantation issues and brain death criteria was observed more often in public MS when compared to their private counterparts, suggesting more room for improvement in Federal and State medicine universities. As previously reported, women were more prone to share their decision favoring PMOD when compared to men. Some attitudes and knowledge were shown to vary according to the geographical area. In general, students from the south and southeast were shown to have talked more frequently to a relative about organ donation, heard more about transplantation issues at MS, and have more knowledge concerning brain death criteria compared to their counterparts from other regions. This may reflect greater general awareness concerning donation and transplantation in those higher HDI regions with higher transplantation activity.

In accordance with previous reports from Brazil, the US, and Europe^{5-7,13}, most of the medical students who were not willing to PMOD were in doubt, and few reported religious or cultural beliefs to justify their decision. This is in contrast to several other reports from Asia and the Middle East, where religion, fear of body mutilation and/or handling, family reaction, and distrust in the transplantation system are the main reasons against PMOD¹⁴.

CONCLUSION

Most medical students in Brazil have above-average attitudes toward organ donation but lack appropriate knowledge concerning brain death criteria to identify possible organ donors after graduation. Several gaps in curricula of MS in Brazil have to be addressed to improve the knowledge of future intensive care physicians about organ donation and, consequently, improve the transplantation activity in Brazil.

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AUTHORS' CONTRIBUTIONS

ELOB, MMPN, ARC: Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **JHPG:** Conceptualization, Formal Analysis. **EBH, LC, PLB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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Role of immature granulocytes and total bilirubin values in the diagnosis of perforated appendicitis in patients over 65 years

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the effectiveness of immature granulocyte count, immature granulocyte percentage, and total bilirubin value in predicting complicated and perforated appendicitis in patients aged 65 years and older with a diagnosis of appendicitis.

METHODS: In this study, 84 patients, aged 65 years and older, who had appendectomy demographic information, preoperative white blood cell count, neutrophil/lymphocyte ratio, immature granulocyte count and immature granulocyte percentage, operation findings, and pathology results were collected retrospectively. They were grouped into 4 categories: complicated, non-complicated, perforated, and non-perforated, according to the data and surgical findings.

RESULTS: Total bilirubin and immature granulocyte count were found to be statistically significant in predicting complicated and perforated appendicitis in patients aged 65 years and older with a diagnosis of appendicitis. The total bilirubin was found to have the following values in differentiating complicated appendicitis: area under the curve=0.883, sensitivity=78.3%, and specificity=88.5%. Total bilirubin had the highest discrimination power with area under the curve=0.804 in differentiating perforation.

CONCLUSION: The immature granulocyte percentage and total bilirubin count are the fast, inexpensive, and reliable parameters that can be used to predict complicated and perforated appendicitis in patients aged 65 years and older.

KEYWORDS: Appendicitis. Aged. Bilirubin.

INTRODUCTION

Acute appendicitis (AA) is the most common cause of acute abdominal pain that requires surgical intervention¹. It is often seen in the second and third decades of life. The incidence of AA after adolescence decreases with age². While the mortality rate due to AA in elderly patients is 8%, it varies between 0 and 1% in young patients³. In addition to applying with an insignificant history and physical examination, they generally progress with a reduced inflammatory response accompanied by less leukocytosis. Therefore, elderly patients may go unnoticed at the time of diagnosis, and they have the possibility of complicated and perforated appendicitis. According to the multivariate analyses in a large observational study, being diagnosed with AA over the age of 65 years is one of the reasons that increase mortality. In addition, the rate of having perforated, abscess, or complicated appendicitis is higher in patients over 65 years of age with a diagnosis of AA⁴.

Studies have demonstrated that in cases such as sepsis and infection, the number of immature granulocytes increases

significantly compared to healthy individuals, and this value can be used as an inflammatory marker⁵.

Although hyperbilirubinemia is usually increased in liver dysfunctions and biliary tract obstructions, increased bilirubin can also be observed in patients due to general peritonitis, AA, and sepsis. *Escherichia coli* and *Bacteroides fragilis* are the most common bacterial causes of appendicitis⁶. Complicated appendicitis causes intestinal edema and slowing of motility. These mechanisms result in increased bilirubin⁷.

In this study, we examined the laboratory parameters that can be used to predict appendicitis perforation in patients aged 65 years and older.

METHODS

Our study was approved by the ethics committee (decision no.: E-21-758). Between January 8, 2017, and January 9, 2021, 84 adult patients over 65 years of age who underwent appendectomy with the preliminary diagnosis of AA in the

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clinic were evaluated retrospectively by examining the hospital records. By examining the pathology reports of the patients, they were grouped as simple (edematous and phlegmon) appendicitis and complicated (perforated and gangrene) appendicitis according to the complication status and as non-perforated appendicitis and perforated appendicitis according to the perforation status.

Statistical analysis

For the statistical analyses of the study data, Statistical Package for Social Sciences (SPSS), version 23.0 for Windows (SPSS Inc. Chicago, IL, USA) software was used. In the descriptive statistics section, categorical variables have been presented as number and percentage, while continuous variables are presented with mean±standard deviation and the median (smallest-largest value). The compliance of continuous variables with normal distribution was evaluated using visual (histogram and probability graphs) and analytical (Kolmogorov-Smirnov and Shapiro-Wilk tests) methods. The Mann-Whitney U test was used for the comparison analysis. To determine the probability of using white blood cell (WBC) count, neutrophil/lymphocyte ratio (NLR), immature granulocyte count (IGC), immature granulocyte percentage (IG%), total bilirubin, direct bilirubin, and indirect bilirubin values in determining complicated appendicitis and perforated appendicitis, a receiver operating characteristic (ROC) curve analysis was performed. Within the scope of this analysis, the area under the curve (AUC) and its statistical significance were evaluated, and it was decided whether to calculate a threshold point for the relevant parameters. The threshold point was determined using the Youden

Index for the appropriate parameters. A $p < 0.05$ was considered statistically significant.

Findings

Of the patients included in our study, 41 (48.8%) were male and 43 (51.2%) were female. The mean age was determined as 70 ± 5.9 years. According to the pathology results, the patients were divided into four groups according to their complication status and perforation status; 61 (72.6%) patients were grouped under the simple appendicitis group, 23 (27.4%) patients were grouped under the complicated appendicitis group, 73 (86.9%) patients were grouped under the non-perforated appendicitis group, and 11 (13.1%) patients were grouped under the perforated appendicitis groups. It was investigated whether WBC count, NLR, IGC, and IG%, total bilirubin, direct bilirubin, and indirect bilirubin values are the parameters that can be used to determine the complication status and perforation status in patients over 65 years of age with a preliminary diagnosis of AA.

Between simple appendicitis and complicated appendicitis groups, a statistically significant difference was found in terms of WBC count, NLR, IGC, and IG%, total bilirubin, direct bilirubin, and indirect bilirubin values ($p < 0.05$).

Between non-perforated appendicitis and perforated appendicitis groups, a statistically significant difference was found in terms of IGC and IG%, total bilirubin, direct bilirubin, and indirect bilirubin values ($p < 0.05$). Between non-perforated appendicitis and perforated appendicitis groups, no statistically significant difference was found in WBC count and NLR ($p < 0.05$) (Table 1).

Table 1. Comparison of laboratory findings between appendicitis groups, 2021.

	Simple appendicitis Median (min-max)	Complicated appendicitis Median (min-max)	p^*	Non-perforated appendicitis Median (min-max)	Perforated appendicitis Median (min-max)	p^*
IGC	0.04 (0.01-0.30)	0.10 (0.04-0.70)	<0.001	0.05 (0.01-0.31)	0.10 (0.04-1.80)	0.012
IG%	0.40 (0.10-0.70)	0.60 (0.30-1.80)	<0.001	0.40 (0.10-1.80)	0.60 (0.30-1.00)	0.048
Total bilirubin	0.48 (0.21-1.28)	1.10 (0.34-1.86)	<0.001	0.55 (0.21-1.86)	0.96 (0.34-1.53)	0.001
Direct bilirubin	0.19 (0.04-0.54)	0.36 (0.04-0.80)	<0.001	0.20 (0.04-0.80)	0.36 (0.04-0.66)	0.005
Indirect bilirubin	0.30 (0.09-0.84)	0.65 (0.25-1.33)	<0.001	0.34 (0.09-1.07)	0.55 (0.25-1.33)	0.015
WBC	11800 (4190-28250)	14590 (5330-25810)	0.001	12210 (4190-28250)	14590 (5330-25810)	0.072
NLR	5.41 (0.56-25.08)	8.02 (1.71-30.64)	0.026	6.20 (0.56-30.64)	6.97 (2.20-27.12)	0.343

*Mann-Whitney U test. IGC: immature granulocyte count; IG%: immature granulocyte percentage; WBC: white blood cell; NLR: neutrophil/lymphocyte ratio.

Accordingly, the highest differential power was found in the total bilirubin parameter. The AUC value was determined as 0.883. Limit values were determined for all parameters according to the ROC curves. It was observed that the parameter with the highest sensitivity was total bilirubin, while the parameters with the highest specificity were IG% and IGC (Table 2).

Accordingly, the highest differential power was found in the total bilirubin parameter. The AUC value was determined as 0.804. Limit values were determined for all parameters according to the ROC curves. It was observed that the parameter with the highest sensitivity was total bilirubin, and the parameter with the highest specificity was the percentage of immature granulocytes (Table 3).

The use of IG%, WBC count, and NLR values in differentiating the perforated AA group from the non-perforated AA group was not found to be statistically significant ($p < 0.05$).

DISCUSSION

Acute appendicitis is the most common surgical emergency, which is seen more rarely in elderly patients, and most frequently in the second and third decades, and its incidence decreases over the years after puberty². Since cardiac, renal, and pulmonary diseases are also more common in this age group, they are more mortal in this age group.

The diagnosis of appendicitis is made by the clinician's physical examination, laboratory tests, and the combined use of visualization methods. It has been reported that the use of ultrasonography and computed tomography makes the diagnosis of AA more accurate in some cases and reduces false negativity by 10%^{8,9}. In addition to this, opaque substances to be given are not preferred in this age group since they are nephrotoxic. Although the use of ultrasound helps to avoid these risks, it is dependent on the person performing it, and the appendix cannot be seen at a rate of 55%, which does not help with diagnosis in some cases¹⁰.

Table 2. Receiver operating characteristic analysis of laboratory parameters in differentiating simple appendicitis and complicated appendicitis, 2021.

Test result variable(s)	AUROC	p	Asymptotic 95% confidence interval		Cutoff value	Sensitivity (%)	Specificity (%)
			Lower bound	Upper bound			
IGC	0.831	<0.001	0.725	0.936	>0.075	73.9	83.6
IG%	0.778	<0.001	0.662	0.894	>0.65	47.8	95.1
Total bilirubin	0.883	<0.001	0.801	0.966	>0.85	78.3	88.5
Indirect bilirubin	0.841	<0.001	0.749	0.934	>0.49	73.9	85.2
WBC	0.735	0.001	0.614	0.856	>13,615	69.6	73.8
Direct bilirubin	0.838	<0.001	0.725	0.951	>0.32	73.9	88.5
NLR	0.658	0.026	0.517	0.799	>6.65	60.9	63.9

IGC: immature granulocyte count; IG%: immature granulocyte percentage; WBC: white blood cell; NLR: neutrophil/lymphocyte ratio; ROC: receiver operating characteristic curve; AUROC: area under the receiver operating characteristics.

Table 3. Receiver operating characteristic analysis of laboratory parameters in the differentiation of non-perforated appendicitis and perforated appendicitis, 2021.

Test result variable (s)	AUROC	p	Asymptotic 95% confidence interval		Cutoff value	Sensitivity (%)	Specificity (%)
			Lower bound	Upper bound			
IGC	0.732	0.013	0.582	0.883	>0.075	72.7	74
IG%	0.682	0.053	0.504	0.860	>0.650	45.5	87.7
Total bilirubin	0.804	0.001	0.671	0.937	>0.875	81.8	80.8
Indirect bilirubin	0.729	0.015	0.573	0.884	>0.515	63.6	76.7
WBC	0.669	0.072	0.490	0.848	>13.785	63.6	79.9
Direct bilirubin	0.762	0.005	0.591	0.934	>0.345	72.7	80.8
NLR	0.589	0.343	0.391	0.787	>14.200	36.4	94.5

IGC: immature granulocyte count; IG%: immature granulocyte percentage; WBC: white blood cell; NLR: neutrophil/lymphocyte ratio; ROC: receiver operating characteristic curve; AUROC: area under the receiver operating characteristics.

Prolonging the time between symptom onset and the surgery increases the risk of appendix perforation. Therefore, early diagnosis is of great importance in the success of treatment¹¹. Studies have demonstrated that older patients are diagnosed with appendicitis less accurately before surgery than younger patients^{4,12}. Considering the relationship of delay in the diagnosis and treatment of severe appendicitis with postoperative mortality, morbidity, and length of hospital stay, new biomarkers are needed to predict the severity of this type of appendicitis¹³.

In our study, the rate of complicated appendicitis is 27.4%, which is compatible with the studies in the literature^{5,6}. It has been demonstrated that total bilirubin value is an important marker in the differentiation of perforated, non-perforated, complicated, and simple appendicitis in patients aged 65 years and older. Due to the increased bacterial load, it invades the portal system directly or by translocation. This causes bacteria both to interact with the liver parenchyma and to increase bilirubin by causing acholia¹⁴. Sevinc et al., after evaluating 3,392 cases of hyperbilirubinemia (>1.0 mg/dL), stated that it has an important place in determining appendicitis perforation, especially in elderly patients¹⁵. Eren et al. also found a significant relationship between perforated or gangrenous appendicitis and total bilirubin¹⁶. In addition to these studies, the results we found are compatible with other studies in the literature^{6,17,18,19}.

The new-generation hemogram devices used in recent years can calculate the number and percentage of immature granulocytes, thus enabling them to be used as an easily and quickly accessible marker without requiring additional costs. There are many studies indicating the prognostic importance of immature granulocyte in patients with sepsis and bacteremia in the literature^{5,20-23}. Soh et al. conducted a study on patients who applied to the emergency department with abdominal pain complaint and stated that immature granulocyte value should be considered in terms of making an emergency abdominal surgery decision or in terms of peritonitis²⁴. Durak et al. stated that immature granulocytes can be used for evaluating intestinal necrosis in mesenteric ischemia²⁵.

Preoperative diagnostic steps gain more importance in geriatric patients aged 65 years and older, both because they are diagnosed late and because of more complications in the

postoperative period. New inflammatory markers are needed that can be used due to the inadequacy of the values such as WBC and NLR that we used routinely in these patients, the limitation in the use of radiological examinations due to the frequency of nephrotoxicity in this patient population, and the inability of these patients to express themselves. In our study, on the contrary, it was concluded that immature granulocyte is a distinguishing parameter in both the differentiation of complicated and simple appendicitis and the differentiation of perforated and non-perforated appendicitis in the geriatric-sensitive population aged 65 years and older. Many studies in the literature support our results.

CONCLUSION

Early prediction of complicated and perforated appendicitis in geriatric patients aged 65 years and older is extremely important in terms of reducing high mortality rates and hospital costs. Total bilirubin and immature granulocyte are reliable parameters that can be used to determine the severity of AA in patients aged 65 years and older.

AUTHORS' CONTRIBUTIONS

SA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **EC:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **BBS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. **MC:** Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing. **IE:** Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing. **AS:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **YU:** Formal Analysis, Visualization, Writing – original draft. **MRP:** Formal Analysis, Visualization, Writing – original draft.

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Functional and biochemical improvement following total knee arthroplasty in early postoperative period

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SUMMARY

OBJECTIVE: There are very few studies about total knee arthroplasty biomechanical and biochemical effects in the early postoperative period. The aim of this study was to investigate the effect of total knee arthroplasty on pain intensity, knee joint valgus angle, malalignment, functional status, knee joint position sense, and cytokine levels.

METHODS: A total of 29 patients (female/male: 24/5) who underwent total knee arthroplasty were included in the late-stage knee osteoarthritis group, and 22 patients (female/male: 13/9) with grade 4 osteoarthritis were included in the early-stage knee osteoarthritis group. The visual analog scale and the Western Ontario and McMaster Universities Osteoarthritis Index were used to evaluate the pain intensity and functional status. Alignment and knee position sense measurements were also calculated. Systemic venous blood samples were taken to evaluate the interleukin-6, tumor necrosis factor-alpha, and interleukin-1 beta cytokine levels.

RESULTS: In the study group, there were positive improvements in pain intensity, functional status, valgus angle, malalignment, amount of joint position sense deviation at 70° knee flexion angle parameters, and interleukin-6 of patients at the postoperative 6th week compared to the preoperative period ($p < 0.05$). The patients in the study group had similar or better results in pain intensity, functional status, valgus angle, malalignment, amount of joint position sense deviation at 35°, 55°, and 70° knee flexion angles parameters, and in interleukin-6, compared to the control group at postoperative 6th week.

CONCLUSION: Total knee arthroplasty provides improvements in pain, function, valgus angle, joint position sense, and interleukin-6 in the early postoperative period.

KEYWORDS: Knee arthroplasty. Interleukins. Proprioception. Radiography. Osteoarthritis.

INTRODUCTION

Osteoarthritis (OA) is one of the most common chronic peripheral joint diseases. Loss of function and pain are important symptoms, and one of the main goals of treatment is to improve the quality of life¹. Total knee arthroplasty (TKA) is a favorite surgical approach aimed at improving joint function and relieving pain intensity. In the United States, the incidence rate of TKA is expected to increase², thus increasing the financial burden. Biomechanical and biochemical changes associated with TKA are being studied but not clearly explained. So, it is important to show the changes in pain intensity, lower extremity alignment, functional status, joint position sense (JPS), and cytokine levels (IL-6, TNF- α , and IL-1 β) of patients who underwent TKA.

Related literature generally focused on biomechanical gains such as reducing pain and increasing functional status after

TKA. However, there is very limited evidence about cytokines, and JPS in the acute phase after TKA. So, this situation requires examining cytokines (IL-6, TNF- α , and IL-1 β) after TKA. The investigation of cytokine levels in terms of response to degenerative processes and surgical stress in the joint can be guided. Biomechanical deteriorations in the knee joint affect patients' knee JPS adversely³. Although many studies show evidence that TKA improves proprioception in suitable surgical candidates, the overall effect of TKA on proprioception is controversial⁴. More studies are still needed on this subject in a holistic approach.

The main hypothesis of the study is to investigate whether there is a functional and biochemical effect of TKA surgery. Another hypothesis is to investigate whether the functional parameters and inflammatory cytokines in the group undergoing

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TKA reach levels similar to those in the OA group 6 weeks after the operation without any indication for surgery. So, the purpose of this study was to indicate the early effects of TKA on pain intensity, knee joint valgus angle, malalignment, functional status, knee JPS, and cytokine levels in patients with late-stage knee osteoarthritis (LSKO), and to compare the early-stage knee osteoarthritis (ESKO) and LSKO in terms of these parameters.

METHODS

This study was designed as a prospective cohort study. It was completed with 51 OA patients (female/male: 37/14): 29 in the LSKO group (female/male: 24/5) and 22 in the ESKO group (female/male: 13/9). They were evaluated with complaints of knee pain by the Department of Orthopaedics and Traumatology, Farabi Hospital, Faculty of Medicine, Karadeniz Technical University. The OA severity level was determined according to the Kellgren-Lawrence classification system. Patients with grade 4 degeneration levels who had surgical indications between the ages of 45 and 75 years were included in the LSKO group (n=29), and they underwent surgery. Patients without surgical indication with lower than 3 OA severity were included in the ESKO group (n=22), and they did not undergo surgery. Patients with surgical history of the ipsilateral side, neuropathic pain, loss of sensation, and systemic, chronic, and infectious diseases were excluded from the study. First, 41 cases were evaluated for the LSKO group in the preoperative period; 11 patients who did not come for follow-up after discharge and 1 patient who was hepatitis C virus positive were excluded.

This study was approved by the Karadeniz Technical University Scientific Research Ethics Committee (May 25, 2018; no. 2018/91). Patients were informed about the study. They read and signed the written informed consent form.

After the sociodemographic features were recorded, pain intensity, valgus angle and malalignment, functional status, JPS, and cytokine levels (IL-6, TNF- α , and IL-1 β) were evaluated. Measurements were taken once from the ESKO group, and twice from the LSKO group in preoperative and postoperative 6th weeks.

Main outcome measures

Pain intensity: The pain intensity was evaluated by the visual analog scale. The patients marked their intensity of pain on rest and walking on a vertical line of 10 cm (0: no pain, 10: unbearable pain)⁵.

Valgus angle and malalignment assessment: They were calculated on the knee anteroposterior direct x-ray image by

an experienced orthopedist. The valgus angle was calculated as the angle facing the lateral between the anatomical axis of the tibia and the anatomical axis of the femur. For malalignment measurement, vertical lines were drawn from the edges to the proximal joint face of the tibia and the distal joint face of the femur. The distance between these perpendicular lines was recorded⁶.

Functional status: The Western Ontario and McMaster Universities Arthritis Index (WOMAC) was used. The validity and reliability of the index in Turkish were determined by Tüzün et al.⁷ This 24-item index has three subdimensions: pain, stiffness, and physical function. A high score means a low level of functional status⁷.

JPS: In our study, the protocol developed by Hurley⁸ was used. The knee was passively brought to 70° at a rate of 10°/s starting from 90° of flexion. After holding it for 3 s at this angle, it was passively brought to its initial position at the same speed. Then, the patient was instructed to actively bring the knee to the targeted angle and hold this position for 5 s. The amount of deviation from the target angle was recorded. The average of their trials was calculated for the final score. The same evaluations were performed for 55° and 35° knee flexion angles, respectively.

Cytokine level: Systemic venous blood samples were taken. IL-6, TNF- α , and IL-1 β cytokine levels were examined. Venous blood samples were taken to separator gel biochemistry tubes and allowed to clot for 30 min at room temperature. The clotted blood samples were centrifuged for 10 min at 3000 rpm. Serum samples obtained as a result of centrifugation were divided into tubes with micro-volume caps and stored at -80°C. All the samples in the LSKO and ESKO groups were analyzed simultaneously using kits that can measure using the enzyme-linked immunosorbent assay method.

Statistical analysis

The SPSS 21.0 (Statistical Package for Social Science, Chicago, IL, USA) package program was used for the analysis. Student's t-test and Mann-Whitney U test were used in the preoperative and postoperative comparisons of the groups. Paired-sample t-test and Wilcoxon test were used to compare the measurements. Mean, standard deviation, and total number were calculated. The significance level was taken as $p < 0.05$. The G*Power 3.1.9.2 program was used for sample size. When the effect size was accepted as 0.5 with 5% type 1 error and 80% power according to repeated measures differences analyses, it was calculated as 28 patients for LSKO. The current power was calculated as 82% for the results.

RESULTS

Sociodemographic features and preoperative outcome measurements were shown in Table 1.

Pain intensity, functional status, valgus angle, malalignment, and JPS deviation in 70° and IL-6 showed significant improvement in the postoperative 6th week compared to the preoperative period in the TKA group ($p < 0.05$). There was no significant difference among TNF- α , IL-1 β , and JPS deviation in

35° and 55° in preoperative and postoperative periods ($p > 0.05$) (as can be seen in Table 2).

According to the results of the relationship between the LSKO and ESKO groups 6 weeks after TKA, TNF- α and IL-1 β values were significantly higher, while pain intensity during walking and malalignment amount data were lower in the LSKO group ($p < 0.05$). There was no significant difference in pain intensity during rest, WOMAC score, valgus

Table 1. Sociodemographic and preoperative outcome measurements.

Parameters		LSKO group X \pm SD	ESKO group X \pm SD	p-value
Age (years)		67.55 \pm 6.55	54.18 \pm 8.25	<0.001*
Body weight (kg)		82.31 \pm 8.64	82.95 \pm 13.73	0.84
Body height (cm)		161.34 \pm 6.69	166.18 \pm 10.60	0.07
Body mass index (kg/m ²)		31.72 \pm 3.79	30.25 \pm 5.59	0.27
Pain intensity	During rest	4.04 \pm 2.87	2.10 \pm 1.78	0.004*
	During walking	6.88 \pm 2.09	4.46 \pm 2.77	0.001*
Valgus angle		184.23 \pm 7.31	177.56 \pm 2.80	<0.001*
Malalignment		5.46 \pm 2.67	2.75 \pm 1.96	<0.001*
WOMAC score		72.72 \pm 13.13	33.64 \pm 12.44	<0.001*
Amount of joint position sense deviation	35°	6.11 \pm 6.06	8.27 \pm 7.39	0.25
	55°	6.08 \pm 4.04	5.43 \pm 3.64	0.56
	70°	10.40 \pm 9.21	5.89 \pm 4.89	0.04*
Cytokine	IL-6	203.90 \pm 73.76	139.04 \pm 90.68	0.007*
	TNF- α	54.54 \pm 3.82	34.18 \pm 14.65	<0.001*
	IL-1 β	113.14 \pm 11.17	86.60 \pm 21.42	<0.001*

LSKO: late-stage knee osteoarthritis; ESKO: early-stage knee osteoarthritis; IL-6: interleukin 6; TNF- α : tumor necrosis factor-alpha; IL-1 β : interleukin-1 beta; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; X: mean; SD: standard deviation. *Statistically significant differences ($p < 0.05$).

Table 2. Differences between preoperative and 6 weeks after TKA for LSKO group.

Parameters		Preoperative	Postoperative 6th week	p-value
Pain intensity	During rest	4.07 \pm 2.87	1.26 \pm 1.35	<0.001*
	During walking	6.88 \pm 2.09	2.10 \pm 1.69	<0.001*
Valgus angle		184.23 \pm 7.31	176.26 \pm 2.91	<0.001*
Malalignment		5.46 \pm 2.67	1.84 \pm 2.66	<0.001*
WOMAC score		72.72 \pm 13.13	27.92 \pm 14.24	<0.001*
Amount of joint position sense deviation	35°	6.11 \pm 6.06	6.42 \pm 5.89	0.85
	55°	6.08 \pm 4.04	5.26 \pm 4.91	0.26
	70°	10.40 \pm 9.21	6.02 \pm 4.96	0.02*
Cytokine	IL-6	203.90 \pm 73.76	161.04 \pm 30.71	0.002*
	TNF- α	54.54 \pm 3.82	52.18 \pm 4.65	0.09
	IL-1 β	113.14 \pm 11.70	114.45 \pm 14.43	0.83

LSKO: late-stage knee osteoarthritis; ESKO: early-stage knee osteoarthritis; IL-6: interleukin 6; TNF- α : tumor necrosis factor-alpha; IL-1 β : interleukin-1 beta; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; X: mean; SD: standard deviation. *Statistically significant differences ($p < 0.05$).

Table 3. Comparison of the variables between the LSKO group postoperative 6th week results and the ESKO group results.

Parameters		LSKO group X±SD	ESKO group X±SD	p-value
Pain intensity	During rest	1.26±1.35	2.10±1.78	0.06
	During walking	2.10±1.69	4.46±2.77	0.001*
Valgus angle		176.26±2.91	177.56±2.80	0.14
Malalignment		1.84±2.66	2.75±1.96	0.01*
WOMAC score		27.92±14.24	33.64±12.44	0.14
Amount of joint position sense deviation	35°	6.42±5.89	8.27±7.39	0.34
	55°	5.26±4.91	5.43±3.64	0.89
	70°	6.02±4.96	5.89±4.89	0.92
Cytokine	IL-6	161.04±30.72	139.04±90.68	0.28
	TNF- α	52.18±4.65	34.18±14.66	<0.001*
	IL-1 β	114.46±14.43	86.60±21.42	<0.001*

LSKO: late-stage knee osteoarthritis; ESKO: early-stage knee osteoarthritis; IL-6: interleukin 6; TNF- α : tumor necrosis factor-alpha; IL-1 β : interleukin-1 beta; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; X: mean; SD: standard deviation. *Statistically significant differences (p<0.05).

angle, and JPS deviation in 35°, 55°, and 70° (p>0.05) (as can be seen in Table 3).

DISCUSSION

In this study, patients who underwent TKA recovered compared to those who underwent pre-surgery in terms of pain intensity, valgus angle, malalignment, functional status, JPS deviation in 70°, and IL-6. They had pain intensity level during rest, valgus angle, functional status, JPS, and IL-6 similar to the ESKO group.

Pain intensity after the TKA is affected by many physical, radiological, and biochemical factors⁹. Patients in the LSKO group had higher preoperative pain intensity than patients in the ESKO group. This may result from higher degenerative conditions in the knee in the surgery group¹⁰. In our study, it was seen that high pain intensity tends to decrease over time in the postoperative period, and the pain intensity at rest in the postoperative 6th week was lower than the individuals in the ESKO group. Similarly, Si et al.¹¹ reported that pain intensity during rest increased on the first postoperative day compared to the preoperative period and decreased in the following days. Pain intensity during activity decreased after the surgery, but it did not decrease to the level at rest. This may be a natural result of patients' efforts to exercise, and emotional conditions such as fear of movement and anxiety. More studies are still needed on this subject.

In our study, similar to the literature, increased valgus angles were corrected by surgery and brought to a similar level with individuals in the ESKO group¹². In addition, the malalignment

was improved to a better level than the ESKO group in the postoperative 6th week.

In our study, functional status in the postoperative 6th week of the LSKO group was improved. They had a similarity to ESKO. After surgery, especially the decrease in pain intensity contributed to the improvement in walking and functional status of the patients¹³. In another study, it was reported that the use of anti-inflammatory drugs may cause a decrease in WOMAC knee scores¹⁴.

It was observed that the patients in the LSKO and ESKO groups have similar knee JPS deviation at postoperative 6th week. In LSKO, more deviation in 70° in preoperative may be due to increased stress in the joint at an advanced knee flexion degree and hence the increased pain. After TKA, these deviations became similar to those in ESKO. The knee joint is very important for proprioception due to the mechanoreceptors it contains. So, in early postoperative period, follow-up could improve functional mobility. Lowering the valgus angle to normal can have positive effects on knee proprioception. However, for these patients, loss of knee proprioception may continue for a year¹⁵. Long-term studies are still needed.

In the LSKO group, IL-6 decreased to a level similar to that of ESKO at the postoperative 6th week. TNF- α and IL-1 β values decreased but were not similar to the level of ESKO. The IL-6 value is the most important cytokine parameter affected by surgery in our study. Indeed, serum IL-6 level is one of the best reflecting indicators of surgical stress and is the laboratory indicator with the greatest diagnostic accuracy for periprosthetic infection.¹⁶⁻¹⁸ In related literature, serum IL-6 levels of individuals who have undergone

TKA show a significant increase in early postoperative days and are almost equal to the preoperative period in the 6th week¹⁸⁻²¹. Cytokine levels can be evaluated as a local inflammatory response or a systemic inflammatory response²². In our study, serum cytokine levels were evaluated. According to the literature, the contralateral side knees of individuals who have undergone TKA mostly have moderate or severe OA²³. Therefore, the positive effects of surgery on current cytokine levels can be limited depending on the other side. More studies are still needed.

The emotional state could be effective in reducing pain and cytokine levels. The emotional states of the patients were not evaluated, which is the limitation of the study. Furthermore, there are some clinical implications. First, TKA provides biomechanical and biochemical improvements. Second, valgus angle and malalignment decrease after TKA. Third, the IL-6 value is one of the most important cytokine parameters affected by surgery. These findings will provide information to physicians, physiotherapists, and health professionals working in this field on medication and rehabilitation approaches in pre- and post-surgical pain control.

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CONCLUSION

The TKA provides improvements in pain, function, valgus angle, JPS, and IL-6 in the early postoperative period. In the postoperative 6th week, patients who had TKA had similar clinical and functional results in terms of IL-6 level, pain intensity at rest and walking, WOMAC score, valgus angle, malalignment amount, and knee JPS to patients in the early stage of knee OA. These findings are also very important in determining the treatment approaches and patients' expectations on knee osteoarthritis.

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AUTHORS' CONTRIBUTIONS

AE, ME, OA: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **KC, SÖY, İB, SCK:** Conceptualization, Data curation, Formal Analysis.

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Is pectoralis muscle index a risk factor for mortality in left ventricular assist device patients?

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SUMMARY

OBJECTIVE: We aimed to investigate whether sarcopenia measured from pectoralis muscles is a risk factor for long-term mortality in left ventricular assist device patients.

METHODS: Patients aged >18 years implanted with a left ventricular assist device in a single center between 2013 and 2019 were retrospectively included. Patients without a thoracic computed tomography scan performed within 3 months of left ventricular assist device implantation and without computed tomography scans appropriate for pectoralis muscle measurement were excluded. Pectoralis muscle measurements were made on thoracic computed tomography slices, and pectoralis muscle indices were calculated for each patient. Sarcopenia was defined as being in the gender-specific lowest tertile of pectoralis muscle index. Survival was compared between patients with and without sarcopenia.

RESULTS: The study was conducted on 64 left ventricular assist device patients who met the inclusion criteria. Notably, 21 (32.8%) of the study patients were sarcopenic. Diabetes mellitus and sarcopenia were more common in patients with 2-year mortality in our cohort. Patients with sarcopenia had a worse 2-year survival ($p<0.001$). Sarcopenia had an adjusted hazard ratio of 4.04 (95% confidence interval (CI) 1.36–12.02, $p=0.012$), while diabetes mellitus was associated with an adjusted hazard ratio of 3.14 (95%CI 1.17–8.39, $p=0.023$).

CONCLUSION: Sarcopenia defined by low pectoralis muscle index increases the risk for 2-year mortality in left ventricular assist device patients.

KEYWORDS: Ventricular assist device. Sarcopenia. Heart failure. Survival.

INTRODUCTION

With the continuing shortage of heart donors, left ventricular assist devices (LVADs) are increasingly implanted for end-stage heart failure (HF) patients¹. Appropriate patient selection and greater operative experience have resulted in up to 80% 1-year survival on LVADs². While the operative risk of LVAD implantation is better understood, factors that influence long-term success are less elucidated.

Frailty is an age-related vulnerability that limits a patient's capacity against disease, injury, or a procedure. It is a significant prognostic factor for HF patients who face a greater risk of frailty due to cardiopulmonary failure and their coexisting comorbidities³. In LVAD patients, frailty negatively impacts long-term survival with a 1.11 to 3-fold increase in overall mortality⁴⁻⁶. Widely used risk scores in current use, such as the Heartmate 2 risk score, take demographic, echocardiographic, laboratory, and catheterization data into account but do not incorporate frailty⁷.

Sarcopenia is one component of frailty that encompasses muscle strength and muscle quantity. Sarcopenia is more

prevalent in HF patients than in healthy subjects in the same age group and impacts survival^{8,9}. Low handgrip strength was associated with worse outcomes in HF patients implanted with an LVAD¹⁰. The definition of sarcopenia varies with many methods used in literature to measure sarcopenia, including muscle quantity, quality, and strength.

While other measurements of sarcopenia are valuable, in clinical practice, thoracic computed tomography (CT) is the most routinely performed preoperative assessment that can quantify sarcopenia in LVAD patients. This study aimed to investigate whether sarcopenia measured from pectoralis muscles is a risk factor for long-term mortality in LVAD patients.

METHODS

Approval was obtained from the institutional academic board (28001928.01.01) and the associated ethics committee (HNHEAH-KAEK 2021/264-3391) for this study. Patients aged >18 years implanted with an LVAD in a single center between 2013 and 2019 were retrospectively included. All operations

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were performed by a single, dedicated HF and transplant team. Patients implanted with a bridge-to-transplant indication or a bridge-to-destination indication were included. Patients without a thoracic CT scan performed <3 months of LVAD implantation and without CT scans appropriate for pectoralis muscle measurement were excluded. Patient demographics, laboratory results before surgery, echocardiographic data, and indication for transplant, survival data, and causes of death were recorded. The outcome of interest was 2-year survival.

Measurement of muscle mass

All CT images recorded as 3 mm slices with a Toshiba Aquilion 64 device were evaluated by a single radiologist experienced in cardiac and thoracic pathologies and blinded to the patient information. Pectoralis muscle measurements were made on a single axial slice immediately above the aortic arch using the Vitrea (Canon Medical Informatics, Minnesota, USA) post-processing software. CT scans were performed with the upper extremities at full extension in the neutral position. Patients were excluded if scans were performed without arms at extension or if a defibrillator prevented measurement of the muscle quantity. Left and right pectoralis muscle areas were measured in cm² with the postprocessing software. The two areas were averaged and divided by the square of the patient's height in meters, standardizing a pectoralis muscle index (PMI) (cm²/m²) for each patient.

Definition of sarcopenia

Tertiles were calculated for PMI separately for female and male patients. Sarcopenia was defined as being in the gender-specific lowest tertile of PMI. Gender-specific tertile method is the most frequently used method for defining sarcopenia groups in the literature¹¹⁻¹³. We grouped patients into two with patients in the lowest tertile as sarcopenic and in the mid and high tertiles as nonsarcopenic.

Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics version 25 (IBM Corp, Armonk, NY). Nominal variables are presented as numbers and percentages, and continuous variables are presented as mean and standard deviation. Groups were compared using the chi-squared test or Fisher's exact test for nominal variables, the Student's t-test for continuous variables with normal distribution, and the Mann-Whitney U test for continuous variables without normal distribution. Survival of patients with and without sarcopenia was analyzed using Kaplan-Meier curves, log-rank test, and Cox regression analysis. The level of statistical significance was set at $p < 0.05$.

RESULTS

During the study period, 79 patients were implanted with an isolated LVAD. Three patients aged <18 years, eight patients without recent preoperative CTs, and four patients whose CTs were not suitable for pectoralis muscle measurement were excluded. The study was conducted on 64 LVAD patients.

The mean age of the study patients was 50.2 ± 8.7 years, and 9 (14.9%) were female. Notably, 21 (32.8%) of our LVAD patients were sarcopenic. Sarcopenic LVAD patients were less likely to have hyperlipidemia (4.8% vs. 30.2%, $p = 0.021$) and had higher bilirubin (2.43 ± 4.68 vs. 1.42 ± 3.2 , $p = 0.012$) and lower blood urea nitrogen levels (21.7 ± 10.9 vs. 26.2 ± 11.9 , $p = 0.044$). Other baseline characteristics were similar between sarcopenic and nonsarcopenic patients.

Overall, 1-year mortality was 26.6% and 2-year mortality was 34.4%. The causes of death for the 22 patients with 2-year mortality were cerebrovascular events for 10 patients, right HF for 5 patients, infectious causes for 3 patients, pump thrombosis for 2 patients, mesenteric ischemia for 1 patient, and acute graft failure after heart transplant for 1 patient. At univariate analysis, diabetes mellitus and sarcopenia were associated with 2-year mortality in our cohort. Male and female patients with 2-year mortality had lower pectoralis muscle indices. Other baseline parameters or type of implanted device did not differ in patients with and without mortality (Table 1).

The effect of sarcopenia on 2-year mortality was assessed. The Kaplan-Meier curves were plotted for patients with and without sarcopenia (Figure 1). The 2-year mortality was 25.6% for nonsarcopenic patients and 52.4% for sarcopenic patients. The survival distributions for the two groups were significantly different ($p < 0.001$). A Cox regression model was created with parameters significant at univariate analysis and Interagency Registry for Mechanically Assisted Circulatory Support profile to assess the effect of sarcopenia on 2-year mortality. The results of the analysis are given in Table 2. Sarcopenia had an adjusted hazard ratio (HR) of 4.04 (95% confidence interval (CI) 1.36–12.02, $p = 0.012$), while diabetes mellitus was associated with an HR of 3.14 (95%CI 1.17–8.39, $p = 0.023$). The model with PMI instead of sarcopenia showed an increase of 1 unit in PMI to be protective with an HR of 0.379. (95%CI 0.214–9.670, $p = 0.001$).

DISCUSSION

Our study demonstrated that sarcopenia measured by PMI is a useful risk factor for long-term mortality in LVAD patients. PMI can be readily measured from CT images routinely captured during preoperative assessment of LVAD patients and be used to assess long-term mortality in LVAD patients.

Table 1. Baseline factors in patients with and without 2-year mortality.

	2-Year Mortality (-) (n=42)	2-Year Mortality (+) (n=22)	p-value
Age	51.4±8.1	47.9±9.5	0.165
Female Gender (%)	5 (11.9)	4 (18.2)	0.480
Body Mass Index (kg/m ²)	26.84±4.76	26.77±4.53	0.972
Diabetes Mellitus (%)	10 (23.8)	11 (50.0)	0.034
Hypertension (%)	14 (33.3)	6 (27.3)	0.619
Hyperlipidemia (%)	9 (21.4)	5 (22.7)	1.000
Creatinine (mg/dL)	1.05±0.39	1.18±0.41	0.157
Pro-BNP (pg/mL)	1069.4±1028.8	1065.5±1074.0	0.966
Albumin (g/dL)	3.56±0.69	3.37±1.06	0.904
Bilirubin (mg/dL)	1.10±0.64	2.98±6.24	0.511
INR	1.32±0.37	1.29±0.35	0.955
Na (mEq/L)	136.4±4.3	135.5±2.8	0.352
K (mEq/L)	4.2±0.5	4.2±0.6	0.659
BUN (mg/dL)	25.2±12.0	23.8±11.2	0.821
Platelet (1000/μL)	219.0±68.9	222.5±83.6	0.858
Hemoglobin (g/dL)	12.6±3.8	11.6±2.3	0.392
ALT (IU/L)	29.4±20.5	22.1±13.6	0.211
AST (IU/L)	25.2±11.9	25.8±19.2	0.772
PVR (Wood)	2.97±1.26	3.24±1.39	0.327
TAPSE	16.6±3.2	16.9±4.4	0.837
Aortic Insufficiency			
None (%)	35 (83.3)	20 (90.9)	0.707
Mild (%)	7 (16.7)	2 (9.1)	
Previous Sternotomy (%)	1 (2.4)	1 (4.5)	1.000
Etiology			
Dilated CMP (%)	23 (54.8)	9 (40.9)	0.292
Ischemic CMP (%)	19 (45.2)	13 (59.1)	
Indication			
BTT (%)	10 (23.8)	9 (40.9)	0.155
Destination (%)	32 (76.2)	13 (59.1)	
Sarcopenia (%)	11 (25.6)	11 (52.4)	0.034
Pectoralis Index (cm ² /m ²)			
Men	4.62±1.26	4.05±0.68	0.033
Women	4.84±1.29	2.85±0.59	0.026
INTERMACS			
1 (%)	4 (9.5)	1 (4.5)	0.518
2 (%)	2 (4.8)	2 (9.1)	
3 (%)	15 (35.7)	4 (18.2)	
4 (%)	18 (42.9)	13 (59.1)	
5 (%)	3 (7.1)	2 (9.1)	
Implanted Device			
Heartware (%)	8 (19.0)	7 (31.8)	0.576
Heartmate 2 (%)	18 (42.9)	7 (31.8)	
Heartmate 3 (%)	15 (35.7)	8 (36.4)	
Heart Asist 5 (%)	1 (2.4)	0 (0)	

Pro-BNP: pro-brain natriuretic peptide; INR: international normalized ratio; BUN: blood urea nitrogen; ALT: alanine aminotransferase; AST: aspartate aminotransferase pulmonary vascular resistance; TAPSE: tricuspid annular plane systolic excursion; CMP: cardiomyopathy; BTT: bridge to transplant. Numbers in bold emphasize statistical significance.

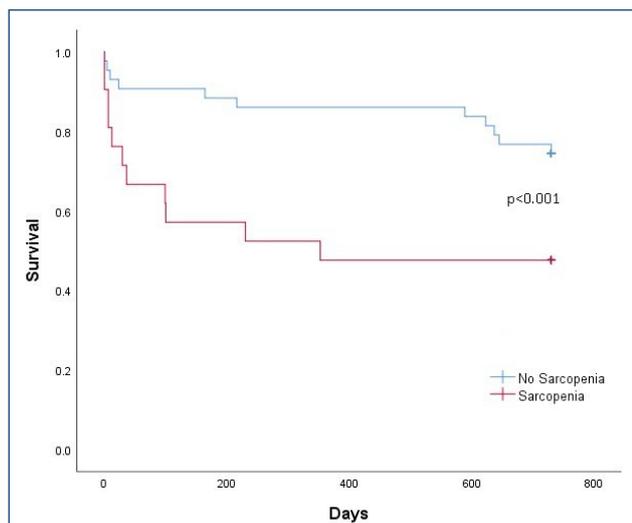


Figure 1. Kaplan-Meier curves of LVADs patients with and without sarcopenia

Table 2. Cox regression model for 2-year mortality.

		Hazard Ratio (95%CI)
Sarcopenia	0.012	4.041 (1.358–12.020)
Diabetes Mellitus	0.023	3.136 (1.172–8.393)
INTERMACS		
1	0.943	0.914 (0.075–11.058)
2	0.195	3.997 (0.491–32.517)
3	0.462	0.526 (0.095–2.915)
4	0.815	1.200 (0.261–5.527)
5 (reference)	0.314	
Bilirubin	0.490	1.033 (0.942–1.134)
Hyperlipidemia	0.645	1.327 (0.398–4.425)
Blood Urea Nitrogen	0.755	1.007 (0.962–1.055)

INTERMACS: Interagency Registry for Mechanically Assisted Circulatory Support. The significant p values are given in bold.

There are several risk scores valuable in assessing operative risk in LVAD patients. These scores have their own shortcomings, such as the destination therapy score being developed before the era of continuous flow assist devices and the Heartmate 2 score lacking external validity¹⁴. These scores serve to identify operative risk but fall short of identifying patients who can benefit from LVAD therapy in the long term¹⁵. Other risk models have been developed for long-term mortality after LVAD implantation. The Penn-Columbia risk score considers age, creatinine, bilirubin, body mass index (BMI), right ventricular dysfunction, and aortic insufficiency for 1-year survival¹⁶. Similarly, other risk models for 1-year mortality have found age, renal function, low hemoglobin, and destination therapy,

besides several other hemodynamic and patient factors, to be predictive of 1- and 2-year survival^{17,18}. No current model for LVAD survival has incorporated frailty or sarcopenia. A major reason for this is that this patient group is not able to perform frailty tests that can comprehensively evaluate sarcopenia. Radiological measurements of muscle quantity or quality can be better suited for this patient population.

Sarcopenia is used as a marker of biological aging and has been shown to have prognostic value in HF¹⁹. Myofibrillar wasting in the skeletal muscles exacerbates exercise intolerance and ventilatory insufficiency, lowering the patient's overall functional status, which can explain the association with surgical and long-term mortality in LVAD patients with lowered muscle mass²⁰. It is speculated that as the patient's HF contributes to frailty, successful LVAD therapy may revert a component of the patient's frail status²¹. One study has shown frailty in LVAD patients to be reversible to a certain extent, and higher quality of life and a better baseline glomerular filtration rate were associated with improved frailty scores²². Data on postoperative PMI were not available for our study patients, and future studies with muscle quality and quantity measures after long-term LVAD therapy can provide more information on the reversibility of sarcopenia with LVADs and the benefits of interventions on sarcopenia before surgery on survival.

Definitions of sarcopenia vary and include physical performance (gait speed), muscle strength, muscle quality (fat infiltration), or muscle quantity⁹. PMI is readily available in most LVAD patients, and most patients are unable to complete performance-based frailty tests, which in itself is associated with worse HF and worse long-term LVAD outcomes²³. A high BMI can mask muscle wasting or early muscle changes that precede cardiac cachexia, and radiological measurements are more valuable in the precise detection of early sarcopenia²⁴. Muscle quantity measurements can aptly capture the risk in this patient group that is too ill to perform frailty tests and have prognostic value. Pectoralis muscle quantity was found to be a stronger indicator of mortality than other traditional risk factors²⁵. In our study patients, sarcopenia and diabetes affected long-term survival, and sarcopenia was the factor with the highest HR.

There are certain limitations to our study. The study design is single center and retrospective. The outcome of interest was all-cause mortality, and conclusions cannot be generalized to specific causes of mortality. Different definitions of sarcopenia can make external validation difficult; however, we have categorized our patients with the lowest tertile definition that is frequently used in the literature. While all implanted devices

are continuous flow LVADs, four different devices were used in our institution during the study period.

CONCLUSION

Sarcopenia, defined by low PMI, increases the risk for 2-year mortality in LVAD patients. Methods that best quantify sarcopenia in end-stage HF patients should be studied, as well as the benefits of preoperative reversal of sarcopenia.

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AUTHORS' CONTRIBUTION

SBE: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **HB:** Data curation, Investigation, Methodology, Writing – original draft. **MB:** Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **MS:** Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **SA:** Conceptualization, Data curation, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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Iron deficiency anemia in infants in Sousa (PB), Brazil: an association with nutritional status

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SUMMARY

OBJECTIVE: The aim of this study was to describe the prevalence of anemia and iron deficiency anemia (IDA) in infants and verify the association of iron deficiency with nutritional status.

METHODS: This cross-sectional and observational study included 104 infants aged between 7 and 9 months, assisted from August to September 2021 by the Family Health Strategy program in Sousa municipality (Paraíba, Brazil). Clinical and anthropometric data were collected, and a 24-h food recall questionnaire was applied using the DietPro software (version 5.0) in order to verify food consumption and assess iron intake. Variables associated with iron deficiency ($p < 0.05$) were analyzed using multiple logistic regression.

RESULTS: Anemia and IDA were observed in 40.4% and 19.2% of infants, respectively. Only one infant was taking prophylactic supplementation (ferrous sulfate). Infants with IDA presented reduced hemoglobin ($p < 0.001$) and ferritin ($p < 0.001$) and increased Z-scores of body mass index-for-age (Z-BMI) ($p = 0.027$), weight-for-height ($p = 0.007$), and weight-for-age ($p = 0.032$). All Z-scores were inversely correlated with ferritin (Z-BMI [ρ : -0.37 ; $p < 0.001$], weight-for-height [ρ : -0.37 ; $p < 0.001$], and weight-for-age [ρ : -0.29 ; $p = 0.002$]). Ferritin was also directly correlated with daily iron intake (ρ : 0.22 ; $p = 0.018$). Finally, multiple logistic regression showed a significant and direct association of iron deficiency with weight-for-height Z-score (odds ratio: 2.86; 95% confidence interval: 1.38–5.64; $p = 0.004$).

CONCLUSION: About 60% of infants presented anemia or IDA. Iron deficiency was associated with the weight-for-height Z-score, showing the vulnerability of infants during the introduction of complementary feeding.

KEYWORDS: Anemia. Iron deficiency anemia. Nutritional status. Infant. Iron.

INTRODUCTION

During the introduction of complementary feeding, infants are vulnerable to micronutrient deficiency, including iron deficiency (ID)¹. According to the World Health Organization (WHO), 42% of children aged between 6 and 59 months have anemia². In Brazil, 18.9% of infants aged between 6 and 23 months have anemia³.

Iron deficiency anemia (IDA) is a major contributor to the global burden of disease, affecting especially children in underdeveloped and developing countries⁴. Iron is an essential micronutrient for several functions, especially growth and development, and ID may result in irreversible deficits in cognition, motor function, and behavior⁵.

Prophylactic supplementation with ferrous sulfate has a good cost-effectiveness ratio for preventing anemia and ID with the stimulation and promotion of breastfeeding and timely and healthy complementary feeding. This supplementation has been encouraged in Brazil since 2005, for which

the Brazilian Society of Pediatrics (SBP) recommends oral intake for healthy and exclusively breastfed infants starting at 6 months^{6,7}. Regarding risk factors for ID, SBP recommends anticipating the supplementation⁸.

Despite the widely described control strategies, anemia is still prevalent in Brazilian infants, especially in the north-east region. In this sense, studying predisposing factors for ID could ensure a better approach to the disease. Therefore, this study aimed to describe the prevalence of anemia and IDA in infants and verify the association of ID with nutritional status.

METHODS

This observational and cross-sectional study included 104 infants aged between 7 and 9 months from Sousa municipality (Paraíba, Brazil). The study was approved by the research ethics committee of the *Centro Universitário FMABC* (number 3.436.978).

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Sousa municipality has an estimated population of 69,997 people, a human development index of 0.668, and 100% Family Health Strategy coverage.

Infants were recruited in July 2021, and clinical and laboratory data were collected from August to September 2021. During this period, 234 infants (7–9 months) were identified. This age group was selected based on the high vulnerability to anemia during the introduction of complementary feeding. The Family Health Strategy attended 137 infants, and 104 caregivers agreed to participate in the study (Figure 1).

Preterm infants, twins, those with chronic diseases, and those presenting any infection up to 1 month before data collection were excluded.

A questionnaire collected the following data: demographic (gender, birth weight, age, and the number of siblings), caregiver (age and maternal educational level), nutritional status and feeding habits of the infant (duration of exclusive breastfeeding, age of introduction of complementary feeding, and presence of disease), and prophylactic supplementation with ferrous sulfate. A 24-h food recall questionnaire was also applied using the DietPro software (version 5.0) to assess iron intake. The dietary reference and recommended dietary allowance guided the adequacy of iron intake⁹. A single researcher (nutritionist and main investigator) applied the questionnaires.

For anthropometric assessments, Z-scores for height-for-age, weight-for-age, weight-for-height, and body mass index-for-age (Z-BMI; weight divided by the squared height) were obtained by a single nutritionist using the WHO Anthro software¹⁰. Weight was obtained using a digital scale (infant without clothes or diapers), and height was obtained using a stadiometer (infant in a supine position).

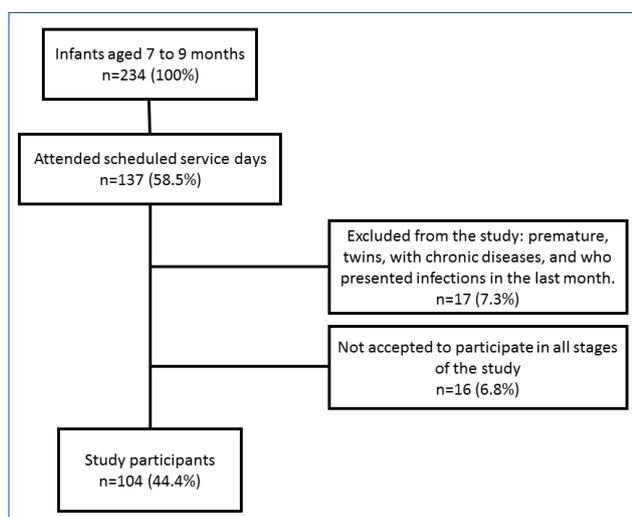


Figure 1. Study flowchart.

Laboratory tests were performed with a 3-h fast 2 days after questionnaires in order to assess anemia and nutritional status of iron. These tests included blood count (automated method), ferritin (chemiluminescence method), serum iron (colorimetric method), and C-reactive protein (CRP; latex agglutination method). CRP was qualitatively classified as normal (≤ 5 mg/L) or abnormal (> 5 mg/L).

Anthropometric and laboratory variables were compared among three groups: without anemia and ID (hemoglobin [Hb] ≥ 11 g/dL and ferritin ≥ 12 μ g/L if CRP ≤ 5 mg/L, or ferritin ≥ 30 μ g/L if CRP > 5 mg/L), with anemia (Hb < 11 g/dL and ferritin ≥ 12 μ g/L if CRP ≤ 5 mg/L, or ferritin ≥ 30 μ g/L if CRP > 5 mg/L)¹¹, and with IDA (Hb < 11 g/dL and ferritin < 12 μ g/L if CRP ≤ 5 mg/L, or ferritin < 30 μ g/L if CRP > 5 mg/L)¹². Serum iron under 30 mg/dL was considered ferropeia¹³.

Data were analyzed using the SPSS software version 25.0 (IBM®). The chi-square test compared qualitative variables, and the Spearman's rank correlation coefficient (rho) analyzed correlations between variables. The multiple logistic regressions used a selection template hierarchy for independent variables and considered ID as a dependent variable¹⁴. Statistical significance was set at 5%.

RESULTS

This study included 104 infants (65.4% female) aged between 7 and 9 months (7.93 ± 0.82 months). We excluded 17 infants (7.3%) who were preterm, twins, presented infection up to 1 month before data collection, and with chronic diseases (Figure 1). Table 1 shows the general characteristics of infants.

Regarding nutritional status, 49% of infants had a Z-BMI over +1 (risk of overweight, overweight, and obesity). Anemia and IDA were observed in 40.4% and 19.2% of infants, respectively. Only one infant took prophylactic supplementation with ferrous sulfate (Table 1).

Table 2 compares general and laboratory data among groups. Infants with IDA presented a higher Z-BMI than infants with anemia or without anemia and ID ($p=0.021$). Moreover, 90% of infants with IDA presented Z-BMI over +1, increased CRP ($p=0.001$), and reduced mean corpuscular volume ($p<0.001$). They also presented increased Z-BMI ($p=0.027$), weight-for-height Z-score ($p=0.007$), and weight-for-age Z-score ($p=0.032$), and reduced Hb ($p<0.001$) and ferritin ($p<0.001$) in continuous variables.

Ferritin was directly correlated with iron intake (rho: 0.229; $p=0.018$) and inversely correlated with Z-BMI (rho: -0.37; $p<0.001$), weight-for-height (rho: -0.37; $p<0.001$), and weight-for-age Z-scores (rho: -0.297; $p=0.002$).

Table 1. General characteristics and laboratory variables of the evaluated infants (n=104).

Variable	n	%
Gender		
Female	68	65.4
Mother's age (years)		
<20	13	12.5
20–30	52	50
30–40	36	34.6
≥40	3	2.9
Maternal education (years)		
0	4	3.9
<4	7	6.7
4–6	38	36.5
6–8	32	30.8
≥8	23	22.1
Attending monthly childcare		
Yes	36	34.6
Received nutritional guidance		
Yes	36	34.6
Infant's age (months)		
7	39	37.5
8	33	31.7
9	32	30.8
Infant's number of siblings		
0	52	50
1	39	37.5
2	11	10.6
3	2	1.9
Birth weight (g)		
≥2,500	103	99
Received exclusive BM up to the 6 months		
Yes	17	16.3
Supplemented BM before 6 months		
Yes	64	61.5
Receiving BM at the moment		
Yes	50	48.1
Receiving powdered milk		
Yes	95	91.4
Receiving cow's milk		
Yes	6	5.8
Receiving beef		
Yes	45	43.3
Receiving pork		
Yes	0	0
Receiving chicken		
Yes	61	58.6
Nutritional status (zBMI)		
Normal weight	53	51
Risk of overweight	31	29.8
Overweight	16	15.4
Obesity	4	3.8
Receiving ferrous sulfate		
Yes	1	1
Hemoglobin (g/dL)		
<11	60	57.7
Mean corpuscular volume (fL)		
<75	14	13.5
Serum iron (µg/dL)		
<40	26	25
Ferritin (µg/L)		
Low	20	19.2
CRP (mg/L)		
>5	10	9.6
Anemia		
–	42	40.4
Iron deficiency anemia		
–	20	19.2

BM: breast milk; zBMI: Z-score body mass index; CRP: C-reactive protein. Low ferritin defined by: <12 µg/L if CRP ≤5 mg/L and <30 µg/L if CRP >5 mg/L.

Multiple logistic regression showed that the weight-for-height Z-score was independently associated with ID in infants (odds ratio [OR]: 2.86; 95% confidence interval [CI] 1.38–5.6; $p=0.004$). The increase of one weight-for-height Z-score was associated with a 2.86-fold chance of ID (Table 3).

DISCUSSION

The present study showed a high prevalence of anemia and IDA in infants from Souza municipality, which was higher than that observed in the Brazilian National Survey of Food and Child Nutrition (ENANI-2020) with 7,473 children aged between 6 and 59 months. According to ENANI-2020, the prevalence of anemia and IDA in Brazil was 18.9% and 8%, respectively, in infants aged 6–23 months. Our cutoff points for anemia and IDA followed the ENANI-2020 recommendation³. In a meta-analysis including 37 Brazilian studies with 17,741 children, the prevalence of anemia in children under 5 years old was an important public health issue, affecting mainly those living in low-income communities and indigenous and quilombola populations¹⁵.

Children with high Z-BMI and growth had reduced iron storage and an increased prevalence of IDA. This result may be due to the low iron absorption and sequestration of reticuloendothelial iron caused by chronic inflammation from adiposity¹⁶. Also, initial iron storage is depleted during child development, which occurs faster in rapidly growing infants¹⁷. In a cross-sectional study with 1,607 children aged 1–3 years, increased Z-BMI was associated with low ferritin (OR: -1.51 µg/L; 95%CI -2.23 to -0.76; $p<0.0001$)¹⁸. Moreover, in a cohort study with 729 infants from birth to 24 months, weight gain in the second year of life was inversely associated with iron storage in apparently healthy children¹⁹.

Ferritin was directly correlated with daily iron intake in infants. In an Indian cross-sectional study with 217,324 children under 5 years, anemia was associated with low iron intake (OR: 0.110; 95%CI 1.084–1.149; $p<0.001$)²⁰. Measures to reduce IDA due to low iron intake include the promotion of breastfeeding and healthy complementary feeding, as recommended by the dietary guidelines for Brazilian children under 2 years old⁷. Furthermore, the Brazilian Ministry of Health suggests the parallel use of prophylactic supplementation with ferrous sulfate and powdered micronutrients⁶.

A controlled Brazilian study compared 462 infants aged 6–8 months receiving powdered micronutrients in complementary feeding with 521 nonsupplemented infants. After 60 days, nonsupplemented infants had a higher prevalence of anemia (23.1% vs. 14.3%; $p<0.001$) and IDA (10.3% vs. 4.9%;

Table 2. Comparison of clinical-demographic and laboratory variables between groups of infants without anemia and iron deficiency, anemia, and iron deficiency anemia (n=104).

Variable	Without anemia and iron deficiency (n=42)	Anemia (n=42)	Iron deficiency anemia (n=20)	p-value
Categorical variables				
Gender				
Male	11 (26.2%)	17 (40.5%)	8 (40%)	0.331*
Age (months)				
7	18 (42.9%)	14 (33.3%)	7 (35%)	0.787*
8	14 (33.3%)	13 (31%)	6 (30%)	
9	10 (23.8%)	15 (35.7%)	7 (35%)	
Number of siblings				
0	19 (45.2%)	23 (54.8%)	10 (50%)	0.386*
1	19 (45.2%)	12 (28.6%)	8 (40%)	
2	3 (7.1%)	7 (16.7%)	1 (5%)	
3	1 (2.4%)	0	1 (5%)	
Birth weight (g)				
<2,500	0	1 (2.4%)	0	0.475*
Received exclusive breastfeeding until 6 months				
Yes	6 (14.3%)	9 (21.4%)	2 (10%)	0.469*
Supplemented breastfeeding before 6 months				
Yes	28 (66.7%)	24 (57.1%)	12 (60%)	0.660*
Receiving breastfeeding now				
Yes	19 (45.2%)	21 (50%)	10 (50%)	0.893*
Receiving infant formula				
Yes	38 (90.5%)	37 (88.1%)	20 (100%)	0.287*
Receiving whole cow's milk				
Yes	3 (7.1%)	3 (7.1%)	0	0.469*
Receiving meat (beef)				
Yes	18 (42.9%)	17 (40.5%)	10 (50%)	0.777*
Receiving poultry				
Yes	28 (66.7%)	24 (57.1%)	9 (45%)	0.261*
Nutritional status (Z-score of body mass index)				
Normal	20 (47.6%)	27 (64.3%)	6 (30%)	0.021*
Overweight risk	17 (40.5%)	8 (19%)	6 (30%)	
Overweight	3 (7.1%)	7 (16.7%)	6 (30%)	
Obesity	2 (4.8%)	0	2 (10%)	
Mother's age (years)				
<20	7 (16.7%)	5 (11.9%)	1 (5%)	0.289*
20-30	16 (38.1%)	26 (61.9%)	10 (50%)	
30-40	17 (40.5%)	11 (26.2%)	8 (40%)	
≥40	2 (4.8%)	0	1 (5%)	

Continue...

Table 2. Continuation.

Variable	Without anemia and iron deficiency (n=42)	Anemia (n=42)	Iron deficiency anemia (n=20)	p-value
Maternal education (years)				
0	2 (4.8%)	2 (4.8%)	0	0.292*
1-4	6 (14.3%)	1 (2.4%)	0	
4-6	14 (33.3%)	16 (38.1%)	8 (40%)	
6-8	13 (31%)	14 (33.3%)	5 (25%)	
≥8	7 (16.7%)	9 (21.4%)	7 (35%)	
Attending monthly childcare				
Yes	11 (26.2%)	17 (40.5%)	8 (40%)	0.125*
Received nutritional guidance				
Yes	11 (26.2%)	17 (40.5%)	8 (40%)	0.331*
Receiving ferrous sulfate				
Yes	0	1 (2.4%)	0	0.475*
Mean corpuscular volume ≥75 fL				
Yes	42 (100%)	36 (85.7%)	12 (60%)	<0.001*
Serum iron ≥40 µg/dL				
Yes	42 (100%)	23 (54.8%)	13 (65%)	<0.001*
C-reactive protein ≤5 mg/L				
Yes	42 (100%)	38 (90.5%)	14 (70%)	0.001*
Continuous variables				
Age				
Months	7.9±0.8	8±0.8	8±0.83	0.459 [†]
Body mass index				
Z-score	1.1 (1.4)	0.76 (1.6)	1.4 (1.9)	0.027 [†]
Weight-for-height				
Z-score	1.1±1	0.7±1	1.7±1.2	0.007 [†]
Height-for-age				
Z-score	0.11 (1.5)	-0.66 (1.3)	-0.32 (1.2)	0.297 [†]
Weight-for-age				
Z-score	0.8±1.2	0.3±1.2	1.1±1.3	0.032 [†]
Hemoglobin				
g/dL	12.2±0.7	10.1±0.5	9.8±0.8	<0.001 [†]
Mean corpuscular volume				
fL	86.4 (2.4)	77.5 (1.1)	80.8 (11.4)	<0.001 [†]
Serum iron				
µg/L	76.3±23.5	51±23.5	59.3±26.9	<0.001 [†]
Ferritin				
µg/L	22.5 (5.6)	23.1 (39.5)	10.4 (4.7)	<0.001 [†]
Iron intake				
mg/day	26.6 (18.2)	19 (31.4)	13.1 (17.2)	0.060 [†]
Protein intake				
g/day	23.8 (26)	23.8 (24.3)	25 (26.1)	0.877 [†]

Anemia: hemoglobin <11 g/dL with normal ferritin. Iron deficiency anemia: hemoglobin <11 g/dL and ferritin <12 µg/L, if C-reactive protein ≤5 mg/L or <30 µg/L, if C-reactive protein >5 mg/L. Significance level of chi-squared test*, Student's t-test[†], and Mann-Whitney test[‡].

Table 3. Logistic regression of variables associated with iron deficiency in infants (n=104).

Variables	OR	95%CI	p-value
Gender			
Female	-0.02	0.32–3.60	0.987
Age			
Months	-0.05	0.49–1.95	0.957
Weight-for-height			
Z-score	2.86	1.38–5.6	0.004
Height-for-age			
Z-score	-1.47	0.36–1.15	0.141
Receiving BF now			
Sim	0.72	0.48–4.74	0.472
Receiving meat			
Sim	-0.81	0.20–1.91	0.415
Iron intake			
mg/day	-1.69	0.92–1.00	0.322

Dependent variable: iron deficiency defined as: ferritin <12 µg/L if C-reactive protein ≤5 mg/L or ferritin <30 µg/L if C-reactive protein >5 mg/L. OR: odds ratio; CI: confidence interval; BF: breastfeeding.

p=0.002) than infants receiving powdered micronutrients²¹. In a meta-analysis including 136 studies, iron supplementation for infants was associated with a reduced risk of ID (relative risk: 0.21; 95%CI 0.12–0.39; heterogeneity: 94%; p<0.00001) and IDA (relative risk: 0.14; 95%CI 0.04–0.54; heterogeneity: 88%; p=0.004)²².

Our study corroborated others suggesting that serum iron was not an isolated biomarker to evaluate iron storage. In a review of 22 guidelines, all of them recommended dosing ferritin to classify ID and IDA. From those, 10 recommended transferrin saturation and none recommended isolated serum iron²³.

CRP increased by 30% in infants with IDA, which we could not explain. CRP is an acute-phase protein used to assess inflammation from different etiologies²⁴. ID impairs immune

function and increases the risk of infections, especially in infants with IDA, exacerbating the inflammatory process and possibly explaining the association found in the study²⁵. Since ferritin also increases during inflammation, the WHO published a guideline in 2020 for dosing CRP with ferritin to evaluate ID and recommended an adjustment in ferritin cutoff points for individuals with CRP over 5 mg/L (infants: <12 µg/L if CRP ≤5 mg/L; <30 µg/L if CRP >5 mg/L)¹². However, we did not find studies associating ID with CRP in infants.

The sample in this study was carefully selected, excluding infants with acute or chronic diseases (i.e., inflammation). In addition, we used ferritin cutoff points considering the inflammation to identify ID. The main limitation of this study was the assessment of a single Brazilian municipality; thus, the data may not represent the general population. In addition, the cross-sectional design hindered the establishment of a cause-effect relationship.

CONCLUSION

This study observed a high prevalence of anemia and IDA (60%) in infants. Also, ID was associated with an increased weight-for-height Z-score.

Therefore, promoting breastfeeding and a balanced, timely, and healthy complementary feeding with iron-rich foods and guiding a prophylactic supplementation of ferrous sulfate and powdered micronutrients are essential to prevent nutritional disorders.

AUTHORS' CONTRIBUTIONS

LKARA: Conceptualization, Data curation, Investigation, Writing – original draft. **JCPF:** Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft. **ROSS:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – review & editing.

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Vitamin and mineral levels during pregnancy

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SUMMARY

OBJECTIVE: Numerous physiological changes occur during pregnancy, which affect both the mother and the fetus. The objective of this study was to evaluate the magnesium, calcium, phosphate, parathormone (PTH), and vitamin D levels in each trimester of pregnancy.

METHODS: In this study, 30 pregnant women in the first trimester, 30 pregnant women in the second trimester, 30 pregnant women in the third trimester, and 30 healthy, non-pregnant women (control) in the same age group were included. The serum magnesium, calcium, phosphate, PTH, and vitamin D levels were measured in all the participants.

RESULTS: No statistically significant difference was found in the PTH and phosphate levels within the groups. In contrast, a significant difference was found in the vitamin D, calcium, and magnesium levels ($p < 0.001$ for all). By analyzing the differences between the groups, the vitamin D, calcium, and magnesium levels were found to decrease with increase in the gestational weeks.

CONCLUSION: For maintaining a healthy pregnancy and fetus, we recommend vitamin D, calcium, and magnesium levels to be included in routine follow-ups for each trimester and supplemented in case of deficiency.

KEYWORDS: Pregnancy. Minerals. Vitamins.

INTRODUCTION

During pregnancy, many physiological changes occur which have an effect on both the mother and fetus. One such transient parameter is vitamin D. 7-Dehydrocholesterol, which is found in the skin, turns into cholecalciferol (vitamin D₃) under the effect of ultraviolet B (UVB) rays and acts as the precursor of vitamin D in the skin. Another vitamin D precursor is ergocalciferol (D₂). These forms of vitamin D are converted into 1,25-dihydroxy-cholecalciferol (calcitriol), the active form of vitamin D, via 25-alpha-hydroxylase in the liver and 1-alpha-hydroxylase in the kidney¹. The most essential function of vitamin D is regulation of the plasma calcium and phosphate levels. Vitamin D is a fat-soluble, antiapoptotic, and anti-inflammatory steroid-structured molecule that facilitates bone development, contributes to the formation of the immune system, and prevents the development of cancer, among other functions². During pregnancy, significant changes occur in the vitamin D and calcium metabolism of the body. For bone development and mineralization, the fetus accumulates 2–3 mg of calcium per day in the first trimester and about twice this amount in the third trimester. Vitamin D deficiency in pregnant women can lead to diseases such as gestational diabetes, preeclampsia, preterm labor, intrahepatic cholestasis, while in children, it can cause low birth weight, premature birth, abnormal skeletal

development, immune system deficiency, and diseases such as rickets, asthma, type 1 diabetes, and cardiovascular disorders³⁻⁵.

When the plasma calcium levels decrease, it stimulates PTH secretion, activates 1-alpha-hydroxylase enzyme, and actively forms vitamin D. Vitamin D increases plasma calcium and phosphate levels by increasing intestinal calcium and phosphate absorption and also by regulating osteoclast activity⁶. Hence, the need for PTH increases during pregnancy⁷. In general, PTH concentrations are found to be in the normal range in the first trimester of pregnancy, and then continue to increase and return back to the normal level toward the end of the third trimester⁸.

Magnesium is also necessary for vitamin D activation; hence, the need for magnesium increases during pregnancy⁹. Magnesium deficiency can lead to preterm labor in pregnant women as well as developmental retardation in the fetus and metabolic syndrome in the later stages of life¹⁰.

The incidence rate of vitamin D deficiency in pregnant women varies between 20–40% in the world and 18.2–45.9% in Turkey^{11,12}. The aim of this study was to determine vitamin D, PTH, magnesium, calcium, and phosphate levels in the first, second, and third trimesters in multiparous pregnant women and to determine whether there is a difference between trimesters and healthy, nonpregnant women in the same age group.

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METHODS

This study included a total of 90 pregnant women and 30 healthy, nonpregnant control who were admitted to the Necip Fazıl City Hospital Gynecology and Obstetrics Department outpatient clinic between March and July 2021. Ethical approval was obtained from the ethics committee of the Kahramanmaraş Sütçü İmam University (decision number: 03-20/04/2022-03). Multiparous pregnant women who did not have any previous risk factors or known diseases, did not smoke, and did not use any vitamin D preparations were included in the study. Patients with metabolic and endocrine diseases were excluded from the study. The study participants were categorized into four groups: Group I comprising 30 pregnant women in their first trimester (8.68 ± 0.57 weeks), Group II comprising 30 pregnant woman in their second trimester (22.72 ± 3.64 weeks), Group III comprising 30 pregnant women in their third trimester (34.66 ± 3.38 weeks), and Group IV comprising 30 nonpregnant women as healthy control. Each group consists of different patients. Fasting venous blood samples were collected from the participants of all the groups. The blood samples were centrifuged to separate the serum and plasma. The resulting serum samples were frozen at -80°C until analysis. The following methods were used in the analysis of the samples. The magnesium, calcium, phosphate, vitamin D, and PTH levels were measured.

The magnesium, calcium, and phosphate levels were measured calorimetrically using Roche Hitachi Cobas C501 (Roche Diagnostics, F. Hoffmann-La Roche Ltd., Japan) autoanalyzer. The vitamin D and PTH levels were measured using Roche Hitachi Cobas e-601 module (Roche Diagnostics, F. Hoffmann-La Roche Ltd.) autoanalyzer device via the electrochemiluminescence immunoassay method.

STATISTICAL ANALYSIS

The SPSS version 25.0 (IBM Statistics for Windows version 25, IBM Corp., Armonk, NY, USA) program was used to analyze the study data. Variance homogeneity was checked with Levene test, and conformity of the data to normal distribution was evaluated by Shapiro-Wilk test. Quantitative data were presented as mean \pm standard deviation. Categorical data were presented as n (number) and percentage (%). Multiple groups were compared using ANOVA, and Tukey HSD was used for intergroup comparisons. The data were examined at 95% confidence interval and $p < 0.05$ was considered statistically significant for all the analyses.

RESULTS

The study included multiparous pregnant women in the first, second, and third trimesters and healthy, nonpregnant controls

with similar characteristics. The mean age was 26.83 ± 5.93 years in Group I, 27.03 ± 5.58 years in Group II, 27.43 ± 5.81 years in Group III, and 30.77 ± 7.22 years in Group IV. There was no statistically significant difference between the groups in terms of age. Demographic variables of patients are provided in Table 1. Furthermore, no statistically significant difference was found in the PTH and phosphate levels within the control group (Group IV) and the pregnant groups (Groups I, II, and III) ($p = 0.495$, $p = 0.173$, and $p = 0.149$, respectively). Conversely, vitamin D, calcium, and magnesium levels were found significantly lower in the pregnant groups (Groups I, II, and III) compared to the control group (Group IV) ($p < 0.001$ for all). When the differences between the groups were analyzed, it was found that the vitamin D, calcium, and magnesium levels decreased as the gestational weeks increased. The vitamin D, calcium, and magnesium levels of Group I were significantly higher than the other groups ($p < 0.05$). There was no statistically significant difference between Groups II and III in terms of the vitamin D, calcium, and magnesium levels ($p > 0.05$). All the biochemical variables are presented in Table 2.

DISCUSSION

Vitamin D possesses essential functions such as regulating calcium and phosphorus metabolism, contributing in the development of a strong immune system, and preventing oxidative DNA damage and cancer development by inducing apoptosis in tumor-forming cells^{13,14}. Studies in the literature have reported vitamin D deficiency to be a common phenomenon in pregnant women^{11,12}. Another study stated that the vitamin D levels increased twofold in pregnant women during the first trimester and decreased 2–3 times during the subsequent trimesters¹⁵. In contrast, other studies have reported no change or increase in the vitamin D levels between trimesters¹⁶. Vitamin D deficiency in pregnant women can cause disorders, including gestational diabetes, preeclampsia, preterm labor, and intrahepatic cholestasis, while in children, it can lead to low birth weight, premature birth, abnormal skeletal development, immune system deficiency, and diseases such as rickets, asthma, type 1 diabetes, and cardiovascular disorders³⁻⁵. In general, the concentration of 25(OH)D in cord blood has been shown to be approximately 50–80% of that of the serum¹⁷. Some studies have found that vitamin D supplementation in pregnant women prevents hypocalcemia, which can cause the softening of the bones, such as in the disorders craniotabes and rickets, dilated cardiomyopathy, and immune system pathologies^{18,19}.

In our study, it was determined that there was no statistically significant difference in PTH and phosphate levels between the

Table 1. Demographic variables of patients (Kemal Hansu).

Variables	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	Group 4 (n=30)	p
Age (years)	26.83±5.93	27.03±5.58	27.43±5.81	30.77±7.22	0.049
Gestational age (weeks)	8.68±0.57	22.72±3.64	34.66±3.38	–	<0.001

Data are expressed as mean±SD or n (%), unless otherwise noted. One-way ANOVA (with Tukey HSD) or Pearson's chi-square test.

Table 2. Comparison of the hematological, biochemical, and hormonal variables of the groups (Kemal Hansu).

Variables	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	Group 4 (n=30)	p
PTH (mIU/mL)	48.61±63.80	70.77±48.55	73.94±42.44	60.55±32.62	0.173
25(OH)D (ng/L)	16.17±10.04*	8.75±4.75 [†]	9.89±7.61 [†]	19.36±6.8064	<0.001
Calcium (Ca) (mg/dL)	9.44±0.54 [‡]	9.34±0.34 [§]	9.04±0.36 [†]	9.82±0.39	<0.001
Magnesium (Mg) (mg/dL)	1.98±0.14 [‡]	1.95±0.38 [‡]	1.90±0.13 [‡]	2.06±0.15	0.021
Phosphate (P) (mg/dL)	3.47±0.64	3.49±0.87	3.69±0.75	3.27±0.45	0.149

Data are expressed as the mean±SD or n (%), unless otherwise noted. One-way ANOVA (with Tukey HSD) or Pearson's chi-square test. *p>0.05 between the control group and the related group; [†]p<0.001 between the control group and the related group; [‡]p<0.05 between the control group and the related group; [§]p<0.01 between the control group and the related group. PTH: parathormone.

control group and pregnant women. PTH is necessary to increase calcium absorption in pregnant women. Serious complications such as intrauterine growth retardation, neonatal tetany, intrauterine deaths, long bones curvature, low birth weight, spontaneous abortions, and fetal deaths can be seen in fetuses of pregnant women with hypoparathyroidism²⁰. Therefore, maternal parathormone levels are important in pregnancy. In various studies, it has been shown that PTH decreases or remains the same in the first trimester of pregnancy, increases in the second trimester, and returns to normal levels in the third trimester^{21,22}. It has been determined that phosphate levels increase very little during pregnancy and it is important in fetal bone development, but high phosphate levels cause fetal complications by vascular calcification or muscle calcification²³.

Magnesium is a cation that is required in numerous biological and cellular functions, such as protein synthesis, nucleotide metabolism, ATP production, neuromuscular transmission, increased vitamin D synthesis, and bone mineralization^{9,24,25}. Magnesium deficiency in pregnant women can lead to preterm labor and metabolic syndrome, growth retardation in the fetus, and insulin resistance in the future stages of life^{10,26}. In the present study, a significant reduction was observed in the magnesium levels in all the trimesters in the pregnant women compared

with the control group (p<0.001). Intergroup examination revealed that the magnesium levels decreased with increase in the gestational weeks, albeit not statistically significant.

CONCLUSION

Based on the results of this study, the vitamin D, calcium, and magnesium levels were significantly lower in the pregnant groups compared with the control group. Although not statistically significant, it was determined that the magnesium levels decreased with increasing number of gestational weeks. In terms of maintaining a healthy pregnancy, we think that controlling the levels of the vitamin D, calcium, and magnesium during pregnancy and completing the deficiency may be beneficial for both maternal and fetal health.

AUTHORS' CONTRIBUTIONS

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

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Translation and validation of the techno-stress questionnaire in Brazil

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SUMMARY

OBJECTIVE: The aim of this study was to translate the techno-stress questionnaire proposed by Ragu Nathan et al into Brazilian Portuguese and to culturally adapt and validate it. For this, 4 of the 11 original questionnaires' domains were used.

METHODS: The questionnaires' domains translated and adapted were as follows: techno-overload, techno-invasion, techno-complexity, and job satisfaction. Initially, the techno-stress questionnaire was translated into Brazilian Portuguese language according to international standards, followed by cultural adaptations. Validation for feasibility and psychometric properties of translated questionnaire was performed on 138 gynecologists and obstetricians who use message applications to communicate with patients. The physicians were divided into groups according to the weekly messaging application usage time for communication with patients: <2 h (GI, n=89), 2–5 h (GII, n=29), and >5 h (GIII, n=23). The questionnaire was applied to all participants twice on the same day, overseen by two different interviewers, at a 15-min interval. After 15 days, it was readministered. The discriminant validity and reliability were calculated to validate the instrument.

RESULTS: Techno-stress subscales showed statistically significant differences between the groups. The Cronbach's alpha coefficient for the techno-stress questionnaire was >0.80, showing good internal consistency. No differences were observed in the test-retest comparison of the techno-stress questionnaire, and the intraclass correlation coefficient results showed excellent reproducibility (²0.75).

CONCLUSION: The techno-stress questionnaire was adequately translated into Brazilian Portuguese, with good discriminant validity, good internal consistency, and adequate test-retest results.

KEYWORDS: Mobile applications. Occupational stress. Validation study. Gynecology. Surveys and questionnaires.

INTRODUCTION

Technological innovation has made rapid and dramatic changes to society. The Internet has altered the way we communicate, exchange information, and develop relationships¹. Increasingly spread through our professional and personal lives, communication technologies make it possible to rapidly and easily keep in touch with colleagues, friends, and family².

The forthcoming of social media has shifted information-seeking behavior in society, and the health sector is not immune from this influence³. The digital revolution had a considerable impact on how doctors interact with patients, and the increased use of smartphones and related software applications has created a new era in the exchange of clinical data between patients and physicians⁴.

Innumerable advantages of using communication applications in healthcare have been reported: free of cost, improvement of communication, time saving, no requirement for a computer, and the possibility of an immediate response. Besides, drawbacks have also been described: increase in workload by staying

online 24 h a day, disparity in the sense of urgency, clinical information not being included in medical records, possible issues of privacy and data protection, ethical aspects of clinical evaluations at a distance, and lack of specific legislation⁴⁻⁸.

It is not surprising that the advent of instant messaging is gradually affecting patient-physician communication and that unrestricted access to physicians via messaging applications creates challenging situations. How these technological transformations are affecting physicians' well-being and mental health is not known⁹.

The techno-stress questionnaire proposed by Ragu Nathan et al. is an effective means to evaluate the techno-stress influence on job satisfaction and professional commitment and provides information that allows making inferences about physicians' responses demanded by the use of communication technology in their professional life¹⁰.

The goal of the present study was to translate and cross-culturally adapt the techno-stress questionnaire proposed by Ragu Nathan et al. into Brazilian Portuguese while studying the

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reliability and validity of this Portuguese-language version. Of the 11 original questionnaires' domains, only 4 were used for this purpose.

METHODS

Study design and population

This study was conducted from August 2019 to July 2020, previously submitted to and approved by the Research Ethics Committee of the University Center FMABC under number 3.528.229. The population sample consisted of gynecologists and obstetricians selected by convenience. The professionals were personally invited to a regional meeting and all agreed to participate in the research.

The inclusion criteria were as follows: gynecologists and obstetricians working in the state of São Paulo, Brazil, who used WhatsApp to communicate with patients and agreement to participate in the study according to the informed consent form, which all participants signed.

Participants were divided into research groups according to the self-reported weekly time of WhatsApp use to communicate with patients: <2 h (GI), 2–5 h (GII), and >5 h (GIII). The categories were arbitrarily defined.

Translation of the techno-stress questionnaire

Written authorization was first obtained from Professor John Q. Tu, one of the authors of the instrument, to translate and validate the questionnaire. We used 4 of the 11 original domains: techno-overload, techno-invasion, techno-complexity, and job satisfaction. Seven domains were excluded because they did not fit the professional context of the study participants.

To validate the instrument, we used the guidelines proposed by Guillemain, Bombardier, and Beaton for the translation and validation of health-related quality-of-life instruments¹¹. The first step was the translation into Brazilian Portuguese by a sworn translator. Then, the questionnaire was back-translated into English by a certified native Portuguese-speaking translator with English language proficiency to compare the newly translated version with the original questionnaire. Next, cultural adaptation was performed by the research team who discussed the questionnaire until a consensus version was reached, which happened when the level of agreement was greater than 50% among the members. The translated version is as follows:

Tecnossobrecarga

*Sou forçado por esta tecnologia a trabalhar com mais rapidez.
Sou forçado por esta tecnologia a fazer mais trabalho do que consigo.*

*Sou forçado por esta tecnologia a trabalhar com prazos muito apertados.
Sou forçado a mudar meus hábitos de trabalho para me adaptar a novas tecnologias.
Tenho uma carga de trabalho maior devido à maior complexidade tecnológica.*

Tecnoinvasão

*Passo menos tempo com minha família devido a esta tecnologia.
Tenho que estar em contato com meu trabalho mesmo durante minhas férias devido a esta tecnologia.
Tenho que sacrificar o tempo de minhas férias e fins de semana para me manter a par das novas tecnologias.
Sinto que minha vida pessoal está sendo invadida por esta tecnologia.*

Tecnocomplexidade

*Não sei o suficiente sobre esta tecnologia para conseguir desempenhar minha função satisfatoriamente.
Necessito muito tempo para entender e usar novas tecnologias.
Não encontro tempo suficiente para estudar e aperfeiçoar minhas habilidades tecnológicas.
Eu concluo que os novos funcionários desta organização sabem mais sobre tecnologia de computador do que eu.
Frequentemente concluo que as novas tecnologias são muito complexas para eu entender e utilizar.*

Satisfação no trabalho

*Eu gosto de fazer o que faço no trabalho.
Eu tenho uma sensação de orgulho ao realizar meu trabalho.
Meu trabalho é agradável.*

Validation of the techno-stress questionnaire

The techno-stress questionnaire was applied at three different times: the first (T0) was overseen by researcher A; the second (T1) occurred 15 min after the end of T0 and was overseen by researcher B; and the third (T2), 15 days later, when the instrument was completed via WhatsApp after being sent by researcher A.

Concerning the psychometric properties for measuring the techno-stress questionnaire, the variables analyzed were internal consistency, test-retest reliability, and intraobserver and interobserver reliability. The internal consistency evaluates the correlation between the items and is determined from the subscale scores and the total score. A higher value indicates a greater correlation between various items on the scale. A retest was performed 15 days after the first application of the questionnaire, assessing the intraobserver reliability, and the interobserver reliability was assessed by applying the instrument 15 min after the first interview. The discriminant

validity was evaluated by applying the questionnaire to the three groups studied.

Statistical analysis

The data were tabulated in the Microsoft Excel 2003 software. The IBM-SPSS for Windows version 20.0 software (IBM Corp., Chicago, IL, USA) was used for analysis.

The normality of the data was analyzed using the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) and the Bonferroni test were used to compare continuous variables. The chi-square test and the likelihood ratio test were used to compare categorical variables.

The scores' questionnaire was analyzed by the mean. The variables worded positively were inverted, thus characterizing the higher the mean, the higher the techno-stress levels.

The internal consistency of the techno-stress questionnaire was evaluated with Cronbach's alpha coefficient (>0.7 : acceptable)¹².

The test-retest reliability was assessed with the intraclass correlation coefficient (ICC), to determine whether the studied tool is reliable for comparing the scores obtained at T0 and T2 (intraobserver) and at T0 and T1 (interobserver) ($ICC \geq 0.75$: excellent reproducibility)¹³.

Statistical tests were two-tailed, with a significance level of 5%.

RESULTS

We included 138 physicians, divided into three research groups according to the weekly WhatsApp usage time for communication with patients: <2 h (GI, $n=86$), $3-5$ h (GII, $n=29$), and >5 h (GIII, $n=23$).

The mean age of the participants in GI, GII, and GIII was 46.6 ± 13.2 , 46.1 ± 12.2 , and 41.9 ± 6.8 years, respectively ($p=0.249$). More than half of the doctors self-identified as a female in all groups ($p=0.234$). All groups had a greater proportion of cohabiting people ($p=0.091$), persons with at least one child ($p=0.633$), and persons living in São Paulo city and neighborhood ($p=0.548$). Most participants in all groups had been working for more than 15 years as a doctor ($p=0.599$). The professionals who had more office work activity ($p=0.019$) and who worked more hours a week ($p=0.048$) communicated more with patients via WhatsApp.

Demographic data are described in Table 1.

Table 2 shows the scores' questionnaire in the groups studied (represents T0, when the three questionnaires were answered). Techno-overload and techno-invasion were related to longer WhatsApp usage time to communicate with patients ($p<0.001$). Techno-complexity and job satisfaction did not differ between

groups. When adjusted for the workload and office work of research participants, the results were similar. The discriminant validity of the techno-stress questionnaire for the sample was demonstrated.

Techno-overload was higher in GIII than in GI and GII ($p<0.05$). Techno-invasion was higher in GIII than in GI and GII and higher in GII than in GI ($p<0.05$).

The Cronbach's alpha coefficient for the techno-stress questionnaire was >0.80 for all domains and for the overall score, showing good internal consistency for the domains translated.

No differences were observed in the test-retest comparison of the techno-stress questionnaire translated into Brazilian Portuguese in the intraobserver or interobserver evaluation ($ICC \geq 0.75$) (Table 3).

DISCUSSION

The techno-stress questionnaire was developed and validated in 2008 in English¹⁰. The findings of the present study demonstrated acceptable psychometric properties of the Brazilian Portuguese version of the instrument, which allows its use in Brazilian physicians. The general understanding of the translated terms was adequate, and only small changes were necessary. There was adequate internal consistency for all domains of the questionnaire, and Cronbach's alpha coefficient showed excellent internal consistency in the overall score.

The present study also confirmed the usefulness of the techno-stress questionnaire to assess the impact of the use of messaging apps as a stressor affecting the well-being of gynecologists and obstetricians who routinely use this tool to communicate with patients, thereby demonstrating the discriminant ability of the instrument.

Our data suggest that techno-invasion and techno-overload were associated with the high frequency of WhatsApp usage for communication with patients. Thus, our findings are in line with the study by Waizenegger, which suggested that techno-overload (constant connectivity) leads to techno-invasion, an important cause of workers' techno-stress¹⁴. The change in the doctor-patient relationship caused by the use of communication technology is considered one of the most stressful factors in the individual exposure profile of physicians¹⁵.

According to previous studies, the additional working hours to which virtual communication exposes doctors are an important cause of exhaustion^{16,17}. Techno-stress at work is related to poor mental health, and the resulting psychological stress can have consequences such as chronic fatigue, relationship conflicts, substance abuse, psychiatric morbidities, and suicidal ideation¹⁸⁻²⁰.

Table 1. Sociodemographic characteristics.

Variable	WhatsApp group			p
	<2 h/week (n=86)	2–5 h/week (n=29)	>5 h/week (n=23)	
Age (years)				
mean±DP	46.6 ± 13.2	46.1 ± 12.2	41.9 ± 6.8	0.249*
Gender, n (%)				
Female	49 (57)	20 (69)	17 (73.9)	0.234
Male	37 (43)	9 (31)	6 (26.1)	
Marital status, n (%)				
Single	30 (34.9)	8 (27.6)	9 (39.1)	0.091#
Married	49 (57)	21 (72.4)	14 (60.9)	
Cohabitation	7 (8.1)	0 (0)	0 (0)	
Children, n (%)				
Yes	55 (64)	21 (72.4)	14 (60.9)	0.633
No	31 (36)	8 (27.6)	9 (39.1)	
Residence, n (%)				
São Paulo city	84 (97.7)	28 (96.6)	22 (95.7)	0.548#
Countryside of São Paulo	1 (1.2)	0 (0)	1 (4.3)	
Other states	1 (1.2)	1 (3.4)	0 (0)	
Graduate year, n (%)				
1–15 years	29 (33.7)	7 (24.1)	8 (34.8)	0.599
>15 years	58 (66.3)	22 (75.9)	7 (65.2)	
Smoking, n (%)				
Yes	5 (5.8)	1 (3.4)	0 (0)	0.285#
No	81 (94.2)	28 (96.6)	23 (100)	
Alcoholism, n (%)				
Yes	4 (4.7)	3 (10.3)	0 (0)	0.155#
No	82 (95.3)	26 (89.7)	23 (100)	
Physical activity, n (%)				
Yes	49 (57)	15 (51.7)	15 (65.2)	0.618
No	37 (43)	14 (48.3)	8 (34.8)	
Weekly workload, n (%)				
<20 h	2 (2.3)	1 (3.4)	2 (8.7)	0.048#
20–30 h	5 (5.8)	4 (13.8)	0 (0)	
31–40 h	27 (31.4)	8 (27.6)	2 (8.7)	
41–60 h	40 (46.5)	13 (44.8)	11 (47.8)	
>60 h	12 (14)	3 (10.3)	8 (34.8)	
Work-duty activity, n (%)				
Yes	35 (40.7)	11 (37.9)	6 (26.1)	0.438
No	51 (59.3)	18 (62.1)	17 (73.9)	
Office work activity, n (%)				
Yes	74 (86)	28 (96.6)	23 (100)	0.019#
No	12 (14)	1 (3.4)	0 (0)	
Work-surgery activity, n (%)				
Yes	45 (52.3)	20 (69)	16 (69.6)	0.148
No	41 (47.7)	9 (31)	7 (30.4)	
Academic activity, n (%)				
Yes	31 (36)	11 (37.9)	8 (34.8)	0.971
No	55 (64)	18 (62.1)	15 (65.2)	

Test: chi-square; #Likelihood ratio test; ANOVA.

Table 2. Discriminant validity of the questionnaire between groups.

Variable	WhatsApp group			P	p [†]
	<2 h/week (n=86)	2-5 h/week (n=29)	>5 h/week (n=23)		
Techno-overload					
Mean±DP	2.67±1.04	3.05±1.1	3.93±0.85	<0.001*	<0.001
Techno-invasion					
Mean±DP	2.78±1.09	3.41±1.04	4.64±0.39	<0.001*	<0.001
Techno-complexity					
Mean±DP	2.42±0.96	2.64±1.11	2.44±1.14	0.593*	0.849
Job satisfaction					
Mean±DP	4.34±0.7	4.31±0.9	4.39±0.84	0.932*	0.953

Test: chi-square; *ANOVA; †value adjusted for the workload and office work activity.

Table 3. Test-retest intraobserver and interobserver reliability of the techno-stress questionnaire.

Questionnaire domain	Intraobserver ICC CI	Interobserver ICC CI
Techno-overload	0.845 (0.782–0.890)	0.926 (0.897–0.947)
Techno-invasion	0.804 (0.724–0.861)	0.904 (0.867–0.931)
Techno-complexity	0.838 (0.774–0.886)	0.963 (0.948–0.973)
Job satisfaction	0.833 (0.767–0.882)	0.988 (0.983–0.991)

ICC: intraclass correlation coefficient; CI: confidence interval.

Besides their negative impact on individuals' quality of life, techno-stress between doctors may also affect the quality of care delivered to patients. It is suggested that there is a proven relationship between poor well-being and poor patient safety, leading to adverse outcomes on patient care and worsening physician-patient relationship^{21,22}. Physicians' emotional exhaustion causes indifference to the patient's needs, compromising the quality of care²⁰.

We believe that the techno-stress questionnaire translated into Portuguese in Brazil is an instrument that can evaluate physicians' perceptions about the influence of the use of communication technology in their professional life as a stressor.

CONCLUSION

Of the 11 original domains of the technological stress questionnaire, only 4 were translated into Brazilian Portuguese,

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culturally adapted, and shown to be a valid and reliable instrument for evaluating the use of messaging apps as a stressor affecting the well-being of gynecologists and obstetricians who routinely use this tool to communicate with patients.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Research Ethics Committee of the University Center FMABC under number 3.528.229 on August 23, 2019. All study participants provided informed written consent.

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AUTHORS' CONTRIBUTIONS

MGV: Conceptualization, Data curation, Writing – original draft. **RTF:** Conceptualization, Data curation, Writing – original draft. **GDT:** Conceptualization, Data curation, Writing – original draft. **DIGC:** Formal Analysis, Methodology, Project administration, Supervision. **EO:** Formal Analysis, Methodology, Project administration, Supervision. **CEF:** Visualization, Writing – review & editing.

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Temporal muscle thickness predicts mortality in prevalent hemodialysis patients

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SUMMARY

OBJECTIVE: Ultrasonographic temporal muscle thickness measurement has recently emerged as a promising method of nutritional assessment in various conditions; hence, we aimed to determine the relationship between temporal muscle thickness and mortality in prevalent hemodialysis patients.

METHODS: Adult patients who were on a regular in-center hemodialysis program for ≥ 3 months were included, and patients with severe nonrenal organ failure or any recent significant disease inception were excluded. Baseline demographic; clinical, laboratory, and anthropometric data, including malnutrition inflammation score; and outcomes data were collected using a standardized form.

RESULTS: A total of 60 patients (32 males, diabetes prevalence: 26.6%) who met the eligibility criteria participated in the study, with a mean follow-up of 33.3 ± 11.5 months, a median age of 66.5 (interquartile range 52.7–74) years, time on hemodialysis of 36 months, and a body mass index of 25.9 kg/m^2 . Infections and cardiovascular events were the most common causes of overall mortality that occurred in 41.6% of the patients. Temporal muscle thickness was significantly lower in nonsurvivors (8.8 vs. 10.6 mm, $p < 0.001$). Multivariate Cox regression analysis involving age, albumin, spKt/V, and malnutrition inflammation score revealed that temporal muscle thickness was a significant predictor of mortality (hazard ratio=0.740, $p=0.035$). Receiver operating characteristic curve analysis has shown 68% of sensitivity and 81.8% of specificity for a cutoff value of 9.4 mm ($p < 0.001$). Temporal muscle thickness was weakly or mildly correlated with hemodialysis vintage, body mass index, albumin, and malnutrition inflammation score and moderately correlated with age ($r = -0.536$, $p < 0.001$).

CONCLUSION: Ultrasonographic temporal muscle thickness has been found as a significant predictor of mortality in prevalent hemodialysis patients. Temporal muscle thickness could be a novel marker of nutritional status and predictor of mortality; hence, further studies are warranted.

KEYWORDS: Temporal muscle. Malnutrition. Hemodialysis. Mortality.

INTRODUCTION

Chronic kidney disease (CKD) is a common disease, with an estimated prevalence of above 800 million across the world¹. The multidimensional costs of CKD are also very high, and according to a recent study, CKD resulted in 35.8 million disability-adjusted life-years in 2017². The burden of CKD becomes even greater in patients with advanced stages of CKD, as the uremic milieu constitutes a unique environment, with multiple consequences, such as CKD, mineral bone disease, cardiovascular disease, and nutritional deterioration³. The course and status of nutrition are quintessential components of the evaluation and treatment of patients with advanced stages of CKD, and yet they are often overlooked in daily practice. The laborious nature of longitudinal follow-up of the components of nutritional status on top of busy routines could partially explain the reason for that.

A nomenclature and a set of diagnostic criteria for protein energy wasting (PEW) were proposed on behalf of the International Society for Renal Nutrition and Metabolism (ISRNM) in 2008, based on four categories, namely, biochemical parameters, body mass, muscle mass, and dietary intake⁴. A multidomain semiquantitative scale of malnutrition inflammation score (MIS) has been used in multiple studies to predict morbidity and mortality in maintenance hemodialysis (HD) patients^{5,6}. Subjective global assessment and French PEW score were also proposed, but rather than using thresholds for any parameter, there is a general agreement to consider the longitudinal course of the nutritional components for the diagnosis and treatment.

Temporal muscle thickness (TMT) measured by ultrasonography has recently been proposed to correlate with nutritional status and changes in TMT to correlate with recent energy adequacy

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in elderly patients^{7,8}. Nutritional deterioration and sarcopenia, in particular, are common in both geriatrics and HD patients, while the underlying metabolic abnormalities are not identical; for example, muscle protein degradation and resting energy expenditure are increased only in HD patients, whereas insulin resistance and inflammation are increased and muscle protein synthesis is decreased in both patient groups⁹. We aimed to investigate the role of TMT in predicting malnutrition in prevalent HD patients.

METHODS

Study design and ethics committee approval: This is a single-center, prospective, observational study. Data were collected within 6 months between December 2018 and May 2019. Written informed consent was obtained from the patients, which included a detailed explanation of the objectives of the study and the methods to be used. An ethics committee approval was obtained from our hospital (decision number 159, dated November 14, 2018).

Inclusion and exclusion criteria: Patients over the age of 18 years who had been regularly receiving chronic HD treatment for at least 3 months due to end-stage renal failure in our hospital were included in this study. Those who were temporarily on dialysis due to acute kidney injury (AKI), those with a history of hospitalization and surgery within the past 3 months, and those with infectious disease, end-stage liver disease, malignancy, or malabsorption syndrome were excluded from the study.

Hemodialysis: High-flux HD was applied to the patients by using biocompatible HD membranes with a dialysate containing standard bicarbonate and 140 mEq/l sodium. While blood flow rates varied between 300 and 350 ml/min, the dialysate flow rate was kept constant at 500 ml/min. To calculate the given dialysis dose (urea reduction ratio [URR] and spKt/V), the post-dialysis plasma urea levels of the same dialysis session were measured by the Daugirdas method.

Data collection: The first evaluation, including demographic, anthropometric, and biochemical data, was performed for all patients in December 2018. Demographic data, laboratory parameters, and dialysis adequacy measurements (URR and spKt/V) were obtained retrospectively by examining the patient files. Anthropometric measurements were calculated by examining the patients 15–30 min after the end of the mid-week HD session. Triceps skinfold thickness (TST) was measured at the back of the arm, from the midpoint between the olecranon and the acromion, with the arms released from the side, by applying standard pressure by clamping the skin with the Saehan Skinfold Caliper. After fasting overnight, predialysis (before the mid-week session) venous blood samples were taken from all subjects in the morning. MIS is a reliable scoring

system that has been used to predict malnutrition-related events and/or serious clinical outcomes. Scoring is done by looking at nutritional history, physical examination, body mass index (BMI), and laboratory data. It consists of 4 parts and 10 questions. Each question is given a score between 0 (normal) and 3 (severe malnutrition). Scoring is done between 0 (normal) and 30 (severe malnutrition). High score reflects the severity of malnutrition and inflammation. It has been proven that high MIS scores are predictors of morbidity and mortality in these patients^{5,6}. A Toshiba (Toshiba Medical Systems Company, Ōtawara, Japan), Aplio 500 device (USA), and a 12-MHz linear probe were used for TMT measurement. The linear transducer was placed in the temporal fossa perpendicular to the muscle plane with gentle pressure, approximately 2 cm below the upper part of the lateral edge of the eyelid bilaterally. The thickness of the middle part of the temporal muscle was measured. During the collection of ultrasonographic images, three measurements were recorded for each muscle. The average of three measurements for the right and left sides was taken as the final data⁷.

Statistical analysis

The SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The suitability of the variables for normal distribution was examined using the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Since the data did not fit the normal distribution, descriptive statistics were presented as median (interquartile range [IQR]: 25–75%), frequency distribution, and percentage. The chi-square and Fisher's exact tests were used to evaluate categorical variables. The Mann-Whitney U test was used for comparing the two independent groups. The relationship between the variables was evaluated using the Spearman's correlation test. The diagnostic decision-making feature of TMT (median) in predicting mortality was evaluated by receiver operating characteristic (ROC) curve analysis. A multivariate Cox regression model was created for the analysis of independent variables associated with all-cause mortality. The Kaplan-Meier survival analysis was used for detecting the mean survival time of all patients and TMT (median) cutoff groups. The statistical significance level was accepted as $p < 0.05$.

RESULTS

The demographic data and laboratory analysis results of the 60 patients (male/female: 32/28) are shown in Table 1. Of the total, 16 (26.6%) patients were diabetic. The median (IQR) age and HD vintage of all patients were 66.5 (52.7–74) years and 36 (15.7–95.2) months, respectively. The vascular access types of the patients were as follows: 37 with an arteriovenous

(AV) fistula, 21 with a tunneled central venous catheter, and 2 with an AV graft. The median (IQR) MIS and TMT values of all patients were 8.5 (6–10.2) and 9.7 (8.7–10.9) mm, respectively. Notably, 25 (41.6%) patients died during the follow-up period (33.3±11.5 months). Causes of death were as follows: 14 (56%) infections (8 COVID-19, 5 sepsis, and 1 infective endocarditis), 5 (20%) ischemic heart diseases, 4 (16%) cerebrovascular accidents, and 2 (8%) complications of HD insufficiency such as hyperkalemia and hypervolemia.

When compared to survived patients, the nonsurvivor group was significantly older (60 vs. 73 years, $p=0.008$), had lower serum albumin (3.8 vs. 3.7 g/l, $p=0.002$), higher MIS (8 vs. 10, $p=0.001$), and lower TMT median values (10.6 vs. 8.8 mm, $p<0.001$). However, there were no significant differences between the two groups regarding gender, HD vintage and adequacy, frequency of patients with diabetes and/or catheters, anthropometric measurements, and laboratory parameters, except serum albumin (Table 1).

Table 1. Demographic and laboratory data of all patients and groups.

	All patients (n=60)	Survivors (n=35)	Nonsurvivors (n=25)	p-value
Demographics				
Age (years)	66.5 (52.7–74)	60 (44–70.5)	73 (56–77.5)	0.008
Gender (male/female)	32/28	20/15	12/13	0.484
HD vintage (months)	36 (15.7–95.2)	24 (14.5–89.5)	48 (20–100.5)	0.342
Diabetes mellitus, n (%)	16 (26.6)	7 (20)	9 (36)	0.169
HD adequacy and access				
spKt/V	1.4 (1.2–1.6)	1.40 (1.3–1.6)	1.30 (1.2–1.4)	0.052
Catheter, n (%)	21 (35)	9 (25.7)	12 (48)	0.074
Anthropometry				
Dry weight (kg)	71.2 (59.2–80.5)	77 (62–80.2)	66.5 (57.7–82.2)	0.475
Body mass index (kg/m ²)	25.9 (22.7–29.2)	26.3 (23.2–29.9)	25.4 (21–28.7)	0.419
Triceps skinfold thickness (mm)	16 (13–18)	16 (14–18)	14 (12–18)	0.135
Laboratory				
Predialysis urea (mg/dL)	128.5 (105.7–151)	131 (108.5–150.5)	122 (100.5–157)	0.795
Predialysis creatinine (mg/dL)	7.4 (5.6–8.9)	7.5 (6.3–9.1)	6.6 (4.7–8.7)	0.063
Hemoglobin (g/dL)	10.9 (9.8–11.9)	10.7 (9.8–12)	11.2 (9.9–11.8)	0.832
Uric acid (mmol/L)	6.2 (5.3–6.6)	6.3 (5.4–7)	5.6 (5.2–6.5)	0.203
Sodium (mmol/L)	138 (136–140)	138 (137–140)	137 (135–139.5)	0.423
Potassium (mEq/L)	4.8 (4.3–5.4)	4.8 (4.3–5.2)	5.1 (4.2–5.4)	0.519
Calcium (mg/dL)	9 (8.7–9.5)	9.1 (8.8–9.4)	9 (8.6–9.5)	0.741
Phosphorus (mg/dL)	5.1 (4–6.5)	5 (4–6.6)	5.2 (3.9–6.3)	0.820
Intact PTH (pg/mL)	468 (231–648)	478 (226–626)	460 (198–742)	0.894
Ferritin (ng/mL)	307 (164–493)	319 (200–486)	217 (143–530)	0.428
Total cholesterol (mmol/L)	162 (145–206)	164 (147–210)	162 (141–194)	0.300
LDL (mmol/L)	93 (81–119)	95 (83–132)	90 (68.5–107)	0.179
Triglyceride (mmol/L)	116 (87–177.7)	128 (87–179)	110 (86–173)	0.499
Hs-CRP (mg/dL)	0.5 (0.1–2)	0.5 (0.1–1.9)	1.3 (0.1–2.2)	0.504
Albumin (g/L)	3.8 (3.4–3.9)	3.8 (3.6–4)	3.7 (3.2–3.8)	0.002
MIS	8.5 (6–10.2)	8 (5.5–9)	10 (8–12)	0.001
TMT-median (mm)	9.7 (8.7–10.9)	10.6 (9.5–11.4)	8.8 (8.5–10)	<0.001

Data were presented as median and interquartile range (25–75%). Statistically significant ($p<0.05$) values were marked in bold.

HD: hemodialysis; spKt/V: single-pool Kt/V; PTH: parathormone; LDL: low-density lipoprotein; Hs-CRP: high-sensitivity C-reactive protein; MIS: malnutrition inflammation score; TMT: temporal muscle thickness.

In correlation analysis (Table 2), we found statistically significantly negative correlations between TMT (median) values and age ($r=-0.536, p<0.001$), HD vintage ($r=-0.328, p=0.012$), and MIS ($r=-0.330, p=0.010$). Significantly positive correlation was present between TMT (median) values and dry weight ($r=0.396, p=0.002$), BMI ($r=0.335, p=0.009$), TST ($r=0.314, p=0.014$), predialysis serum uric acid ($r=0.368, p=0.004$), and albumin ($r=0.286, p=0.027$).

The multivariate Cox regression model was created for the analysis of independent variables associated with all-cause mortality (Table 3). Low TMT (median) ($p=0.035$), low serum albumin ($p=0.010$), and high MIS ($p=0.037$) were found to be independent predictors of mortality.

In the ROC curve analysis (Figure 1), we found that the optimal cutoff value of TMT (median) for predicting death was 9.4 mm ($p<0.01$, AUC 0.782, 95%CI 0.663–0.901). The sensitivity and specificity of this cutoff value were 68% and 81.8%, respectively. Positive and negative predictive values were 0.48 and 0.91, respectively, positive and negative likelihood ratios were 3.74 and 0.39, respectively, while the diagnostic odds ratio was 8.70 (95%CI 2.13–35.21).

Table 2. Bivariate correlation analysis of temporal muscle thickness (median) with demographic, anthropometric, and laboratory variables.

TMT (median)	Correlation coefficient (r)	p-value
Age	-0.536	<0.001
HD vintage	-0.328	0.012
Dry weight	0.396	0.002
BMI	0.335	0.009
TST	0.314	0.014
Uric acid	0.368	0.004
Albumin	0.286	0.027
MIS	-0.330	0.010

TMT: temporal muscle thickness; HD: hemodialysis; BMI: body mass index; TST: triceps skinfold thickness; MIS: malnutrition inflammation score. Bold characters indicate statistically significant results.

Table 3. Multivariate cox regression model created for the analysis of independent variables associated with all-cause mortality.

Predictors of mortality	p-value	Hazard ratio	95%CI	
			Lower	Upper
Age (years)	0.641	1.009	0.972	1.047
TMT (median)	0.035	0.740	0.559	0.980
spKt/V	0.176	0.250	0.033	1.862
Albumin	0.010	0.214	0.066	0.694
MIS	0.037	1.180	1.010	1.380

TMT: temporal muscle thickness; spKt/V: single-pool Kt/V; MIS: malnutrition inflammation score. Bold characters indicate statistically significant results.

The overall mortality rate and mean survival time of all patients were 41.6% and 33.6±1.45 months, respectively. Out of 60 patients, 24 patients had TMT (median) under the cutoff value (≤ 9.4 mm), while 36 patients had TMT (median) above the cutoff value (>9.4 mm). The mortality rate was significantly higher in TMT (median) ≤ 9.4 mm group compared to the TMT (median) >9.4 mm group [70.8% (17/24) vs. 22.2% (8/36), respectively, p (log-rank) <0.001]. Likewise, as compared to TMT (median) >9.4 mm, the mean survival time of the patients with TMT (median) ≤ 9.4 mm was found significantly lower [37.4 ± 1.34 vs. 27.9 ± 2.65 months, respectively, p (log-rank) <0.001] during the follow-up period. Figure 2 illustrates the Kaplan-Meier patient survival curve according to the TMT (median) cutoff value.

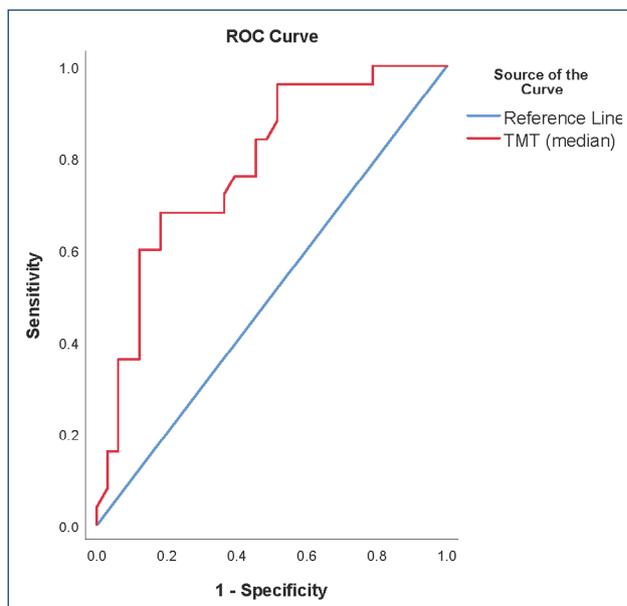


Figure 1. Receiver operating characteristic (ROC) analysis of the relationship temporal muscle thickness (median) and mortality.

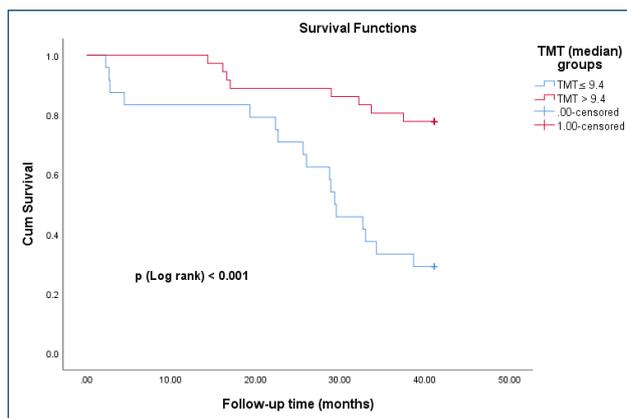


Figure 2. Kaplan-Meier patient survival curve according to temporal muscle thickness (median) groups.

DISCUSSIONS

Mortality rates are unacceptably high in patients with end-stage renal disease receiving chronic HD. The presence of PEW is a modifiable risk factor that is associated with mortality¹⁰. There is evidence in the recent literature that a decrease in temporal muscle mass may be a strong predictor of malnutrition and mortality in various conditions^{11,12}. To the best of our knowledge, for the first time in HD patients, we have shown that the TMT measured by ultrasonography could predict moderate-to-severe malnutrition and all-cause mortality.

The preference to study TMT as a surrogate measure of nutrition and muscle mass may be twofold logical: first, the temporal muscle is a striated muscle and has a strong correlation with calf circumference, and second, the temporal muscle is an important masticatory muscle that could reflect the exercise of chewing and hence dental health and nutritional intake^{13,14}. In addition, the measures of TMT have a low tendency to be affected by exercise or rest versus clench¹⁵.

Reports on the prevalence of PEW in dialysis patients vary largely, but in a recent meta-analysis that included 90 studies from 34 countries, the IQR for PEW prevalence was found to be 28–54%¹⁶. In this study, mild, moderate, and severe malnutrition was found in 31.7, 36.7, and 31.7% of the patients, respectively. Strict criteria, older age, high rates of catheter use, and single-center bias may account for the high prevalence of malnutrition. The overall mortality during a mean follow-up of 33 months was 46%, and the main causes of mortality were infections and cardiovascular diseases. These figures are comparable with the large analyses of HD patients¹⁷. An ROC analysis for TMT to predict mortality have shown an AUC of 0.782 with moderate sensitivity (68%) and specificity (81.8%), but notably these findings were comparable with those of MIS and all-cause mortality analysis. In contrast to MIS, however, TMT is relatively more practical to perform, which could improve the attendance of nutritional status surveillance and treatment of HD patients. Furthermore, TMT and MIS have independently predicted mortality as shown in the multivariate Cox regression analysis; hence, a combination of TMT and MIS may further improve the predictive power of the singular use of each test.

Another important finding from this study was that TMT was weakly correlated with more conventional nutritional indicators, notably serum albumin, MIS, and BMI. The interpretation of these data does not seem straightforward, but at least this is roughly similar to the results of a previous study of the elderly reported by Hasegawa et al.⁷ These data suggest that TMT has a discriminative validity. A large anthropometric study aimed at determining the normal ranges of TMT in

healthy individuals and a method of correction according to body surface area or height, etc. to generate more comparable data that could arguably provide more refined results.

There are several limitations of this study. The sample size is small, and the study was conducted at a single HD center. The ultrasonography of TMT was performed by one experienced radiologist who was unaware of the patients' clinical data; this fact bypasses the interobserver inconsistency, whereas intraobserver consistency was not tested. The normal ranges of TMT were not determined in healthy controls, a cutoff value was identified based on ROC analysis, and the follow-up was relatively short. The measurement of TMT was done at the beginning of the study and follow-up measurements were not done; hence, the data rely solely on the initial measurements. However, to prevent an observer-related systematic error, we preferred the arithmetic average of six measurements (three on the right side and three on the left side) for each patient. Notably, even though our study population was relatively small, the statistical analysis has shown a significant relationship between TMT and mortality, which is contrary to what we were more likely to find out (a wide range of CI for TMT in a small sample size should reduce the power of the study). We believe that such a finding suggests that the TMT may truly be a strong predictor of adverse outcomes and its variability across patients could be high. For both reasons, the characteristics and value of TMT in patient monitoring for nutritional status and clinical outcomes merit further studies. The utilization of widely recognized MIS and anthropometric parameters simultaneously in this study strengthens the validity of the results.

CONCLUSION

As a method for evaluating the nutritional status and outcomes of prevalent HD patients, TMT measurement by ultrasonography has shown promising results in predicting all-cause mortality. The correlation of TMT with other parameters, such as BMI, serum albumin, MIS, HD vintage, and TST, was rather weak, which raises the possibility that TMT could be a new independent marker of nutritional status. Further studies on larger HD patients with longer follow-up and the inclusion of healthy individuals are needed to validate the results of this study.

AUTHORS' CONTRIBUTIONS

EK: Conceptualization, data curation, and formal analysis. **TŞ:** Writing – original draft and writing – review & editing. **SD:** Data curation and writing – review & editing. **MB:** Conceptualization and data curation.

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Association between acanthosis nigricans and overweight with hypertension in children and adolescents from low-income families

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SUMMARY

OBJECTIVE: This study aimed to describe the prevalence of acanthosis nigricans and high blood pressure in children and adolescents from low-income families, and to verify the association of elevated blood pressure with nutritional status and the presence of acanthosis nigricans.

METHODS: This is a cross-sectional and controlled study with 232 children and adolescents from an institution for low-income families. Pubertal stage, body mass index Z-score, waist-to-height circumference ratio (increased waist-to-height circumference ratio >0.5), the presence of acanthosis nigricans, and blood pressure were assessed.

RESULTS: The prevalence of excess weight and the change in waist-to-height circumference ratio was 37.9%. Acanthosis nigricans and increased blood pressure occurred in 20.3 and 34.8%, respectively. The prevalence of acanthosis nigricans and hypertension was higher in individuals with excess weight ($p < 0.001$; $p < 0.001$) and with an increased waist-to-height circumference ratio ($p = 0.009$; $p < 0.001$). Logistic regression showed a significant and independent association of body mass index Z-score (OR 2.35; 95%CI 1.52–3.65; $p < 0.001$) and the presence of acanthosis nigricans (OR 2.43; 95%CI 1.12–5.23; $p = 0.023$) with elevated blood pressure.

CONCLUSION: Acanthosis nigricans and elevated blood pressure occurred in one-fifth and one-third of the individuals in an institution for children from low-income families. Overweight and the presence of acanthosis nigricans increased the risk of high blood pressure more than twofold.

KEYWORDS: Acanthosis nigricans. Arterial pressure. Pediatric obesity. Cardiometabolic risk factors.

INTRODUCTION

The prevalence of childhood obesity has increased tenfold in the last four decades worldwide¹. In Brazil, excess weight (overweight and obesity) was observed in 30% of children aged between 5 and 9 years and in 19.4% of adolescents².

Children and adolescents with obesity may present cardiometabolic risks, including arterial hypertension, which present insulin resistance as a central mechanism. Acanthosis nigricans (AN) occurs more frequently in conditions such as hyperinsulinism, insulin resistance, type 2 diabetes, and obesity at all ages, including pediatric age. The assessment of AN can be performed in a practical way and is part of the routine pediatric physical examination, assisting in the early diagnosis of these conditions³⁻⁵.

A meta-analysis including 47 cross-sectional studies with children and adolescents showed that overweight and obesity increased the risk of hypertension by 5 and 15 times, respectively⁶.

Studies evaluating the association between AN and arterial hypertension with excess weight in children and adolescents from low-income families in our country are rarely reported in the literature⁷.

The aims of this study were to describe the prevalence of AN and high blood pressure (BP) in children and adolescents from low-income families and to verify the association of elevated BP with nutritional status and the presence of AN.

METHODS

Since this is a cross-sectional study, 232 children and adolescents from an institution for children from low-income families in situations of social vulnerability in the city of Santo André, Brazil were included. The study was conducted from January to December 2019.

The exclusion criteria were as follows: patients with chronic diseases (except overweight, asthma, and rhinitis),

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such as genetic, cardiovascular, endocrine, and renal diseases, acute infections, and those using hormonal and non-hormonal anti-inflammatory drugs in 3 months prior to the clinical evaluation.

The anthropometric assessment was performed according to protocols standardized by the World Health Organization (WHO) and the Ministry of Health of Brazil^{8,9}. Weight and height measurements were expressed as body mass index Z-score (zBMI), calculated using the WHO Anthro and WHO Anthro Plus 3.2.2 software. For the anthropometric classification, the cutoff points recommended by the WHO were adopted¹⁰. The children's waist measurement was also obtained using an inextensible measuring tape, positioned at the midpoint between the superior border of the iliac crest and the last fixed rib cage, which was used to calculate the waist-to-height circumference ratio (WHtR). WHtR was classified as altered when the value was equal to or greater than 0.5¹¹.

The pubertal stage was defined by a single professional (pediatrician) and classified according to what was proposed by Marshall and Tanner^{12,13}.

BP was measured in the right arm relaxed, at heart height, with the palm facing up and supported on a rigid surface, with the individual seated and at rest. A sphygmomanometer was used with a cuff suitable for arm circumference. Three measurements were obtained with a minimum interval of 2 min between them, and the mean of the values was calculated. BP was classified according to the criteria of the Manual de orientação de hipertensão arterial na infância e adolescência da Sociedade Brasileira de Pediatria – 2019¹⁴: altered blood pressure levels (elevated BP): ≥ 90 th percentile (P90) for age, sex, and height (BP $\geq P90$ and $< P95$: elevated BP; PB $\geq P95$ and $< P95+12$ mmHg: hypertension stage 1; BP $\geq P95+12$ mmHg: hypertension stage 2).

The skin of the cervical and bilateral axillary regions was evaluated in search of AN, the regions where acanthosis occurs most frequently¹⁵. Each child was evaluated simultaneously by two pediatricians.

The data were entered, revised, and consolidated in an Excel spreadsheet (Office®). For statistical analysis, Epi Info™, version 7.2.2.6 was used. Qualitative variables were presented as absolute numbers and percentages and compared through the χ^2 test: logistic regression (dependent variable: elevated BP ($\geq P90$); independent variables: zBMI, WHtR) and the presence of acanthosis. The significance level adopted was 5%.

This study was approved by the Research Ethics Committee of the Centro Universitário FMABC, under opinion number: 3.058.583.

RESULTS

The study included 232 children and adolescents aged 4–14 years (mean 8.4 ± 3.2 years). The general characteristics of the study population are presented in Table 1. Excess weight (overweight and obesity) and a change in WHtR occurred in 37.9% of the participants. Cervical and/or axillary AN and elevated blood pressure ($\geq P90$) were observed in 20.3% and 34.8% of participants, respectively.

Table 2 shows the general characteristics stratified according to the presence of AN and BP. There was no difference between the overweight and non-overweight groups regarding sex, age, or pubertal stage. Individuals with AN had a higher prevalence of overweight ($p < 0.001$) and altered WHtR ($p = 0.009$).

There was no difference between the groups of individuals with normal BP in relation to sex, age, and pubertal stage. The group of children with altered BP presented a higher prevalence of overweight ($p < 0.001$), change in WHtR ($p < 0.001$), and AN ($p < 0.001$) (Table 2).

Logistic regression showed a significant and independent association of zBMI (OR 2.35; 95%CI 1.52–3.65; $p < 0.001$) and the presence of AN (OR 2.43; 95%CI 1.12–5.23; $p = 0.023$) with elevated BP. There was no association between WHtR and the risk of elevated BP ($p = 0.567$) (Table 3).

Table 1. General characteristics of the study population (n=232).

		n	%
Sex	Male	124	53.5
	Female	108	46.5
Age	<10 years	151	65.1
	≥ 10 years	81	34.9
Puberty	Prepubescent	164	70.7
	Pubescent	68	29.3
zBMI	Normal weight	144	62.1
	Overweight	49	21.1
	Obesity	28	12.1
	Severe obesity	11	4.7
WHtR	<0.5	144	62.1
	≥ 0.5	88	37.9
Acanthosis nigricans	Absent	185	79.7
	Present	47	20.3
Blood pressure	Normal	151	65.1
	Elevated	37	15.9
	Hypertension stage 1	37	15.9
	Hypertension stage 2	7	3

zBMI: body mass index Z-score; WHtR: waist-to-height circumference ratio.

Table 2. Comparison of clinical variables in relation to acanthosis nigricans (absent or present) and blood pressure (normal or increased).

		Absent AN (n=185)	Present AN (n=47)	p-value	Normal BP (n=151)	Elevated BP (n=81)	p-value
Sex	Male	101	23	0.595	79	45	0.738
	Female	84	24		72	36	
Age	<10 years	118	33	0.512	103	48	0.222
	≥10 years	67	14		48	33	
Puberty	Prepubescent	131	33	1	110	54	0.403
	Pubescent	54	14		41	27	
zBMI	Normal weight	131	13	<0.001*	116	28	<0.001*
	Overweight	54	34		35	53	
WHtR	<0.5	123	21	0.009*	107	37	<0.001*
	≥0.5	62	26		44	44	
AN	Absent				134	51	<0.001*
	Present				17	30	

AN: acanthosis nigricans; BP: blood pressure; zBMI: body mass index Z-score; WHtR: waist-to-height circumference ratio. Elevated BP group: children with BP ≥90th percentile for sex, age, and height. *Significance level of χ^2 test.

Table 3. Logistic regression of variables associated with elevated blood pressure (n=232).

	OR	95%CI		p-value
zBMI	2.35	1.52	3.65	<0.001*
WHtR	1.23	0.60	2.49	0.567
Presence of AN	2.43	1.12	5.23	0.023*

zBMI: body mass index Z-score; WHtR: waist-to-height circumference ratio; AN: acanthosis nigricans. Dependent variable elevated BP group: children with BP ≥90th percentile for sex, age, and height. Independent variables: zBMI classification, classification of WHtR, and classification of the presence of AN. *Significance level of χ^2 test.

DISCUSSION

Overweight and the presence of AN increased the risk of elevated BP in the study population by 2.3 and 2.4 times, respectively. The association between these risk factors (overweight, presence of AN, and elevated BP) increases the risk of future cardiovascular events¹⁶. More than one-third of the participants had overweight and/or altered WHtR. This study was conducted in an institution for children in situations of social vulnerability from low-income families. Food disorders may justify the higher prevalence of overweight and obesity in this population. Childhood obesity is increased by low socioeconomic status and place of residence^{17,18}. A cross-sectional study conducted on 175 children and adolescents aged 6–17 years, from a low-income community in the city of Santa Rita do Sapucaí, Brazil, observed 37.2% of overweight, the levels similar to that observed in this study⁷.

The prevalence of AN in this study was one in five children. Sex, age, and pubertal stage did not influence the risk of AN. Children with AN had higher zBMI and WHtR levels. A study conducted in Turkey evaluated 160 overweight children and adolescents (10.4±3.3 years old), and also found no significant difference in the presence of AN in relation to these variables. Apparently, the only predictive factors for AN are hyperinsulinism and the severity of obesity¹⁹. A systematic review showed that AN is associated with insulin resistance and risk for type 2 diabetes mellitus²⁰. A study on 8,371 children from territories belonging to the United States in the Pacific demonstrated that intervention measures addressing eating habits, lifestyle, and sleep and screen time control significantly reduced excess weight, waist circumference, and the presence of AN when compared to the control group (overweight [effect size] $d = -3.95\%$; 95%CI -7.47 to -0.43); waist circumference ($d = -0.71\%$; 95%CI -1.37 to -0.05), and AN prevalence ($d = -2.28\%$; 95%CI -2.77 to -1.57)²¹.

One-third of the participants had increased blood pressure (≥P90). Sex, age, and pubertal stage did not influence the risk of altered BP. Children with elevated BP had a higher prevalence of increased zBMI, WHtR, and the presence of AN. A meta-analysis of 41 studies on hypertension in Africa, involving 52,918 participants aged 3–19 years from 10 countries, described a total prevalence of 18.8% of elevated BP and hypertension, with a significant predominance in overweight children ($p < 0.001$)²². Another meta-analysis of 64 studies conducted in India, with 187,990 participants aged 4–19 years, described a similar prevalence of 17% of elevated BP and hypertension, also with significant predominance in overweight children ($p < 0.001$)²³. Song et al.

conducted a meta-analysis on the worldwide prevalence of childhood hypertension with 186,630 participants under 19 years of age and described that overweight (OR 4.99; 95%CI 2.18–8.81, $p < 0.001$) and obesity (OR 15.27; 95%CI 7.31–25.38, $p < 0.001$) significantly increase the risk of elevated BP⁶. A systematic review and meta-analysis study with 18,925 children showed that an intervention combining diet and physical activity significantly reduced children's blood pressure [effect size $d = -1.64$ mmHg (95%CI -2.56 to -0.71; $p = 0.001$) for systolic BP and $d = -1.44$ mmHg (95%CI -2.28 to -0.60; $p = 0.001$) for diastolic BP]²⁴.

The high prevalence of elevated blood pressure in this study can be attributed to low socioeconomic status. Martinovic et al. evaluated 434 children aged 6–13 years from two schools in Montenegro and found that the worst socioeconomic level is a risk factor for increased blood pressure ($p = 0.021$)²⁵. In addition, although BP was verified three times in each child, measurements were obtained on the same day. The measurements were performed at the institution, with the presence of teachers and caregivers, but without the family members. Some children may have felt uncomfortable with transient elevated BP, not allowing us to affirm that this alteration represents a definitive diagnosis.

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CONCLUSIONS

This study demonstrated that AN and BP increased in one-fifth and one-third of the individuals in an institution for children in situations of social vulnerability with low family income. Overweight and the presence of AN increased the risk of high blood pressure more than twofold.

BP measurement and skin assessment for AN add less time to a pediatric consultation and may help in the early diagnosis of comorbidities associated with childhood obesity, anticipating measures to control overweight and reduce future cardiometabolic risks.

AUTHOR CONTRIBUTION

CAV: Data curation, Formal analysis, Writing – original draft. **JCPF:** Data curation, Methodology, Investigation, Project administration, Writing – original draft. **LSS:** Formal analysis, Methodology. **FISS:** Conceptualization, Methodology, Validation, Writing – review & editing. **ROSS:** Conceptualization, Methodology, Visualization, Writing – review & editing.

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Causes of acute abdomen, preferred imaging methods, and prognoses in geriatric patients presenting to the emergency department with abdominal pain

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SUMMARY

OBJECTIVE: Abdominal pain is one of the most common reasons for admission to the emergency department in the geriatric population. The aim of this study was to investigate the diseases frequently detected in elderly patients diagnosed with acute abdomen in the emergency department, the imaging methods used in the diagnostic processes of these diseases, and the prognosis of the patients.

METHODS: In all, 175 patients who visited the emergency department due to abdominal pain and were hospitalized with a diagnosis of acute abdomen were evaluated. The patients were categorized into seven groups according to their diagnosis as biliary diseases, pancreatitis, appendicitis, gastrointestinal system perforation, ileus, mesenteric ischemia, and atypical causes.

RESULTS: The mean age of the patients was 76.3 ± 7.7 years (range 65–93), and 96 (54.9%) were women. The most common causes of acute abdomen were biliary diseases and pancreatitis. Ultrasonography (88.6%) was the most frequently preferred imaging method in the emergency department, and it was most frequently used for biliary diseases. Notably, 20 (11.4%) patients were treated in the intensive care unit, and 9 (5.1%) patients died.

CONCLUSION: The most common causes of acute abdomen in the geriatric population were biliary diseases and pancreatitis, and ultrasonography imaging was the most common choice for the diagnosis of these diseases. In elderly patients with abdominal pain, rapid and accurate diagnosis and selection of the correct imaging method are extremely important.

KEYWORDS: Acute abdomen. Aged. Radiological imaging.

INTRODUCTION

Acute abdominal pain is one of the most common reasons for admission to the emergency department in the geriatric population¹. With the increase in the elderly population, elderly patients constitute an increasing proportion of patients presenting to the emergency department due to acute abdominal pain². However, this situation causes additional difficulties for emergency physicians. In an elderly patient with abdominal pain, the clinical manifestations may be very different and nonspecific³.

However, the diagnosis may be difficult or delayed in elderly patients due to the different manifestations of the disease, cognitive problems, and communication difficulties, which may increase overall mortality. Therefore, the early and accurate diagnosis of acute abdomen in elderly patients is critical and significantly affects the outcomes of these patients.

The aim of this study was to investigate the diseases frequently detected in elderly patients diagnosed with acute abdomen in

the emergency department, the imaging methods used for the diagnosis, and the prognosis of the patients.

METHODS

This retrospective study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Malatya Turgut Özal University Clinic Ethics Committee (2021, decision no. 83).

All patients aged 65 years and older who were diagnosed with acute abdomen and hospitalized in the Malatya Training and Research Hospital emergency department between June 1, 2021, and January 31, 2022, were included in the study. The number of patients diagnosed with acute abdomen was 175. No patient was excluded from the study for any reason. The images and reports of radiological examinations, reasons for hospitalization, treatments applied, length of hospital stay, and patient outcomes hospitalized in the emergency room were retrieved from the

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hospital data processing system. Ultrasonography (USG) and computed tomography (CT) reports of the patients that were previously recorded in the hospital data processing system were used in the evaluation. Direct abdominal radiographs (DAR) were retrieved from the hospital imaging archive system and evaluated retrospectively by a radiologist. The patients were categorized into seven groups according to their diagnosis as biliary causes (acute cholecystitis, cholangitis, etc.), pancreatitis, appendicitis, gastrointestinal system (GIS) perforation, ileus, mesenteric ischemia, and atypical causes (pyelonephritis, malignant causes, etc.).

Statistical Analysis

IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Descriptive statistics were expressed as mean±standard deviation and minimum-maximum values for continuous variables depending on their distribution. The chi-square test was performed to evaluate the relationship between the diagnosis groups, demographic data, and radiological examinations. The significance level (p-value) was set at 0.05.

RESULTS

A total of 175 patients aged ≥65 years who were diagnosed with acute abdomen were included in the study. The mean age of the patients was 76.3±7.7 years (range 65–93 years), and 96 (54.9%) were women.

Biliary diseases were the most common cause of acute abdomen (65 patients, 37.1%). Other causes were pancreatitis in 50 (28.6%) patients, atypical causes in 24 (13.7%) patients, and appendicitis in 12 (6.9%) patients. In all, 129 (73.7%) patients had at least one chronic disease. The most common chronic disease detected in the patients was hypertension, followed by diabetes mellitus. The hospitalization period of the patients ranged between 1 and 34 days, and the average hospitalization period was 5.6±4.7 days. Notably, 20 (11.4%) patients were treated in the intensive care unit, and 9 (5.1%) patients died. GIS perforation, in which 4 out of 8 patients died, had the highest mortality rate (50%). The demographic characteristics and diagnosis groups of the patients are shown in Table 1.

As radiological imaging in the emergency department, USG was performed on 155 (88.6%) patients, DAR on 90 (51.4%) patients, and CT on 69 (39.4%) patients. Magnetic resonance imaging (MRI) was not performed on any patient in the emergency department. However, magnetic resonance cholangiopancreatography (MRCP) was performed on seven patients during their hospitalization.

The most common examination was USG, which was performed on 155 (88.6%) patients. DAR was the second most common examination with 51.4%, and CT (39.4%) was the least common examination. When examination modalities were investigated according to the diagnosis groups, DAR was the most frequently used modality in patients with ileus and the least frequently used modality in those with biliary diseases. USG was most frequently performed in biliary pathologies and pancreatitis and least in ileus. CT was performed most frequently in patients with appendicitis and GIS perforation and least frequently in those with biliary diseases and pancreatitis (Figure 1). A significant difference was found in the presence or absence of DAR, USG, and CT examination according to the diagnosis group (p=0.002, p<0.001, and p<0.001, respectively). The percentage distribution and p-values of the examinations are presented in Table 2.

DISCUSSION

Currently, imaging modalities have become an important diagnostic tool for acute abdomen in the elderly, and CT is the most commonly used method⁴. CT may be considered the primary technique for the diagnosis of acute abdominal pain, except in patients with clinically suspected acute cholecystitis⁵. Intravenous contrast-enhanced CT provides superior anatomical detail and diagnostic

Table 1. Percent distribution of patients' demographic characteristics, prognostic status, and diagnosis groups.

	n (%)
Female	96 (54.9)
Male	79 (45.1)
No chronic diseases	46 (26.3)
One chronic disease	72 (41.1)
More than one chronic disease	57 (32.6)
Hospital ward	155 (88.6)
Intensive care unit	20 (11.4)
Discharged	166 (94.9)
Exitus	9 (5.1)
Biliary disease	65 (37.1)
Pancreatitis	50 (28.6)
Appendicitis	12 (6.9)
Ileus	14 (8.0)
GIS perforation	8 (4.6)
Mesenteric ischemia	2 (1.1)
Atypical causes	24 (13.7)

n: number of patients; GIS: gastrointestinal system.

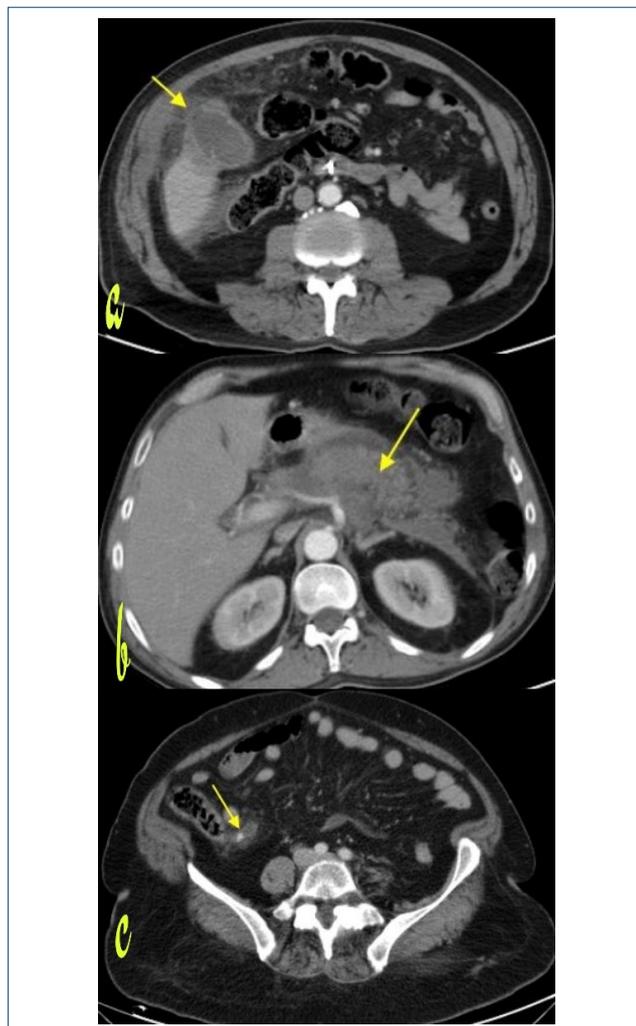


Figure 1. Computed tomography images of the patients with gallbladder perforation showing irregularity in the wall of the sac and adjacent fat stranding (a); with pancreatitis showing an increase in thickness and adjacent fat stranding (b); and with appendicitis showing an increase in the diameter, edema in the wall, intraluminal appendicoliths, and adjacent fat stranding (c).

specificity by imaging the intestinal wall, detecting primary and secondary pathologies in the surrounding mesentery, and even showing small amounts of intestinal ischemia and extraluminal air in the peritoneal cavity⁶. CT not only supports the diagnosis but also provides information about the treatment method to be chosen⁷. In a prospective study by Esses et al.⁸, the proportion of cases in which physicians reported a high degree of certainty in the suspected diagnosis increased from 36% before CT to 77% after CT. In the study of Rosen et al.⁹, with patients admitted to the emergency department with nontraumatic abdominal pain, pre- and post-CT diagnoses were found to be compatible only in 37% of the patients. In addition, CT eliminated the need for hospitalization in 17% of patients and the need for emergency surgery in 75% of patients. In our study, CT examination was performed on 69 (39.4) patients. CT examination was performed on patients with the most common diagnoses of GIS perforation (87.5%), ileus (85.7%), and appendicitis (75.0%). All of these patients were definitively diagnosed with CT. Abdominal USG is the first method of choice, especially when evaluating biliary diseases, due to its accessibility and rapid results, low cost, and lack of ionizing radiation³. It has been reported that ultrasound has a sensitivity and specificity of over 70% in the diagnosis of appendicitis¹⁰. In addition, abdominal USG is an excellent rapid screening method for aortic aneurysm, liver tumor or abscess, kidney stones, and hydronephrosis. USG was performed in 88.6% of the patients in the present study. Although USG was preferred most frequently in patients with biliary pathology and pancreatitis, it was least commonly used for ileus. USG was sufficient for diagnosis in patients with biliary pathology.

Radiographic examination, including upright and supine abdominal and upright chest x-rays, is useful in detecting intestinal obstruction, adynamic ileus, kidney stones, and perforation of hollow organs. It is an easily accessible radiological

Table 2. Presence of DAR, USG, and CT examination according to the diagnosis group.

	DAR+ n (%)	USG+ n (%)	CT+ n (%)
Biliary (n=65)	25 (38.5)	65 (100)	14 (21.5)
Pancreatitis (n=50)	25 (50.0)	49 (98.0)	15 (30.0)
Appendicitis (n=12)	9 (75.0)	11 (91.7)	9 (75.0)
Ileus (n=14)	14 (100)	4 (28.6)	12 (85.7)
GIS perforation (n=8)	4 (50.0)	5 (62.5)	7 (87.5)
Mesenteric ischemia (n=2)	1 (50.0)	1 (50.0)	1 (50.0)
Atypical (n=24)	12 (50.0)	20 (83.3)	11 (45.8)
Total (n=175)	90 (51.4)	155 (88.6)	69 (39.4)
p-value	0.002	<0.001	<0.001

n: number of patients; GIS: gastrointestinal system; DAR: direct abdominal radiograph; USG: ultrasonography; CT: computed tomography. + indicates the presence of imaging method.

examination in the evaluation of patients with acute abdomen, but it has little place¹¹.

Although MRI is used less frequently in the initial diagnosis⁵, today there are studies on its importance in the diagnosis of acute abdomen^{12,13}. In this study, no patient underwent an MRI examination in the emergency department. MRCP was performed on 7 (4%) patients during the hospitalization period. The MRCP examinations of these patients did not contribute to the diagnosis.

In this study, the highest mortality rate was recorded in patients with GIS perforation. GIS perforation is an emergency and life-threatening condition that requires immediate intervention. Additional treatment strategies may be needed depending on the underlying causes¹⁴. On x-ray, a translucent crescent-shaped air under the diaphragm is the finding to be examined⁵. Currently, CT is the imaging method of choice in cases of suspected perforation. Extraluminal air that is too small to be detected with conventional radiography can be demonstrated with CT. CT also shows signs such as phlegmon, abscess, and the presence of fluid¹⁵. In addition, the location of the perforation can be determined using CT¹⁶. CT was performed in six and DAR in five of eight patients with GIS perforation in

the present study. X-ray detected subdiaphragmatic free air in two patients. Perforation and accompanying findings were reported in all CT examinations, but the location of the perforation was not specified.

CONCLUSION

In this study, the most common causes of acute abdomen in the elderly were biliary diseases and pancreatitis. USG imaging was the most common choice in patients diagnosed with biliary diseases and pancreatitis, and CT was the most common choice in the diagnosis of appendicitis, ileus, and perforation. Considering that the diagnostic accuracy is lower and the mortality is higher in elderly patients with acute abdomen compared to younger patients, rapid and accurate diagnosis and choosing the correct imaging method are extremely important.

AUTHORS' CONTRIBUTIONS

MI, MA, ME: Conceptualization, data curation, formal analysis, writing – original draft, writing – review & editing.

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Potentially inappropriate medications based on TIME criteria and risk of in-hospital mortality in COVID-19 patients

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SUMMARY

OBJECTIVE: This study aimed to evaluate the relationship between hospital admission potentially inappropriate medications use (PIM) and in-hospital mortality of COVID-19, considering other possible factors related to mortality.

METHODS: The Turkish inappropriate medication use in the elderly (TIME) criteria were used to define PIM. The primary outcome of this study was in-hospital mortality.

RESULTS: We included 201 older adults (mean age 73.1±9.4, 48.9% females). The in-hospital mortality rate and prevalence of PIM were 18.9% (n=38) and 96% (n=193), respectively. The most common PIM according to TIME to START was insufficient vitamin D and/or calcium intake per day. Proton-pump inhibitor use for multiple drug indications was the most prevalent PIM based on TIME to STOP findings. Mortality was related to PIM in univariate analysis (p=0.005) but not in multivariate analysis (p=0.599). Older age (hazards ratio (HR): 1.08; 95% confidence interval (CI): 1.02–1.13; p=0.005) and higher Nutritional Risk Screening 2002 (NRS-2002) scores were correlated with in-hospital mortality (HR: 1.29; 95%CI 1.00–1.65; p=0.042).

CONCLUSION: Mortality was not associated with PIM. Older age and malnutrition were related to in-hospital mortality in COVID-19.

KEYWORDS: COVID. Older adult. Potentially inappropriate medication use. Criteria.

INTRODUCTION

Coronavirus disease-2019 (COVID-19) started in China in December 2019 and it has caused mortality in approximately 6 million people and infected about 448 million people worldwide, as accessed at the time of writing this manuscript¹. The predictors of poor outcomes in COVID-19 have been reported as male sex, older age, immunodeficiency, and having comorbidities (coronary artery disease, congestive heart failure [CHF], chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus, hypertension, and/or obesity)²⁻⁴.

Aging poses comorbidities and, accordingly, it is correlated with multiple drug use (polypharmacy). Potentially inappropriate medication (PIM), closely linked to polypharmacy, contributes to many problems such as falls, syncope, malnutrition, frailty, delirium, and also cost burden⁵. PIM is responsible for one-fifth of the mortality in the elderly; additionally, it is probably responsible for more deaths if unrecognized drug adverse effects are taken into account⁶. Globally, approximately 40% of outpatients over the age of 65 years have PIM at least once⁵.

PIM is defined as having a safer alternative drug or drug dose, using drugs without an indication or any benefit, or not using the appropriate drug despite an indication^{5,7}. There are many different screening tools for detecting PIM (e.g., the Beers criteria⁸, the Screening Tool of Older Persons' potentially inappropriate Prescriptions/Screening Tool to Alert to Right Treatment (STOPP/START) criteria⁹, and country-specific criteria such as those seen in Austria¹⁰, China¹¹, and the Turkish inappropriate medication use in the elderly (TIME) criteria¹²). The TIME criteria were published in 2019 and composed of 112 TIME to STOP criteria and 41 TIME to START criteria, with a total of 153 criteria¹². Recently, the TIME criteria have also been internationally validated for use in European countries⁷.

Previous research has shown that PIM is related to mortality. However, there is little known about PIM and COVID-19 mortality in hospitalized patients^{13,14}. Mortality may be associated with PIM in elderly individuals; this situation is often ignored and not studied by physicians other than geriatricians. This study aimed to provide for this deficiency. To the best of our knowledge, no studies have been published on PIM and

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in-hospital mortality related to COVID-19. Therefore, we aimed to investigate the relationship between PIM and in-hospital mortality due to COVID-19 and other factors that predict in-hospital mortality.

METHODS

A single-center cross-sectional study was designed at the Marmara University Medical School Hospital, which is a referral hospital for patients with COVID-19, comprising patients admitted between February and June 2021. This research was conducted in accordance with the Helsinki World Medical Association Declaration. Written informed consent was obtained from patients or proxies. Those who did not give consent were excluded. The study was approved by the Local Ethics Committee of Marmara University (Marmara University Clinical Research Ethics Committee/Decision no: 09.2021/68).

All older adults aged ≥ 60 years who had a positive real-time reverse transcriptase-polymerase chain reaction (RT-PCR) of COVID-19 and/or positive radiologic involvement of COVID-19 were included in the study. The primary outcome of this study was in-hospital mortality.

Age, sex, weight (kg), height (cm), body mass index (BMI), smoking habits, comorbidities, the number of drugs, specific drugs or drug contents, admission to the intensive care unit (ICU), ICU stay time (days), and presence of in-hospital mortality were collected. The length of hospital stay or time until in-hospital mortality was used as the follow-up time. Medication use on admission was recorded from the electronic records of the Turkish Ministry of Health. In this study, polypharmacy is defined as the regular use of five or more drugs⁵. The TIME criteria were used to define PIM¹². On admission, an experienced geriatrician checked the patients' drugs, determined PIM, and analyzed overprescribed and underprescribed drugs. The nutritional status of the participants was determined using the Nutritional Risk Screening 2002 (NRS-2002) screening tool¹⁵. Patients with ≥ 3 points were defined as at nutritional risk and those with < 3 points were assessed as well-nourished.

The SARS-CoV-2 infection was detected using RT-PCR assay of samples collected with nasopharyngeal swabs. We included participants with probable and confirmed COVID-19¹⁶. Confirmed disease was described as a positive result of the COVID-19 RT-PCR. The severity of infection was categorized as mild, moderate, severe, and critical¹⁷.

At the time of hospital admission, laboratory parameters were measured and assessed. Thorax CT was performed on participants who had polypnea (30 cycles per minute with

90% of blood oxygen saturation on room air) and/or hypoxia (oxygen saturation level ≤ 92). A specialist radiologist evaluated all the CT imaging. All patients were treated with favipiravir (first day: 1600 mg twice daily, 600 mg twice daily for 4 days), prophylactic enoxaparin (1 mg/kg), and proton-pump inhibitors (PPIs). If the patients had hypoxia (oxygen saturation level $\leq 92\%$), dexamethasone and oxygen-supportive treatment were started.

Statistical analysis

We determined the normality of the variables using visual (histograms and probability plots) and the Kolmogorov-Smirnov test. Categorical variables are shown as numbers and percentages (n, %). These analyses were compared using the chi-square test or Fisher's exact test, if appropriate. Normally distributed continuous variables are reported as a mean and standard deviation; group comparisons were performed using the independent sample t-test. When the distribution of continuous variables was normal, the data were expressed as median (minimum-maximum) and compared using the Mann-Whitney U test. The relationships between the variables and mortality were investigated using the Cox regression analysis. Multicollinearity was checked among independent variables. Results are shown as 95% confidence intervals (CI) and hazard ratios (HR). Statistical analyses were performed using the SPSS software package version 22.0 (IBM, Armonk, NY). p-values < 0.05 were considered significant.

RESULTS

A total of 201 hospitalized participants (73.1 ± 9.4 , 48.3 female) were involved in the study. The medians and ranges for the numbers of drugs and numbers of PIM were 4.0 (1–11) and 2.0 (1–6), respectively. The in-hospital mortality rate was 18.9% (n=38). Table 1 presents the baseline characteristics and laboratory parameters of the 201 participants.

The prevalence of PIM, as determined using the TIME criteria, was 96% (n=193). Of note, 84% of PIM was categorized as TIME to START, and 29.4% was categorized as TIME to STOP. Table 2 shows the top five ranked PIMs.

Nonsurvivors were older (median age 80.5 vs. 70.0 years, $p < 0.001$) and had more PIMs ($p = 0.005$) compared with survivors of COVID-19. In addition, mortality was associated with the presence of CHF ($p < 0.001$), dementia ($p = 0.040$), admission to the ICU ($p < 0.001$), long hospital stay ($p = 0.026$), and the presence of malnutrition ($p < 0.001$) (Table 1).

In multivariate Cox regression analysis, we investigated variables that were associated with mortality in univariate

Table 1. Characteristics and laboratory parameters of participants (n=201) and univariate analysis of survivors and nonsurvivors.

	All participants (total n=201) n (%)	Survivors (total n=163) n (%)	Nonsurvivors (total n=38) n (%)	p-value
Sex				
Female	97 (48.3%)	81 (49.7)	16 (42.1)	0.399
Male	104 (51.7%)	82 (50.3)	22 (57.9)	
Age*	73.0 (60–96)	70.0 (60–95)	80.5 (61–96)	<0.001†
BMI*	27.5 (16.3–44.1)	27.8 (16.3–44.8)	26.9 (18.4–40.0)	0.109
Smoking	62 (30.8%)	53 (32.5)	9 (23.7)	0.279
HT	131 (65.2%)	107 (65.6)	24 (63.2)	0.772
DM	87 (43.3%)	71 (43.6)	16 (42.19)	0.871
CAD	54 (26.9%)	44 (27)	10 (26.3)	0.932
COPD	36 (17.9%)	28 (17.2)	8 (21.1)	0.581
Malignancy	25 (12.4%)	23 (14.1)	2 (5.3)	0.105
CKD	24 (11.9%)	17 (10.4)	7 (18.4)	0.193
Dementia	22 (10.9%)	14 (8.6)	8 (21.1)	0.040†
CHF	19 (9.5%)	8 (4.9)	11 (28.9)	<0.001†
COVID severity				
Mild	9 (4.5%)	9 (5.5)	0 (0.0)	0.077
Moderate	48 (23.9%)	39 (23.9)	9 (23.7)	
Severe	135 (67.2%)	110 (67.5)	25 (65.8)	
Critical	9 (4.5%)	5 (3.1)	4 (10.5)	
COVID severity				
Mild+moderate	57 (28.4)	48 (29.4)	9 (23.7)	0.478
Severe+critical	144 (71.6)	115 (70.6)	29 (76.3)	
Number of chronic diseases*	3.0±1.5 (1–7)	2.9±1.5 (1–7)	3.4±1.7 (1–7)	0.174
Number of drugs*	4.0 (1–11)	4.0 (1–11)	4.0 (1–10)	0.663
Polypharmacy	76 (37.8%)	63 (38.7)	15 (39.5)	0.814
Number of PIM*†	2.0 (1–6)	2.0 (1–6)	2.5 (1–6)	0.005†
PIM†	193 (96)	155 (95.1)	38 (100)	0.064
TIME to START	180 (89.6)	142 (91.6)	38 (100)	0.015†
TIME to STOP	59 (29.4)	47 (30.5)	12 (31.6)	0.549
Length of hospital stay (days)*	14.0 (3–68)	13.0 (3–68)	22.0 (4–67)	0.026†
ICU stay	47 (23.4%)	19 (11.7)	28 (73.7)	<0.001†
ICU stay (days)*	5.0 (1–28)	5.0 (2–27)	6.0 (1–28)	0.924
Score of NRS-2002*	3.4±1.5 (0–7)	3.2±1.5 (0–7)	4.3±1.2 (2–7)	<0.001†
NRS-2002				
Nonmalnutrition	62 (30.8%)	60 (36.8)	2 (5.3)	<0.001†
Malnutrition risk	139 (69.2%)	103 (63.2)	36 (94.7)	
White blood cell ($\times 10^3/\mu\text{L}$)*	7.2 (1.4–23.7)	7.1 (1.4–23.7)	7.3 (1.6–17.8)	0.856
Lymphocyte ($\times 10^3/\mu\text{L}$)*	1.1 (0.1–11.0)	1.1 (0.1–3.9)	1.0 (0.2–11.0)	0.445
Neutrophil ($\times 10^3/\mu\text{L}$)*	5.5 (0.7–23)	5.5 (0.7–23.0)	5.6 (0.2–13.2)	0.924
Hemoglobin (g/dL)	12.5 (4.1–16.9)	12.5 (4.1–16.9)	12.1 (8–16.6)	0.600
Thrombocyte ($\times 10^3/\mu\text{L}$)*	201 (27–588)	204.0 (35–538)	162.0 (27–414)	0.007

Continue...

Table 1. Continuation.

	All participants (total n=201) n (%)	Survivors (total n=163) n (%)	Nonsurvivors (total n=38) n (%)	p-value
LDH (U/L)*	383.0 (105–1329)	474.0 (149–1192)	425.0 (105–1329)	0.315
Glucose (mg/dL)*	127.0 (59–538)	128.0 (59–538)	108.5 (76–303)	0.042†
GFR (mL/m)*	72.1 (4.2–159.6)	76.8 (4.2–159.6)	48.8 (11.7–112)	<0.001†
C-reactive protein (mg/L)*	85.0 (0.6–342.0)	81.4 (0.6–342.0)	102.5 (3.3–300)	0.242
Prothrombin time (s)*	14.6 (10.6–85.2)	14.5 (10.6–47.1)	15.4 (11.6–85.2)	0.071
INR*	1.1 (0.9–6.9)	1.1 (1.02–1.21)	1.2 (0.94–6.94)	0.090
aPTT (s)*	30.6 (19.9–75.1)	30.4 (0.9–3.7)	31.8 (21.8–74.1)	0.049†
Fibrinogen (mg/dL)*	544.0 (198–999)	547 (19.8–75.1)	533.5 (198–792)	0.376
D-dimer (mg/dL)*	0.99 (0.05–20)	0.91 (0.1–20.0)	1.5 (0.05–5.3)	0.230
Ferritin (µg/L)*	413.0 (14–3484)	397 (29.0–3484.0)	441.0 (14.0–1754.0)	0.946
Procalcitonin (µg/L)*	0.14 (0.02–31.4)	0.1 (0.1–29.9)	0.3 (0.1–31.4)	<0.001†

BMI: body mass index; HT: hypertension; DM: diabetes mellitus; CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; CHF: congestive heart failure; PIM: potentially inappropriate medications; TIME: Turkish Inappropriate Medication use in the Elderly; ICU: intensive care unit; LDH: lactate dehydrogenase; GFR: glomerular filtration rate; INR: international normalized ratio; aPTT: activated partial thromboplastin time. *Numeric variables were presented as median (minimum-maximum) or mean±SD. †PIM was determined based on TIME criteria; ‡significant p-value.

Table 2. Top five ranked potentially inappropriate medications of participants based on TIME criteria.

Participants with PIM n (%)	TIME to START n (%)	TIME to STOP n (%)
	193 (96.0)	59 (37.6)
1	E1. n=170 (84.6%) Vitamin D if vitamin D intake <800–1000 IU per day and/or calcium if elementary calcium intake <1000–1200 mg per day	C4. n=10 (6.4%) PPIs for multiple drug use indication (no benefit, potential harm)
2	I1. n=127 (63.2%) ONS with MN or MNR if nutritional counseling/dietary supplementation are not sufficient to achieve nutritional goals	C2. n=10 (6.4%) Aspirin, clopidogrel, NSAIDs or corticosteroids in patients with peptic ulcer history/dyspepsia-gastroesophageal reflux symptoms or with concurrent antiplatelet/anticoagulant/corticosteroid treatment(s) without PPI prophylaxis
3	A2. n=29 (14.4%) Statin therapy for secondary prevention in patients with documented atherosclerotic coronary artery disease (previous acute coronary syndrome/coronary artery angioplasty or stenting/coronary artery bypass grafting/abdominal aortic aneurysm), documented atherosclerotic cerebrovascular disease (presence of ischemic stroke/TIA/previous carotid endarterectomy or stenting) or peripheral arterial disease	H1. n=9 (5.7%) High potency anticholinergic drugs [e.g. tricyclic antidepressants, chlorpromazine, thioridazine, clozapine, olanzapine, hyoscine, oral oxybutynin, first generation antihistamines (pheniramine, chlorpheniramine, hydroxyzine, cyproheptadine, dimenhydrinate, diphenhydramine, meclizine, etc.), paroxetine] in patients with falls/constipation/narrow angle glaucoma/delirium/dementia/urinary retention/obstructive LUTS symptoms/concurrent use of anticholinergic drugs
4	A1. n=21 (10.4%) Antiplatelet therapy (aspirin or clopidogrel) for secondary prevention in patients with documented atherosclerotic coronary artery disease (previous acute coronary syndrome/coronary artery angioplasty or stenting/coronary artery bypass grafting/abdominal aortic aneurysm), documented atherosclerotic cerebrovascular disease (presence of ischemic stroke/TIA/previous carotid endarterectomy or stenting) or symptomatic lower extremity artery disease	E1. n=7 (4.5%) Long-term use of NSAIDs (>3 months) in the presence of alternative treatment
5	A6. n=20 (10.0%) Beta-blocker with ischemic heart disease (antianginal effect in chronic ischemic heart disease/mortality reduction effect in post-MI era) or systolic heart failure (EF≤40%) (bisoprolol/prolonged release metoprolol succinate/carvedilol/nebivolol in systolic heart failure; any beta blocker in ischemic heart disease)	B18. n=7 (4.5%) Piracetam except for myoclonic convulsion therapy (with no proven clinical efficacy, cost burden, and side effect potential)

TIME: Turkish Inappropriate Medication use in the Elderly; PIM: potentially inappropriate medications; IU: international unit; PPI: proton-pump inhibitors; ONS: oral nutritional supplements; MN: malnutrition; MNR: malnutrition risk; NSAID: non-steroidal anti-inflammatory drug; TIA: transient ischemic attack; LUTS: lower urinary tract symptoms; MI: myocardial infarction; EF: ejection fraction.

analysis. Older age (HR: 1.07; 95%CI 1.03–1.11; $p < 0.001$) and higher NRS-2002 scores (HR: 1.20, 95%CI 1.01–1.68; $p = 0.045$) were related to in-hospital mortality. Different models were analyzed for assessing the relationship between PIM and mortality, as shown in Table 3.

DISCUSSION

In this study, older age and malnutrition were independently associated with in-hospital mortality in geriatric patients with COVID-19. Although the number of PIMs was statistically significantly higher in nonsurvivors compared with survivors, there was no longer a significant relationship with mortality after adjustment for confounders in multivariate analysis. To the best of our knowledge, this is the first study to analyze the relationships between PIM and in-hospital mortality of older adults with COVID-19.

In previous studies, the in-hospital mortality rate of COVID-19 in older adults was reported to be higher than in our study (30–50% vs. 18.9%)^{18,19}. A possible explanation for these differences is that the mean age of our population was younger than those in other studies^{18,19}. The other explanation is that the hypoxic patients received oxygen supplement treatment but not steroids in previous studies^{18,19}. As in the report of the RECOVERY group²⁰, death rates were lower in patients with hypoxia who received dexamethasone treatment. In this study, all patients with hypoxia were treated with dexamethasone. In addition, experienced geriatricians were involved in the follow-up and treatment of all patients

in this study. This may have resulted in better care for older patients during hospitalization, and a decrease in drug interactions and PIM, thus reducing the mortality rates. In this study, we recognized and discontinued PIM drugs during hospital admission time. There are different nutritional risk screening tools in clinical practice. In this study, we assessed malnutrition using the NRS-2002. A study, which compared four different nutritional risk screening tools, found that the NRS-2002 was more successful than others in recognizing malnutrition in COVID-19²¹. Although the number of studies evaluating the relationship between COVID-19 and malnutrition is small, most of these studies found that malnutrition was an important risk factor for COVID-19-related mortality². Early implementation of nutritional support may have reduced the mortality rate of our patients.

In a study in Italy²², 95% of participants had at least one PIM based on the Beers Criteria at admission. Cattaneo et al.²² evaluated the drug-drug interactions (DDIs) and included them in PIM, so its prevalence was very high. However, the TIME criteria do not include DDIs. The prevalence of PIM in this study was slightly higher than in other studies¹³. This may be because the PIM prevalence was only considered deprescribing and not underprescribing in these studies. An important advantage of the TIME criteria is that PIM use should not only be limited to overuse of medications but also include a lack of use of beneficial medications. In our study, most of the participants who had untreated malnutrition were captured in the underprescribing group, and one of three in the overtreatment group based on the TIME criteria. The relationship between the

Table 3. Cox regression model for mortality with potentially inappropriate medications.

	Model 1		Model 2		Model 3		Model 4	
	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
Age	1.07 (1.03–1.11)	<0.001†	1.07 (1.03–1.12)	0.001†	1.07 (1.03–1.12)	0.001†	1.07 (1.02–1.13)	0.005†
Sex: male	1.40 (0.71–2.76)	0.329	1.28 (0.64–2.56)	0.483	1.46 (0.72–2.98)	0.293	1.32 (0.67–2.85)	0.383
Number of PIM*	1.02 (0.79–1.32)	0.869	0.99 (0.75–1.30)	0.914	0.91 (0.67–1.23)	0.543	0.92 (0.68–1.25)	0.929
CHF			0.47 (0.21–1.08)	0.074	0.51 (0.22–1.19)	0.118	0.46 (0.17–1.20)	0.112
Dementia			1.05 (0.42–2.61)	0.922	1.23 (0.48–3.12)	0.669	1.11 (0.42–2.92)	0.834
Score of NRS-2002					1.26 (0.99–1.60)	0.056	1.20 (1.011–1.68)	0.045†
Glucose (mg/dL)							1.00 (0.99–1.01)	0.895
Procalcitonin (µg/L)							1.04 (0.97–1.11)	0.294
GFR (mL/m)							1.01 (0.99–1.02)	0.487
aPTT (s)							1.02 (0.98–1.06)	0.375

Model 1: adjusted by sex, age, and PIM (based on TIME criteria); Model 2: adds CHF and dementia to Model 1; Model 3: adds score of NRS-2002 to Model 2; Model 4: adds 4 laboratory values to Model 3. HR: hazard ratio; CI: confidence interval; PIM: potentially inappropriate medications; CHF: congestive heart failure; GFR: glomerular filtration rate; aPTT: activated partial thromboplastin time; TIME: Turkish Inappropriate Medication use in the Elderly. *PIM was determined based on TIME criteria; †significant p-value.

number of PIMs that was significant in the univariate analysis but did not show significance in the multivariate analysis in this study, and mortality may be better explained with long-term follow-up studies, but due to the nature of the evolving pandemic, we wanted to publish our results as soon as possible for wide availability. In addition, although the effects of drug cessation are seen in a shorter period, longer follow-up is required to see the effect on mortality when drugs/support products are started.

Many studies reported that older age was the main risk factor for COVID-19 mortality^{2,3}. With aging, the immune system is more prone to infections, impaired cell-mediated and humoral immunity, and pro-inflammation.

The other factor related to in-hospital mortality was malnutrition in the present study. Studies in Turkey², China²¹, and other countries showed that malnutrition was related to in-hospital mortality in patients with COVID-19. In this study with the NRS-2002, 7 out of 10 patients were diagnosed as having malnutrition, and malnutrition increased the in-hospital mortality rate by 29%. Therefore, older patients with COVID-19 should receive nutrition screening.

This study has some limitations. This is a single-centered study performed at a referral COVID-19 center with a short follow-up period, which restrains the generalization of our results. With long-term mortality, drug effects can be observed better in patients with undertreatment. We only included hospitalized patients in this study, and most of the participants

had severe COVID-19. Therefore, the results do not reflect the real effect of PIM on patients with COVID-19.

CONCLUSION

Older age and malnutrition were related to in-hospital mortality in COVID-19 in this study. Mortality is more common in older individuals with higher numbers of PIM; however, we could not show its effect on mortality in the early period, and the effect of PIM on mortality may be better revealed in long-term studies. The TIME criteria recommend diagnosing malnutrition and initiating treatment. Early intervention may have an impact on mortality in COVID-19 patients.

AUTHORS' CONTRIBUTIONS

NŞD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **AT:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **BC:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **ŞO, DK:** Data curation, Investigation. **Bİ:** Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **GB:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Anogenital index and bone mineral density associations after natural and surgical menopause: a preliminary study

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate postmenopausal women to determine whether an anogenital index (AGI) is associated with bone mineral density (BMD) based on the hypothesis that the effects of menopause are similar for both.

METHODS: A total of 338 generally healthy postmenopausal women who were referred for a routine annual check and 140 women who met the inclusion criteria were enrolled in the study. Based on the menopausal status, the women were classified into natural menopause and surgical menopause. AGI was calculated by dividing anogenital distance by body mass index. The BMD of the femoral neck, body of the femur, and lumbar spine (L1 and L2) was measured using dual-energy x-ray absorptiometry.

RESULTS: There was a statistically significant and same-directional correlation between age and AGI for all cases ($r=0.234$ and $p=0.005$). The AGI level decreased as the parity increased ($r=-0.582$ and $p<0.001$). The AGI level decreased significantly as the menopause duration was prolonged ($r=0.288$ and $p<0.001$). While there was no statistically significant correlation between L2-L4 BMD and AGI ($p=0.128$), as the femur and femoral neck BMD levels increased, the AGI level increased statistically significantly ($r=0.330$ and $p<0.001$, $r=0.292$ and $p<0.001$).

CONCLUSION: The AGI levels in healthy postmenopausal women give preliminary information about their BMD status. A decrease in AGI levels may predict lower BMD in postmenopausal women. Further larger and well-controlled studies may be required to determine the relationship between AGI and BMD in the future.

KEYWORDS: AGI. BMD. Postmenopausal osteoporosis.

INTRODUCTION

Menopause is actually the period after a woman's cessation of menstruation is certain. Although it is a term used, the most important fact in this period is the end of woman's reproductive ability her reproductive period¹. Reproductive aging is influenced by many factors. Along with the gonads, aging of the entire endocrine system and systemic aging are also in question. Although menopause happens at a certain time, various changes begin many years in advance. This period brings with it a series of unique complaints as well as pathological changes that can cause serious diseases in the long term². Regardless of the cause, surgical menopause and loss of estrogens and androgens before the normal age of menopause affect a lot of systemic functions and have long-term health consequences^{3,4}.

Osteoporosis is a systemic skeletal disease characterized by low bone mass, deterioration in the microarchitecture, quality of bone tissue, and decreased bone strength leading to an increased risk of fracture⁵. Postmenopausal osteoporosis is an important public health disease. Prolongation of life

expectancy, development of osteoporosis, and osteoporotic fracture impairment in daily living activities have become more important problems⁶. The most important factors in the development of the disease are age and hormonal changes⁷. Similar to bones, changes occur in skin structure and collagen production in the postmenopausal period. Decreases in estrogen levels, production of collagen changes with an increase in collagen strength, skin elasticity reduction, a severe decline in genital water content, and anatomical changes in female genital organs may also occur. Both the vagina and the external female genitals are affected⁸.

The anogenital distance (AGD) is identified as the length (in mm) between the labia posterior commissure and the center of the anus. The anogenital index (AGI) was used to control two variables, namely, height and weight. AGI was calculated by dividing AGD by body mass index (BMI)⁹. Recent studies have found that the AGD of postmenopausal women is significantly shorter than that of premenopausal women¹⁰. Several studies on adult men, as in women, have provided

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strong evidence of the relationship between AGD length and male reproductive function^{11,12}.

This study evaluated postmenopausal women to determine whether AGI is associated with BMD based on the hypothesis that the effects of menopause are similar for both.

METHODS

This prospective study was performed between January 2021 and May 2021. Ethical approval was obtained from the Ministry of Health Ankara City Hospital, Ethical Review Board (No. E2/21/79). In all, 338 generally healthy postmenopausal women who were referred for a routine annual check to the Menopause Clinic of the Ministry of Health Ankara City Hospital, and 140 women who met the inclusion criteria were enrolled in the study. According to the analysis of 95% confidence ($1-\alpha$), 95% test power ($1-\beta$), and $d=0.5$ effect size, the number of samples to be taken in each group was determined as 54, according to the independent samples t-test analysis¹³. This study was conducted following the principles of the Declaration of Helsinki. All patients were extensively informed of the study design and hypothesis. Written informed consent was obtained from all patients enrolled.

Menopausal status was defined as the absence of menstruation for at least 12 months, according to the World Health Organization's definition of menopause. Based on the menopausal status, the women were classified into natural menopause and surgical menopause. All women included in the study were asked to complete the researcher's administered questionnaire regarding age, parity, height, weight, type of birth, menopausal status, time since menopause, surgical history, medical illness history, and drug intake, including hormone therapy (HT). Healthy postmenopausal women who did not have any additional systemic disease and did not receive hormone replacement therapy were included in the study. None of our subjects were using hormone replacement therapy or were receiving medications affecting bone mineralization. The exclusion criteria were as follows: patients with secondary causes of osteoporosis (primary hyperparathyroidism, Cushing's syndrome, rheumatoid arthritis, etc.), liver and chronic kidney diseases, bone diseases, metabolic disorders, or other systemic diseases.

Patients' data (height, weight, body mass index, AGD, and AGI) were collected on the same day during the outpatient examination. An AGD measurement was conducted using a paper ruler in the lithotomy position to define the distance between the posterior commissure of the labia and the anus center. BMI was calculated as female weight (kg) divided by squared height (m^2). AGI was calculated by dividing AGD by BMI.

Bone mineral density measurements

The BMD of the femoral neck, body of the femur, and lumbar spine (L1 and L2) was measured by dual-energy x-ray absorptiometry using a QDR 4500A (Hologic, Bedford, MA, USA) densitometer and was expressed in absolute values as grams of mineral content per square centimeters of bone area (g/cm^2).

Statistical analysis

Statistical analysis was performed using the SPSS software (Statistical Package for the Social Sciences) version 22 (SPSS Inc., Chicago, IL). Data are presented as mean \pm standard deviation (SD) and 95% confidence interval (CI). The Kolmogorov-Smirnov test was used to investigate whether the normal distribution assumption was met. Descriptive statistics were given as mean \pm SD or median (25–75th) percentiles, where applicable. While the mean differences between types of menopause were compared using Student's t-test, the Mann-Whitney U test was applied for the comparisons of non-normally distributed data. Degrees of association between continuous variables were evaluated by Spearman's rank-order correlation analysis. Multiple linear regression analysis via stepwise procedure was applied for determining the best predictor(s), which affects the AGI levels. Any variable whose univariable test had a p-value of <0.10 was accepted as a candidate for the multivariable model along with all variables of known clinical importance. Coefficient of regression, 95% confidence interval, and t-statistic for each independent variable were also calculated. Data analysis was performed using the IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, USA). A p-value of <0.05 was considered statistically significant.

RESULTS

For our study, 338 postmenopausal women were initially examined. After the exclusion criteria, 140 women met the inclusion criteria and formed the study population; 86 patients were in the natural menopause group and 54 patients were in the surgical menopause group.

The demographic characteristics of the participants are listed in Table 1. There was no statistically significant difference in terms of mean age, BMI, duration of menopause, and BMD levels between the surgical menopause group and the natural menopause group ($p>0.05$). Compared to the natural menopause group, the parity of the surgical menopause group was statistically significantly higher, and the AGD level was statistically significantly lower ($p<0.001$). The mean AGI level was also statistically significantly lower in the surgical menopause group compared to that in the natural menopause group ($p<0.001$).

Table 1. Demographic and clinical characteristics for menopause types.

	Total (n=140)	Natural (n=86)	Surgical (n=54)	p-value
Age (years)	57.6±4.9	57.7±4.4	57.4±5.6	0.668*
Body mass index (kg/m ²)	29.2 (26.6–31.0)	28.8 (26.4–30.8)	29.7 (26.6–31.7)	0.103 [†]
Parity	2.0 (2.0–3.0)	2.0 (1.0–3.0)	3.0 (2.0–3.0)	<0.001 [†]
Duration of menopause (years)	7.5 (5.0–11.0)	7.5 (5.0–11.0)	7.5 (3.7–10.0)	0.512 [†]
AGD	27.0 (21.0–30.0)	29.0 (22.0–31.0)	23.0 (20.0–28.0)	<0.001 [†]
AGI	0.89±0.21	0.95±0.20	0.79±0.19	<0.001 *
L2-L4	1.13 (0.99–1.28)	1.10 (0.99–1.27)	1.15 (0.99–1.34)	0.368 [†]
Femur	1.00 (0.92–1.08)	1.01 (0.91–1.09)	1.00 (0.92–1.06)	0.480 [†]
Femur neck	0.97 (0.90–1.03)	0.99 (0.90–1.04)	0.96 (0.90–1.01)	0.689 [†]

Data were shown as mean ± standard deviation or median (25–75th) percentiles. AGD: anogenital distance; AGI: anogenital index. *Student's t-test; [†]Mann-Whitney U test. Statistically significant p-values are shown as bold characters.

The results of the correlation analysis between AGI levels and other demographic and clinical characteristics were shown in Table 2. There was a statistically significant and same-directional correlation between age and AGI for all cases ($r=0.234$ and $p=0.005$). On the contrary, as the parity increased, the AGI level was decreasing ($r=-0.582$ and $p<0.001$). The AGI level decreased significantly as the menopause duration was prolonged ($r=0.288$ and $p<0.001$). While there was no statistically significant correlation between L2-L4 BMD and AGI ($p=0.128$), as the femur and femoral neck BMD levels increased, the AGI level increased statistically significantly ($r=0.330$ and $p<0.001$; $r=0.292$ and $p<0.001$).

In the next step, the most determinant factors in predicting the change in AGI level were investigated with multivariate stepwise elimination linear regression analysis (Table 3). As a result of univariate statistical analysis, all variables that were found to be $p<0.10$ were included in the linear regression model as candidate factors. As a result of stepwise elimination, the best predictors that affect the AGI levels are parity, type of menopause, duration of menopause, and femur BMD. In other words, when corrected for other factors, being in surgical menopause continued to decrease the AGI level statistically significantly compared to natural menopause ($B=-0.116$, 95%CI -0.176 to -0.056; $p<0.001$).

DISCUSSION

This cross-sectional design study investigated the relationship between BMD and AGI in different categories of postmenopausal women: surgical menopause and natural menopause. To the best of our knowledge, this study is the first attempt to investigate AGI and explore the relationship between AGI and BMD in postmenopausal women.

Table 2. The results of correlation analysis between anogenital index levels and other demographic and clinical characteristics.

	Coefficient of correlation	p-value*
Age	0.234	0.005
Parity	-0.582	<0.001
Duration of menopause	-0.288	<0.001
L2-L4	0.129	0.128
Femur	0.330	<0.001
Femur neck	0.292	<0.001

*Spearman's rank-order correlation analysis. Statistically significant p-values are shown as bold characters.

Table 3. The best predictors that affect anogenital index levels, the results of multiple linear regression analysis.

	B	95%CI for B		t	p-value
		LL	UL		
Parity	-0.062	-0.086	-0.039	-5.224	<0.001
Surgical menopause	-0.116	-0.176	-0.056	-3.827	<0.001
Duration of menopause	0.010	0.003	0.017	2.706	0.008
Femur neck	0.222	0.057	0.387	2.665	0.009

B: coefficient of regression; CI: confidence interval; LL: lower limits of CI; UL: upper limits of CI. Statistically significant p-values are shown as bold characters.

It is known that bone loss due to estrogen deficiency starts after menopause. Multiple clinical studies have established that surgical menopause carries a higher risk of osteoporosis than natural menopause due to long-term low estrogen levels^{14,15}. Compared with natural menopause, in surgical menopause due to bilateral oophorectomy, there are no premise hormonal changes because of its instantaneous nature¹⁶. In addition,

there is a sudden decrease in androgen levels in addition to estrogen in women in surgical menopause, which negatively affects bone density¹⁷. Dimitrios et al. showed that BMD values were decreased, whether in women with natural or surgical menopause, and found that women with surgical menopause had lower BMD as time progressed than normal menopausal women¹⁸. In our study, BMD levels were similar in women with natural or surgical menopause, but AGI levels were lower in the surgical menopause group and associated with femur and femoral neck BMD levels.

The decrease in circulating estrogen has similar effects on the skin, muscle, and connective tissue as well as on the bone. Low estrogen levels are associated with accelerated skin aging due to thinning, loss of collagen, and reduced elasticity¹⁹. There have been a lot of studies on female external genitalia to understand the change after menopause^{20,21}. However, the use of AGI in human studies is still rare. Lee et al. have shown that changes in AGD and AGI have the potential to be used as a scale to predict physical changes in the skin after menopause²². Similarly, we investigated the change of AGI in surgical and natural menopause groups in our study. It was observed that the decrease in AGI levels was higher in the surgical menopause group compared to the natural menopause group.

We developed our research with the hypothesis that postmenopausal AGI and BMD change might be related, and a statistically significant relationship was found between BMD and AGI levels in our study. The AGI was associated with femur and femoral neck BMD levels. The best predictors that affect the AGI levels are parity, type of menopause, duration of menopause, and femur BMD. We observed significantly negative correlations between AGI and the duration of menopause and parity. But AGI was not associated with L2-L4 BMD levels.

Our study had several strengths. First, it is a prospective study. We have eliminated possible confusion due to exogenous hormonal drugs. This is important because using exogenous HT is associated with higher BMD. In addition, AGI measurements were performed by a gynecologist who was not aware of the patient's condition, thus eliminating the interobserver bias.

Our study has some limitations. The study and the control group consisted of women admitted to the same hospital. Our study is the cross-sectional nature of its design. Longitudinal data from an extended follow-up on a large cohort would be more definitive. Our results cannot be generalized to postmenopausal women using exogenous HT, who were excluded from our analysis.

CONCLUSION

A statistically significant relationship was found between bone mineral density and AGI levels in our study. The AGI levels in healthy postmenopausal women give preliminary information about their BMD status. Decreased AGI level is a potential predictor of bone loss in postmenopausal women. This study is the first attempt to investigate AGI and explore the relationship between AGI and BMD in postmenopausal women. The observational nature and limited sample size of the present study do not allow for generalizations. Therefore, our results should be considered suggestive for larger studies to investigate the relationship between AGI and BMD in the future.

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 Declaration of Helsinki and its later amendments or comparable ethical standards.

AUTHORS' CONTRIBUTIONS

GNB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **EUO, MKK:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. **IH:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **MGO:** Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

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Thorax computed tomography findings and anti-SARS-CoV-2 immunoglobulin G levels in polymerase chain reaction-negative probable COVID-19 cases

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SUMMARY

OBJECTIVE: This study aimed to evaluate the SARS-CoV-2 immunoglobulin G (IgG) levels after 6 months of polymerase chain reaction (PCR) negative but assumed to be COVID-19 positive cases to investigate the relationship between IgG levels and thoracic computed tomography (CT) findings.

METHODS: This was a single-center study that included patients whose PCR test results were negative at least three times using nasopharyngeal swabs but had clinical findings of COVID-19 and thoracic CT findings compatible with viral pneumonia. Six months after discharge, the IgG antibodies were analyzed. The cutoff value for negative and positive serology was defined as <1.4 (index S/C) and ≥1.4 (index S/C), respectively. In addition, the patients were categorized according to their thoracic CT findings as high (typical) and low (atypical). Also, the patients were grouped into classes as <5% lung involvement versus ≥5% lung involvement.

RESULTS: The patients' mean age was 49.78±12.96 years. PCR was negative, but patients with COVID-19 symptoms who had SARS-CoV-2 IgG positive were 81.9% (n=95). The antibody titer and lung involvement ≥5% were statistically significantly higher in SARS-CoV-2 IgG positive cases (p<0.001 and p=0.021). Age and chest CT findings were the risk factors for lung involvement (OR=1.08, p<0.001 and OR=2.19, p=0.010, respectively).

CONCLUSION: This study is valuable because increasing severity (≥5%) of lung involvement appears to be associated with high and persistent IgG antibody titers. In probable cases of COVID-19, even if the PCR test is negative, high IgG titers 6 months after discharge can predict the rate of lung parenchymal involvement.

KEYWORDS: COVID-19. SARS-CoV-2. Reverse transcriptase-polymerase chain reaction. Computed tomography. Chest. Immunoglobulin G, Antibody.

INTRODUCTION

Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is a respiratory disease caused by a novel coronavirus-2019 (COVID-19) that has rapidly spread worldwide and affected every aspect of life. The definitive diagnosis is made based on viral testing (nucleic acid amplification or antigen tests) in patients having clinical signs and symptoms or close contacts¹. Determining viral genome targets by reverse-transcription polymerase chain reaction (RT-PCR) is the gold standard in COVID-19 diagnosis. However, the sensitivity (i.e., the ability of a test to correctly identify those with the disease) and specificity (i.e., the ability of a test to correctly identify those without the disease) of RT-PCR testing through nasopharyngeal swabs are relatively low with a varying degree of false negativity^{2,3}. As in

all laboratory tests, inadequate sampling, improper transport, improper ribonucleic acid extraction process, and the presence of amplification inhibitors may yield false-positive or false-negative results^{4,5}. A negative test result does not rule out the possibility of COVID-19, while a positive test often indicates the presence of the infection⁶.

Previous studies have shown a specificity of >95% for enzyme-linked immunosorbent assay-based immunoglobulin (Ig) M and G antibody testing in the diagnosis of COVID-19⁷. Although antibody response to SARS-CoV-2 infection usually occurs in the first 7–11 days after infection, delayed antibody production may be seen in some cases. Therefore, antibody testing is not helpful and not recommended in diagnosing current infection⁶. Serological tests also have limited utility in

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diagnosing acute infections, as they depend on the duration of symptom onset⁸. Therefore, antibody testing is not feasible in diagnosing acute infections⁶.

Due to the limited utility of RT-PCR and antibody testing in COVID-19, imaging studies have become an integral part of the diagnostic work-up of these patients. Although thoracic computed tomography (CT) findings showing ground-glass opacities in the bilateral, multifocal, and peripheral ground or interlobular septal consolidation suggest COVID-19 pneumonia in patients with lower respiratory tract involvement, these findings may be seen in non-COVID-19 viral pneumonia. The definitive diagnosis can be more challenging in the absence of typical lung involvement and the presence of RT-PCR negativity⁹.

The antibody status and titers are unknown 6 months after the initial diagnosis in probable COVID-19 cases, and the protective role of IgG antibodies is uncertain. Therefore, in this study, we aimed to evaluate the SARS-CoV-2 immunoglobulin G (IgG) levels at 6 months in probable COVID-19 cases and investigate the relationship between anti-SARS-CoV-2 IgG levels and thoracic CT findings.

METHODS

Study design and study population

In this single-center, retrospective, cross-sectional study, a total of 380 hospitalized patients with probable COVID-19 infection who had persistent fever ($>38^{\circ}\text{C}$), hypotension ($<90/60$ mmHg), tachycardia (>100 bpm), tachypnea (>30 breaths per min), an oxygen saturation (SpO_2) of $<92\%$, and elevated acute phase response (C-reactive protein >40 mg/L, ferritin >500 ng/mL, and D-dimer $>1,000$ ng/mL) were screened. Those having antigen positivity for influenza, heart failure, bacterial pneumonia, and restrictive lung disease were excluded from the study. Medical data of the patients who were hospitalized with the diagnosis of PCR-negative probable COVID-19 infection between March 2020 and May 2020 were reviewed.

Inclusion criteria were as follows: >18 years of age at the time of admission, at least three consecutive negative results of RT-PCR assay using nasopharyngeal swabs, having at least one thoracic CT scan during the hospital stay, having no need for intensive care, and discharge with full recovery. Those unwilling to participate in the study, deceased patients, pregnant and breastfeeding women, and those lost to follow-up were excluded. Finally, 116 patients whose RT-PCR test results were negative using oropharyngeal and nasopharyngeal swabs but had clinical findings of COVID-19 and thoracic CT findings

compatible with viral pneumonia were included. Six months after discharge, all patients' SARS-CoV-2 IgG antibodies and thoracic CT findings were analyzed.

According to this scheme, the patients were classified into two categories of thoracic CT findings: a high (typical) and a low (atypical) level of suspicion of consistency with COVID-19. We also evaluated the possible relationship between anti-SARS-CoV-2 IgG levels and the thoracic CT findings. The patients were categorized into two groups: $<5\%$ lung involvement and $\geq 5\%$ lung involvement. Similarly, we compared the chest CT findings of the patients with $<5\%$ lung involvement versus $\geq 5\%$ lung involvement to elucidate the effect of lung involvement on anti-SARS-CoV-2 IgG antibodies.

Data collection

The patients who were included in the study were reached by phone calls and scheduled for a visit at 6 months in the outpatient setting. Clinical data, COVID-19 reinfection, and comorbidities of the patients were recorded. Venous blood samples were collected from each patient for antibody testing. Baseline thoracic CT scans were assessed by a single radiologist who was blinded to the clinical findings and antibody test results of the patients, and the severity of lung involvement was examined. Demographic, clinical, laboratory, and imaging characteristics of the patients were recorded.

Antibody testing

The chemiluminescence microparticle immunoassay was used for the qualitative detection of anti-SARS-CoV-2-specific IgG antibodies in human serum and plasma. In clinical practice, the SARS-CoV-2 IgG antibody testing is used to detect IgG antibodies against the nucleocapsid protein of the SARS-CoV-2 in human serum and plasma in suspected COVID-19 cases having signs and symptoms or confirmed cases. In this study, the antibody testing was performed using the ARCHITECT autoanalyzer (Abbott Laboratories, IL, USA). The cutoff value for negative and positive serology was defined as <1.4 (index S/C) and ≥ 1.4 (index S/C), respectively. The sensitivity and specificity of the assay were calculated as 99.63% (Abbott ARCHITECT™ SARS-CoV-2 IgG Instructions for Use. H14806R01. April 2020).

Imaging studies

Baseline thoracic CT scans of all patients during the hospital stay were retrospectively evaluated. Chest computed tomography score, in which each lobe could be awarded a CT score from 0 to 5, depending on the percentage of the involved lobe, can be presented as follows:

- Score 0: 0% involvement;
- Score 1: less than 5% involvement;
- Score 2: 5–25% involvement;
- Score 3: 26–49% involvement;
- Score 4: 50–75% involvement;
- Score 5: greater than 75% involvement¹⁰.

Exemplary thorax CT findings demonstrating COVID-19 infiltration in the axial view of our patients are shown in Figure 1. Thoracic CT findings of patients with <5% lung involvement versus ≥5% lung involvement were compared.

Ethical consideration

Prior to the study, all patients were informed about the nature of the study and a written informed consent was obtained. The study protocol was approved by the institutional Ethics Committee with Approval No. 71306642-050.05.04. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Descriptive statistics were given median (minimum-maximum), frequencies with percentages, and mean±standard deviation. Fisher’s exact test, chi-square test, Mann-Whitney U test, and independent samples t-tests were performed for comparisons. The risk factors of lung involvement were analyzed with binary logistic regression analysis using the enter method. The SPSS (IBM Corp., Released 2021, IBM SPSS Statistics for Windows, Version 28.0, Armonk, NY) software was used for analyses. The level of significance was taken as α=0.05.

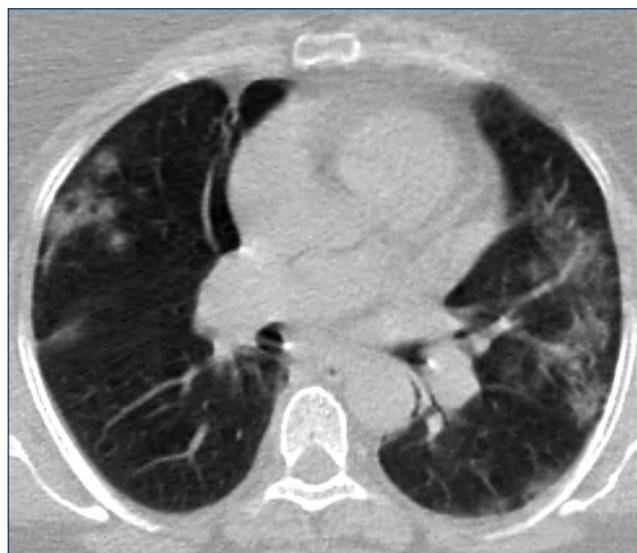


Figure 1. Thorax CT findings showing COVID-19 infiltration in the axial view.

RESULTS

In this study, we included only 116 PCR-negative patients, i.e., 64 (55.2%) men and 52 (44.8%) women, who had COVID-19 symptoms with a mean age of 49.78±12.96 years. COVID-19 symptoms were compared with the patients’ investigation results that were presented in Table 1 and the risk factors of lung involvement that were presented in Table 2. Chest CT

Table 1. COVID-19 symptoms comparisons with patients’ investigation results.

	SARS-CoV-2 IgG	
	Positive (n=95)	Negative (n=21)
Antibody titer (S/C)	6.59 (1.40–49.71)	0.16 (0.01–0.85)
p-value	<0.001	
Age (year)	50.08±12.35	48.43±15.72
p-value	0.654	
Gender		
Male	51 (53.7%)	13 (61.9%)
Female	44 (46.3%)	8 (38.1%)
p-value	0.493	
Lung involvement of chest CT findings		
<5%	29 (30.5%)	12 (57.1%)
≥5%	66 (69.5%)	9 (42.9%)
p-value	0.021	
Chest CT findings		
Typical COVID-19	80 (84.2%)	14 (66.7%)
Atypical COVID-19	15 (15.8%)	7 (33.3%)
p-value	0.073	

Data were presented as median (minimum-maximum), mean±standard deviation, and frequencies with percentages. IgG: immunoglobulin G; CT: computed tomography.

Table 2. Risk factors of lung involvement.

	OR (95%CI)	p-value
Age	1.08 (1.03–1.12)	<0.001
SARS-CoV-2 IgG		
Positive (reference category)		0.357
Negative	0.75 (0.40–1.39)	
Chest CT findings		
Inconsistent with COVID-19 (reference category)		0.010
Consistent with COVID-19	2.19 (1.21–3.96)	
Constant	0.022	<0.001
Hosmer-Lemeshow test		0.834
Omnibus test		<0.001

Nagelkerke R-square=0.310. CI: confidence interval; IgG: immunoglobulin G; CT: computed tomography.

findings did not have a statistically significant difference between SARS-CoV-2 IgG groups ($p=0.073$). Patients who had $\geq 5\%$ lung involvement were statistically significantly high in SARS-CoV-2 IgG positive group ($p=0.021$). Age, gender, and chest CT findings did not have a statistically significant difference between SARS-CoV-2 IgG groups ($p=0.654$, $p=0.493$, and $p=0.073$, respectively). Conversely, age and chest CT findings consistent with COVID-19 were the risk factors for lung involvement. As the age increased, the risk of $\geq 5\%$ lung involvement increased 1.08 times; while the chest CT findings were consistent with COVID-19, the risk of $\geq 5\%$ lung involvement increased 2.19 times compared to inconsistent with COVID-19 (OR (95%CI)=1.08 (1.03–1.12) $p<0.001$ and OR (95%CI)=2.19 (1.21–3.96) $p=0.010$, respectively).

In most of the patients with negative RT-PCR, there were typical chest CT findings consistent with COVID-19. However, atypical findings were also observed in some cases. In addition, we found no significant difference in anti-SARS-CoV-2 IgG antibody titers between the typical and atypical cases.

All patients were evaluated in terms of COVID-19 reinfection at 6 months. RT-PCR positivity was detected in three patients. Of these, anti-SARS-CoV-2 IgG negativity was detected in two patients, despite RT-PCR positivity. In the other patient, anti-SARS-CoV-2 IgG antibody titers were significantly higher (6.18 S/C) at the time of diagnosis as confirmed by RT-PCR positivity. However, as the antibody testing was performed after the diagnosis of COVID-19, it remained unclear whether antibody production occurred after the initial infection.

DISCUSSION

Several reports in the literature have shown viral pneumonia based on clinical and/or chest CT findings, despite having RT-PCR negativity using oropharyngeal and nasopharyngeal swabs. In parallel with the practices of the world, these patients were considered probable COVID-19 cases and treated with current treatment protocols in the in-hospital setting. This study evaluated the possible relationship between anti-SARS-CoV-2 IgG levels and thoracic CT findings⁹⁻¹¹. Our study results showed that 81.9% of the probable COVID-19 cases had significantly higher anti-SARS-CoV-2 IgG titers 6 months after the diagnosis. These results indicate that a negative RT-PCR test result does not exclude the possibility of COVID-19 and that a high number of probable COVID-19 cases are, indeed, COVID-19-positive cases. In addition, we observed antibody production in the probable COVID-19 cases at 6 months, despite negative RT-PCR results, and anti-SARS-CoV-2 IgG titers remained high in 81.9% of the cases.

Dimeglio et al.¹² reported that 30 patients had positive chest findings with a negative RT-PCR test result. Two months after the symptom onset, 14.3% of these patients had positive anti-SARS-CoV-2 antibodies. The authors suggested that non-COVID-19 pneumonia should be investigated in these patients. However, this finding can be attributed to this study's low number of RT-PCR-negative patients. In addition, non-COVID-19 pneumonia cases were not excluded from the study, and clinical evaluation was performed later. Unlike this study, we observed anti-SARS-CoV-2 IgG antibody positivity in most probable COVID-19 cases, indicating that these patients recovered from COVID-19.

In April 2020, a group of radiologists from the University of Southern California proposed a 5-point scale of suspicion (low, moderate, and high) for pulmonary involvement of COVID-19 on thoracic CT, namely, the COVID-19 imaging reporting and data system (COVID-RADS)¹¹⁻¹². According to this scheme, probable COVID-19 patients may present with typical and atypical chest CT findings¹³. Our study's relatively high number of patients may have provided sufficient power to achieve reliable statistical results. Of note, our findings are consistent with the report of De Smet et al.¹⁴.

In this study, we compared the chest CT findings of patients with $< 5\%$ lung involvement versus $\geq 5\%$ lung involvement to elucidate the effect of lung involvement on anti-SARS-CoV-2 IgG antibodies and titers. A significantly higher number of patients with anti-SARS-CoV-2 IgG positivity with high antibody titers had $\geq 5\%$ lung involvement. Based on these findings, we can speculate that antibody production and titers may be high in patients with severe COVID-19 infection. Also, anti-SARS-CoV-2 antibodies may persist after infection even in the long term, although it is an issue to be elucidated due to the scarcity of data in the literature.

Furthermore, our study evaluated all patients in terms of COVID-19 reinfection at 6 months. RT-PCR positivity was detected in three patients. In these cases, the RT-PCR test results were positive using nasopharyngeal swabs. Despite RT-PCR negativity at the time of the initial admission, reinfection was confirmed by RT-PCR positivity. Similarly, previous studies have shown varying amounts of RT-PCR positivity using nasopharyngeal swabs³⁻⁶. In two of three patients, anti-SARS-CoV-2 IgG negativity was detected. In the other patient, anti-SARS-CoV-2 IgG antibody titers were significantly higher at the time of diagnosis, as confirmed by RT-PCR positivity. Consistent with our findings, the COVID-19 reinfection rate was reported as 1.09/10,000 in antibody-negative cases and 0.13/10,000 in antibody-positive cases in the literature¹⁵. Ozturk et al. did a retrospective study on 320 patients

who had COVID-19 symptoms and were admitted to the hospital. The importance of this study was to show the accurate and equivalent performance of serological antibody assays and chest CT in diagnosing COVID-19 within 0–7 days from the onset of COVID-19 symptoms¹⁶.

CONCLUSION

This study is valuable as it highlights the evidence that increasing severity ($\geq 5\%$) of chest CT findings of lung involvement appears to be associated with high and persistent anti-SARS-CoV-2 IgG antibody titers 6 months after patients' discharge. In conclusion, even if the RT-PCR test is negative, anti-SARS-CoV-2 specific

IgG positivity may indicate previous evidence of COVID-19. After 6 months, high SARS-CoV-2 IgG titers can predict the rate of lung parenchymal involvement in probable cases of COVID-19 with negative PCR testing.

AUTHORS' CONTRIBUTIONS

IY: Data curation, Formal analysis, Writing – review & editing. **CK:** Data curation, Writing. **BS:** Data curation, Formal analysis, Software. **MK:** Data curation, Writing – original draft. **MT, BD:** Data curation, Supervision. **MZ, HO:** Data curation, Writing – review & editing. **OT:** Formal analysis. **MC:** Conceptualization, Data curation, Methodology, Writing – review & editing.

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Does pulmonary hypertension affect early-term outcomes of off-pump coronary artery bypass surgery?

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SUMMARY

OBJECTIVE: This study aimed to investigate the effect of preoperative pulmonary hypertension (PHT) on postoperative early mortality and morbidity in patients undergoing off-pump coronary artery bypass grafting (CABG).

METHODS: A total of 1107 patients undergoing elective first-time off-pump CABG between January 2011 and April 2022 were included in this retrospective observational cohort study. The patients were categorized into two groups according to their preoperative systolic pulmonary artery pressure (SPAP) values. The PHT group (n=104) consisted of patients with a SPAP value >30 mmHg, while the non-PHT group (n=1003) consisted of patients with a SPAP value ≤30 mmHg. Patients' preoperative demographics and clinical features, operative data, and postoperative outcomes were recorded and then compared between the groups.

RESULTS: In the PHT group, the median age was significantly higher (66 vs. 63 years, p=0.001) and the median left ventricular ejection fraction level was significantly lower (45 vs. 50%, p=0.045) as compared to the non-PHT group. Additionally, the PHT group included a significantly greater percentage of patients with chronic obstructive pulmonary disease (22.1 vs. 7.4%, p=0.019). As perioperative early-term outcomes, complications, and mortality were considered, the groups were statistically similar, and there were no significant differences between the groups, except for the development of atrial fibrillation.

CONCLUSION: For the first time in the literature, this study revealed that mild PHT (mean SPAP=38.9±8.7 mmHg) did not significantly affect early-term outcomes of off-pump CABG.

KEYWORDS: Pulmonary hypertension. Outcomes. Mortality. Morbidity. Coronary artery bypass grafting.

INTRODUCTION

Pulmonary hypertension (PHT) is defined as mean pulmonary arterial pressure (MPAP) ≥25 mmHg at rest, assessed by right cardiac catheterization¹. While various pathological conditions such as left-sided heart diseases, chronic pulmonary diseases, chronic thromboembolic PHT, and collagen tissue diseases are responsible for the etiology of PHT, it is always virtually related to worsening symptoms, increased morbidity, and mortality, regardless of underlying pathological conditions. PHT is reported to influence about 1% of the world's population, and more than half of the patients with cardiac failure might be influenced by this clinical entity^{2,3}. Thus, physicians may frequently expect to encounter patients with PHT in daily clinical practice. Moreover, cardiac surgeons may also frequently encounter patients with PHT in their surgical practice and operate on them as well.

The existence of PHT in patients scheduled for coronary artery bypass grafting (CABG) surgery could be easily

determined using cardiac catheterization or, more often, transthoracic echocardiography, which is a noninvasive, radiation-free, and contrast-free method. These aforementioned methods give an idea on possible perioperative risks and are also important to take preventive measures against potential adverse events. Although PHT has historically been considered as a significant risk factor for poor outcomes in patients undergoing CABG, there are a limited number of studies examining the effect of PHT on the outcomes of CABG surgery. Moreover, the limited studies on this subject in the literature are inconsistent and also contain some unanswered queries^{4,5}. On the contrary, almost all of these limited studies have been conducted in conventional on-pump CABG patients, and, to the best of our knowledge, there is no study in the literature investigating the effect of PHT on early-term outcomes of off-pump CABG. Thus, we designed this study to investigate whether PHT affects early-term surgical outcomes in patients undergoing off-pump CABG.

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METHODS

Ethical considerations

The study was started once approval was obtained from the institutional clinical research ethics committee (decision number: 2022/136, dated 10.05.2022) and carried out according to the ethical principles of the Declaration of Helsinki. All patients were informed about the operation and perioperative process, and their verbal and written consents were obtained.

Study population and design

The study was a retrospective observational cohort study and conducted on patients undergoing elective isolated first-time off-pump CABG from January 2011 to April 2022. The study population consisted of a total of 1107 patients whose medical data were available, and the patients were categorized into two groups according to their preoperative systolic pulmonary artery pressure (SPAP) values. One group consisted of patients with SPAP value >30 mmHg (PHT group, $n=104$) while the other group consisted of patients with SPAP value ≤ 30 mmHg (non-PHT group, $n=1003$), based on the 2015 European Society of Cardiology and the European Respiratory Society Guidelines⁶. Patients' preoperative basic demographics and clinical characteristics, operative data, postoperative outcomes, and complications were screened, recorded for the analysis, and then compared between the groups. Primary outcomes of the study included in-hospital mortality and major postoperative complications such as low cardiac output syndrome, myocardial infarction, cerebrovascular event, mediastinitis, pneumonia requiring reintubation, and new-onset renal failure requiring hemodialysis, while secondary outcomes of the study included mechanical ventilation time, inotrope and intra-aortic balloon pump requirements, lengths of intensive care unit (ICU), and hospital stays. Patients who underwent on-pump, emergency or redo CABG, conversion from off-pump to on-pump CABG during the surgery, concomitant cardiac or noncardiac surgery, and those with a history of primary or chronic thromboembolic PH were excluded from the study.

Determination of SPAP values

Estimates were based on the averages of three cardiac cycles in sinus rhythm and five in atrial fibrillation for each Doppler and M-mode-based measurement in preoperative transthoracic echocardiography. The simplified Bernoulli Equation was used to determine SPAP values from the continuous wave Doppler peak tricuspid regurgitation velocity with right atrial pressure (RAP). We included the densest tricuspid regurgitation jet with an apparent selected peak in the continuous wave Doppler.

The diameter and respiratory fluctuation in the diameter of the inferior vena cava (IVC) were used to calculate RAP: an IVC diameter of 21 mm that collapses $>50\%$ with a sniff indicated a typical RA pressure of 3 mmHg, an IVC diameter of >2.1 mm that collapses $>50\%$ with a sniff was considered an intermediate value of 10 mmHg, and an IVC diameter of >21 mm that collapses 50% with a sniff or 20% with silent inspiration was considered to have a high RA pressure of 15 mmHg.

Surgical procedure

All patients were operated on under general anesthesia and via standard median sternotomy. After the sternotomy and pericardiotomy, coronary arteries and ascending aorta were evaluated. The mostly harvested bypass grafts were internal thoracic artery (ITA), radial artery, and great saphenous vein. In patients with severe calcific and porcelain ascending aorta, the "no-touch aorta" technique was performed. Coronary anastomoses were initiated following intravenous heparin administration of 200 IU/kg and obtaining a target-activated clotting time of above 300 s. During the off-pump CABG technique, an Octopus IV tissue stabilizer was used to ensure the proper position of the beating heart. Left ITA-left anterior descending artery anastomosis was usually the first performed anastomosis to perfuse a significant myocardial area and avoid a sudden myocardial failure. Following an arteriotomy of the targeted coronary artery, a bulldog clamp and air blowing were used to provide a blood-free space during distal anastomosis. Distal and proximal anastomoses were performed with either 7/0 or 8/0, and either 6/0 or 7/0 propylene sutures, respectively. After all anastomoses were completed, the effect of unfractionated heparin was neutralized by protamine infusion, and then the operation was terminated in a standard manner.

Postoperative follow-up and medications

All patients were transferred to the ICU after the operation. During ICU follow-up, patients' invasive arterial and central venous pressures, heart rhythm, oxygen saturation, urine output, and mediastinal drainage were monitored continuously, and arterial blood gas analyses were performed at frequent intervals. Patients' hemodynamic data determined, if necessary, the initiation and maintenance of inotropic therapy and the choice of inotropic agents. Within the first 24 h after the operation, 100 mg acetylsalicylic acid, 75 mg clopidogrel, and low-molecular-weight heparin were prescribed if not contraindicated. The patients were transferred from the ICU to a standard ward once their hemodynamics were stabilized. If not contraindicated, other routine medications, including statins and β -blockers, were prescribed. Moreover, if a clinical indication

arises, angiotensin-converting enzyme inhibitors, diuretics, etc., were also added to the medical treatment.

Statistical analysis

The R software was used to perform the statistical analyses⁷. The Shapiro-Wilk test was used to evaluate the normality of variables. Continuous variables with normal and abnormal distribution were presented as mean±standard deviation and median (min-max) values, respectively. Categorical variables were expressed as numbers (percentages). Continuous variables were analyzed using the Mann-Whitney U test, while categorical variables were analyzed using the chi-square test. A p-value <0.05 was regarded as statistically significant for all analyses.

RESULTS

Patients with PHT comprised 9.4% of whole study population (n=104/1107). The mean SPAP value in the PHT group was detected as 38.9±8.7 mmHg (range: 32–70).

When preoperative basic clinical features of the groups were evaluated, the median age in the PHT group was significantly higher as compared to that in the non-PHT group (66 vs. 63 years, p=0.001). The median left ventricular ejection fraction (LVEF) level in the PHT group was significantly lower than that in the non-PHT group (45% vs. 50%, p=0.045). Additionally, the PHT group included a significantly greater percentage of patients with chronic obstructive pulmonary disease (COPD) as compared to the non-PHT group (22.1% vs. 7.4%, p=0.019). In terms of other clinical characteristics and comorbid diseases, both groups were statistically similar, and there were no significant differences between the groups (Table 1).

When operative and postoperative data of the groups were compared, it was detected that only percentage of new-onset atrial fibrillation (AF) in the PHT group was significantly greater than that in the non-PHT group (41.3% vs. 22.1%, p=0.033). Apart from this, no significant differences were found between the groups in terms of intraoperative variables, perioperative outcomes, major postoperative complications, and in-hospital mortality (Table 2).

DISCUSSION

In this study, we investigated whether PHT affects the early-term outcomes of off-pump CABG by comparing the groups of patients with and without PHT. Patients' preoperative basic clinical and demographic data as well as the intraoperative and postoperative early results were compared between the groups. The most important finding of the study was that PHT did

Table 1. Preoperative clinical characteristics of groups.

Variable	PHT group (n=104)	Non-PHT group (n=1003)	p-value
Age (year)	66 (40–82)	63 (35–87)	0.001*
Gender (male)	43 (41.3%)	376 (37.5%)	0.067
Weight (kg)	77 (50–118)	77 (48–140)	0.646
Height (cm)	170 (150–190)	170 (145–190)	0.815
BMI (kg/m ²)	27.2 (18.5–42.5)	27.0 (18.0–46.2)	0.560
Obesity	24 (23.1%)	219 (21.8%)	0.277
LMCA disease	20 (19.2%)	172 (17.1%)	0.218
LVEF level (%)	45 (25–70)	50 (25–70)	0.045*
Hypertension	75 (72.1%)	647 (64.5%)	0.246
Diabetes mellitus	44 (42.7%)	375 (37.4%)	0.322
Hyperlipidemia	46 (44.2%)	392 (39.1%)	0.284
Myocardial infarction	32 (30.8%)	331 (33.0%)	0.468
Chronic renal dysfunction	10 (9.6%)	67 (6.7%)	0.330
Chronic liver disease	1 (0.9%)	2 (0.2%)	1.000
Peripheral arterial disease	13 (12.5%)	95 (9.5%)	0.383
COPD	23 (22.1%)	74 (7.4%)	0.019*
Previous PCI	17 (16.3%)	132 (13.2%)	0.352
Previous CVE	13 (12.5%)	70 (7.0%)	0.514
Smoking	35 (33.6%)	283 (28.2%)	0.380

PHT: pulmonary hypertension; BMI: body mass index; LMCA: left main coronary artery; LVEF: left ventricular ejection fraction; COPD: chronic obstructive pulmonary disease; PCI: percutaneous coronary intervention; CVE: cerebrovascular event. *Significant p-value.

not adversely affect early perioperative results, significant morbidity, and mortality in patients undergoing off-pump CABG, except for new-onset AF.

The existence of PHT has been generally considered to be an important risk factor affecting short- and long-term outcomes following heart surgery. Considering the recent advancements in the management of PHT following heart surgery, a greater comprehension of its effect on the results might help in the perioperative clinical management of patients with PHT. Determining the existence of PHT by simply measuring the pulmonary artery pressures with transthoracic echocardiography is important in terms of providing an insight concerning possible operative and postoperative risks and taking necessary precautions against these risks⁸.

Some risk factors for cardiovascular diseases as well as comorbidities may be present in patients with PHT. Fan et al.⁹

Table 2. Intraoperative and postoperative data of groups.

Variable	PHT group (n=104)	Non-PHT group (n=1003)	p-value
LITA usage	97 (93.3%)	943 (94.0%)	0.820
Complete revascularization	96 (92.3%)	947 (94.4%)	0.484
Number of distal bypass	4 (1-5)	4 (1-7)	0.556
Extubation time (h)	6 (2-12)	6 (2-48)	0.145
Length of ICU stay (h)	24 (24-216)	24 (24-504)	0.083
Length of hospital stay (day)	5 (4-21)	5 (4-39)	0.227
Inotrope requirement	24 (23.1%)	183 (18.2%)	0.103
IABP requirement	9 (8.6%)	49 (4.9%)	0.249
Low cardiac output syndrome	7 (6.7%)	53 (5.3%)	0.508
Myocardial infarction	5 (4.8%)	32 (3.2%)	0.465
Cerebrovascular event	2 (1.9%)	35 (3.5%)	0.520
Reintubation	5 (4.8%)	35 (3.5%)	0.214
Pneumonia	7 (6.7%)	34 (3.4%)	0.177
Mediastinitis	4 (3.8%)	32 (3.2%)	1.000
Re-exploration for bleeding	4 (3.8%)	40 (4.0%)	1.000
ARD requiring hemodialysis	2 (1.9%)	28 (2.8%)	0.563
Gastrointestinal bleeding	1 (0.9%)	9 (0.9%)	1.000
New-onset atrial fibrillation	43 (41.3%)	222 (22.1%)	0.033*
In-hospital mortality	2 (1.9%)	20 (2.0%)	1.000

PHT: pulmonary hypertension; LITA: left internal thoracic artery; ICU: intensive care unit; IABP: intra-aortic balloon pump; ARD: acute renal dysfunction. *Significant p-value.

conducted a retrospective study on a large sample size involving a total of 5401 patients with acute myocardial infarction (AMI), and patients were grouped according to their SPAP values at the time of admission. The study revealed that SPAP was related to age, low LVEF, Killip classification, and AMI site, and was also detected to be significantly associated with 6-month cardiac death after AMI. The authors eventually concluded that elevated SPAP values could be a useful marker to predict early-term prognosis following AMI. We determined that elevated SPAP value, namely PHT, was related to age and low LVEF in our study, consistent with the aforementioned study. In addition to these, COPD was also detected to be a frequent comorbid disease in the PHT group in our study population.

When the limited number of studies investigating the effect of pulmonary artery pressures and PHT on surgical outcomes

after cardiac surgery were reviewed, it was observed that the studies included some methodological differences, and the results of the studies were inconsistent with each other. In the literature, some of these limited studies investigating the effect of PHT on surgical outcomes were conducted in patients undergoing conventional on-pump CABG^{5,10,11}, while others were conducted on patients undergoing valvular heart surgery^{12,13}. In a study conducted on 177 patients undergoing conventional on-pump CABG by Çatav et al.¹⁰, the patients were divided into three groups according to their SPAP values. Patients with an SPAP <30 mmHg were assigned to the normal SPAP group, those with an SPAP between 30 and 50 mmHg were assigned to the mild PHT group, and those with an SPAP >50 mmHg were assigned to the severe PHT group. The rates of in-hospital mortality were found to be 4.7%, 10%, and 18.9% for normal SPAP, mild PHT, and severe PHT groups, respectively, and the difference between the hospital mortalities of normal SPAP and severe PHT groups was significant. Akça et al.⁵ conducted a study with a larger sample size involving a total of 1244 patients undergoing conventional on-pump CABG, and the patients were divided into two groups according to the existence of PHT (SPAP ≥30 mmHg) or absence of PHT (SPAP <30 mmHg). The study revealed that early-term mortality rates following on-pump CABG were statistically similar, and there was no significant difference between both groups with respect to perioperative early mortality, while the PHT group had a greater inotrope requirement, a longer mechanical ventilation time, and an increased length of ICU stay. Moreover, Akca et al.¹¹ assessed the effect of preoperative PHT on not only early-term but also long-term results by screening a larger patient population. Among 2325 patients undergoing elective isolated conventional on-pump CABG, 287 patients with high preoperative SPAP ≥30 mmHg were evaluated, and of them, 69 patients with complete data who were on follow-up were included in their study. The authors found that the long-term mortality rate was 5.79% during 33.9±17 (9-100) months follow-up period, and life expectancy was calculated as 94.7 months. As a result of these findings, PHT was indicated to be not associated with poor long-term outcomes, and CABG could be safely performed in patients with PHT. Melby et al.¹² studied whether PHT remains a risk factor for adverse outcomes on a total of 1080 patients undergoing surgical aortic valve replacement (AVR) for primary severe aortic valve stenosis. The patients were prospectively assessed and then divided into two groups according to the existence of PHT (SPAP ≥35 mmHg) or absence of PHT (SPAP <35 mmHg). The study demonstrated that operative mortality, prolonged ventilation, and length of hospital stay were significantly higher in the PHT

group, and preoperative PHT was an independent risk factor for decreased long-term survival in patients undergoing AVR. Ghoreishi et al.¹³ examined the impact of preoperative PHT on short- and long-term results in 873 patients undergoing mitral valve surgery for mitral regurgitation. In their study, PHT was classified as none (SPAP value <40 mmHg), mild ($40 \leq$ SPAP value <50 mmHg), moderate ($50 \leq$ SPAP value <60 mmHg), or severe (SPAP value \geq 60 mmHg), and preoperative SPAP was detected to be a strong predictor of both early mortality and late survival following mitral valve surgery. In another study reviewing a total of 3343 cardiac surgery patients, the effect of PHT on perioperative morbidity and mortality, and the accuracy of the Society of Thoracic Surgeons (STS) risk model for patients with PHT were assessed. In the study, MPAP was used to determine the existence or absence of PHT, and MPAP was defined as normal (<25 mmHg), or as mild (25–34 mmHg), moderate (35–44 mmHg), or severe (\geq 45 mmHg) PHT. The study showed that perioperative complications and mortality increased with higher MPAP values, and both moderate and severe PHT were significantly related to increased mortality, even after accounting for STS risk. In addition, a subgroup analysis for only isolated CABG patients showed significantly increased mortality for all PHT categories (mild, moderate, and severe). The authors consequently deduced that the observed mortality rate was significantly greater than predicted by the STS model for patients with moderate and severe PHT, and the addition of PHT to the STS risk model should be considered especially in isolated CABG cases⁴.

In the aforementioned studies, it was noted that the determined pulmonary artery pressure values for dividing the patients into groups were different for each study, and there was no standardization for PHT classification and definition in the existing literature. This lack of standardization can be considered as one of the possible reasons why the results of these studies are different and inconsistent. In our study, the 2015 European Society of Cardiology and the European Respiratory Society Guidelines⁶ were considered when grouping patients

according to whether they had PHT or not, and pulmonary artery pressure values in the guidelines were taken into account.

The most important feature of our study that can make a significant contribution to the existing literature was that it was conducted in a relatively large patient population undergoing off-pump CABG. To the best of our knowledge, this is the first study that investigates the effect of PHT on early-term outcomes of off-pump CABG. On the contrary, our study also has several limitations. The main limitations of the study are its single-centered design and retrospective nature. Another important limitation is the lack of a subgroup analysis in the form of mild, moderate, or severe PHT in the group of patients with PHT.

CONCLUSION

This study demonstrated for the first time in the literature that mild PHT had no significant effect on early-term surgical outcomes, including mortality and major postoperative complications, in patients undergoing off-pump CABG. However, further prospective well-designed studies with larger patient participation are required to support the results of our study and obtain more evident scientific information.

AUTHORS' CONTRIBUTIONS

YV: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft. **AY:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft. **DT:** Data curation, Investigation, Methodology, Supervision, Validation, Writing – original draft. **UTKK:** Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. **ID:** Data curation, Investigation, Methodology, Validation, Writing – review & editing. **SB:** Investigation, Supervision, Validation, Writing – review & editing. **ERU:** Methodology, Supervision, Writing – review & editing.

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Associations between muscle strength, dyspnea and quality of life in post-COVID-19 patients

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SUMMARY

OBJECTIVE: Patients with severe coronavirus disease 2019 (COVID-19) develop high muscle weakness. The objective of this study was to analyze the physical fitness of post-COVID-19 patients and its relationship with dyspnea and health-related quality of life (HrQoL).

METHODS: This observational, retrospective, cross-sectional study was conducted between October and November 2021 in the Universidad Europea de Madrid (Spain), with 32 post-COVID-19 patients aged 63.2 (14.1) years. Muscle strength, aerobic capacity, maximal respiratory mouth pressures, dyspnea, and HrQoL were analyzed 6–12 months after discharge for COVID-19. To analyze the relationship between continuous variables, Spearman's correlation test and Pearson's correlation test were performed.

RESULTS: The participants had a mean handgrip strength of 22.1 (9.0) kg and very poor HrQoL. Negative moderate correlations were found between handgrip strength and length of hospital and intensive care unit stay ($r=-0.37$; $p=0.002$). In addition, muscle strength was negatively correlated with dyspnea ($r=-0.37$; $p=0.008$) and HrQoL, and moderate-large negative correlations were found between dyspnea and HrQoL.

CONCLUSION: Higher handgrip strength was associated with lower COVID-19 severity and less sequelae. Therefore, either the patients with severe COVID-19 suffered greater muscle breakdown, or higher muscle strength acted as a mitigating factor for the disease. It is suggested that post-COVID-19 rehabilitation programs should focus on increasing muscle strength. Also, adequate physical fitness could mitigate the physical and mental post-COVID-19 sequelae.

KEYWORDS: COVID-19. SARS-CoV-2. Muscle strength. Hand strength. Quality of life.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a viral infection that causes severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and, in severe cases, induces severe systemic inflammation, causing lung damage and respiratory distress, requiring hospitalization, and sometimes even admission to intensive care units (ICUs)¹.

The chronic fatigue syndrome is one of the more frequent post-COVID sequelae, possibly due to the significant muscle mass reductions that have been observed in COVID-19 patients after 20 days admitted to the ICU². These findings have also been reported in hospitalized patients during the first SARS-CoV epidemic, almost two decades ago, with significant decreases in handgrip strength and the distance covered during the 6-min walk test (6MWT), several months after hospital discharge³. Significant muscle mass and muscle strength loss have also been reported in other patients admitted to the ICU, possibly due to a decrease in protein synthesis and an increase in muscle breakdown, resulting in a drastic increase in protein catabolism⁴.

At present, COVID-19 infection is thought to cause muscle breakdown due to systemic inflammation, the so-called cytokine storm. Indeed, C-reactive protein, IL-6, IL-1b, or alpha-TNF can directly induce muscle proteolysis and decrease protein synthesis, especially when the inflammation is associated with the lung⁵. COVID-19 patients admitted to the ICU present higher C-reactive protein levels compared to other patients admitted to the ICU and also a direct correlation between inflammation and muscle breakdown⁶.

Patients affected by a more severe COVID-19 are those who develop higher muscle weakness⁷ and also those with higher levels of inflammatory markers⁸. Another fact to highlight is that elderly patients, who are the majority of patients with severe COVID-19, already have a situation of muscle breakdown and systemic inflammation, even reaching sarcopenia⁹. On the contrary, physical exercise exerts an anti-inflammatory effect¹⁰, so possibly more active people or people with better physical fitness suffer a milder COVID-19 and less severe sequelae, such as dyspnea or a decrease in the health-related quality of life (HrQoL)¹¹.

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The objective of this study was to analyze the physical fitness of post-COVID-19 patients and its relationship with dyspnea and HrQoL.

METHODS

Study design

This observational, retrospective, cross-sectional study was conducted between October and November 2021.

Settings and participants

A total sample of 32 post-COVID-19 patients were recruited via email between October and November 2021 in the Universidad Europea de Madrid (Spain). A code was assigned to participants prior to statistical analysis, thus guaranteeing the confidentiality of their data. The inclusion criteria were having being discharged from COVID-19 infection (diagnosed with a PCR test) 6–12 months ago and not having fever or symptoms of re-infection at the present moment.

Ethical considerations

This study respected the Helsinki guidelines at all times and followed the ethical standards of Spain. All the participants read and signed an informed consent statement before becoming part of this investigation.

Measurements

Anthropometric variables: Height and weight were measured using a stadiometer and a scale. Then, the body mass index (BMI, in kg/m²) was calculated.

Muscle strength was assessed through the Medical Research Council (MRC) scale, which grades the patient's strength from 0 (no muscle activation) to 5 (muscle activation against examiner's full resistance, full range of motion) on 12 different muscle groups¹². Also, *handgrip strength* was measured using the Takei 5001 Hand Grip analog dynamometer. Handgrip strength has a large correlation with general strength, functionality, and all-cause mortality. A score <27 kg is considered a risk factor for all-cause mortality¹³.

Aerobic capacity was assessed through the 6MWT, which is a sub-maximal exercise test, 126 where the distance covered walking over a time of 6 min is used as the outcome (in m)¹⁴.

To assess respiratory muscles function, *maximal inspiratory pressure* (MIP) and *expiratory mouth pressure* (MEP) were measured using the Micro Respiratory Pressure Meter (FS985, Micro Medical, Los Angeles, CA, USA). The participants performed the maneuvers in a sitting position with a stuffy nose through

a clamp that prevented air leaks and a straight back, following the protocol of the “Sociedad Española de Pneumología y Cirugía Torácica.” The highest values of MIP and MEP were recorded and expressed in cm of H₂O¹⁵.

Dyspnea was assessed through the modified MRC scale, which is a self-rating tool to measure the degree of disability that breathlessness poses on day-to-day activities on a scale from 0 (no breathlessness, except on strenuous exercise) to 4 (too breathless to leave the house, or breathless when dressing or undressing)¹⁶.

Health-related quality of life: To assess HrQoL, the participants filled out the Short Form-36 Health Survey Questionnaire v2 (SF-36). It consists of 36 items, which gives a score from 0 (worst self-perception of HrQoL) to 100 (best self-perception of HrQoL) in 8 sections, which are regrouped into two main components: Physical Component Summary and Mental Component Summary¹⁷.

A 30-day mortality probability during the COVID-19 infection was measured using the SEIMC COVID-19 score, which consists of five items that give a total score from 0 (lowest risk) to 30 (highest risk), where age of the participants >40 years is the more relevant item¹⁸.

Respiratory severity score during the COVID-19 infection was measured with the Brescia respiratory severity scale, which simplifies the clinical summary of a patient's status, providing a score from 0 (lowest severity) to 8 (highest severity)¹⁹.

The *length of hospital stay and the length of stay in the ICU* were recorded, expressed in days. These variables, along with the 30-day mortality probability and the respiratory severity score, were given by the hospital discharge report, which was provided by the participants.

Statistical analysis

Shapiro-Wilk test was employed to assess the normality. Then, a descriptive analysis was developed for all the subjects using mean (standard deviation) (SD) for the parametric variables and median (interquartile) for nonparametric variables. In addition, to analyze the relationship between continuous variables, Spearman's correlation test and Pearson's correlation test were performed for the nonparametric and parametric variables, respectively. The magnitudes of correlation between continuous variables were qualitatively interpreted using the following criteria: trivial ($r \leq 0.1$), small ($r = 0.1 - 0.3$), moderate ($r = 0.3 - 0.5$), large ($r = 0.5 - 0.7$), and very large ($r = 0.7 - 0.9$). After Bonferroni correction was applied, the statistical significance was set at an alpha level of <0.01. All analyses were conducted using IBM SPSS for Windows (version 27, IBM Corp., Armonk, NY, USA).

RESULTS

Sociodemographic data of the sample

First, 43 patients were recruited via email, and 11 (26%) were excluded because the COVID-19 infection had occurred more than 12 months ago. Finally, a total of 32 participants aged 63.2 (14.1) years (59% female) were analyzed. Their mean BMI was 26.6 (3.7) kg/m². Most of the participants (78%) required hospital stay, with a mean length of 37.0 (48.0) days, while 12.5% of the participants required ICU stay for 45.8 (18.4) days (Table 1).

Muscle strength and aerobic capacity

The participants had a mean handgrip strength of 22.1 (9.0) kg. Regarding aerobic capacity, the mean distance covered in the 6MWT was 373.5 (114.0) m (Table 2).

Table 1. Characteristics of the study population (n=32) and COVID-19 severity.

Variables	Mean (SD) or median (IQR)
Age	63.2 (14.1)*
Gender	n=13 male n=19 female
Body mass index (kg/m ²)	26.6 (3.7) [†]
Respiratory severity score (Brescia scale)	2.1 (2.5)*
30-Day mortality probability (SEIMC score)	7.8 (5.7)*
Duration of hospital stay (days) (n=25 patients)	37.0 (48.0)*
Duration of intensive care unit stay (days) (n=4 patients)	45.8 (18.4)*

IQR: interquartile range. *Values are represented as mean (standard deviation) (SD). [†]Values are represented as median (IQR).

Table 2. Muscle strength, aerobic capacity, dyspnea, and quality of life in 32 post-COVID-19 patients.

Variables	Mean (SD) or median (IQR)
Dyspnea score	1.0 (0.0)*
Maximal inspiratory pressure (cm H ₂ O)	53.0 (27.3) [†]
Maximal expiratory pressure (cm H ₂ O)	61.5 (26.0) [†]
Handgrip strength (kg)	22.1 (9.0) [†]
Muscle strength (MRC scale)	60.0 (0.0)*
6-Min walk test (m)	373.5 (114.0) [†]
SF-36 Physical Component Summary	36.8 (9.2) [†]
SF-36 Mental Component Summary	36.6 (13.3) [†]

IQR: interquartile range. *Values are represented as mean (standard deviation) (SD). [†]Values are represented as median (IQR).

Respiratory muscles function

The participants had an MIP of 53.0 (27.3) cm H₂O and an MEP of 61.5 (26.0) cm H₂O (Table 2).

Health-related quality of life

Regarding HrQoL, the mean values of the summary scores were as follows: Physical Component Summary 36.8 (9.2) and Mental Component Summary 36.6 (13.3) (Table 2).

30-Day mortality and respiratory severity score

The mean SEIMC COVID-19 score was 7.8 (5.7), and the Brescia respiratory severity score was 2.1 (2.5) (Table 1).

Correlations between the continuous variables

Moderate-large negative correlations were found between dyspnea and muscle strength MRC ($r=-0.37$; $p=0.008$) and between dyspnea and the Mental Component Summary (HrQoL) ($r=-0.41$; $p=0.006$) (Table 3).

Handgrip strength showed negative moderate correlations with 30-day mortality ($r=-0.36$; $p=0.001$), length of hospital stay ($r=-0.37$; $p=0.002$), and length of ICU stay ($r=-0.37$; $p=0.002$). Also, there was a negative moderate correlation between the duration of ICU stay and muscle strength MRC ($r=-0.42$; $p=0.007$) (Table 3).

Age showed positive large correlations with length of hospital stay ($r=0.56$; $p=0.001$) and 30-day mortality ($r=0.84$; $p=0.000$) (Table 3).

Finally, the 6MWT score showed a positive moderate correlation with the Physical Component Summary (HrQoL) ($r=0.36$; $p<0.001$) (Table 3).

DISCUSSION

Some participants of this study had a mild COVID-19 and recovered at home, while most of the participants had a severe COVID-19 and required hospital stay or ICU stay for a month, probably because they were older adults. The participants were evaluated 6–12 months after discharge for COVID-19.

Also, the participants had low handgrip strength, below the cutoff point of 27 kg, which is considered a risk factor for all-cause mortality¹³. Also, they had a poor performance in the 6MWT. In addition, according to the predictive equation of the MIP and MEP values of the participants should be 67.6 and 76.2 cm H₂O, respectively. So, the participants presented 22% lower values of MIP and 19% lower values of MEP, compared to the predictive values of the equation of Souto-Miranda et al.²⁰ These findings have also been reported in other observational studies with post-COVID-19 patients²¹.

Table 3. Correlations between the continuous variables.

		Brescia	SEIMC-score	Length of hospital stay	Length of ICU stay	Dyspnea	Age	Hand grip	6MWT	SF-36 physical	SF-36 mental
Brescia	Pearson r		0.27	0.76*	0.85*		0.27	-0.24	-0.37	-0.22	0.34
	p-value		0.122	0.000	0.000		0.128	0.185	0.048	0.193	0.285
SEIMC-score	Pearson r			0.33	0.11		0.84*	-0.36*	-0.42	0.17	0.15
	p-value			0.063	0.547		0.000	0.001	0.025	0.587	0.247
Length of hospital stay	Pearson r				0.82*		0.342	-0.37*	-0.52	-0.62	-0.43
	p-value				0.000		0.051	0.009	0.041	0.023	0.034
Length of ICU stay	Pearson r						0.56*	-0.37*	-0.35	-0.68	-0.46
	p-value						0.001	0.002	0.063	0.051	0.061
Dyspnea	Spearman r	-0.21	-0.31	-0.41	-0.05		-0.24	0.14	-0.11	-0.46	-0.41*
	p-value	0.131	0.092	0.020	0.800		0.188	0.461	0.566	0.043	0.006
Age	Pearson r							-0.23	-0.51	-0.11	-0.13
	p-value							0.204	0.041	0.275	0.185
Hand grip	Pearson r								0.39	0.36	0.41
	p-value								0.038	0.028	0.078
MRC scale	Spearman r	-0.11	-0.04	-0.56	-0.42*	-0.37*	0.08	0.22	0.41	0.26	0.34
	p-value	0.509	0.853	0.151	0.007	0.008	0.646	0.219	0.027	0.098	0.28
6MWT	Pearson r									-0.36*	0.39
	p-value									0.000	0.13

ICU: intensive care unit; 6MWT: 6-min walking test. Bold indicates statistically significant values. *Statistical significance was set at an alpha level of <0.01.

Handgrip strength showed inverse moderate correlations with the 30-day mortality, length of hospital stay, and length of ICU stay. In addition, there was a negative moderate correlation between the duration of ICU stay and muscle strength MRC. Interestingly, muscle weakness was seen mainly in patients with a more severe COVID-19 disease, as reported in other studies⁷. In contrast, physical exercise and handgrip strength are associated with lower inflammation¹⁰, so perhaps patients with better physical fitness had some protection against COVID-19 since the most severe forms of this disease develop in patients with higher levels of inflammation⁵.

Most severely affected COVID-19 patients are elderly who have frequently high levels of systemic inflammation and muscle breakdown, even reaching sarcopenia⁸. Interestingly, in this study, the age of the participants showed a positive large correlation with the length of hospital stay and a negative moderate correlation with the handgrip strength, so the older patients presented a more severe COVID-19 and had lower levels of muscular strength.

According to the dyspnea scale, the participants had “shortness of breath when hurrying on the level or walking up a slight

hill,” which showed an inverse negative correlation with muscle strength MRC and could have had a great negative impact on HrQoL, as negative moderate-large correlations were found between dyspnea and HrQoL. In turn, 6MWT showed a positive moderate correlation with HrQoL. The participants of this study were in the 10th percentile of Physical and Mental Component Summaries, compared to the reference values of their age²², so they had very poor HrQoL.

Therefore, the physical and mental sequelae that the post-COVID-19 patients present may depend largely on muscular strength and aerobic capacity. Physical exercise could, therefore, be a mitigating factor for this disease. In turn, post-COVID-19 rehabilitation programs should focus on muscle strength to reduce dyspnea and increase the HrQoL.

Despite the relevant findings found in this study, the main limitation is that it is an observational cross-sectional study, and causalities cannot be established. In addition, some variables were measured with self-reported tools, which can lead to potential bias or subjectivity. However, muscle strength should be addressed in the rehabilitation of the

post-COVID-19 sequelae and in the prevention of severe affections of the disease.

CONCLUSION

Higher handgrip strength was associated with lower COVID-19 severity and lower mortality. Therefore, either the patients with severe COVID-19 suffered greater muscle breakdown, or higher muscle strength acted as a mitigating factor for the disease. Also, lower muscle strength was associated with higher dyspnea, and higher dyspnea was associated with lower HrQoL. Adequate physical fitness could mitigate the physical and mental post-COVID-19 sequelae. In turn,

post-COVID-19 rehabilitation programs should focus on increasing muscle strength.

AUTHORS' CONTRIBUTIONS

GGPS: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, supervision, validation, visualization, writing – original draft, writing – review & editing.

BSP: Conceptualization, data curation, investigation, methodology, project administration, resources, supervision, validation, supervision, validation, visualization, writing – original draft, writing – review & editing.

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Effects of therapeutic ultrasound and paraffin with or without vacuum massage on biomechanical properties of grafted skin after burn: a randomized controlled trial

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SUMMARY

OBJECTIVE: This study aimed to compare the effects of therapeutic ultrasound and paraffin with or without vacuum massage on the biomechanical properties of grafted skin after a burn.

METHODS: A total of 44 patients with deep second- and third-degree burns, with a mean age of 35.89 (± 11.53) years, who visited the Hospital Burn Unity, were included in the study. The therapeutic interventions were randomly defined by drawing lots, with a crossover design (crossover), and a minimum interval of 7 days (washout) between interventions. Skin biomechanical parameters such as distensibility (R0) and viscoelasticity (R6) were noninvasively evaluated by Cutometer before and after 0, 10, 20, and 30 min of intervention with therapeutic ultrasound and paraffin alone, as well as associated with negative pressure therapy of the skin (vacuum therapy). In this study, all groups showed increased distensibility (R0) in the period immediately after the application of the resources and a progressive reduction in the effects in the consecutive tests. Participants with skin grafts showed a decrease in viscoelasticity (R6) in all groups, except therapeutic paraffin and therapeutic ultrasound and vacuum massage.

CONCLUSION: The biomechanical properties of grafted skin after a burn are altered after therapeutic intervention with ultrasound alone or associated with vacuum massage, such as intervention with paraffin associated with vacuum massage, for both parameters evaluated, skin distensibility (R0) and skin viscoelasticity (R6). However, the same did not occur for the intervention with isolated paraffin. There was no significant difference between the interventions therapeutic ultrasound and therapeutic paraffin.

KEYWORDS: Skin transplantation. Burns. Physical therapy modalities. Ultrasonic therapy.

INTRODUCTION

Therapeutic interventions in the burned individual aim at reducing sequelae and disability, involving an increase in the distensibility of the patient and a consequent increase in functionality¹. The healing process that occurs post-burn is known to cause a reduction in tissue flexibility. Even in grafted areas, retractions are common in particular regions, resulting in contractures. Although burned skin does not exhibit the same biomechanical characteristics as normal skin, therapeutic approaches may help to minimize these sequelae, improving the quality of life for these patients².

The rehabilitation of burned patients involves specific behaviors in both the prevention and recovery of certain movements, using different therapeutic techniques^{3,4}. Therapeutic

ultrasound (TU) is used in the rehabilitation of scar tissue when there is a high concentration of collagen. There is a thermal effect, promoting an increase in the extensibility of the collagen, increasing the distensibility of the skin and the range of motion of the affected joints^{5,6}. This effect is also attributed to the use of therapeutic paraffin (TP), which increases superficial blood flow and the extensibility of collagen fibers, improving tissue mobility⁷. Vacuum massage is a noninvasive mechanical massage technique performed with a device that lifts the skin using suction. This is thought to promote mobilization of the skin, increasing tissue distensibility^{8,9}. This technique produces additional stretching of the skin when combined with heating using either ultrasound or paraffin. Therapeutic interventions with ultrasound often precede other therapeutic modalities

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such as kinesiotherapy as well as manual and assisted massage, aiming to increase tissue distensibility first. However, the durability of the effects seen with therapeutic interventions has not yet been established.

The importance of mobilization of the burned patients in the rehabilitation process is known; however, if performed inappropriately, it can produce lesions in the areas affected by the burn, which are naturally rigid, increasing the recovery period. Thus, it is important to know the effects of those therapeutic interventions on tissue distensibility^{10,11}. Therefore, the objective of this study was to compare the effects of TU and TP with or without vacuum massage on the biomechanical properties of skin and grafted skin after a burn.

METHODS

Study design and ethical aspects

A randomized controlled trial was conducted at the Burn Unity of the Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Brazil. The protocol was approved by the University's Institutional Ethic Committee for the research (13386/2011) and registered in Clinical Trials (NCT02185950). Patients were duly informed about the procedures used in the study and signed the consent form.

Inclusion criteria were patients aged between 18 and 60 years presenting burns in the upper limbs, with at least 3 months of postoperative healing of a skin graft (0.2 mm thick), normal skin site in the contralateral limb, without signs of infection, and with no limitations of upper limb movements. Noninclusion criteria were burn patients aged over 60 years and infection (fever in the last 24 h) and limitation of upper limb movements.

In this study, the Cutometer 580® (Courage-Khazaka electronic GmbH, Cologne, Germany) was used to assess the biomechanical characteristics of the skin, such as distensibility and viscoelasticity. Participants and their skin graft areas were divided into four groups according to the different therapeutic interventions applied: Group 1, TU—therapeutic ultrasound; Group 2, TP—paraffin therapy; Group 3, TUV—therapeutic ultrasound and vacuum massage; and Group 4, TPV—therapeutic paraffin and vacuum massage.

The design of this study of therapeutic interventions was a crossover, and the evaluated areas were submitted to all therapeutic procedures with the application of the resources randomly assigned, with an interval of 7 days (washout) to avoid interference between each intervention. The sample was calculated using the software *Ene*, version 3.0 (Autonomous University of Barcelona, Barcelona, Spain).

Therapeutic methods

Therapeutic interventions with ultrasound (TU) and paraffin (TP) alone or associated with vacuum massage (TUV and TPV) were applied in delimited areas with a size of 9×5 cm, followed by evaluations immediately after the applications and after 10, 20, and 30 min. The TU was applied for 4 min at a frequency of 3 MHz, the intensity of 1 W/cm², in continuous mode with the transducer in a circular motion. Paraffin was applied in four layers, heated to 38.5°C in specific equipment (Carci, São Paulo, Brazil), and remaining for 20 min in the place to be evaluated.

For TUV and TPV, the vacuum massage was performed with the Dermotonus Slim® equipment (IBRAMED, Amparo, São Paulo, Brazil), with continuous negative pressure of 250 mmHg and a 1.8-cm diameter glass accessory for 4 min with longitudinal slips.

Biomechanical properties of the skin

The biomechanical properties of the skin were evaluated in areas with skin sheet grafting at least 3 months postoperative in the upper limbs of the patient. The evaluations were carried out in a controlled environment, in the supine position, always in the morning, to avoid chronobiological interference in the skin characteristics¹².

Biomechanical characteristics of the skin were evaluated before the application of the resources in both areas: the area of the scar (grafted) and in the control area (contralateral intact, normal skin) and after 0 (P0), 10 (P10), 20 (P20), and 30 (P30) min after intervention. The evaluation of the biomechanical properties of the skin, such as distensibility and viscoelasticity, was performed with the Cutometer MPA 580® equipment (Courage-Khazaka electronic GmbH, Cologne, Germany), calibrated with a 2-mm diameter probe and a 500 mbar (375 mmHg) vacuum. The probe was attached to the area to be evaluated with 1 s of suction and 1 s of relaxation, in three cycles, configuring three tissue deformation curves. The vertical deformation was evaluated in mm using constant negative suction. The parameters R (relative) are automatically calculated in mm, where R0 corresponds to distensibility and represents the passive behavior of the skin to force, with low values representing greater firmness, and R6 to viscoelasticity.

Statistical analyses

The data for the variables, distensibility and viscoelasticity, were normalized by the Shapiro-Wilk test. The Friedman test was used for intragroup comparisons over time, and the Kruskal-Wallis test followed by the Dunn post-hoc test was used for intergroup comparisons between the different interventions and had a significance level of 5% ($p < 0.05$). All processing was

performed in the *Statistical Package for Social Sciences (SPSS)*, version 17.0 (Chicago, IL, USA).

RESULTS

A total of 44 burned patients participated in the study, with a predominance of men (59.1%) and a mean age of 35.8 (± 11.5) years. We evaluated 140 areas with skin grafts with $p < 0.05$, stimulated with different therapeutic interventions: intervention with ultrasound (UT), intervention with paraffin (PT), intervention with ultrasound and vacuum (TUV), and intervention with paraffin and vacuum (TPV).

Although controversial, factors that may interfere in the assessment of the biomechanical characteristics of the skin, such as color, age, and sex, are pointed out¹³⁻¹⁶. Skin color showed no significant difference for variables R0 (0.31 ± 0.08 , 0.30 ± 0.09 , and 0.3 ± 0.1 , $p = 0.44$) and R6 (0.5 ± 0.08 ; 0.4 ± 0.1 , and 0.4 ± 0.1 , $p = 0.43$), considering the control group for black, brown, and white, respectively.

A significant increase in total distensibility was observed in the normal skin of the TU group immediately after application (P0), when compared to the pre-intervention; however, there was a reduction in this variable over time (P30) (Table 1). The TPV treatment group showed a significant difference between pre-intervention and immediately after application (P0) in normal skin, with increased skin distensibility. However, a significant decrease in distensibility was found between P10 and P20 when compared to the data collected at P0 (Table 1). There was a progressive reduction in the values at all times observed for all groups, in the area of the skin graft (P0, P10, P20, and P30). However, this post-intervention reduction was significant only in the TUV treatment group when comparing P30 with that immediately after the intervention (Table 1).

The viscoelasticity of the normal skin for the TU and TP groups showed a significant increase from pre-treatment to shortly after the application of the intervention (P0). Furthermore, a significant increase for the TU treatment group between P20 and P30 compared to P0 was also observed. The TUV and P10 treatment groups had a significant increase (Table 2). For skin graft areas, there was a significant decrease between pre-intervention and P0 in viscoelasticity observed in the TU and TPV groups. Furthermore, in the TUV and TPV groups, a significant increase in viscoelasticity was observed for P30 and P10, respectively (Table 2). In general, it can be observed that for both parameters, skin distensibility (R0) and skin viscoelasticity (R6), both for the normal area and the skin graft area, there was no significant difference between the interventions (Table 3).

DISCUSSION

The rehabilitation of the burned individual is complex and challenging, whose main objective is to promote functionality, which is often compromised due to biomechanical changes in the scar tissue, with consequent movement limitation. The results of this study show the effects produced after the application of the therapeutic intervention with TU and TP on the biomechanical properties of the skin, such as skin distensibility and viscoelasticity.

It is established that the application of TU in a continuous mode increases the tissue temperature, associated with an increase in the distensibility of collagen in scar tissue⁵. This justifies the use of TU to increase tissue distensibility¹⁷. Previously, the effect of TU has been evaluated on the scars and skin grafts of burn victims, and although the authors did not find a significant increase in distensibility

Table 1. Comparison of tissue distensibility values in control and skin graft area at different times.

Area	Group	Pre	P0	P10	P20	P30
Normal skin	TU (n=39)	0.32 (0.27–0.38)	0.36 ^a (0.31–0.45)	0.35 (0.29–0.40)	0.33 (0.30–0.40)	0.31 ^b (0.29–0.38)
	TP (n=38)	0.30 (0.25–0.36)	0.31 (0.27–0.37)	0.34 (0.26–0.39)	0.34 (0.28–0.38)	0.32 (0.26–0.38)
	TUV (n=32)	0.35 (0.27–0.42)	0.36 (0.30–0.44)	0.33 (0.30–0.41)	0.34 (0.28–0.40)	0.31 ^b (0.26–0.40)
	TPV (n=32)	0.33 (0.25–0.38)	0.35 ^a (0.27–0.40)	0.32 ^b (0.27–0.37)	0.30 ^b (0.26–0.36)	0.32 (0.26–0.37)
Skin graft area	TU (n=39)	0.13 (0.10–0.16)	0.14 (0.10–0.19)	0.13 (0.10–0.18)	0.12 (0.09–0.17)	0.13 (0.09–0.16)
	TP (n=38)	0.11 (0.09–0.16)	0.13 (0.10–0.16)	0.12 (0.10–0.16)	0.11 (0.10–0.14)	0.12 (0.10–0.16)
	TUV (n=32)	0.13 (0.10–0.16)	0.14 (0.09–0.18)	0.11 (0.08–0.16)	0.12 (0.09–0.16)	0.11 ^b (0.08–0.15)
	TPV (n=32)	0.11 (0.09–0.16)	0.13 (0.10–0.17)	0.10 (0.08–0.15)	0.10 (0.08–0.15)	0.11 (0.09–0.16)

TU: ultrasound group; TP: paraffin group; TUV: ultrasound and vacuum massage group; TPV: paraffin and vacuum massage group and vacuum. ^aIt differs from Pre ($p < 0.05$); ^bIt differs from P0 ($p < 0.05$). Tissue distensibility values (R0, in mm), pre-intervention (Pre), immediately (0 min, P0), 10, 20, and 30 min after intervention (P10, P20, and P30). Values presented in median (first quartile-third quartile).

Table 2. Comparison of relative values for viscoelasticity, in control and skin graft area at different times.

Area	Group	Pre	0	10	20	30
Normal skin	TU (n=39)	0.45 (0.40–0.50)	0.36 ^a (0.31–0.42)	0.42 (0.37–0.48)	0.44 ^b (0.38–0.50)	0.45 ^b (0.36–0.50)
	TP (n=38)	0.47 (0.43–0.50)	0.42 ^a (0.37–0.47)	0.43 (0.40–0.50)	0.44 (0.39–0.49)	0.46 (0.38–0.53)
	TUV (n=32)	0.43 (0.38–0.48)	0.39 (0.32–0.45)	0.43 ^b (0.40–0.51)	0.48 ^b (0.41–0.53)	0.50 ^b (0.39–0.55)
	TPV (n=32)	0.45 (0.39–0.49)	0.44 (0.36–0.47)	0.46 ^b (0.42–0.53)	0.47 ^{ab} (0.43–0.52)	0.47 (0.39–0.54)
Skin graft area	TU (n=39)	0.59 (0.53–0.71)	0.53 ^a (0.45–0.64)	0.59 (0.48–0.69)	0.57 (0.51–0.67)	0.63 (0.48–0.72)
	TP (n=38)	0.57 (0.50–0.71)	0.55 (0.50–0.64)	0.55 (0.51–0.63)	0.56 (0.52–0.67)	0.55 (0.49–0.66)
	TUV (n=32)	0.60 (0.50–0.73)	0.50 (0.43–0.59)	0.57 (0.50–0.71)	0.59 (0.49–0.65)	0.67 ^b (0.53–0.82)
	TPV (n=32)	0.58 (0.55–0.66)	0.52 ^a (0.42–0.59)	0.60 ^b (0.51–0.70)	0.60 (0.52–0.71)	0.58 (0.49–0.63)

TU: ultrasound group; TP: paraffin group; TUV: ultrasound and vacuum massage group; TPV: paraffin and vacuum massage group and vacuum. ^aIt differs from Pre ($p < 0.05$); ^bIt differs from P0 ($p < 0.05$). Viscoelasticity (R6), pre-intervention (Pre), immediately (0 min, P0), 10, 20, and 30 min after intervention (P10, P20, and P30). Values presented in median (first quartile-third quartile).

Table 3. Comparison of values related to tissue distensibility, control area, and skin graft in the different resources.

Area	Time (min)	TU	TP	TUV	TPV
Normal skin	Pre	0.32 (0.27–0.38)	0.30 (0.25–0.36)	0.35 (0.27–0.42)	0.33 (0.25–0.38)
	0	0.36 (0.31–0.45)	0.31 (0.27–0.37)	0.36 (0.30–0.44)	0.35 (0.27–0.40)
	10	0.35 (0.29–0.40)	0.34 (0.26–0.39)	0.33 (0.30–0.41)	0.32 (0.27–0.37)
	20	0.33 (0.30–0.40)	0.34 (0.28–0.38)	0.34 (0.28–0.40)	0.30 (0.26–0.36)
	30	0.31 (0.29–0.38)	0.32 (0.26–0.38)	0.31 (0.26–0.40)	0.32 (0.26–0.37)
Skin graft area	Pre	0.13 (0.10–0.16)	0.11 (0.09–0.16)	0.13 (0.10–0.16)	0.11 (0.09–0.16)
	0	0.14 (0.10–0.19)	0.13 (0.10–0.16)	0.14 (0.09–0.18)	0.13 (0.10–0.17)
	10	0.13 (0.10–0.18)	0.12 (0.10–0.16)	0.11 (0.08–0.16)	0.10 (0.08–0.15)
	20	0.12 (0.09–0.17)	0.11 (0.10–0.14)	0.12 (0.09–0.16)	0.10 (0.08–0.15)
	30	0.13 (0.09–0.16)	0.12 (0.10–0.16)	0.11 (0.08–0.15)	0.11 (0.09–0.16)

Pre: pre-intervention; 0: immediately after; 10: after 10 min; 20: after 20 min; 30: after 30 min. No significant difference ($p > 0.05$) in the comparisons. Tissue distensibility (R0, in mm), TU (ultrasound group); TP (paraffin group); TUV (ultrasound and vacuum massage group); TPV group (paraffin and vacuum massage group). Values presented in median (first quartile-third quartile).

with TU, they found a significant improvement in the active and passive amplitude of the movement⁷. These results can be related to tissue distensibility and therefore support the results of this study.

Despite this, TU is frequently used in therapeutic interventions, the ineffectiveness indicates that the wrong parameters are being used, such as the size of the treated area, duration, intensity, frequency, tissue type, transducer movement, and the “therapeutic window”^{5,18}.

The literature about the use of paraffin therapy and vacuum massage, including the specific parameters such as temperature, time, or type of application, is scarce^{6,19}. It has been found previously that different methods of applying paraffin therapy may influence the depth of heating, having

different responses in different pathologies^{20,21}. Previously, it has been observed that an increase in the elasticity of a burn scar that was treated with TP could remain for 4 h¹⁸. In this study, the maintenance of the effect did not remain for 30 min.

The vacuotherapy or vacuum massage applied to the skin leads to mechanical stress on the biological structures that make them up²². It is thought that the combination of interventions with vacuum massage can produce an impact on the collagen found in scar tissue and elastic fiber remodeling²³. However, different from other studies, in this study, with only one application of the intervention, no significant increase was noted for distensibility of both the normal and skin graft for either combination therapy, TUV and TPV^{8,9}.

Despite no significant difference being seen in this study between normal skin and skin graft after the intervention, it should be considered that the distensibility pre-intervention of the skin graft was on average 0.12 mm, corresponding to an increase of 0.01–0.02 mm post-intervention, which is a 10% increase in the total distensibility. With the mean thickness of 0.20-mm grafts²⁴ and that in the evaluated period, between 3- and 6-month postoperative, the complete maturation of this scar had not yet occurred²⁵, a distensibility around 0.12 mm may in fact correspond to more than 50% of the initial thickness of the graft.

All groups evaluated in this study demonstrated a profile of increased distensibility in the period immediately after the application of the intervention, with a progressive reduction in the effects over time, most likely due to the probable “therapeutic window.” There was no significant difference found between normal and skin graft, though, with values approximately 50% lower for scar skin. These results highlight the need for a distensibility gain in scar tissue, showing changes in the biomechanics properties of scar tissue in relation to normal skin.

Additionally, the effect of the interventions can increase the elastic properties more than the viscoelastic properties of normal skin, considering it was found that the viscoelasticity diminished immediately after the intervention. The viscoelasticity changes had no durability and did not remain more than 10 min after both TUV and TPV. In the skin graft, the viscoelasticity was observed to decrease in all the groups, except after intervention with TP, which showed practically no change. These data showed that TUV and TPV are more associated with changes in the elastic properties of the skin than the viscoelastic properties of the scar tissue.

In this study, it was observed that intervention with TU presented significant results in both normal and skin graft (between pre-intervention and P0), with a decrease in viscoelasticity. The skin graft areas that received the TP did not show

significant differences at any time (P0, P10, P20, and P30). However, 30 min after the application of TUV to the skin graft, a return to the pre-intervention state was seen, which is characteristic of the elastic property of the tissue. It could be hypothesized that the nonsignificant changes in the distensibility and the viscoelasticity of the skin graft studied may be due to the intervention being carried out on a tissue type that already has greater rigidity.

The nonuniform behavior of the responses found in this study may be due to the anisotropic and nonlinear responses of the skin in terms of its biomechanical characteristics. The skin under stretching stress exhibits a nonlinear behavior²⁶. The skin is complacent and large deformations can occur with a relatively low applied load. This is because a large portion of the fibers is not aligned, and the stiffness of the skin gradually increases as the fibers line up in the direction of the applied load.

CONCLUSION

Therapeutic intervention with ultrasound or paraffin alone or associated with vacuum massage alters the biomechanical properties of distensibility and viscoelasticity of the grafted skin after burns.

AUTHORS' CONTRIBUTIONS

ACG: Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **RRJG:** Methodology, Software, Writing – original draft, Writing – review & editing. **LAR:** Methodology, Resources, Writing – review & editing. **JAFJ:** Methodology, Writing – review & editing. **CSC:** Visualization, Writing – original draft. **APF:** Visualization, Writing – original draft, Writing – review & editing. **ECOG:** Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Impact of COVID-19 on the prescription of contraceptives in a city in São Paulo

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SUMMARY

OBJECTIVE: In the beginning of the pandemic, measures, such as social distancing, lockdown strategies, and restrictions on mobility, as well as the fear of transiting through health facilities, raised concerns about the impact of COVID-19 on women's ability to continue using contraceptives.

METHODS: This is a retrospective cohort study, which evaluated reports of medication distribution spreadsheets in Bauru – SP, from January 2019 to June 2021.

RESULTS: Our study showed that the municipal dispensation of contraceptives in the SUS was markedly impacted by the COVID-19 pandemic, suffering reductions that can impact on an increase in unplanned pregnancy rates. It is possible to note a significant decrease in the distribution of combined oral contraceptives (44.18%), combined injectable contraceptives (47.58% reduction), and medroxyprogesterone acetate (13.98%). This fact may be associated with the reduction in offers of face-to-face consultations in gynecology, due to the social isolation necessary at the time of the pandemic.

CONCLUSION: Ensuring access to contraceptives during health emergencies should be a public health policy priority. Thus, it is essential to draw up strategic plans to encourage full access to reproductive planning services even in times of health emergency, so that the occurrence of unplanned pregnancies can be adequately prevented.

KEYWORDS: COVID-19. Contraception. Public health.

INTRODUCTION

In January 2020, the World Health Organization (WHO) declared the SARS-CoV-2 outbreak as a public health emergency of international concern, having declared the situation a worldwide pandemic on March 11, 2020¹. Brazil registered its first case of COVID-19 in February 2020, with more than 663,000 deaths in the country resulting from this infection so far². In this war against an invisible and potentially fatal enemy, many countries have had to make difficult decisions to balance the demands of trying to contain the spread and contagion of COVID-19 while simultaneously planning in a coordinated way to maintain the delivery of essential health services. Governmental and private organizations in many parts of the world have had to suspend many routine and elective services in their quest to control the pandemic³.

In the beginning of the pandemic, measures, such as social distancing, lockdown strategies, and restrictions on mobility, as well as the fear of transiting through health facilities, raised concerns about women's ability to continue using contraceptives. Global production and disrupted supply chains, as well as overstretched healthcare facilities, also threatened to reduce the availability of family planning supplies and services. Infectious outbreaks, as reported in previous epidemic situations, can have the potential to devastate family planning programs⁴. Since patients

are confined and healthcare workers are at risk of infection⁵, it would be expected to note a reduction in the supply of health services and care to the population. The United Nations Population Fund (UNFPA) estimates that 12 million women had their contraceptive methods interrupted or discontinued in this period, leading to 1.4 million unplanned pregnancies worldwide⁶.

In Brazil, the health system is organized according to the complexity of services and procedures, seeking universal access, equity, and equality⁷. The right to family planning is guaranteed in the Brazilian Federal Constitution (Law No. 9,263 of 1996). Everyone has the right to information, specialized assistance, and access to contraceptive methods. Choosing the most suitable contraceptive method is an option that every woman should have. Providing free contraceptives and promoting the most effective contraceptive methods reduce unwanted pregnancies⁸. Women who experience an unplanned pregnancy may in fact represent greater health costs than those who plan to become pregnant. In Brazil, in 2014, it was estimated that the costs and outcomes associated with an unwanted pregnancy would result in a cost of R\$ 2,293 per unwanted pregnancy⁹. Sexual and reproductive rights (SRH) are a significant public health issue and must be a priority. It is essential for the scientific community to build clinical, epidemiological, psychosocial, and behavioral links between COVID-19 and SRH¹⁰.

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Therefore, in addition to the clinical scope of the disease caused by SARS-CoV-2, we must not neglect the impact of COVID-19 on sexual and reproductive health¹⁰. Thus, evaluating the impact of the pandemic on the prescription and distribution of contraceptives is essential so that strategies can be devised to prevent unplanned pregnancies.

METHODS

This is a retrospective cohort study, which evaluated reports of medication distribution spreadsheets from the pharmacies of the Basic Health Units in Bauru – SP, from January 2019 to June 2021. The product dispensing data were compiled in Excel tables and condensed for further analysis. The article was approved by the local ethics committee (CAAE 50011921.9.0000.5417).

RESULTS

Our study analyzed the number of units dispensed and the type of contraceptives (combined oral contraceptives, monthly injectable contraceptives, quarterly injectable contraceptives, progestogen-only oral contraceptives, and copper IUD) distributed by municipal pharmacies, comparing data obtained from January 2019 to February 2020 (before the advent of the pandemic in Brazil), with data obtained between March 2020 and June 2021, as shown in Table 1.

DISCUSSION

In the beginning of the pandemic, WHO published guidelines suggesting that women who were well adapted to their contraceptive methods should maintain their use. In addition, it sought to encourage the use of long-acting reversible contraceptives (LARCs) (even beyond the labeling period, according

to safety studies in extended use) and the use of telemedicine for contraceptive counseling, allowing more people to have access to family planning guidelines, despite the social isolation measures¹¹. Despite being a necessary measure in controlling the spread of the pandemic, isolation guidance as a measure to control the transmission of the SARS-CoV-2 virus has a huge impact on unmet needs for family planning: the United Nations health agency estimated that every three months of lockdown worldwide could lead to 2 million more women losing access to modern methods⁶. The COVID-19 pandemic has brought the Brazilian healthcare system to a halt, and as many family planning clinics and clinics have been temporarily closed, sales and distributions of most modern contraceptives have plummeted¹².

Since there is an important risk of increasing unplanned pregnancies during the COVID-19 pandemic, there have been movements to encourage women, health professionals, policy makers, and the whole society to discuss reproductive planning and contraception services as a priority service¹³. Ensuring access to contraceptives during health emergencies should be a public health policy priority. However, some researchers have shown that many people had access to reproductive counseling services during the lockdown period; however, some specific population groups, such as young people and the vulnerable population, reported difficulties in accessing services^{14,15}. Such groups, in this case, are among those with the highest demand for contraceptives.

The COVID-19 pandemic had a strong impact on contraceptive dispensing in several scenarios other than Brazil, with varying degrees of reduced dispensing according to the type of contraceptive, age group, and the level of restrictions related to the pandemic. When evaluating the data from our study, it is possible to note a significant decrease in the distribution of combined oral contraceptives (44.18%), combined injectable contraceptives (47.58% reduction), and medroxyprogesterone acetate (13.98%). This fact may be associated with the reduction in offers of face-to-face consultations in gynecology, due to the social isolation necessary at the time of the pandemic. Other international studies also showed these same trends, such as a French study that showed a 2% reduction in the dispensing of oral contraceptives during the lockdown period, with prescriptions for the use of long-term contraceptives and women under 25 years of age being the most impacted¹⁶. This behavior was also observed in a South African study that reported reduced use of LARCs during the first 5 weeks of social isolation¹⁷. On the other hand, contrary to these South African data, we observed in our results that there was an increase in the use of copper IUDs (an increase of 43.75%). Taking into account that in a single face-to-face consultation, it is possible to carry out contraceptive guidance and IUD insertion and that this

Table 1. Monthly distribution of contraceptives by pharmacies of SUS health units in Bauru.

Contraceptive	AMC (01/2019 to 02/2020)	AMC (03/2020 to 06/2021)	Variation (%)
Cooper IUD 380A	16	23	+43.75
MPA 150 mg/mL	565	486	-13.98
LNG 0.15 mg/EE 0.03 mg	740	413	-44.18
VE 5 mg/NETA 10 mg	435	228	-47.58
NETA 0.35 mg	25	65	+160

AMC: average monthly consumption; DIU: dispositivo intrauterino de cobre; MPA: medroxyprogesterone acetate; LNG: levonorgestrel; EE: etinylestradiol; EV: estradiol valerate; NETA: norethisterone acetate.

method has high contraceptive efficacy and long action, we can say that this increase can greatly contribute to the reduction of unplanned pregnancy rates in this population.

It was observed that there was a 160% increase in the dispensing of progestogen-only contraceptives (norethisterone) during the period evaluated. However, this fact can be explained by the removal of desogestrel from the dispensation line of health units in the same period, which was replaced by norethisterone.

Despite these increases, when comparing pre- and post-pandemic data in a unified way, a total reduction of 31.77% in the dispensing of contraceptives by the units studied was found. It should also be noted that one of the possible causes for the 160% increase in the use of progestogen-only oral contraceptives (norethisterone 0.35 mg) is the recent de-standardization of another type of progestogen-only oral contraceptive (desogestrel 0.075 mg), which was taken from the pharmacies of these health units. Therefore, in view of this general context, it is possible to infer that the COVID-19 pandemic negatively affected the distribution of contraceptives in the municipality of Bauru. This fact may, in the future, impact the rates of unplanned pregnancies in the region. During the COVID-19 pandemic, the estimated reduction in contraceptive use in low-income countries was substantial^{18,19}.

The impact of these restrictions on unwanted pregnancy at a population level remains undetermined¹⁶. However, the negative effects seem to be more evident in low- and middle-income countries and among low-income people^{17,20,21}.

In Brazil, the prevalence of modern contraceptive methods is 75–80% among women of reproductive age^{9,22}. Furthermore, 74% of the population depends on the national health service (Sistema Único de Saúde) as their main source of care, including the provision of free contraception²³. The Brazilian health system provides its users free of charge only with female and male condoms, combined oral contraceptives, a progestogen-only pill (norethisterone 0.35 mg), monthly and quarterly injectables, and a copper IUD. Access to contraceptives in the private sector may indicate inequalities in their supply and distribution, evidencing growth in sales of contraceptives not provided free of charge¹².

Reproductive choice is one of the most fundamental human rights, and contraception represents a big step toward greater gender equality²⁴. In addition to the impact on lifestyle changes and women's mortality, unplanned pregnancy also imposes costs. The National Commission Specialized in Contraception of the Brazilian Federation of Gynecology and Obstetrics (FEBRASGO) recommends that women who request new contraceptive methods be guided by face-to-face medical consultation or, when available, by telemedicine, for the use of effective contraceptive methods, in addition to a

condom. In addition, it suggests that those efforts should be directed toward the continued use of methods already chosen by women, through active screening of users and provision of contraceptives by health professionals. Since the provision of LARCs as well as the scheduling of sterilization surgery methods have been delayed during the pandemic, highly effective self-administered methods can be offered meantime²⁵.

As strengths of this study, we have that it is an unprecedented regional assessment, with assessment of indirect measures of the impact of the pandemic on the attention to family planning and reproductive services. The main limitations of this study are as follows: data are scarce and only represent the reality of the municipal population that uses the SUS, not including data on the dispensing of contraceptive products purchased in private health services. In addition, when evaluating general data from pharmaceutical units, it is not possible to characterize the population that benefited from such contraceptives, as the data are not linked to users. Data referring to definitive sterilization procedures, such as tubal ligation and vasectomy surgeries, were not evaluated in this study. However, it is expected that, if there has been any change in the rates of performance of these surgeries, it will be in the sense of reducing procedures, given that non-emergency surgical procedures were largely reduced during the most critical periods of the COVID-19 pandemic.

CONCLUSION

Our study showed that the municipal dispensation of contraceptives in the SUS was markedly impacted by the COVID-19 pandemic, suffering reductions that can impact an increase in unplanned pregnancy rates. Ensuring access to contraceptives during health emergencies should be a public health policy priority. Thus, it is essential to draw up strategic plans to encourage full access to reproductive planning services even in times of health emergency, so that the occurrence of unplanned pregnancies can be adequately prevented.

AUTHORS' CONTRIBUTIONS

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

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Evaluation and follow-up of antibody formation after CoronaVac vaccine

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SUMMARY

OBJECTIVE: The aim of this study was to monitor the time-dependent change by evaluating the antibody levels at the 4th, 7th, 10th, 13th, and 16th weeks after the second dose of the CoronaVac vaccine.

METHODS: The study group (n=65) were between 21 and 60 years old and received two doses of the CoronaVac vaccine. Blood samples were collected after 4th, 7th, 10th, 13th, and 16th weeks of the second dose of the vaccine administration. There was a coronavirus disease 2019 recovered group (n=29) who were SARS-CoV-2 real-time PCR test result positive before the vaccination period, and no coronavirus disease 2019 history group (n=36). Age, BMI, gender, smoking, comorbidity, coronavirus disease 2019 contact history, and working in the coronavirus disease 2019 service history of the individuals were recorded.

RESULTS: No statistically significant difference was found in the descriptive findings of the individuals according to coronavirus disease 2019 recovered group and no coronavirus disease 2019 history group. It was observed that antibody levels in the coronavirus disease 2019 recovered group were found to be higher for each period of serum collection compared to the no coronavirus disease 2019 history group, which were statistically significant. The distribution curves of the antibody levels according to the timing of blood collection in coronavirus disease 2019 recovered group, no coronavirus disease 2019 history group, and total subjects were extrapolated, and it was observed that the estimated time for the antibodies to reach the threshold value of the test was 214, 145, and 166 days after vaccination.

CONCLUSION: It is important to make booster doses, as the CoronaVac vaccine will lose its effect after the fifth month due to the decrease in Ab levels. In addition, since the antibody levels decrease later in those who have a history of coronavirus disease 2019 infection and are vaccinated, individuals who have no previous history of coronavirus disease 2019 should be given priority for vaccination.

KEYWORDS: COVID-19. Vaccines, inactivated. Immunity, humoral.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) has become a serious public health problem, causing a pandemic. It has led to high morbidity and mortality worldwide¹. Vaccines have been seen as the most important solution to end the COVID-19 pandemic. CoronaVac is a type of inactivated vaccine used against COVID-19. It is prepared by injecting pathogen strains that are killed by chemical (formaldehyde) or physical (UV and heat) methods that do not cause disease, and the body is made to produce antibodies against them². After demonstrating the safety and effectiveness of the vaccine in Phase 1/2 studies conducted in various countries, Phase 3 studies were initiated³. In the Phase 3 study conducted in Turkey, the effectiveness of the

CoronaVac vaccine was found to be 83.5%⁴. Considering the rapidly increasing number of cases and deaths, the CoronaVac (Sinovac Life Sciences, Beijing, China) vaccine was given an emergency use approval on January 13, 2021, by the Medical Medicines and Devices Agency of Turkey, and vaccination started with healthcare professionals in Turkey. The vaccine was administered as two doses, 28 days apart³.

The vaccine has been shown to provide strong protection against COVID-19; however, it is thought that there may be a loss of protection due to decreased immunity over time and viral variation due to various mutations⁵. Studies of the immune response in people with a prior history of COVID-19 infection have shown that antibody levels drop 4 months after infection⁶.

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In this study, the aim was to monitor the time-dependent change by evaluating the antibody levels at the 4th, 7th, 10th, 13th, and 16th weeks after the second dose of the CoronaVac vaccine in healthcare workers. It was also aimed to evaluate whether there is a difference between antibody levels by dividing healthcare workers who received the CoronaVac vaccine into two groups: those with a previous history of COVID-19 and those without evidence of previous infection. Thus, it is aimed to provide a prediction about how long the expected benefit from the vaccine will last by monitoring the antibody levels and to provide data for the booster dose requirement of the vaccine.

METHODS

Study design and participants

This prospective study was conducted in our hospital between March and June 2021. The study group included 65 healthcare workers who were 21–60 years old and received two doses of the CoronaVac (Sinovac Life Sciences) vaccine. Besides, the study group was selected from subjects whose antibody level was above the threshold at the fourth week after the vaccination in a previous study. Blood samples were collected after the 4th, 7th, 10th, 13th, and 16th weeks of the second dose of the vaccine administration. After the serum samples were centrifuged, the samples were stored at -20°C until testing. Among the individuals, there were COVID-19 recovered group (CRG) ($n=29$), who were SARS-CoV-2 real-time PCR test result positive before the vaccination period, and no COVID-19 history group (NCHG) ($n=36$). The descriptive properties of the

individuals were collected through a questionnaire. The queries were about age, BMI, gender, smoking, comorbidity (hypertension, diabetes, asthma/chronic obstructive lung disease, malignancy, autoimmune disease, thyroid patient, hyperlipidemia, and coronary artery disease), COVID-19 contact history, and working in COVID-19 service history (Table 1).

SARS-CoV-2 immunoglobulin G II quant test (anti-spike immunoglobulin G)

Serum samples were studied using the SARS-CoV-2 immunoglobulin G (IgG) II Quant (Abbott; IL, USA) Chemiluminescent Microparticle Immunoassay, which was developed to detect IgG antibodies against the spike protein S1 subunit of the COVID-19. The assays were carried out on the analyzers Architect I2000sr (Abbott) according to the manufacturer's instructions. Test results of ≥ 50 AU/mL were evaluated as positive.

Statistical analysis

All statistical analyses were performed using the SPSS 22.0 (IBM SPSS Statistics, IBM Corp., Chicago, IL, USA) software for Windows. The accordance of the numeric variables with the normal distribution was assessed with the Kolmogorov-Smirnov test. Demographic and clinical characteristics of CRG and NCHG were compared using Kruskal-Wallis tests for continuous variables, chi-square tests, and Mann-Whitney U tests for categorical variables. The median (interquartile range, IQR) was used to define the nonparametric variables, while frequency and percentage were used for categorical variables. In all of the analyses, the statistical significance level was accepted as a $p < 0.05$.

Table 1. Descriptive findings of subjects according to coronavirus disease 2019 recovered group and no coronavirus disease 2019 history group.

Variables	CRG (N=29)	NCHG (N=36)	Total (N=65)	p	
Age	42 (31–46)	37 (32–44,75)	38 (32–45.5)	0.387 ^m	
BMI (kg/m ²)	27.54 (26.14–30.18)	26.20 (23.34–29.55)	27.35(24.32–29.99)	0.359 ^t	
Gender	Male	14 (42.4)	19 (57.6)	33 (50.8)	0.718 ^k
	Female	15 (46.9)	17 (53.1)	32 (49.2)	
Smoking	Yes, n (%)	9 (40.9)	13 (59.1)	22 (33.8)	0.667 ^k
	No, n (%)	20 (46.5)	23 (53.5)	43 (66.2)	
Alcohol consumption	Yes, n (%)	4 (44.4)	5 (55.6)	9 (13.8)	0.991 ^k
	No, n (%)	25 (44.6)	31 (55.4)	56 (86.2)	
Comorbidity	Positive, n (%)	11 (57.9)	8 (42.1)	19 (29.2)	0.166 ^k
	Negative, n (%)	18 (39.1)	28 (60.9)	46 (70.8)	
COVID-19 contact history	Yes, n (%)	20 (44.4)	25 (55.6)	45 (69.2)	0.967 ^k
	No, n (%)	9 (45)	11(55)	20 (30.8)	
Working in COVID-19 service history	Yes, n (%)	17 (54.8)	14 (45.2)	31 (47.7)	0.113 ^k
	No, n (%)	12 (35.3)	22 (64.7)	34 (52.3)	

^mMann-Whitney U test; ^tindependent sample t test; ^kchi-square test.

Ethics committee approval

Ethical approval was obtained for the study from the Medical Research Ethical Committee of the Kastamonu University Faculty of Medicine (approval no: 2020-KAEK-143-70).

RESULTS

No statistically significant difference was found for the descriptive findings of the individuals according to CRG and NCHG (Table 1). In the study group, median age was 38 years, median BMI was 27.35, there were no obese subject, nearly half of the individuals had worked in COVID-19 service history, smoking was prevalent in 33.8%, comorbid conditions were present in 29.2%, and COVID-19 contact history made up 70.8% of the sample.

There were five individuals whose antibody levels sharply increased at the 13th week and decreased at the 16th week but were still higher than at the 4th week, which may indicate acute COVID-19 infection. Three of them were in CRG, and two were from NCHG. These subjects were excluded from the further analysis.

The distribution of antibody levels according to the time of blood collection after CoronaVac vaccination and the comparison of CRG and NCHG are presented in Table 2. It was observed that antibody levels in the CRG were found to be higher for each

period of serum collection compared to the NCHG, and they were statistically significant. Likewise, the change of the antibody levels from the fourth week to the other weeks of serum collection were analyzed according to CRG and NCHG, and it was observed that the difference was statistically significant in the 4th–10th weeks and the 4th–13th weeks. In the 10th week, there was an increase in antibody levels in 12 and 17 subjects from CRG and NCHG, respectively, and 10 and 13 of the subjects, respectively, had either COVID-19 contact history or were working in COVID-19 service in the last 3 months history.

The distribution curves of the antibody levels according to the timing of blood collection in CRG, NCHG, and total subjects are presented in Figure 1. These curves were extrapolated, and it was observed that the estimated time for the antibodies to reach the threshold value of the test were 214, 145, and 166 days after vaccination for CRG, NCHG, and total subjects, respectively.

DISCUSSION

The reduction in efficacy of the COVID-19 pandemic is dependent on the success of widespread vaccination; however, the efficacy and resilience of immune responses vary depending on the type of vaccine and the characteristics of the vaccinated population. Data on the duration of the protective effect of

Table 2. The distribution of antibody levels according to the time of blood collection after CoronaVac vaccination and the comparison of coronavirus disease 2019 recovered group and no coronavirus disease 2019 history group.

	Time after CoronaVac vaccination	CRG (n=25)		NCHG (n=34)		p
		Average±s.d.	Median (min–max)	Average±s.d.	Median (min–max)	
COV-2IgG (AU/MI)	4th week	1734.0±1406.5	1458.9 (265.3–6637.9)	1104.7±727.6	836.1 (214.2–3835.0)	0.044^m
	7th week	1399.9±1671.7	866.9 (176.0–7921.8)	607.0±499.8	448.2 (153.8–2815.9)	0.004^m
	10th week	1443.2±1584.3	1051.8 (156.8–7684.4)	607.6±403.0	536.0 (115.4–1997.2)	0.002^m
	13th week	1476.1±2008.1	977.1 (122.9–10095.8)	508.9±399.2	429.9 (82.8–2071.6)	0.001^m
	16th week	872.4±1235.7	473.9 (51.3–6145.6)	283.0±206.8	204.7 (47.8–1005.9)	0.001^m
Change p	4th week / 7th week	-334.1±677.4	-268.5 (-2069–1283.9) 0.009 ^w	-497.7±395.4	-414.8 (-1526.7 to -20.4) <0.001 ^w	0.276 ^m
	4th week / 10th week	-290.7±481.6	-195.8 (-1198.8–1046.5) 0.001 ^w	-497.1±395.5	-400.8 (-1837.8 to -4.9) <0.001 ^w	0.049^m
	4th week / 13th week	-257.9±867.3	-302.9 (-1375.2–3457.9) <0.001 ^w	-595.8±415.5	-458.2 (-1763.4 to -32.5) <0.001 ^w	0.033^m
	4th week / 16th week	-861.6±621.0	-705.5 (-2297.5 to -90.2) <0.001 ^w	-821.7±570.5	-640.0 (-2829.1 to -166.4) <0.001 ^w	0.890 ^m

^mMann-Whitney U test; ^wWilcoxon test; s.d.: standard deviation. Bold indicates significant value.

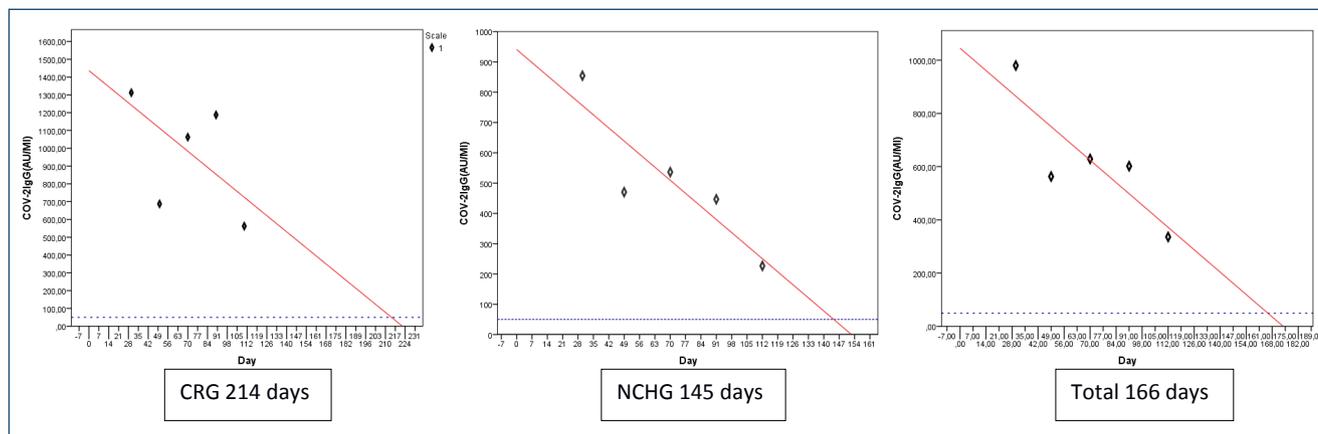


Figure 1. The distribution curves of the antibody levels according to the timing of blood collection from coronavirus disease 2019 recovered group, no coronavirus disease 2019 history group, and total subjects.

the vaccine and the timing of the booster dose are still needed. In this study, time-dependent changes in antibody titers were monitored in a total of 65 healthcare workers vaccinated with two doses of the CoronaVac (Sinovac), 29 of whom had a history of pre-vaccination COVID-19 infection. Follow-up after two doses of the CoronaVac vaccine showed dramatic decreases in median antibody levels over time. During the entire study period, antibody levels in the group with a previous COVID-19 infection were found to be significantly higher than in the group without a COVID-19 infection. When the descriptive findings of the two groups were examined, it was seen that there was no significant difference between the two groups in terms of age, gender, BMI, smoking status, comorbidity, history of contact with COVID-19, and history of working in COVID-19-related services.

Although Ab levels were observed to be over the threshold value of the test for at least 4.8 months by extrapolation, COVID-19 infection developed in 5/65 (7.7%) cases at the 13th week after vaccination. It seems that Ab alone is not sufficient to prevent infection. It is well known that not only spike antibodies but also T-cell- and B-cell-mediated immune factors play a role in the response against SARS-CoV-2⁷. The fact that three of these people have CRG indicates that the protection is limited after a natural infection. In a study conducted on 20,000 healthcare workers in the United Kingdom, the rate of protection from reinfection for the first 5 months after COVID-19 infection was reported as 83%⁸. In a study conducted in China, 25 (4.5%) people were found to be infected with COVID-19 in a 6-month follow-up after two doses of CoronaVac vaccine. Of these cases, 20 were within the first 4 months⁹.

In Table 2, the antibody levels in the serum taken from the fourth week after the second dose of vaccination and their differences between CRG and NCHG were shown, excluding

those who had acute infections. According to the table, antibodies from CRG were found to be significantly higher than those from NCHG at all serum collection times. A sudden increase in the 10th week suggested an acute infection. When we extrapolate Ab levels, at 241, 145, and 166 days the Ab levels remain above the threshold for CRG, NCHG, and total. The decrease in Ab levels to the test threshold means the end of the immune response obtained from the vaccine. Ab persists for an average of 5.5 months, which can vary between 4.8 and 8 months. A study conducted in Thailand showed a significant decrease in antibody levels 3 months after the second dose of CoronaVac vaccine¹⁰. In another CoronaVac study, a 56.7% decrease was observed between antibody levels measured at the first and third months after the second dose¹¹. Several other studies have reported that the antibody response after two doses of CoronaVac greatly protects against re-infection for 6 months^{12,13}. In another study, antibody responses at the fourth month decreased by 61% compared to responses at the first month and even became seronegative by 10%⁹. In another study, a decrease in antibody titers was observed 3 months after the CoronaVac vaccine. Similar to our study, this decrease was more rapid and significant in those with no previous history of COVID-19 infection^{14,15}. In a study of 850 vaccinated participants in Hong Kong, it was reported that the median antibody titers in the BNT162b2 mRNA vaccine group remained above the threshold for 6 months, while the median antibody titers in the CoronaVac vaccine group dropped significantly after 2 months¹⁶. Ab presence durations are similar between vaccine and natural infection. Several studies of the immune response in people with a history of COVID-19 infection have shown that antibody levels drop 4 months after being infected^{17,18}.

The limitations of the study are that the RT-PCR test, which is the gold standard in the acute diagnosis of COVID-19,

cannot be routinely performed for screening during follow-up periods and therefore asymptomatic infected individuals may have been overlooked. In addition, longer term follow-up of cases with a larger sample size will increase the reliability of the study.

CONCLUSION

It is important to apply booster doses, as the CoronaVac vaccine will lose its effect after the fifth month due to the decrease in Ab levels. In addition, since the antibody levels decrease later in those who have a history of COVID-19 infection and

are vaccinated, individuals who have no previous history of COVID-19 should be given priority in vaccination, and it was thought that the period between booster doses may be longer in those with a history of COVID-19.

AUTHORS' CONTRIBUTIONS

BC: Investigation, Methodology, Software, Supervision. **NC:** Methodology, Supervision, Validation, Writing – review & editing. **MYD:** Investigation, Software, Supervision. **CK:** Investigation, Software, Supervision. **RO:** Methodology, Validation. **CD:** Methodology, Investigation. **ZE:** Investigation, Supervision.

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