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EDITORIAL

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The benefits and side effects of gadolinium-based contrast agents in multiple sclerosis patients

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Gadolinium offers extra insight into our body's condition. But, is it safe?

Gadolinium-based contrast agents (GBCAs) were safely delivered to millions of patients throughout the world since 1988, and their usage has definitely benefitted many individuals by allowing doctors to detect neurological disorders earlier and more accurately. GBCAs frequently have minor side effects. Injection-site discomfort, nausea, itching, rash, headaches, and dizziness are the most prevalent adverse effects. Patients with significant renal issues are more likely to experience serious, but uncommon side effects, including gadolinium poisoning and nephrogenic systemic fibrosis[1].

One of the biggest radiological concerns in recent years is the safety of GBCAs used in magnetic resonance imaging (MRI). As a study showed that gadolinium deposited in the brain and remained there, radiologists began to question the safety of gadolinium[2]. Results showed that the high signal intensity in patients' brains is correlated with the number of GBCAs administrated. The new findings have sparked a major debate in radiology about the safety of these agents. For all GBCAs of MRI, the Food and Drug Administration (FDA) mandates a new class warning and additional safety precautions in terms of gadolinium staying in the patients' bodies, including the brain, for months to years after taking these medications[3].

It is known that patients with renal insufficiency cannot filter the gadolinium from their body, so it is included as a FDA warning label on the contrast packaging. However, there was less evidence showing patient safety issues in those with normal renal function. Boxed warnings are also mentioned for recognized hypersensitivity relationships that can arise in individuals, especially in those with allergic diseases. Using GBCA in MRI has been questioned in recent years as evidence has emerged linking gadolinium to nephrogenic systemic fibrosis (NSF) and gadolinium deposition. Although most gadolinium is removed by urine after an MRI scan, a minimal amount stays and can accumulate over time, according to research published in 2017. This is an important concern for people who need to have MRI scans on a frequent basis[1]. Linear and macrocyclic agents are the two types of GBCAs depending on their chemical forms. Several studies indicated that the linear agents remain more in the brain than macrocyclic agents. However, new research has revealed that all treatments, including macrocyclic, leave some gadolinium in the brain. While gadolinium accumulation in the brain has been the focus of attention in recent years, the authors claimed that, in animal tests, 100 times more gadolinium was found to be retained in the skin and bones than in the brain[4]. The patients with multiple sclerosis (MS) should have brain MRIs both during relapse and remission (every few months) to assess disease activity. In the individuals with MS, the dentate nucleus (DN) T1 hyperintensity was detected[5].

However, certain studies on the effects of various kinds of GBCA administrations on gadolinium accumulation in MS patients have yielded conflicting results. In these investigations, some researchers[6] found that repeated macrocyclic GBCAs administrations enhanced DN-to-pons signal intensity ratios, while others[4] found that numerous doses of macrocyclic chelates administrations did not affect brain signal intensity variations in MS patients. To date, no specific clinical evidence was reported for complications associated with the deposition of GBCAs in the patients' brains. However, it is possible that based on the deposition of GBCAs in different areas of the brain, some symptoms, such as neurological and motor disorders, may occur in patients, which requires further studies. Gadolinium deposition in the humans appears to be linked to a wide range of diseases. One of the studies observed the difference in gadolinium deposition between patients

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with neuromyelitis optica spectrum disorder (NMOSD) and those with MS and found that patients with MS were more prone to gadolinium accumulation compared to patients with NMOSD. The authors hypothesized that changes in disease pathophysiology and gadolinium structure could influence gadolinium deposition[7]. There are certain potential biases in examining and evaluating the health/clinical consequences of gadolinium deposition[8]. The bulk of clinical investigations on GBCAs were unable to examine direct acute toxicity and diagnostic effectiveness of the agents, and hence the long-term health impacts, according to the research. Despite no neurological symptoms or parenchymal damage being associated with gadolinium deposition in the retrospective studies published to date, it seems necessary to have a selective approach in MS patients requiring contrast-enhanced MRI[9-15]. Therefore, it is better to monitor MS patients regularly under GBCAs administration to prevent complications if GBCAs are deposited in patients' brains[6,16-20].

AUTHORS' CONTRIBUTIONS

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

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Use of absorbable versus nonabsorbable anchors in the treatment of glenohumeral instability

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INTRODUCTION

In cases of shoulder instability, the placement of anchors, with the open or arthroscopic technique, can be used to repair the types of lesions denominated Bankart [disinsertion of the labrum and ligament – mainly the inferior glenohumeral ligament of the anterior border of the glenoid (most frequent), which may be an isolated detachment of the labrum and ligament or associated with a bone fragment].

A significant improvement in instability and a decrease in recurrence have been achieved as a result of improvements in the arthroscopic technique, better selection of candidate patients for intervention, and improved quality of the implants. The characteristics of the patients that have directly contributed to a positive outcome include age, sex, number of dislocations, sports activities, presence or absence of significant Hill-Sachs lesions, and a better assessment of glenoid bone loss.

In turn, the quality of the implants could influence the effectiveness of the surgery. In recent decades, many types of suture anchors have been introduced to the market and classified as absorbable/biodegradable anchors, in order to differentiate them from the metallic material that constituted the previous models.

Although the metallic anchor is considered safe and promotes firm fixation to the tissue, it can generate complications such as migration and chondral damage, impair the surgical review, and limit imaging studies, as well as facilitate the incarceration of the metallic implant in the bone. In turn, bioabsorbable anchors provide fixation for a limited time and healing may occur incompletely.

Few studies have prospectively compared the effectiveness of these two types of anchors in the treatment of unstable shoulder syndrome, through arthroscopic Bankart lesion repair.

METHODOLOGY

In the methodology, we define the clinical question, the structured question (PICO), the eligibility criteria of the studies, sources of information consulted, search strategies used, critical evaluation method (risk of bias), data to be extracted, measures to be used to express results, and the method of analysis.

Clinical question

Is the use of absorbable anchors in the treatment of glenohumeral instability more effective and safer when compared to nonabsorbable anchors, especially in relation to the occurrence of secondary arthrosis?

Structured question

- P (population): Arthroscopically treated glenohumeral instability;
- I (intervention): Absorbable anchors;
- C (comparison): Nonabsorbable anchors;
- O (outcome): Pain, function, quality of life, secondary arthrosis, and recurrence.

Eligibility criteria

- PICO components.
- Randomized clinical trials (RCTs) and/or observational cohort studies that complement the information with

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a relevant number of patients, follow-up time, or outcomes not covered in the RCTs.
- No period or language restrictions.
- Full text or abstract with necessary data.

Exclusion criteria

- *In vitro* and/or animal studies.
- Case series or case reports.
- Narrative or systematic reviews.

Information sources consulted and search strategies

Medline via PubMed, manual search, and Embase

(Bankart OR Shoulder Dislocation OR Shoulder Joint OR Shoulder instability) AND (Absorbable Implants OR Metal OR Metals OR Biocompatible Materials OR Biodegradable OR bioabsorbable OR nonabsorbable) AND (Therapy/broad[filter] OR Comparative study OR Comparative studies OR Epidemiologic Methods OR Systematic[sb]).

Risk of bias and quality of evidence

For RCTs, the following items are evaluated: focal question, randomization, blinded allocation, double blinding, losses, intention-to-treat (ITT) analysis, definition of outcomes, sample size calculation, and JADAD score[1].

Data extracted

Author, year of publication, study design, characteristics and number of patients, intervention, comparison, outcomes (pain, function, recurrence, secondary arthrosis, other complications, and quality of life), and follow-up time.

Outcome measures

For categorical variables, we use absolute numbers, percentage, absolute risk, reduction or increase in risk, number needed to treat or harm, and 95% confidence interval (95%CI). For continuous variables, we use mean or median, standard deviation, and difference between means.

Expression of the results

The results are presented as follows: diagram of recovery and selection of studies (Figure 1), characteristics of the studies, risk of bias (Table 1), results by outcome, and synthesis of evidence.

When it is possible to aggregate the results of the included studies in relation to one or more common outcomes, a meta-analysis is performed using the RevMan 5.3 software (Cochrane)[2].

Analysis of the quality of evidence

The quality of the evidence is assessed using the GRADEpro software[3].

RESULTS

The results are presented as follows: flowchart (Figure 1) and selection of studies, summaries of RCTs, risk of bias, results by outcome, quality of GRADE[3,10] evidence, and synthesis of evidence.

Characteristics of the included studies

The included studies were two randomized controlled trials.

Milano G et al., 20104

Inclusion criteria: patients with traumatic anterior glenohumeral instability and recurrent dislocation, presence of intra-articular lesions such as anteroinferior glenoid labrum lesion (Bankart or ALPSA lesion [Anterior Labroligamentous Periosteal Sleeve Avulsion, which is a variant of Bankart lesion]), anteroinferior glenohumeral ligament (AIGHL) injuries, and the presence of superior labral anterior and posterior (SLAP) lesion. Exclusion criteria: instability without dislocation, bone glenoid defect greater than 20% according to the "PICO" criterion, and Hill-Sachs lesion greater than 30% of the humeral head. Intervention and comparison: patients underwent arthroscopic surgical repair of the lesion using biodegradable or metallic anchors. Outcomes analyzed: subjective quality of life (DASH) and function (Rowe score and Constant score) of the shoulder evaluated after arthroscopic repair of anterior shoulder instability with biodegradable or metallic anchors. The follow-up time was 2 years.

Tan CK et al., 20065

Inclusion criteria: patients with traumatic anterior glenohumeral instability and recurrent dislocation. Exclusion criteria: patients with previous surgery or a single episode of shoulder dislocation. Intervention and comparison: the patients underwent arthroscopic surgical repair of the Bankart lesion (detachment of the glenoid labrum from the anteroinferior border of the glenoid) using biodegradable or metallic anchors. When an associated SLAP lesion was diagnosed during surgery, this was also corrected. Outcomes analyzed: patients were evaluated preoperatively and postoperatively for shoulder instability, pain, and quality of life. Clinical improvement was represented by a reduction in the Oxford Instability Shoulder Score (OISS – maximum possible score 60) and Visual Analogue Scale (VAS for pain; VAS for instability – maximum possible score 10),

and an increase in SF-12 OS (Short Form-12 Questionnaire Physical Score) and SF-12 MS (Short Form-12 Questionnaire Mental Score). Follow-up time was 1.5–5 years (mean 2.6 years).

Risk of bias and quality of evidence

Neither study was double blind nor examined by intention-to-treat analysis. In particular, in the study by Tan 2006[5], randomization and prognostic variables were not clearly described and the JADAD score was less than 3. The estimated overall strength of evidence was low. When evaluating the GRADE[3] evidence for recurrence, the outcome was moderate.

Analysis of results by outcome

We were able to perform the meta-analysis in only one outcome because it was included in the two selected works, and the evaluation was also performed for the quality of the evidence using the GRADE method.

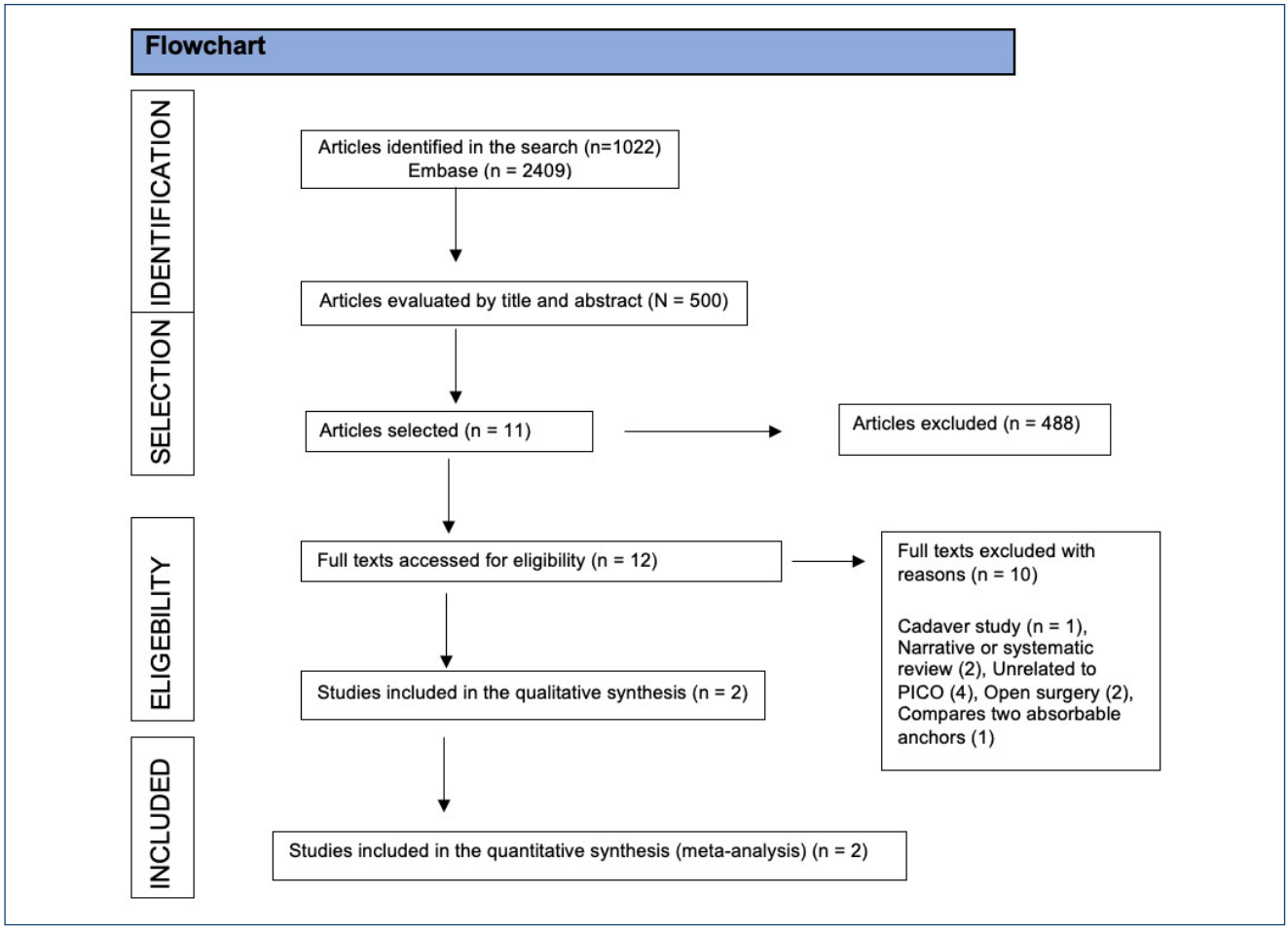

Figure 1. Flowchart of selected works.

Table 1. Descriptive table of the biases of the included randomized clinical trials.

Study	Focal question	Proper randomization	Concealed allocation	Double blinding	Losses (<20%)	Prognostic or Demographic characteristics	Outcomes	Intention-to-treat analysis	Sample calculation	JADAD
Milano G 2010	YES	YES	YES	NO	YES	YES	YES	NO	YES	3
Tan CK 2006	YES	?	YES	NO	YES	?	YES	NO	NO	2

Cochrane Systematic Reviews Database [Year], Number [Issue].

Milano 2010[4]

There was no difference in the subjective assessment of disease-related quality of life using the DASH questionnaire (p>0.05).

There was no difference in shoulder function related to joint stability, or in its global function, evaluated using the Rowe and Constant scores, respectively (p>0.05).

Tan CK 2006[5]

There was no statistically significant difference (p>0.05) between the two types of anchors in terms of clinical improvement assessed using the Oxford Instability Shoulder Score, Visual Analogue Scale for pain and instability, and Short Form-12 (see Table 2 for results).

Recurrence

There was no difference in the risk of recurrence between the two forms of treatment.

Summary of the evidence

The use of absorbable anchors in the treatment of recurrent traumatic shoulder instability does not show differences in terms of pain, function, quality of life, and recurrence outcomes, when compared to treatment with nonabsorbable anchors, with a minimum follow-up of 2 years. A moderate quality of evidence was found.

There is no consistent evidence for the study of the osteoarthritis outcome.

DISCUSSION

The aim of this study was to assess whether absorbable anchors are effective, as well as whether their use reduces the risk of chondrolysis and, consequently, secondary glenohumeral arthrosis, when compared with nonabsorbable anchors.

In the review by Papalia 2014[6], whose objective was to evaluate the clinical outcomes and complications between absorbable and nonabsorbable anchors in the surgical treatment of shoulder instability, the author concluded that "*given the good overall results reported after shoulder stabilization surgery with different types of anchors, it is not possible to comment on which type of anchor is best recommended for routine use.*"

The review of Brown 2017[7], with the objective of evaluating several factors involved in the shoulder instability surgery, including types of anchors, number of anchors used in the procedure, and absorbable versus nonabsorbable, found no difference in the risk of recurrent instability after arthroscopic Bankart reconstruction.

A retrospective study, Uluyardımcı 2021[8], comparing JuggerKnot® anchors, Biomet Inc., Warsaw, IN, USA, with metallic anchors, concluded that satisfactory results were obtained with the use of full suture anchors in the arthroscopic Bankart repair for traumatic anterior shoulder instability. "*The total suture anchors and the metallic suture anchors have similar results in the medium term and the total suture anchors are a reliable and effective option for the arthroscopic Bankart repair. The authors present as a result in one of the evaluated outcomes that according to the Samilson-Prieto classification*[9]*, there was no evidence of glenohumeral osteoarthritis in any of the patients in either group (JuggerKnot® anchors group 41.1±10.4 [ranging from 30 to 60 months] and metallic anchors group 39.6 ± 9.4 [ranging from 28 to 60 months]])*."

In this study, we found few clinical trials comparing bioabsorbable anchors in relation to metallic anchors, as in previous reviews[6,7]. Our data coincide with the literature with no significant differences in the evaluated scores or the rate of recurrence of dislocation in the operated shoulders. We did not find RCTs reporting osteoarthritis/glenohumeral osteoarthrosis secondary to the arthroscopic procedure in the treatment of instability.

CONCLUSIONS

The use of absorbable anchors is as effective as the use of metal anchors in the arthroscopic treatment of glenohumeral

Table 2. Strength of evidence assessed by GRADE.

Certainty assessment							No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	ABSORBABLE ANCHORS	NONABSORBABLE	Relative (95%CI)	Absolute (95%CI)		
Recurrence												
2	Randomized clinical trials	Serious[a,b]	Not serious	Not serious	Not serious	None	5/104 (4.8%)	5/104 (4.8%)	Not estimable	0 less per 1,000 (from -60 to +60)	⊕⊕⊕◯ Moderate	

CI: Confidence interval. [a]No intention-to-treat analysis. [b]Absence of double blinding.

instability, with a low risk of recurrence. The strength of the global evidence for the other outcomes evaluated is low due to the high risk of bias.

To evaluate the osteoarthritis/shoulder osteoarthrosis outcome secondary to the procedure, further studies are necessary.

AUTHORS' CONTRIBUTIONS

AA: Data curation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **AU**: Data curation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **HK**: Data curation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **IAZS**: Data curation, Formal Analysis, Software, Visualization, Writing – original draft, Writing – review & editing. **MMN**: Data curation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **MA**: Data curation, Formal Analysis, Visualization, Writing – original draft, Writing – review & editing. **PRNS**: Data curation, Formal Analysis, Visualization, Writing – review & editing. **PO**: Data curation, Formal Analysis, Visualization, Writing – review & editing. **WMB**: Data curation, Formal Analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Apropos of quality for fine-needle aspiration cytology of thyroid nodules with 22-, 23-, 25-, even 27-gauge needles and indeterminate cytology in thyroidology: an aide memory

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Thyroidology, a dynamic discipline, deals with a crucial and, in the meantime, delicate butterfly-shaped gland, which may demand a gracious approach[1-6]. Up-to-date management of nodular thyroid diseases necessitates the availability of several diagnostic and therapeutic modalities in order to obtain an accurate diagnosis and recommends appropriate treatment options. To this end, image-guided interventional techniques have globally been noticed and increasingly harnessed over the past four decades in thyroidology[1]. Nevertheless, an optimal needle size in order to provide an adequate and accurate thyroid fine-needle aspiration (FNA) cytology has not been established distinctly to date. We read with a great deal and respect the article by Dong and colleagues[7] entitled "Comparison of Ultrasound-Guided Fine-Needle Cytology Quality in Thyroid Nodules with 22-, 23-, and 25-Gauge Needles." The authors compared the cytology quality of sonography-guided FNA in thyroid nodules with the 22-, 23, and 25-Gauge (G) needles prospectively in a total of 480 nodules in 437 consecutive outpatients for 17 months. They declared that the 25-G needles obtained the highest scores of FNA sample quality compared with 22- and 23-G needles. Herewith, they stated that the 25-G needle should be the first choice for thyroid FNA in routine work. To the best of our knowledge, a well-accepted universal guideline for an ideal procedural technique, such as US-FNA, US-guided fine-needle capillary sampling, US-guided core needle biopsy, and optimal needle size in FNA procedures, has not been declared in thyroidology to date. Therefore, a wide range of, 20–27-G in size, needles have been used for FNA applications in different geographic regions, that is, 25–27-G in most Western countries and 21–22-G in Japan[8]. Some authors propounded that the nondiagnostic/unsatisfactory, Category I, the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), rates of 22- and 25-G needles were 18.5 and 21.0%, respectively[9]. However, many authors have demonstrated no significant difference in the adequacy rates of the samples, achieved with finer and thicker needles[10]. We reported a retrospective study, a sum of 500 nodules in 425 eligible consecutive outpatients for 38 months, involving US-FNA with a surgeon-performed US (SUS) in thyroid nodules with 27-G fine-needles with a reasonable low rate, 9.0%, of Category I, TBSRTC[11]. Although Dong et al.[7] stated that they have determined the cytology/smear qualities with four parameters by Haddadi-Nezhad et al.[12], we demonstrated, as an output of a SUS-based serial, that the delicate needle with the finest gauge[3] had possessed a reasonably low, 9.0%, nondiagnostic/unsatisfactory rate, which has been accepted globally as the crucial and significant marker for the quality of thyroid cytopathology, thereby thyroid FNA, utilizing TBSRTC, 1st[13] and 2nd[14] editions, and 2015 American Thyroid Association (ATA) Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer[5] ([A11], Recommendation 9, [A12], Recommendation 10)[15]. For this, revisiting optimal needle size for thyroid FNA to display whether not much finer and less nondiagnostic is an essential issue in thyroidology[4]. The 27-G needle, *minimum minimorum*[5], may provide cytologic quality, big gain[6], while bringing peace and quiet, no pain[6], particularly combining with our proposal of new terminology, Thy MIFNA[2,6]. Less is more?[5] *Volens nolens?*[5]

In addition, Dong et al.[7] stated that they had handled "indeterminate cytology" as Categories III and IV, TBSRTC. Nevertheless, many authorities, even the 2015 ATA Management Guidelines for Adult Patients with Thyroid Nodules and

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Differentiated Thyroid Cancer[15], determined "indeterminate cytology" as thyroid nodules with the cytology of Categories III, IV, and V, TBSRTC, 2nd ed[14], which additionally have the higher risk of malignancies (ROMs), regarding TBSRTC, 1st ed[13], in thyroidology. *A posteriori*, would the mentioned outcomes of the respectable study be affected in case of incorporating the thyroid nodules with Category V, TBSRTC, which have a higher ROM, into their study design, initially, in terms of the terminology of "indeterminate cytology"? In fact, the issue of optimal needle size, hereinabove, merits further investigation. *Ubi dubium ibi libertas.* We thank Dong et al.[1] for their valuable study.

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AUTHORS' CONTRIBUTIONS

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

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Comment on "Prevalence of subhealth status and its effects on mental health and smartphone addiction: a cross-sectional study among Chinese medical students"

Zixuan Zhou[1], Cuiping Li[1*]

Dear editor,

We are pleased to read an article entitled "Prevalence of subhealth status and its effects on mental health and smartphone addiction: a cross-sectional study among Chinese medical students" by Zhang et al.[1]. The findings of this study suggest that anxiety, depression, and smartphone addiction among Chinese medical students are associated with subhealth status. This study is of great significance in the prevention and treatment of the physical and mental health of medical students. However, in our opinion, there are some questions that are still unanswered and worth discussing.

The main problem with this study is that Suboptimal Health Status Questionnaires-25 can only be used to assess suboptimal health status but not to diagnose suboptimal health status. Suboptimal health was defined in this study by a total subhealth status score ≥35, which is obviously subjective. Clearly, such diagnostic criteria for suboptimal health are not widely accepted by scientists. In fact, there are clear diagnostic criteria for suboptimal health[2]. According to the definition of subhealth, subjects with mental subhealth, overweight, prehypertension, pre-diabetes, serum blood lipids (triglycerides or total cholesterol) above the borderline high level, renal subhealth, hepatic subhealth, or thiobarbituric acid-reactive substances ≥5.09 μmol/L (based on the reference range of 95%) were categorized as subhealthy[2].

Another problem is that this study is a non-probabilistic sample (web-based questionnaire). Thus, the sample is not representative. The limitations of the article include the non-probability sampling technique and sampling structure that is limited to a single university. In addition, there are many factors that affect subhealth. For example, the novel coronavirus disease 2019 (COVID-19) epidemic can also have deleterious consequences on depression and anxiety of college students[3].

CONCLUSION

The Suboptimal Health Status Questionnaires-25 can only be used to assess suboptimal health status but not to diagnose suboptimal health status. In addition, the causal relationship between subhealth and anxiety and depression needs to be further investigated.

DATA AVAILABILITY

The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTION

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

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Comment on "Hyperglycemia in pregnancy: sleep alterations, comorbidities and pharmacotherapy"

Rui Guo[1], Xiaoyu Zhang[1*]

Dear editor,

We are pleased to read the article entitled "Hyperglycemia in pregnancy: sleep alterations, comorbidities and pharmacotherapy." This cross-sectional study examined sleep alterations and related factors in pregnant women with diabetes mellitus[1]. In this study, the authors found that poor sleep quality was detected in 58.8% of patients, regardless of the cause of hyperglycemia. In addition, another interesting finding was that metformin treatment and higher parity were associated with poorer sleep quality only in patients with gestational diabetes. Undoubtedly, the conclusions of this study will help further improve the sleep quality of diabetics. However, in our point of view, there are still some issues that deserve further discussion.

First, the purpose of this study was to explore the factors associated with poor sleep quality in patients with diabetes. However, some potential important factors have been overlooked. Obviously, the duration of diabetes is related to sleep quality, which has been confirmed in previous studies[2,3]. In a study[2] involving 198 participants, the authors found that the duration of diabetes was significantly associated with poor sleep quality (AOR 4.88; 95%CI 1.27–18.66; p=0.021), implying that longer duration of diabetes results in a significant reduction in sleep quality. Furthermore, although this study reported glycemia level, it was still inadequate. Another retrospective study[3] indicated that nocturnal glycemic variability was associated with poor sleep quality in patients with type 1 diabetes, indicating that it is still obviously insufficient to use only glycemia level to judge the sleep quality of patients. Therefore, it is suggested that it is necessary to include the duration of diabetes and nocturnal blood glucose variables for subsequent analysis when exploring the influencing factors related to sleep quality in diabetic patients.

Second, metformin treatment was found to be associated with poorer sleep quality only among patients with gestational diabetes. However, given that type 1 diabetes, type 2 diabetes, and gestational diabetes belong to different disease subtypes, there may also be significant differences in the dose and use of metformin. One possible hypothesis is that metformin affects sleep quality in all three diabetic subtypes, but is ignored due to different doses and usages. Thus, from our perspective, to further determine the effects of metformin on sleep quality in patients with different diabetes subtypes, it is necessary to provide more information about metformin.

AUTHORS' CONTRIBUTION

RG: Conceptualization, Writing – original draft. **XZ:** Conceptualization, Writing – original draft

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SHORT COMMUNICATION

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Six-minute walk test predicts future decompensation in patients with compensated liver cirrhosis

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INTRODUCTION

Patients with chronic diseases tend to present a reduction in their exercise capacity and consequently to the impairment of several physiological functions of the body[1,2]. This is also the case with cirrhotic patients whose exercise capacity is measurable through tests already validated, such as the 6-min walk test (6MWT)[2,3]. Since it is known that clinical decompensation is a landmark for the reduction of survival in cirrhotic patients[4], our research group aimed to examine the relationship between the exercise capacity, measured by means of the 6MWT, of clinically stable patients with liver cirrhosis and the development of clinical decompensation related to the disease[5]. However, the odds ratio showing the impact of the distance covered in the 6MWT on the risk of decompensation has not yet been determined.

The rationale for this study was to verify the plausibility of using a simple, easy-to-perform, low-cost, and reliable test that does not require advanced training or special equipment, such as the 6MWT[6], to predict possible decompensation in clinically stable patients with liver cirrhosis. Once screening for the possibility of more severe outcomes can advance early clinical decision-making, the use of an accessible and reliable assessment (6MWT) can bring a safety advantage in the management of these patients. In this sense, one way to assess this possibility is to recruit compensated cirrhotic patients for the 6MWT and to follow them, prospectively, through a prospective longitudinal observational study. Therefore, the aim of this study was to assess the impact of the distance reached in the 6MWT on the risk of clinical decompensation of clinically stable patients with cirrhosis over 1 year.

METHODS

This is an observational, prospective, longitudinal study conducted at the Hepatology Outpatient Clinic of the Gastroenterology Service of the University Hospital of the Juiz de Fora Federal University, Brazil, involving male and female adult patients with an established diagnosis of liver cirrhosis. The inclusion of patients occurred from January to December 2018. A total of 55 volunteers with compensated liver cirrhosis completed an initial clinical evaluation, performed the 6MWT, and were followed up for 12 months. Patients with a history of hepatic decompensation in the past 12 months, such as those with jaundice, ascites, esophageal variceal bleeding, or hepatic encephalopathy, were excluded from the study.

After the initial approach, patients were followed up on an outpatient basis every 2–3 months or when not possible by telephone contact at 3rd, 6th, 9th, and 12th months. A questionnaire was used to inquire about the appearance of signs and symptoms of clinical decompensation of liver cirrhosis, or hospitalization on account of ascites, jaundice, esophageal variceal bleeding, or spontaneous bacterial peritonitis. The differences between the groups with and without clinical decompensation in the follow-up period were analyzed using the independent samples t-test. A parametric test (t-test) was performed after verifying that the data distribution was normal (Shapiro-Wilk test; $p>0.05$) and that its variances were homogeneous (Levene's test; $p>0.05$). To evaluate the effect of the distance walked on the 6MWT on the outcome variable (clinical decompensation), we used a binary logistic regression model (*stepwise*), in which clinical decompensation was the dependent variable. We included gender and age in the model as potential intervening variables to be controlled. We also included the variable Child-Pugh Score[7] (which is known to be related to the

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risk of decompensation) to assess the clinical consistency of the model. The fit of the regression model was assessed by the area under the ROC curve of the regression and the Hosmer-Lemeshow test. All tests were two-tailed, and the significance level was set at 5%. Analyses were performed using MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014).

RESULTS

Of the 55 patients followed for 12 months, 65% were male, with a mean age (±SD) of 56.3 (±10.5) years. The main etiologies found for cirrhosis were alcohol, virus C, and non-alcoholic steatohepatitis. Also, 36 (65%) patients were of Child's class A and 19 (35%) were of Child's class B. Clinical and anthropometric characteristics are summarized in Table 1. There was a significant difference in the 6MWT walking distance between the group that did not present clinical decompensation of cirrhosis (33 patients) and the group that presented clinical decompensation (22 patients) during the 12-month follow-up (470±76 m vs. 378±19 m; p<0.01). There was no statistically significant difference in walking distance between Child-Pugh class A and B patients (451±95 m vs. 401±117 m; p=0.90).

The logistic regression analysis showed that, considering all the variables of the model, the increase of 1 m in the walking distance in the 6MWT reduced the risk of clinical decompensation at follow-up by approximately 2%. This result was adjusted by the regression model for sex, age, and Child-Pugh Score. The *goodness of fit* of the model was ensured by calculating the area under the curve (0.91 [95%CI 0.70–0.97]) and the Hosmer-Lemeshow test (p=0.24 [>0.05]). The result of logistic regression is shown in Table 2, which shows the *odds ratio* with the respective 95%CI for the predictor variable (6MWT) and the variable Child-Pugh Score in order to quantify how much this variable would increase the risk of decompensation.

DISCUSSION

Our research points to a strong association between a shorter walking distance on the 6MWT and a higher risk of clinical decompensation in clinically stable patients with liver cirrhosis at 1-year follow-up, and an approximate 2% reduction in this risk for each meter increase in the walking distance at the end of the test. This corresponds to a 20% reduction in the risk of decompensation for a 10-m increase in the 6MWT result.

Regarding clinical decompensation, liver cirrhosis presents an initial phase, clinically silent, called "compensated cirrhosis," followed by the phase of the appearance of signs and symptoms resulting from portal hypertension and/or hepatic dysfunction, called "decompensated cirrhosis." It is well known that, as the pressure in the portal system increases, the disease evolves to the decompensated phase, when the clinical decompensation of hepatic cirrhosis appear[4]. Survival is related to the appearance of clinical decompensation, with liver-related mortality occurring almost exclusively after the progression of the patient's condition to "decompensated cirrhosis"[8]. In parallel, cirrhosis causes extrahepatic derangements that affect several organic systems and are related to the reduction of exercise capacity, an alteration that is directly related to the degree of hepatic function impairment[9].

In our sample, there was a clear association between a lower exercise capacity, evaluated by the lower distance walked on the 6MWT, and a higher risk of clinical decompensation during the 12-month follow-up. In accordance with the studies that point out that low physical fitness is associated with higher morbidity and mortality by all causes in the presence of several chronic diseases, other studies with cirrhotic patients also point to a direct relationship between physical fitness and prognosis in these individuals[5,10].

Alameri and colleagues showed that the exercise capacity was an independent marker of survival in patients with liver cirrhosis[3]. Another study[11] identified the distance in the 6MWT

Table 1. Clinical and anthropometric characteristics of the population.

Variables	Odds ratio	95%CI
Child-Pugh Score	6.9066	1.3411–35.5676
Distance 6MWT	0.9793	0.9679–0.9909

Data are presented as mean±standard deviation and frequency (%). NASH: nonalcoholic steatohepatits.

Table 2. Results of the logistic regression model evaluating the impact of the Child-Pugh score on the distance in the 6-min walk test.

Variables	n=55
Sex (M/F)	36/19
Age (years)	56.3±10.5
Etiology of cirrhosis	
Alcohol	20 (36.4%)
C virus	13 (23.6%)
NASH	11 (20%)
Others	8 (14.5%)
Indeterminate	3 (5.5%)
Child-Pugh score (A/B)	36 (65.4%)/19 (34.6%)

CI: confidence interval; 6MWT: 6-min walk test.

as an independent predictor of mortality in the candidates for liver transplantation; this physical test can identify candidates at higher risk of death on the waiting list. Our group has previously demonstrated that the 6MWT is a significant predictor of clinical decompensation in patients with cirrhosis and pointed to a cutoff point of 401.8 m as related to an increased risk of clinical decompensation in clinically stable cirrhotic patients[5].

Although several studies with cirrhotic patients show an association between lower exercise capacity and higher mortality rate, to date, the approach that relates this impairment to the risk of the clinical decompensation of the disease is limited in the literature. In this study, the logistic regression adjusted for the model variables revealed a 2% reduction in the risk of clinical decompensation of cirrhosis for each gain of 1 m in walking distance on the 6MWT. One finding that corroborates the result found for the 6MWT in the logistic regression is the approximately seven times higher risk for the most severe patients (Child B) to present decompensation, as expected; this serves to demonstrate the consistency of the findings of the statistical model.

We believe that the strongest point of our study was that we showed the possibility of relating, and even predicting, a severe clinical outcome (cirrhotic decompensation) with an accessible and easy-to-perform test, such as the 6MWT. The impact of impaired exercise capacity in cirrhotic patients emerges as a promising area of study. Although a parallel between impaired hepatic function and exercise capacity is expected, there is a variation in clinical characteristics and measurements that makes the identification of accessible markers that help to indicate the evolution of the disease urgent. Another strength of our research includes the real-world data set that represents a cohort of ambulatory patients with compensated cirrhosis followed in the long term. In addition, all patients had detailed clinical data collected using a standardized questionnaire.

Notwithstanding, our study also has drawbacks that must be considered. Perhaps, the follow-up time used for the main outcome (i.e., hepatic decompensation) in this study is not long enough for meaningful analysis. Furthermore, if our study had been carried out with a larger sample, perhaps the results would have an even more robust clinical significance for clinical practice. Despite we have found a measurable statistical significance in our analysis. In fact, observational studies can always be more informative as samples increase in size and observation time lengthens.

Future prospective longitudinal studies involving a broader population of patients with compensated cirrhosis are needed to determine the accuracy of 6MWT to predict clinical decompensation related to underlying liver disease. Additionally, it will be important to investigate whether a standardized physical activity protocol directed for patients with compensated cirrhosis aiming to gradually increase physical activity will result in better outcomes.

CONCLUSION

The results show a strong association between shorter distance walked in the 6MWT and higher risk of clinical decompensation in clinically stable patients with liver cirrhosis. Therefore, it allows us to assume that the 6MWT, a relatively simple and low-cost test, appears as a viable tool to predict decompensation in this population over a year. These findings add important information about cirrhosis decompensation and the measurement of exercise capacity in the routine evaluation of patients with the disease that should be considered.

AUTHORS' CONTRIBUTIONS

DMNH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Visualization, Supervision, Writing – original draft, Writing – review & editing. **CAMJ:** Data Curation, Formal Analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **FHLP:** Investigation, Resources, Supervision, Validation, Visualization, Writing – original draft. **TMDO:** Data curation, Investigation, Validation, Visualization, Writing – original draft. **CM:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **JMFC:** Conceptualization, Methodology, Project administration, Resources, Visualization, Writing – review & editing.

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SHORT COMMUNICATION

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Depression as a major determinant of PASS (Patient's Acceptable Symptoms State) in rheumatoid arthritis: a cross-sectional study in Brazilian patients

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease with profound repercussions on the patient's well-being. Chronic pain, restriction of activities, and fatigue after the chronic articular inflammatory process interfere with the patient's daily activity and are frequently associated with disability, depression, and anxiety[1,2].

PASS (Patient's Acceptable Symptoms State) is a single question that measures the value beyond which patients consider themselves well[3]. It is obtained from patients responding YES or NO to a question if their current condition is satisfactory, considering the general functioning and current pain. Additionally, this question reflects patients' perceptions about their disease, including their beliefs, and emotional and cognitive responses that are important to determine their resilience and consequent quality of life[4].

The PASS study is vital in understanding the consequences of the disease from the patients' viewpoint, as doctors and patients may have different perspectives and expectations on good health status[5]. According to Puyraimond-Zemmour et al.[5], when a rheumatic patient judges well-being, he values five areas that can be classified into three categories: physical (pain, function, and sleep), mental (coping), and mixed (that includes fatigue). Generally, clinicians tailor treatment according to objective inflammatory signs of disease activity and such an approach may not be satisfactory as it leaves other health domains uncovered.

Here, a group of Brazilian patients with RA was studied to determine the relationship of their answers to the PASS question with disease activity, pain, functionality, and mood conditions.

METHODS

Ethical issues

This study was approved by the local committee of ethics in research – CAAE-32073120.8.0000.0103 under protocol 4.079.233 from June 9, 2020, and all participants signed consent.

Sample and study design

This is a cross-sectional study with a convenient sample that includes patients with RA from a single rheumatology unit from a university hospital that agreed to participate in the study and came for regular consultation during the 1-year period (July 2020–July 2021). Patients were invited to participate according to appointment order for regular consultations and were included according to willingness to participate in the study. This rheumatology unit belongs to a university hospital that treats patients using public health care in Brazil.

Inclusion and exclusion criteria

To be included, patients should be older than 18 years and fill at least six points on the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA[6]. Patients with secondary fibromyalgia, associated inflammatory and neoplastic disorders, and neurological or orthopedical problems that impaired functioning were excluded.

Data collection

Epidemiological (sex, age, age at disease onset, auto-declared ethnic background, and years of formal study), clinical, and serological data were obtained through chart review and direct questioning.

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The disease activity was assessed using SDAI (simple disease activity index) and CDAI (clinical disease activity index); functionality was judged by the HAQ (health assessment questionnaire) and pain through a VAS (visual analog scale; from 0 to 10, where 0=no pain and 10=maximum pain). Anxiety was evaluated using the Beck inventory (p) and depression by the CES-D (Center for Epidemiologic Studies Depression Scale).

The CDAI was measured through a tender and swollen 28-joint count, the patient's global disease activity (from 0–10), and the evaluator's global disease activity (from 0–10). The following cutoff points were used for interpretation: remission ≤2.8, low disease activity >2.8 – ≤10, moderate disease activity >10 – ≤22, and high disease activity >22[7].

The SDAI was measured by the arithmetic sum of tender and swollen 28-joint count, patient's and evaluator's global assessment (from 0–10), and C-reactive protein in mg/dL[7]. Patients with SDAI values ≤3.3 were in remission, with values >3.3 – ≤11 in low disease activity, and values >11 – ≤26 and >26 in high disease activity[7].

The HAQ has questions of about 20 specific activities assessed on a 4-point Likert scale, where 0=without difficulty, 1=with some difficulty, 2=with much difficulty, and 3=unable to do. The 20 activities are grouped into eight functional categories with each category given a single score equal to the maximum value of its component activities. Thus, the final value ranges from 0=no impairment to 3=maximum impairment[8].

The PASS question was expressed as follows: "Think about all the ways your RA has affected you during the last 48 hours. If you were to remain in the next few months as you were during the last 48 hours, would this be acceptable to you?" The YES/NO answer was considered an indicator of satisfaction with the present symptom's state[9].

The CES-D is a 20-item self-report measure that accesses current symptoms of depression on a Likert scale (0=rarely or none of the time; 1=some or little of the time; 2=occasionally or a moderate amount of the time; 3=most or all the time). Values less than 15 are normal, values from 15 to 21 suggest mild to moderate depression, and values over 21 indicate possibility of major depression[10].

The Beck Anxiety Inventory (BAI) is a 21-question multiple-choice self-reported inventory that is used for measuring anxiety severity. Each answer is scored from 0 (not at all) to 3 (severely), and higher total scores indicate more severe anxiety symptoms. The standardized cutoffs are: 0–7=minimal; 8–15=mild; 16–25=moderate; 26–63=severe anxiety[11].

All used instruments were translated and validated into Portuguese[9-11].

Statistical analysis

Data were collected in frequency and contingency tables. Numerical data central tendency were expressed in means and standard deviation (SD) if data were parametric and the medians and interquartile range were nonparametric. Data distribution was judged by the Shapiro-Wilks test. Patients answering YES to PASS were compared with those answering NO using Fisher's and chi-square tests for nominal data and Mann-Whitney and unpaired *t* tests for numerical data. Correlation studies of VAS of pain with depression (CES-D) and anxiety (BAI) were done using the Spearman test. Variables that were associated with the PASS with p>0.1 were studied through multivariate forward logistic regression to evaluate the variable independence, and CDAI, SDAI, HAQ, VAS of pain, CES-D, and BAI were studied as numerical variables. The adopted significance was 5%, and the tests were calculated using the MedCalc Statistical Software v.20.007 (MedCalc Software Ltd, Ostend, Belgium).

RESULTS

Description of the studied sample

In total, 116 patients were included. The sample had a predominancy of middle-aged Caucasian women (Table 1), reflecting the epidemiology of the disease. Median disease activity indexes were compatible with low disease activity, and most patients expressed some degree of anxiety and depression.

In this sample, 34/116 (29.3%) answered NO to the PASS, and 82/116 (70.6%) answered YES.

Comparison of patients with YES and NO responses to Patient's Acceptable Symptoms State

A comparison between the samples with responses YES and NO to PASS is summarized in Table 2.

A logistic regression study using the PASS as a dependent and independent variable: age, SDAI, CDAI, HAQ, VAS of pain, CES-D, and BAI results showed that the only independent variable was depression (p=0.003; OR 1.06; 95%CI 1.02–1.1).

A positive correlation of VAS of pain with CES-D (r=0.29; 95%CI 0.03–0.51; p=0.02) and BAI (r=0.41; 95%CI 0.16–0.60; p=0.001) was found.

DISCUSSION

These results have shown that less than one-third of the patients with RA from this sample (29.3%) were not satisfied with the treatment results. It was also found that patients who answered

Table 1. Epidemiological data, functional and inflammatory indexes, and results of anxiety and depression questionnaires in rheumatoid arthritis patients (n=116).

Female sex/males (n) (%)	99/17	85.3/14.6
Age (years)	30–78	Mean 56.5 (10.4)
Disease duration (years)	1–32	Median 10 (6–17.7)
Auto-declared ethnic background (n)		(%)
Caucasian (n)	109/116	93.9
Afro descendants (n)	5/116	4.3
Asian descendants (n)	2/116	1.7
Positive rheumatoid factor	77/116	66.3
CDAI	0–58	Median 8.5 (2.5–16.4)
SDAI	0–66	Median 10.1 (4.0–21.7)
HAQ	0–2.7	Median 1.0 (0.5–1.7)
Beck Anxiety Inventory (%)	0–52	Median 11 (7–20.0)
No anxiety (n)	34–29.3	
Mild anxiety (n)	39–33.6	
Moderate anxiety (n)	25–21.5	
Severe anxiety (n)	18–15.5	
CES-D	3–55	Median 18.0 (10–26)
Normal (n%)	45–38.7	
Mild to moderate depression (n%)	29–25	
Possibility of major depression (n%)	42–36.2	
VAS of pain	0–10	Median 5.0 (3.0–7.0)

CDAI: Clinical Disease Activity Index; SDAI: Simple Disease Activity Index; HAQ: Health Assessment Questionnaire; CES-D: Center for Epidemiologic Studies Depression Scale; VAS: visual analog scale; n: number, between brackets; IQR: interquartile range.

NO to PASS had more disease activity, felt more pain, had higher levels of depression and anxiety, and had worse clinical performance than those who answered YES.

The proportion of patients in the PASS in this study was similar to that found in a Swedish sample of patients treated and followed by 5 years, reaching low disease activity (of 22.5%)[2]; it was lower than that found in a Norwegian study that encompassed 1,496 patients (36.8%)[12]. In this study, disease activity, loss of function, pain, anxiety, and depression are associated with this dissatisfaction.

The disease activity has also been linked to PASS in several other studies[2,13]. Cutoff values for composite indices were examined in this context and found compatible with moderate disease activity. Values of DAS-28 <4.21 at week 12 and <3.90 at week 52 in a cohort of patients with RA and established disease were considered acceptable by the patients in the study by Heiberg et al. that analyzed the longitudinal stability of the PASS cutoff points[5]. Another study in patients with early RA followed up for 1 year showed that unsatisfactory PASS outcomes were associated with high or moderate disease activity since the patients also had associated high PROM (patient-reported outcome measures) scores[13]. Eberhard et al.[2] further explored this aspect and found that patients with "unacceptable pain" had low swollen joint counts and a high VAS for pain. Discrepancies between the evaluation of inflammatory disease activity and patient's expectations have been reported earlier by others[14,15], highlighting the greater importance of symptoms over inflammatory findings in this setting. Thus, although important, the inflammatory component of the disease may not be the main determinant of PASS.

Arthritis pain is, at least partially, secondary to the action of pro-inflammatory cytokines, which activate nociceptors in

Table 2. Comparison of rheumatoid arthritis patients with answers YES and NO to PASS (Patient's Acceptable Symptoms State).

	PASS: Yes n=82 (%)	PASS: No n=34 (%)	p-value
Female sex (n)	68–82.9	31–91.1	0.38
Age – mean (SD) (years)	57.6 (10.3)	53.9 (10.5)	0.08
Disease duration – median (IQR)	10.0 (6.0–18.0)	12.5 (7.7–17.2)	0.37
Positive rheumatoid factor (n)	55/80–68.7	22/33–66.6	0.82
SDAI – median (IQR)	8.8 (2.8–19.0)	16.15 (6.0–25.6)	0.08
CDAI – median (IQR)	14.0 (5.5–21.5)	8.0 (2.0–15.0)	0.03
HAQ – median (IQR)	0.87 (0.2–1.4)	1.37 (0.9–2.1)	0.001
Beck Anxiety Inventory – median (IQR)	11.0 (6.0–17.0)	13.5 (7.0–25.5)	0.05
VAS pain – median (IQR)	5.0 (2.7–6.2)	6.0 (3.7–7.2)	0.03
CES-D – median (IQR)	17.0 (9.0–22.0)	22.5 (16.2–34.5)	0.001

PASS: Patient's Acceptable Symptoms State; CDAI: Clinical Disease Activity Index; SDAI: Simple Disease Activity Index; HAQ: Health Assessment Questionnaire; CES-D: Center for Epidemiologic Studies Depression Scale; VAS: Visual analogic scale; n: number; SD: standard deviation; IQR: interquartile range.

the synovium[16]. However, it has been documented that a significant group of patients with RA continues experiencing pain despite good inflammatory control of their disease due to neuropathic mechanisms secondary to central sensitization[2,17]. Consequently, directing the RA treatment according to the measurement of objective outcomes may not be enough to achieve good results from the patient's viewpoint. A central sensitization pain treatment requires treating neural pain with drugs, such as gabapentin, or antidepressive agents, such as duloxetine or imipramine, and may benefit from a multidisciplinary team management[18]. In this context, McWilliams et al.[19] observed the importance of early intervention in pain treatment, as patients with high pain levels in the beginning of the disease have an increased risk of long-lasting pain. Considering these, the study on the impact of mood disorders may be important and is poorly explored in the PASS setting. Depression was associated independently with PASS in this study. Moreover, at present, the pain scale is correlated with depression and anxiety, as already observed by others[20]. A systematic review of osteoarthritis pain showed that pain severity was correlated with emotional impairment (anxiety/depression) severity in these patients[20]. Although depression and pain may have common physiopathological links, both have a biochemical basis, focusing on the serotonergic and norepinephrine system and suffers modulation by the same brain structures, that is, the prefrontal cortex[20,21]. Furthermore, mood disorders interfere with pain acceptance, an important mechanism for coping with pain and channeling patients' thoughts and sensations toward valuable goals and purposes[22]. Pain is also considered to predict the level of fatigue and work disability[23], amplifying patients' dissatisfaction.

Epidemiological variables have been studied in the PASS. Also, Salaffi et al.[24] found that patients in the PASS were older, similar to what has been observed in spondyloarthritis[25]. Additionally, Duarte et al. observed that being older than 50 years was associated with PASS in RA[26]. Therefore, the patients in the PASS in this study were older than those not in the PASS, although this difference was not statistically significant.

This study is limited by the small number of participants and its cross-sectional design. In addition, socioeconomic factors and drugs used for the RA treatment were not studied and could have had some influence on the patient's well-being. Nevertheless, all study patients were from a public health-care center that attends to individuals with low socioeconomic status, and this could have given some homogeneity in this context between those answering YES and NO to the PASS. Its main value is that, in this sample, depression was an independent variable in the patient's acceptance of the disease. Clinicians caring for RA patients should be alert for depression signs, introducing early treatment in order to improve their quality of life.

In conclusion, disease activity, loss of function, pain, anxiety, and depression were linked to answering NO to the PASS question. Finally, depression was the only independent variable.

AUTHORS' CONTRIBUTION

PHS: Conceptualization, Writing – original draft. **MHJ:** Conceptualization, Writing – original draft. **BK:** Conceptualization, Writing – original draft. **RN:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **TS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft.

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ORIGINAL ARTICLE

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Determination of aflatoxin M1 in breast milk and related factors

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SUMMARY

OBJECTIVE: Breastfeeding in women with aflatoxin M1 exposure may be a risk factor for the newborn. Thus, it is crucial to determine aflatoxin M1 levels in breast milk and raise mothers' awareness about nutrition in lactation and other periods. This study was carried out to determine aflatoxin M1 contamination in milk samples taken from mothers who gave birth.

METHODS: The study was carried out in the postpartum department of Training and Research Hospital between December 31, 2018, and June 31, 2019, and 90 breastfeeding mothers were included in the study.

RESULTS: A total of 75 (83.3%) of the examined samples were found positive. The mean aflatoxin M1 ratio in positive samples was 12.16 pg/mL (5.00–23.18 pg/mL). Mothers' consumption of processed food was associated with aflatoxin M1 levels (p=0.043). It was determined that the aflatoxin M1 levels of mothers who consumed processed food products 1 or 2 times a month were 3.22 times lower than those who consumed 1–2 times a week.

CONCLUSIONS: This study emphasized the importance of monitoring aflatoxin M1 levels in breast milk for infant health. It is thought that nutrition education given to mothers during pregnancy will significantly impact aflatoxin M1 results. In addition, the dangers of mycotoxins in mother-infant nutrition should be emphasized regularly in health education.

KEYWORDS: Aflatoxin M1. Human milk. ELISA. Infant.

INTRODUCTION

Breast milk alone contains all the nutrients necessary for optimal growth and development of infants for the first 6 months[1]. Depending on the mother's nutritional style, the milk contains nutritive and immunologically beneficial components as well as the contaminants it is exposed to. Thus, it is transferred to the baby in contaminants along with the beneficial components. One of these contaminants is mycotoxins, which occur naturally under appropriate heat and humidity and produce various toxic metabolites by fungi[2,3].

Among the aflatoxins, the most potent hepatocarcinogen and hepatotoxic is AFB_1[4]. This aflatoxin is produced by the fungus *Aspergillus flavus*. Lactation women and animals consuming food contaminated with AFB_1 undergo hydroxylation by the AFB_1 cytochrome P450 enzyme system. AFB_1 is known to transform into its primary metabolite, aflatoxin M1 (AFM_1), which is 10 times less carcinogenic than itself in 12–24 h. It is also accepted that AFM_1 is excreted from the body with urine and breast milk[4]. The hydroxylated metabolite of aflatoxins, AFM_1, is observed in human breast milk[5]. Considering the importance of breastfeeding in the development of newborn infants, the presence of mycotoxins and other contaminants in breast milk should be continuously evaluated. Exposure to aflatoxin can have devastating consequences, especially in newborns whose immune system has not yet developed. In this respect, infants who consume contaminated breast milk containing AFM_1 are at risk of developing hepatocellular carcinoma, stunted development, encephalopathy, and visceral fatty degeneration[5,6]. Breast milk, which is essential for the growth and development of newborns' immune systems, should be as far away from these mycotoxins as possible[7].

Aflatoxin M1 (AFM_1) concentrations in breast milk were measured in Colombia[8], Australia[9], the United Arab Emirates (UAE)[10], Egypt[11], Turkey[2-4], Iran[12], Brazil[13], Italy[14], and Norway[15]. AFM_1 has been detected in human breast milk at various concentrations in various research[5]. Sierra Leone (0.80 ng/L) and the UAE had the lowest and highest quantities of AFM_1 in human breast milk, respectively. The Gambia, Tanzania and Jordan had the lowest prevalence of AFM_1 in human breast milk (2%), whereas the Gambia, Tanzania, and Jordan had the highest (100%). The continents of America (10.30 ng/L) and South-East Asia (358.99 ng/L) have the lowest and highest quantities of AFM_1 in human breast milk, respectively. In addition, the West Pacific (7%) and Africa (52%) continents have the lowest and highest incidence of AFM_1 in human breast milk, respectively[5].

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Breastfeeding has been shown to be a risk factor for babies in women who have been exposed to AFM_1[5]. Thus, it is crucial to determine AFM_1 levels in breast milk and raise mothers' awareness about nutrition in lactation and other periods. In addition, it is essential to reduce the toxic effects of AFM_1 by providing information on protection from AFM_1 exposure. In the literature review, few studies investigating AFM_1 exposure in breast milk were found in Turkey[2-4]. When these studies were examined, no study was found in the Black Sea region. The Black Sea region is a geographical region that receives constant precipitation. It has been reported that the prevalence of AFM_1 in breast milk increases significantly with increasing annual mean precipitation[5]. Considering the regional variation of AFM_1 exposure, investigating the presence of AFM_1 in breast milk in the Black Sea, which is a region that receives continuous rainfall, will contribute to the literature. The aims of this study were to measure the levels of AFM_1 in the breast milk of mothers who had given birth in the area of Black sea and to identify demographic and dietary factors associated with these levels.

METHODS

Study design

This is a toxicological analysis laboratory study. Permission was obtained from the Human Research Ethics Committee (dated May 30, 2018 and no. 6/15) and the institute where the research was conducted.

Participants

Milk samples were collected between December 31, 2018, and June 31, 2019. Data were collected by face-to-face interview technique. A questionnaire consisting of 22 questions was used to collect milk samples, including sociodemographic information about the mothers, nutritional habits, living environment characteristics, and 24-h food consumption information. A total of 90 lactating women who gave birth were included in the present study to analyze AFM_1 levels in breast milk.

Data analysis

Aflatoxin M1 analysis in breast milk samples

Aflatoxin M1 (AFM_1) levels were determined using competitive ELISA and AFM_1 Ridascreen (R1121) commercial assay kits. The lowest detection limit of the AFM_1 Ridascreen commercial test kit is 5 ppt for milk samples. Hence, breast milk samples with AFM_1 levels below 5 ppt were considered negative.

Statistical analysis

The SPSS version 20 software was used for the statistical evaluation of the data. The data indicated by counting in the applied questionnaire form were evaluated as numbers and percentages. It was determined that various variables showed normal distribution according to Kolmogorov-Smirnov test in the differences between AFM_1 levels in breast milk. In this distribution, the independent t-test was used for binary variables, and the ANOVA test was used for multiple variables. AFM_1 levels, age, educational status, and nutritional characteristics of mothers were examined by linear regression method. The data were analyzed within the 95% confidence interval, and the significance level was taken as $p<0.05$.

RESULTS

The mean age of the women participating in the study was 28±5.56. Educational status varies, where 27.8% are primary school graduates and 26.7% are university graduates. Notably, 30% of women had a spontaneous vaginal delivery, and 70% had a cesarean section. It was determined that 12.2% of them took iron medicine, and 6.7% took vitamin D as supplements during the lactation period. In addition, it was seen that 4.4% of them used the herbal mixture, mainly lemon water and milk tea, in this period (Table 1).

In this study, 12.2% of the women defined their houses as damp and 2.2% as quite damp. It was determined that 54.4% of the women used ready-made dairy products, and 81.1% boiled them. It was determined that 81.1% of them consumed spices, and 10% of the spices they used in their homes were moldy. It was determined that 64.4% of women consumed dried fruits and vegetables. The rate of those who read on the packages in grocery shopping is 77.8%. It was determined that 71.1% of them read the expiration date, and 4.4% read the content. It was observed that 54.4% of the women participating in the study did not consume any acidic beverage products, 51.1% of them did not consume any ready-made food products, and 37.8% of them never consumed any processed food products.

The AFM_1 levels in breast milk were found to be positive in 83.3% and negative in 16.7%. The mean AFM_1 ratio in positive samples was measured as 12.16±5.85 pg/ml (5.00–23.18 pg/ml). The relationship between mothers' AFM_1 levels and age, education level, region of residence, and house dampness was analyzed by linear regression analysis. The regression model was statistically insignificant ($F=1.099$; $p<0.373$, Table 2).

The relationship between mothers' AFM_1 levels and their consumption of ready-made, processed, and acidic foods were analyzed by linear regression analysis. The regression model

Table 1. Sociodemographic characteristics of mothers.

Variable	Value
Participants (N)	90 (100)
Age	
Mean±SD	28±5.56
Type of birth, n (%)	
Vaginal delivery	27 (30.0)
Caesarean delivery	63 (70)
Educational status, n (%)	
Primary education	25 (27.8)
Middle school	15 (16.7)
High school	19 (21.1)
College	24 (26.7)
Master's-PhD	7 (7.8)
Living place, n (%)	
Village-town	10 (10.1)
District	32 (35.6)
City center	48 (53.3)
Working condition, n (%)	
Housewife	70 (77.8)
Public officer	8 (8.9)
Employee	8 (8.9)
Academician	4 (4.4)
Smoking, n (%)	
Yes	6 (6.7)
No	84 (93.3)
Chronic disease, n (%)	
Yes*	15 (16.7)
No	75 (83.3)
Using vitamins, n (%)	
Yes	17 (18.9)
No	73 (81.1)
Vitamins used, n (%)	
None	73 (81.1)
Vitamin D	6 (6.7)
Iron	11 (12.2)
Use of herbal mixture, n (%)	
Yes	4 (4.4)
No	86 (95.6)

*Thyroid (6.7%), hypertension (3.3%), diabetes (2.2%), asthma (2.2%).

was statistically insignificant (F=1.091; p<0.375). Although the model was insignificant, a relationship was found between the AFM_1 level and the frequency of consuming processed food (p=0.04). It was observed that the AFM_1 value decreased by 3.22 times when consuming processed products 1–2 times a month. It has been observed that consuming ready-made and acidic foods is not effective in the AFM_1 level.

The relationship between the mothers' AFM_1 levels and their nutritional status in the last 24 h was analyzed by linear regression analysis, showing statistically insignificant (F=0.269; p<0.847, Table 3).

DISCUSSION

In this study, AFM_1 was detected in 83.3% of 90 breast milk samples. The literature review found that it is seen in 93.8% in Lebanon, 98.1% in Iran, 92 and 99.5% in the UAE, 10.5% in Turkey, 90% in Colombia, and 89% in Mexico[4,8,9,12,16-21]. AFM_1 was found to be positive (≥5 pg/mL) in those aged 26–35years, primary school graduates, and living in the city center more than women with a mean age of 28±5.56 years. This situation is similar to the study conducted in Lebanon; it was lower than Iran, Mexico, and the UAE rates[9,12,17-19].

Since mycotoxins are carcinogenic, mutagenic, teratogenic, and toxic substances, it is crucial to determine their levels[2]. Thus, many related studies have been carried out in Turkey. Cherkani-Hassani et al. scanned the studies between 1984 and 2015 to determine the prevalence, levels, and exposure conditions of mycotoxins and their metabolites in breast milk and found that 63 studies were conducted worldwide[22]. The majority of these studies were conducted in Egypt, Iran, Italy, Sudan, and Turkey; however, it is reported that the highest AFM_1 values are observed in Egypt, Ghana, UAE, Nigeria, and Sierra Leone[22]. In a study examining the relationship between maternal dietary habits and mycotoxin in Italy, only 4 out of 82 milk samples were AFM_1 positive. It was concluded that mycotoxin was significantly higher in the milk of those who consumed bread, bakery products, and processed pork[14]. In a study conducted in Egypt, 138 of 388 breast milk samples were AFM_1 positive[20]. In a study conducted in Sudan, 51 of 94 breast milk samples were AFM_1 positive. The main sources of this are peanut butter, vegetable oils, and rice[23]. In a study conducted in Jordan, all 80 breast milk samples were positive for AFM_1; the average of AFM_1 is 67.78 ng/kg. It has been determined that consumed grain products cause this contamination[24]. In a study conducted in China to determine the effects of seasonal differences on the AFM_1 level of milk, 43 of 72 raw milk samples were found to

be AFM_1 positive. It was noted that the AFM_1 concentration increased during the winter months, and seasonal variation was noted. Therefore, people who consume this raw milk are affected in the same way[25]. In a study conducted in Northern Iran, 39 of 250 breast milk samples were AFM_1 positive. AFM_1 levels in rural areas were higher than in the city center. It has been estimated to be due to dietary habits in rural areas that tend to consume more bread, rice, and non-alcoholic beer[26]. In another related study conducted in Morocco, 43 out of 82 breast milk samples were positive for AFM_1[22,27].

Table 2. Regression analysis results for AFM_1 levels and nutritional status of the last 24 h.

	β_0	β_1	SH.	Confidence interval (95%CI)	Test statistics	p	r_1	r_2
Constant	13.47	0	4.29	4.92, 22.01	3.13	0.00	0	0
Age	-0.00	-0.00	0.11	-0.24, 0.22	-0.07	0.94	-0.04	-0.00
Educational status (Primary education)								
Middle school	3.03	0.19	2.06	-1.06, 7.13	1.47	0.14	0.23	0.16
High school	1.10	0.07	1.83	-2.53, 4.74	0.60	0.54	0.09	0.06
College	-2.29	-0.17	1.73	-5.74, 1.15	-1.32	0.18	-0.25	-0.14
Master's/PhD	-0.47	-0.02	2.56	-5.59, 4.63	-0.18	0.85	-0.04	-0.02
Living place (Village-town)								
District	-1.78	-0.14	2.19	-6.14, 2.57	-0.81	0.41	0.02	-0.09
City center	-1.79	-0.15	2.10	-5.91, 2.40	-0.85	0.39	-0.1	-0.09
Dampness at home (No damp)								
Damp	-0.00	0	1.94	-3.87, 3.86	-0.00	0.99	0.02	0
Quite damp	0.45	0.01	4.24	-8.00, 8.90	0.10	0.91	-0.02	0.01

β_0: unstandardized coefficient; β_1: standardized coefficient; r_1: simple correlation; r_2: partial correlation.

Table 3. Regression analysis results for AFM1 levels and nutritional status of the last 24 h.

	β_0	β_1	SH.	Confidence interval (95%CI)	Test statistics	p	r_1	r_2
Constant	14.64		2.45	9.77, 19.52	5.97	0.000		
Consuming dairy products in the last 24 ho (Yes)								
No	-0.03	-0.00	1.33	-2.68, 2.62	-0.02	0.98	0.01	-0.00
Consuming fruit in the last 24 h (Yes)								
No	-0.93	-0.08	1.42	-3.77, 1.89	-0.65	0.51	-0.08	-0.07
Consuming fat in the last 24 h (Yes)								
No	1.61	0.12	1.44	-1.26, 4.48	1.11	0.26	0.05	0.12
Consuming bread in the last 24 h (Yes)								
No	-0.10	-0.00	2.10	-4.28, 4.08	-0.04	0.96	-0.06	-0.00
Consuming vegetable in the last 24 h (Yes)								
No	-0.05	-0.00	1.32	-2.68, 2.56	-0.04	0.96	0.00	-0.00
Consuming meat in the last 24 h (Yes)								
No	-2.06	-0.15	1.50	-5.04, 0.92	-1.37	0.17	-0.13	-0.15
Consuming liquid food in the last 24 h (Yes)								
No	-2.35	-0.16	1.62	-5.59, 0.88	-1.44	0.15	-0.16	-0.15

β_0: unstandardized coefficient; β_1: standardized coefficient; r_1: simple correlation; r_2: partial correlation.

In the meta-analysis examining the prevalence and concentration of AFM_1 in breast milk based on the socioeconomic indices and precipitation of mothers, it was reported that the prevalence of AFM_1 in breast milk increased significantly with increasing annual mean precipitation and poverty[5]. This result is similar to our study. It was determined that the high levels of AFM_1 in the mothers' milk were due to the high annual precipitation rate of the region where the research was conducted.

Studies related to this case in Turkey cover the provinces of Afyonkarahisar[4], Istanbul[28], Ankara[2], Erzurum[29], Şanlıurfa[27], Eskişehir[30], and the district of Fethiye[3]. According to the results of these studies, the highest value in milk samples collected in these regions was determined in Ankara, with a range of 60.90–299.99 ng/L. In the study conducted in Afyonkarahisar, 10.5% of 200 breast milk samples were found positive; positive samples had a mean of 8.45 pg/mL AFM_1[4]. In the study conducted in Istanbul to measure AFM_1 levels in breast milk and raw milk, 8 out of 61 breast milk samples were found to be AFM_1 positive; in positive samples, the mean score was 5.68 ng/L. In this study, no relationship was found between the sociodemographic characteristics of women and their AFM_1 exposure, and this study concluded that dried fruits, nuts, spices, and tea are the primary sources of AFM_1 in breast milk, contradicting the common assumption. In addition, it was concluded that the positive cases were due to the high consumption of carton milk in Istanbul[28]. In the study conducted in Ankara, AFM_1 was positive in all 75 breast milk samples. In the study conducted to determine the relationship between moldy cheese consumed in Erzurum and AFM_1 levels in breast milk samples, AFM_1 was positive in 18 of 73 breast milk samples. AFM_1 was positive in 12 of 44 women who consumed moldy cheese. However, they concluded that there was no difference between AFM_1 exposure in those who consumed and did not consume moldy cheese[29]. In the study conducted in Sanliurfa, it was investigated whether there is a seasonal difference in AFM_1 levels in breast milk. Of the 74 breast milk samples, 66 were positive for AFM_1, and a mean of 19.0±13.0 ng/l AFM_1 was found in positive samples. Notably, 91.2% of 34 breast milk samples taken in June and 87.5% of 40 breast milk samples taken in December were AFM_1 positive. They concluded that the exposure was higher in June[27]. In the study in Eskisehir, in which breast milk AFM_1 levels were evaluated along with their relationship with the mother's nutritional habits, only three of the samples were positive for AFM_1 (mean; 3.59 ng/L). No relationship was found between mothers' dietary habits and AFM_1 level[30]. In the study conducted in Fethiye, 53 of 100 breast milk samples were positive for AFM_1, and the mean of positive cases was 6.36 ng/L AFM_1. A significant difference (58.8%) was found in the breast milk samples of homemakers compared to the workers[3]. In the present study, while the educational status of women was associated with AFM_1 levels, their place of residence and age were not associated with AFM_1 levels. Unlike all other studies, the AFM_1 level was higher in women who consumed ready-to-eat food than those who did not. Whatever the reason, the high prevalence (83.3%) of AFM_1 found in human milk in the present study shows the need to assess further the extent of exposure to aflatoxins in Turkey and the impact that this has on public health.

In the literature, it has been observed that the foods that affect the AFM_1 level in breast milk include contaminated corn oil, peanuts, raw milk, peanut butter, vegetable oil, rice, cow's milk consumption, soybean, and wheat flour[5]. In this study, however, no relationship was found between the consumption of ready-made milk and dairy products and acidic food consumption, and the level of AFM_1. On the contrary, consumption of processed food (sausage) was associated with AFM_1 level. This result in our study added a new product to the food contaminated with AFM_1 level.

CONCLUSIONS

The AFM_1 result in this study was found to be higher than the studies conducted in Turkey. The reason for this is thought to be due to the heavy rainfall in the region where the study was conducted. It has also been observed that mothers are effective in consuming processed food. To minimize the possible harms of aflatoxin to women and newborns, safe food, storage conditions, and possible harm should be mentioned in pre- and post-pregnancy education.

ETHICAL APPROVAL

Permission was obtained from the Human Research Ethics Committee of the Karabuk University (dated May 30, 2018, and no. 6/15) and the institute where the research was conducted. The study was based in accordance with the Declaration of Helsinki.

AVAILABILITY OF DATA AND MATERIALS

All data are publicly available.

AUTHORS' CONTRIBUTIONS

RAD: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **MA:** Data curation, Writing – review & editing. **MO:** Conceptualization, Data curation, Methodology, Writing – review & editing. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

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Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios of overweight children and adolescents

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SUMMARY

OBJECTIVE: This study aimed to compare neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio of overweight children and adolescents with the eutrophic ratios and to verify whether these ratios are associated with age, inflammation, Z-score of body mass index, and waist-to-height ratio.

METHODS: This is a cross-sectional study involving 64 overweight and 106 eutrophic children and adolescents. Data on weight, height, and waist circumference (body mass index and waist-to-height ratio), blood count (neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio), and high-sensitivity C-reactive protein were collected.

RESULTS: The mean age of participants was 8.4±3.2 years. The ratios did not differ between the overweight and non-overweight groups. The platelet-to-lymphocyte ratio has shown a direct and independent association with body mass index (p=0.031) and waist-to-height ratio (p=0.018), a fact not observed for neutrophil-to-lymphocyte ratio. The ultrasensitive C-reactive protein level was higher in the obesity group (p=0.003). Both ratios had a direct and independent association with age.

CONCLUSION: The ratios did not differ between the overweight and non-overweight groups. There was a direct and independent association of platelet-to-lymphocyte ratio with overweight, not observed in neutrophil-to-lymphocyte ratio. The ratios have significantly increased according to the age of the participants.

KEYWORDS: Pediatric obesity. Inflammation. Neutrophils. Lymphocyte count. Platelet count.

INTRODUCTION

Childhood obesity has reached epidemic proportions and is currently a serious public health issue. Its global prevalence has increased 10-fold in the past four decades[1]. In Brazil, the prevalence of overweight in the general population has increased from 43 to 60% between 2002 and 2019, currently reaching 30% of children between 5 and 9 years of age and 19.4% of adolescents[2]. It is known that overweight children are more likely to become obese adults[3].

The pathophysiology of excessive weight gain is complex with interactions between genetic and environmental factors[4]. Excessive adiposity causes a low-grade chronic systemic inflammation, responsible for several comorbidities associated with it[5].

Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) obtained from blood count are recognized as simple ways to assess inflammation and prognosis of several diseases as infections, mental disorders, and neoplasic diseases[6-11]. Although it is easily accessible and low-cost markers, there are few studies that assess NLR and PLR in children and adolescents, especially in our field. In the overweight pediatric age group, in which a low-grade inflammatory state predominates, such markers can be important as predictive factors for the development of complications such as insulin resistance and cardiovascular events.

The aims of this study were to compare NLR and PLR of overweight children and adolescents with the eutrophic ratios and to verify the association of these ratios with age, inflammation, Z-score of body mass index (BMIz), and waist-to-height ratio (WHtR).

METHODS

This is a cross-sectional, controlled study, involving 64 overweight children and adolescents, and 106 eutrophic and healthy individuals grouped by age and gender as a comparison group. The study was conducted at the Cidade dos Meninos Maria

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Imaculada Institution, which serves underprivileged children in the city of Santo André, Brazil.

Inclusion criteria were as follows: children/adolescents enrolled at the institution in 2019, aged over 4 years. Exclusion criteria were as follows: family members and children who did not consent to participate in the study, people with short stature or thinness, children aged 4–5 years classified as at risk of overweight, children with chronic diseases except asthma and allergic rhinitis, children who made use, within 30 days prior to blood collection, of systemic corticosteroids or non-steroidal anti-inflammatory drugs, and children who were ill within 30 days prior to collection.

Participants and their family members signed a free and informed consent form and an assent form, with detailed explanations on the research protocols.

A questionnaire was applied to the people responsible for the individuals, covering information about preexisting diseases, medications in use, and current health conditions.

The application of the questionnaire, physical examination, anthropometric measurements, and blood collection were performed in appropriate spaces in the institution.

Regarding anthropometric measurements, weight (kg), height (m), and waist circumference (cm) were measured. Waist circumference was obtained at the midpoint between the 10th rib and the iliac crest[12]. Subsequently, BMIz and WHtR were calculated. WHtR was classified as altered when the value was ≥0.5[13]. For the BMIz classification, the cutoff points recommended by the World Health Organization (WHO) were adopted[14].

The school had 541 enrolled students, of which 173 were excluded for being under 4 years of age and 23 for not consenting to participate. A questionnaire was applied and a physical examination was performed in 345 participants, of which 57 were excluded due to changes in the physical examination and eight due to the questionnaire. Of 280 considered eligible for the study, 60 did not consent to blood collection and 50 were not invited to be paired with the participants. At the end, 170 children and adolescents participated in all stages of the study.

Blood samples were obtained via vacuum venipuncture. The complete blood count was evaluated by automated flow cytometry and the ultrasensitive C-reactive protein (us-CRP) was analyzed by turbidimetric method. Laboratory tests were performed at the Clinical Analysis Laboratory of the Centro Universitário FMABC. NLR was obtained by dividing the number of neutrophils and the number of lymphocytes. PLR was obtained by dividing the platelet count and the number of lymphocytes.

Data were entered into an Excel spreadsheet (Microsoft) and analyzed using the Stata (r) Software (StataCorp, LC) version 11.0. Qualitative variables were presented as absolute and percentage numbers. Parametric data were expressed as mean±-standard deviation, with p-value calculated using Student's t-test. Non-parametric data expressed as median (minimum–maximum), with p-value calculated by the Mann-Whitney test. To compare the classifications of variables between eutrophic and overweight children, we used the χ^2 test. For correlation analysis, the Spearman's test was applied. For regression analysis, multivariate linear regression was performed. The significance level adopted was 5%.

The study was approved by the Research Ethics Committee of the Centro Universitário FMABC under opinion number: 3,058,583, CAAE: 02670518.7.0000.0082.

RESULTS

A total of 170 children and adolescents were evaluated. The general characteristics of the study participants are shown in Table 1. The mean age was 8.4±3.2 years, 71.2% were prepubescent, and there was a slight predominance of males (52.4%).

Regarding nutritional status, 106 (62.4%) were eutrophic and 64 (37.6%) were overweight (overweight, obesity, and severe obesity). As for WHtR, 64 (37.6%) had values considered to be increased (Table 1). WHtR was high in 21.7% of eutrophics and 64.1% of the overweight group (p<0.001).

Table 2 shows that there was no difference in the count of leukocytes, neutrophils, lymphocytes, platelets, red cell distribution width (RDW) (anisocytosis index), NLR, and PLR when comparing the medians of eutrophic, overweight, and obese individuals. The median of us-CRP was higher in the obesity group (p=0.003).

Spearman's correlation was performed between age, BMIz, and WHtR with laboratory variables. There was a direct and significant correlation between age and mean platelet volume

Table 1. General characteristics of the study population.

Variable		N	%
Sex	Male	89	52.4
Age, years	4–5	32	18.8
	5–10	78	45.9
	≥10	60	35.3
Pubertal development	Prepubescent	121	71.2
Nutritional diagnosis	Eutrophy	106	62,4
	Overweight	39	22.9
	Obesity	19	11.2
	Severe obesity	6	3.5
Waist-to-height ratio	≥0.5	64	37.6

(MPV) (r=0.2077; p<0.001), NLR (r=0.3903; p<0.001), and PLR (r=0.2713; p<0.001) and an inverse correlation with leukocyte count (r=-0.2039; p=0.007), lymphocytes (r=-0.4672; p<0.001), and platelets (r=-0.3445, p<0.001). BMI and WHtR showed significant and direct correlation with us-CRP (r=0.1619; p<0.001 and r=0.2518; p<0.001, respectively). WHtR was directly associated with platelet count (r=0.1508; p<0.001) and lymphocyte count (r=0.2328; p=0.002).

Spearman's correlation between us-CRP, NLR, and PLR with laboratory variables was also applied. There was a direct and significant correlation between us-CRP and RDW (p<0.001), which did not happen for NLR and PLR.

Multivariate linear regression analysis was performed, considering NLR and PLR as dependent variables (Table 3). NLR was directly and independently associated with age (p<0.001), while PLR was directly and independently associated with age (p<0.001), BMIz (p=0.031), and WHtR (p=0.018).

DISCUSSION

This study showed that NLR and PLR did not differ between overweight and non-overweight children and adolescents. Both ratios have increased according to the age of the participants. There was a direct and independent association of PLR with BMI and WHtR, the fact not observed for NLR.

A study performed in Turkey with 187 children and adolescents aged 6–15 years (130 obese and 57 eutrophic) have shown that NLR and CRP concentrations were significantly higher in the obese group compared to healthy controls. PLR did not differ between the groups. Some hypotheses can be suggested to explain the difference in relation to our findings: the study group from Turkey did not include overweight individuals, only obese; in addition, the mean age (12.84±2.04 years) was higher than that of our study (8.4±3.2 years)[15].

The ratios were also assessed in adults. A cross-sectional and controlled study with 90 participants from Turkey (45 severely

Table 2. Comparison of laboratory variables between groups of obesity (n=25), overweight (n=39), and eutrophic (n=106) children and adolescents.

Variable	Eutrophia (n=106)	Overweight (n=39)	Obesity (n=25)	p
Leukocytes (k/mm^3)	6.95 (2.9–14.6)	6.4 (3.3–10.8)	7.4 (3–23.4)	0.23
Neutrophils (k/mm^3)	2.5 (0.32–8.71)	2.47 (0.46–4.78)	3.51 (0.2–15.64)	0.30
Lymphocytes (k/mm^3)	2.81 (1.02–15.64)	2.79 (1.28–6.06)	2.79 (1.62–7.1)	0.80
Platelets (k/mm^3)	297 (70–517)	284 (219–459)	304 (209–487)	0.92
RDW (%)	13.2 (11,9–15,6)	13.1 (11.8–14.9)	13.3 (12–14.6)	0.50
NLR	0.82 (0.11–3.99)	0.89 (0.12–0.61)	1.09 (0.06–0.66)	0.30
PLR	102.07 (35.35–249.02)	113.2 (46–236.5)	106.8 (38–224)	0.68
us-CRP (mg/L)	0.3 (0.1–13.5)	0.5 (0.1–10)	0.9 (0.1–11)	0.003

Non-parametric data are expressed as median (minimum–maximum). RDW: anisocytosis index; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio, us-CRP: ultrasensitive C-reactive protein.

Table 3. Linear regression of variables associated with neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio (n=170).

Variable	Coefficient	Standard error	p	Confidence interval
NLR-dependent variable				
Age	0.005	0.001	0.000	0.003 to 0.008
BMIz	0.087	0.049	0.083	-0.011 to 0.185
WHtR	-1.591	1.119	0.186	-3.954 to 0.771
us-CRP	0.035	0.020	0.084	-0.004 to 0.767
PLR-dependent variable				
Age	0.324	0.089	0.000	0.147 to 0.501
BMIz	7.636	3.519	0.031	0.688 to 14.585
WHtR	-201.356	84.341	0.018	-367.8 to -34.82
us-CRP	1.113	1.456	0.446	-1.762 to 3.990

Independent variables: age, BMIz, WHtR, and us-CRP. NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; BMIz: Z-score of body mass index; WHtR: waist-to-height ratio; us-CRP: ultrasensitive C-reactive protein.

obese and 45 eutrophic) with mean ages of 33±7 and 33±5 years, respectively, showed a statistically significant difference between groups for PLR (p=0.033) and RDW (p<0.001). Severely obese individuals with overweight/obesity have showed PLR values significantly higher comparing to euthropic[16]. However, three studies conducted with adults (n=1,054) have found no association between BMI and PLR[17-19]. A Korean study evaluated the medical records of 15,654 individuals (median age 46 years, age range 14–90 years) observed that leukocyte, neutrophil, and lymphocyte counts, even within their normal range, are closely related to the presence of metabolic syndrome components after adjustment for age, sex, smoking, alcohol consumption, education, and income. The study did not assess NLR and PLR. Low-grade inflammation associated with metabolic syndrome may be the cause of the increased leukocyte count[20].

Regarding NLR, three studies (n=2,090) did not observe an association between BMI and ratio[17,18,21]. A study with 231 participants (mean 57.1±16.3 years) observed lower NLR (p=0.04) in obese compared to eutrophic individuals. According to the Spearman's test, there was a significant and negative correlation, although weak, between BMI and NLR (r=-0.145; p=0.029). The mean lymphocyte count was higher in the group of overweight and obese individuals (p=0.011), influencing lower NLR values in overweight[19].

A retrospective study conducted in Taiwan with 34,013 participants (10,475, 30.8% with metabolic syndrome) have demonstrated that increased NLR is a significant risk factor for metabolic syndrome (p<0.001), demonstrating that this ratio can help identify individuals at risk for this syndrome[22].

One study evaluated 60 adolescents aged 11–16 years (obese group: 30; control group: 30) in 2021 and found no significant difference between the groups regarding leukocyte, neutrophil, lymphocyte, platelet, NLR, and PLR counts (p>0.05)[23].

In this study, only PLR showed an association with BMI and WHtR in children and adolescents. The association with WHtR, which reflects visceral adiposity and is related to insulin resistance, and the fact that PLR is simply obtained from the blood count, suggests the importance of this assessment and the investigation of its relationship with associated morbidities in children and adolescents, in future studies.

The highest median values of us-CRP in overweight individuals point to the low-grade chronic inflammation present in this group. Other studies also describe the association between overweight and inflammation detected by us-CRP[18,21,24]. The presence of inflammation in overweight individuals is admittedly a risk factor for comorbidities. A study with 1,376 children and adolescents from Greek schools (mean age of 11.19±0.66 years) showed that CRP levels can early identify metabolic syndrome[25].

A strong point of the study was the careful selection of the sample, excluding children and adolescents with acute or chronic diseases that present with inflammation from the collection. The main limitations of the study were the assessment of a single institution on the outskirts of the city of Santo André/SP, not allowing to state that the data can be extrapolated to the general population, and also the cross-sectional design, not allowing the assessment of cause-effect.

CONCLUSIONS

Neutrophil-to-lymphocyte ratio (NLR) and PLR did not differ between the groups of overweight and non-overweight children and adolescents. PLR was independently associated with BMI and WHtR, a fact not observed for NLR. The ratios increased significantly and independently with the age of the participants.

ETHICAL ASPECTS

All authors declare that there is no professional or financial conflict of interest. This study was approved by the Research Ethics Committee of the FMABC University Center (dated December 5, 2018, CAAE: 02670518.7.0000.0082, opinion number: 3.058.583). Families received information, risks, and benefits from the study. Literate children received a TALE and their guardians a TCLE.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Effects of perilipin-5 on lipid metabolism and high-sensitivity cardiac troponin I

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SUMMARY

OBJECTIVE: Heart attack is one of the most common causes of sudden death in adults. Therefore, early detection of heart attack and investigation of potential new biomarkers are of great importance. We investigated whether perilipin-5 is a potential biomarker by examining changes in perilipin-5 serum levels along with high-sensitivity cardiac troponin I during a heart attack.

METHODS: The subjects were divided into two groups: (1) control group and (2) patients with heart attack, with 150 people in each group. High-sensitivity cardiac troponin I, perilipin-5, total oxidant status, malondialdehyde, reduced glutathione, and superoxide dismutase levels in serum samples were measured. In addition, perilipin-5 mRNA expressions and protein levels were analyzed.

RESULTS: There was no overall statistical difference between the demographic characteristics of the groups. However, high-density lipoprotein, creatine kinase, Creatine kinase myocardial band, aspartate amino transferase, lactate dehydrogenase, and calcium levels were higher in the heart attack group compared to the control group. We found that the high-sensitivity cardiac troponin I and perilipin-5 levels increased in the patients with heart attack ($p<0.0001$) compared to control. Although there was an insignificant increase in malondialdehyde levels in the heart attack group ($p>0.05$), there was a 35.9% increase in total oxidant status levels and a 33.5 and 24.1% decrease in glutathione and superoxide dismutase levels, respectively ($p<0.01$), compared to control. Perilipin-5 mRNA and protein levels in heart attack patients increased by 48.2 and 23.6%, respectively, compared to the control group ($p<0.01$).

CONCLUSION: Our results showed that perilipin-5 together with high-sensitivity cardiac troponin I could be a promising biomarker in heart attack.

KEYWORDS: Perilipin-5. Troponin I. Heart disease. Oxidative stress. Lipid peroxidation.

INTRODUCTION

Cardiac troponins (cTns) and creatine kinase (CK) MB are the most sensitive and specific biochemical markers used in the diagnosis of myocardial damage, cardiac risk stratification, and prognosis[1]. cTns are protein structures responsible for performing the physiological functions of the heart muscle[2]. In the presence of myocardial damage, serum cTn levels are above the reference range[3]. In addition, elevated troponin levels are universally an important component of the diagnosis and treatment of heart conditions, such as myocardial infarction (MI), coronary syndrome, and cardiac ischemia.

High cTns levels can be caused by various non-coronary causes, such as alkaline-phosphatase interaction, fibrin interaction, hemolysis, kidney failure, and instrumentation failure[4]. Along with analytical improvements, high-sensitivity cTn (hs-cTn) allows early detection of cardiomyocyte damage accurately at lower levels and in a shorter time[5]. Although hs-cTn values are important in terms of diagnosis and prognosis, they may lead to elevated hs-cTn and false-positive results in many cardiac and non-cardiac failures[6].

In most tissues, fatty acids (FAs) are converted to triacylglycerol (TAG) and stored as lipid droplets (LDs), comprising a single layer of phospholipid[7]. Perilipin (PLIN) family proteins, which have a tissue-specific expression, consist of five members (i.e., PLIN1, PLIN, PLIN3, PLIN4, and PLIN5) with ~100 amino acid sequence homology at the N-terminals[8]. Of these, PLIN5 plays a significant role in regulating FA metabolism in oxidative tissues, such as the heart, liver, and skeletal muscle[9]. In in vivo study, PLIN5 expression, which enables the storage of LDs in the heart under normal physiological conditions, was shown to cause an increase in pathological conditions and abnormal lipid accumulation in the heart[10].

Biomarkers, such as CK-MB, troponins, and lactate dehydrogenase (LDH), which cause an increase in serum with cardiomyocyte damage accompanying a heart attack, sometimes cause false-positive results. This case prevents the detection of the current situation or causes misdiagnosis. Since PLIN5 is a heart-specific molecule, it is possible to expect an increase in serum levels with cellular damage during heart attack once other

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conditions (as stated in the exclusion criteria) that may cause interference are eliminated. Thus, in this study, we hypothesized that PLIN5 levels during heart attack increased and released into the circulation with myocardial damage.

METHODS

This study included 300 subjects (age median [min–max]: 45 [36–58] years) who visited Duzce University Medical Faculty Hospital in the period January to November 2019. The subjects are divided into two groups, with 150 participants in each group: (1) control group (healthy subjects) and (2) patients with heart attack. Subjects in Group 1 were selected from those who came to the hospital for routine control, had no acute or chronic disease, and were not previously diagnosed with the disease or were not using drug. In Group 2, we included patients who are over 30 years old and who have had a heart attack suspicion with chest pain (acute MI) applied to the emergency department 6 h after symptom onset. Patients with heart attacks coming to the emergency department with clinical symptoms were identified by surface electrocardiogram (ECG) and/or biochemically cardiac biomarkers. In the diagnostic criteria, there was chest pain lasting for about an hour, the Q-wave was 25% wider than the R wave, and the ST elevation was greater than 1 mm on ECG. According to ECG and biochemistry results, 150 patients with non-ST-elevation MI were included in the study. All subjects were informed verbally and in writing about the experimental design and possible risks and voluntarily included in the study. The Clinical Research Ethics Committee of the Duzce University approved the study protocol (no: 2019/273), and the study was carried out following the current Helsinki Declaration and Good Clinical Practice principles. Informed consent was obtained from the patients included in the study.

The blood samples were centrifuged at 5000×*g* for 10 min at room temperature and then routine biochemistry analyses [i.e., glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, CK, CK-MB, aspartate amino transferase (AST), alanine amino transferase (ALT), gamma-glutamyl transferase (GGT), LDH, creatinine, blood urea nitrogen (BUN), urea, calcium, alkaline phosphatase (ALP), and total bilirubin) were performed using the Cobas 600 autoanalysis. hs-cTnI levels were measured using Elecsys 2010 system (Roche Diagnostics, Germany).

The PLIN5 levels in serum samples were measured with Uscn Life Sciences PLIN5 ELISA kit according to the manufacturer's instructions (ELISA Kit for PLIN5 – Human SEE039Hu). Absorbance reading was done on Chromate 4300 brand ELISA reader device.

Malondialdehyde (MDA), total oxidant status (TOS), reduced glutathione (GSH), and copper/zinc superoxide dismutase (Cu/Zn SOD) levels in serum samples were determined using a commercially available (LS-F27741, Rel Assay Diagnostics, MBS265674 and BMS222, respectively). Measurements were made colorimetrically with a microplate reader according to the manufacturer's instructions.

Perilipin-5 (PLIN5) expression and protein levels were analyzed by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analyses, respectively, in whole blood samples according to our previous work[11]. The PLIN5 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were as follows:

PLIN5 forward 5′-TGT GCA CAG TGC AGC CGA GGA-3′ and PLIN5 reverse 5′-GCT GCA CGA GCA AGG GAA GAC-3′; GAPDH forward 5′-AGC CAC ATC GCT CAG ACA C-3′ and GAPDH reverse 5′-GCC CAA TAC GAC CAA ATC C-3′. The fold changes in PLIN5 and GAPDH mRNA levels were determined according to the $2^{-\Delta\Delta CT}$ formula after amplification. The GAPDH mRNA levels were used as an internal standard.

Two independent samples t-test was used for univariate analyses as well as for normally distributed variables. Pearson's chi-square test was used for the analysis of categorical data. A p-value <0.05 was considered statistically significant. Results were analyzed using the GraphPad Prism software 7 (GraphPad Software, San Diego, CA, USA).

RESULTS

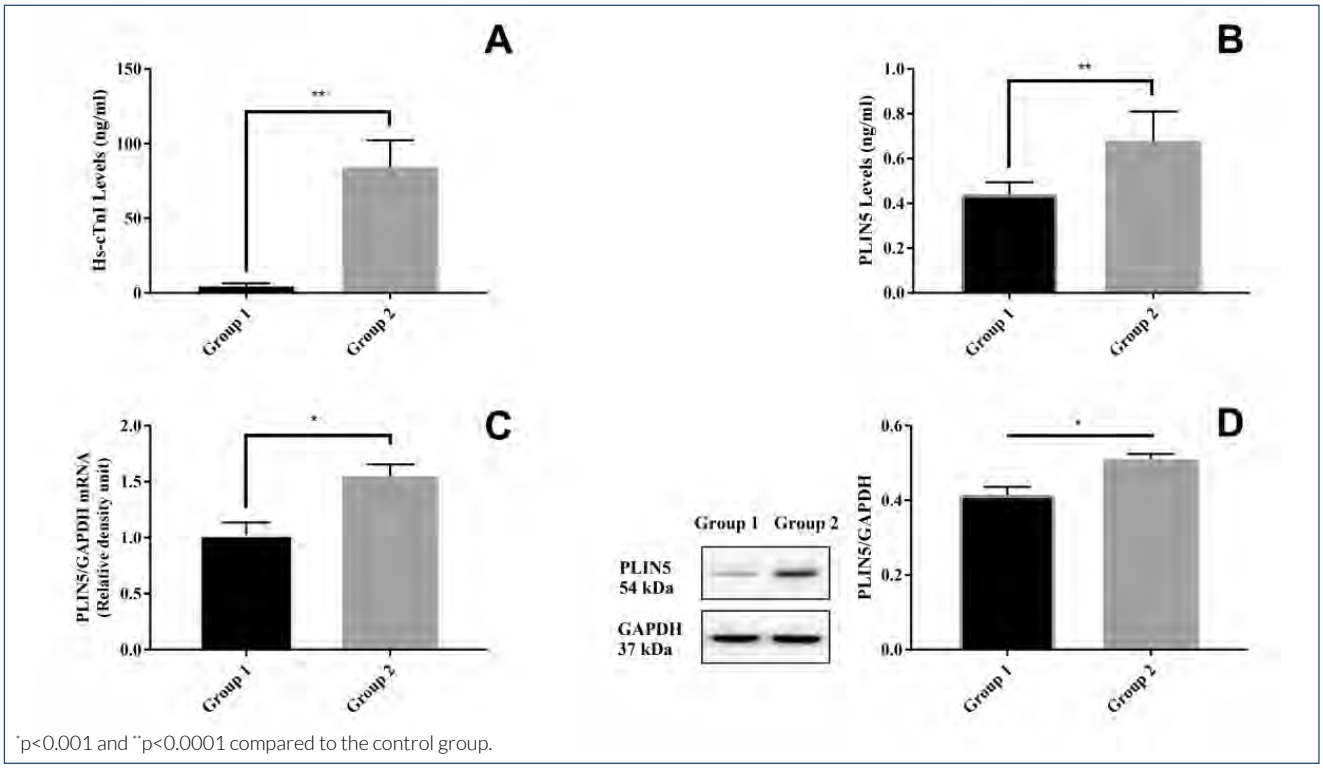
The demographic characteristics and biochemistry results of the subjects are shown in Table 1. We found that HDL, CK-MB, AST, and calcium levels in the patients with heart attack were higher than in the control group (p<0.01). There was an increase in HDL, CK, CK-MB, AST, LDH, and calcium levels in Group 2 (35.8, 27.9, 644.5, 73.7, 41.8, and 87.5%, respectively) compared to the control group.

The patients with heart attack showed a significant increase in hs-cTnI levels compared to the control group (Figure 1). In the patients with heart attack, an increase in hs-cTnI levels is accompanied by an increase in serum PLIN5 levels (Figures 1A, B). The enhancement in hs-cTnI and PLIN5 levels in the patients with heart attack was 1361 and 58.2%, respectively, compared to the control group (p<0.0001). In addition, hs-cTnI and PLIN5 levels showed a positive correlation in both Group 1 and Group 2 (r=0.714; p<0.01).

Table 1. Basic characteristics and biochemical parameters of the subjects.

	Control group (n=150)	Patients with heart attack (n=150)	p-value
Age#	45 (37–58)	41 (36–55)	<0.01
Male*	50 (58)	50 (50)	>0.05
Female*	50 (42)	50 (50)	>0.05
Body mass index	25±2.51	24±1.08	>0.05
Glucose (mg/dl)	80.45±5.72	78.52±6.09	>0.05
Total cholesterol (mg/dl)	153.44±26.84	155.61±21.38	>0.05
HDL (mg/dl)	39.81±4.95	53.36±2.58	<0.01
LDL (mg/dl)	50.33±12.91	51.06±13.82	>0.05
Triglycerides (mg/dl)	104.68±21.75	106.24±20.56	>0.05
Creatine kinase (CK)	79.92±11.61	101.42±15.84	<0.01
CK-MB (IU/L)	8.62±4.95	58.11±8.25	<0.01
AST (U/mL)	15.48±10.53	26.69±8.06	<0.01
ALT (U/mL)	19.24±8.15	20.36±5.65	>0.05
GGT (U/L)	38.51±10.18	40.42±11.17	>0.05
LDH (U/L)	177.39±21.64	248.55±17.49	<0.01
Creatinine (mg/dl)	0.7±0.14	0.8±0.25	>0.05
BUN (mg/dl)	13.62±5.08	12.75±3.71	>0.05
Urea (mg/dl)	27.13±4.15	28.59±6.12	>0.05
Calcium (mg/dl)	8.04±1.67	15.02±3.84	<0.01
ALP (U/L)	71.35±16.58	69.55±6.43	>0.05
Total bilirubin (mg/dl)	0.61±0.14	0.58±011	>0.05

#Numbers are presented as median. *Numbers are presented as (%).



*p<0.001 and **p<0.0001 compared to the control group.

Figure 1. hs-cTnI and PLIN5 levels during heart attack. (A) hs-cTnI levels, (B) PLIN5 levels, (C) PLIN5 mRNA levels, (D) PLIN5 protein levels. Group 1: Control group. Group 2: Patients with heart attack. hs-cTnI: high-sensitivity cardiac troponin I; PLIN5: perilipin-5; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

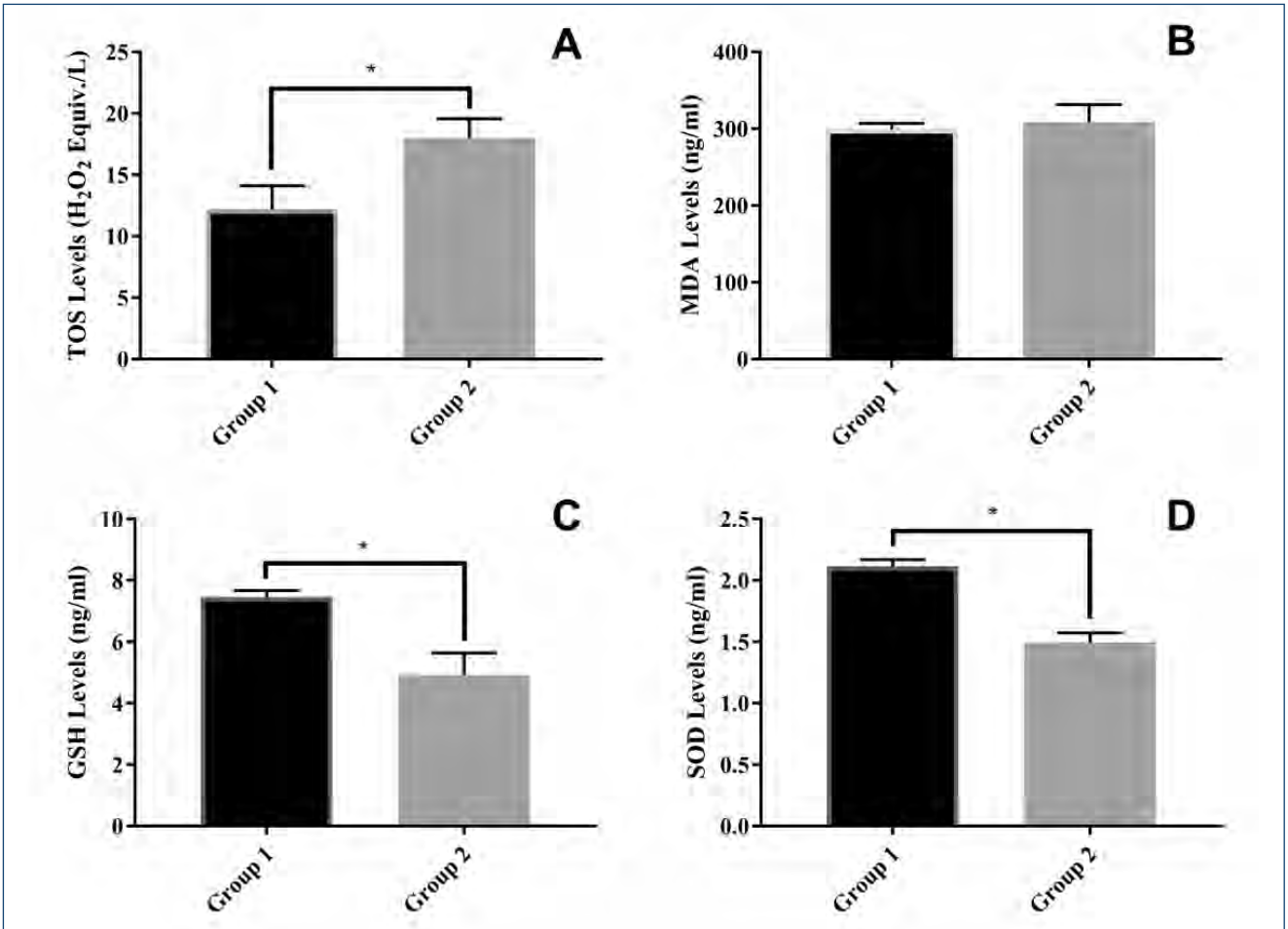
Perilipin-5 (PLIN5) mRNA levels in Group 1 and Group 2 were measured by RT-PCR. PLIN5 mRNA levels in the Group 2 were significantly higher than that in Group 1 (Figure 1C). PLIN5 mRNA levels in the patients with heart attack increased by 48.2% compared to the control group ($p<0.01$). PLIN5 protein levels in healthy subjects (Group 1) and heart attack patients (Group 2) were visualized by Western blot analysis. We found a significant increase in PLIN5 protein levels in Group 2 compared to the Group 1 (Figure 1D).

The TOS and MDA levels in both Group 1 and Group 2 were shown in Figure 2. TOS levels in the patients with heart attack demonstrated a statistically significant increase of 35.9% compared to the control group ($p<0.01$; Figure 2A). The MDA levels in the patients with heart attack showed a statistically insignificant increase of 3.7% compared to the control group ($p>0.05$; Figure 2B). In addition, patients with heart attack in Group 2 exhibited statistically a decrease in GSH and SOD levels by 33.5 and 24.1%, respectively, compared to the control group ($p<0.01$; Figures 2C, D).

DISCUSSION

One of the key findings of this research was that the expression levels of PLIN5 during heart attacks increased with hs-cTnI. Since the increase in hs-cTnI levels was also accompanied by increased serum PLIN5 levels, it is reasonable to say that PLIN5 may be important for detecting cardiac damage status. It is also suggested that despite increased oxidative stress during a heart attack, the lack of an insignificant increase in MDA levels was due to the increase in PLIN5 levels.

Perilipin-5 (PLIN5) has an important role in maintaining heart functions by providing the energy balance of the heart[12].



TOS: total oxidant status; MDA: malondialdehyde; GSH: reduced glutathione; SOD: superoxide dismutase. *p<0.01 compared to the control group.

Figure 2. Oxidation and antioxidant levels during heart attack. (A) TOS levels, (B) MDA levels, (C) GSH levels, (D) SOD levels. Group 1: Control group. Group 2: Patients with heart attack.

Wang et al. demonstrated that the increase in PLIN5 expression levels in cardiac diseases regulated cardiac energy metabolism by stabilizing cardiac lipid droplets[13]. In addition, heart function and metabolism in PLIN5-deificied mice decreased significantly after MI, accompanied by significantly decreased survival[14]. In this study, we showed that PLIN5 expression levels increased in patients with heart attacks, and circulating PLIN5 levels enhanced with cardiac damage.

High-sensitivity cardiac troponin I (hs-cTnI), which is free in myocyte cytosol and bound to myofilaments, is released into the circulation after myocyte damage caused by various heart diseases[15]. The presence of heterophile antibodies and auto-antibodies in serum may affect the analysis process, leading to false hs-cTnI quantification[16]. In this study, we found that patients with heart attack showed an increase in hs-cTnI and PLIN5 levels. This suggested that PLIN5 levels in the serum increased with cardiac damage, because the subjects involved in this study did not have muscle or liver disease. Besides, combined results of blood analysis indicated a high positive correlation between PLIN5 and hs-cTnI. According to our results, when hs-cTnI and PLIN5 results were evaluated together, we clearly found that PLIN5 expression levels were increased in all patients with heart attack. Based on the test results, increased hs-cTnI and PLIN5 levels might provide more accurate prediction in the evaluation of cardiac problems.

Oxidative stress induced by reactive oxygen and reactive nitrogen species (ROS and RNS) has been shown to play a key role in the pathogenesis of various cardiac diseases such as acute MI, coronary syndrome, atherosclerosis, and cardiac ischemia17. A previous study reported that oxidative mechanisms were induced in cases of various cardiac problems18. Similar to our study, Karabacak et al. revealed that there was a strong correlation between cardiac dysfunction and oxidative stress levels19. In the study, we showed that TOS levels in the patients with heart attack were higher than the control. Polidori et al. reported that lipid peroxidation showed a positive correlation with oxidative stress in patients with heart disease20. Wang et al. suggested that the increase in PLIN5 expression may protect against cardiac dysfunction by upregulating the Nrf2 antioxidative pathway13. Likewise, in this study, we found that there was an increase in TOS levels and a decrease in GSH and SOD levels in patients with heart attack, but there was no significant difference in MDA levels. These results may be related to the effect of increased PLIN5 expression levels in the patients with heart attack.

Since our study is a preliminary study, we first tested the accuracy of our hypothesis. Notably, the fact that no statistically significant difference was observed in lipid peroxidation despite the oxidative damage caused by the pro-oxidant/oxidant imbalance during heart attack is also an important data for our study. In addition, when we eliminated interferences (as in the exclusion criteria), we found that PLIN5 increased with troponins in the acute phase during heart attack. Therefore, one of the most important limitations of our study is that although the relationship between PLIN5 and hs-cTnI levels in patients with heart attack in the acute phase was investigated, PLIN5 and hs-cTnI levels in patients hospitalized after heart attack were not evaluated. Another is to assess whether results from a larger patient population to which exclusion criteria were gradually included are consistent with current results. Ultimately, the clinical applicability of our study is not yet available.

CONCLUSION

Although there are significant data showing that PLIN5 increases in serum during heart attack, it should be measured in more patients and over a wider time period to investigate whether it will be a potential biomarker in heart diseases.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Vitamin C (Ascorbic acid) protects from neuropathy caused by cisplatin, through enhanced heat shock protein-70 and reduced oxidant effect

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SUMMARY

OBJECTIVE: We aimed to determine whether vitamin C has a protective effect on cisplatin-induced neuropathy in rats.

METHODS: In total, 24 rats were included in the study of which 8 rats (no drug administered) were categorized as the control group. The remaining 16 rats were given a total dose of 20 mg/kg cisplatin to induce neuropathy. These drug-administered rats (16 rats) were randomly divided into two groups, namely, group-1 (n=8): cisplatin+saline and group-2 (n=8): cisplatin+vitamin C (500 mg/kg/day). All rats were tested for motor function and electromyographic activity 3 days after cisplatin. Motor performance was evaluated by an inclined-plane test. Compound muscle action potential was evaluated. Plasma malondialdehyde, glutathione, tumor necrosis factor-α, interleukin 6, and sciatic nerve HSP 70 levels were measured. Axon diameter and nerve growth factor expression levels were analyzed.

RESULTS: Plasma malondialdehyde, tumor necrosis factor-α, and interleukin 6 levels were higher in the cisplatin+saline group than control group ($p<0.001$). But vitamin C significantly reduced malondialdehyde and inflammatory cytokine levels when compared with the cisplatin+saline group ($p<0.001$). Glutathione levels were lower in both cisplatin+saline and cisplatin+vitamin C groups than control group, but vitamin C significantly ameliorated the glutathione levels ($p<0.05$). Sciatic heat shock protein-70 levels were significantly higher in the cisplatin+vitamin C group than cisplatin+saline group. Compound muscle action potential amplitude and inclined plane test scores were significantly improved in the vitamin C group ($p<0.05$). Axon diameter and nerve growth factor expression ameliorated with vitamin C ($p<0.05$).

CONCLUSIONS: We demonstrated the ameliorated effects of vitamin C on cisplatin-induced neuropathy through increased heat shock protein-70, nerve growth factor levels, and reduced inflammatory and oxidant effects. The results are promising to improve the neurotoxic effects of cisplatin in cancer patients.

KEYWORDS: Cisplatin; Drug related side effects and adverse reactions; Ascorbic acid; Heat shock proteins.

INTRODUCTION

Cisplatin is a platinum-based antineoplastic drug used for the treatment of lung, testicular, ovarian, bladder, head-neck, cervical, and endometrial cancers[1]. The biological activity and toxicity of cisplatin are due to the metal ion compounds binding the proteins, nucleic acids, and other cellular components[2]. Cisplatin binds DNA and inhibits replication, cell cycle, and DNA repair[3]. DNA damage, oxidative stress, free oxygen radicals, and inflammatory cytokines lead to cisplatin-induced cytotoxicity[4].

Dose-related major side effects of cisplatin are nephrotoxicity and neurotoxicity[5]. Common neurological side effects are paresthesia, dysesthesia, loss of tendon reflexes, hearing loss, visual disturbance, muscle weakness, loss of vibration, tremor, and ataxia[6]. Mitochondrial dysfunction, oxidative stress, and alterations of dorsal root ganglia are proposed mechanisms for neurotoxicity[7].

Vitamin C [ascorbic acid (AA)] is a water-soluble antioxidant with various functions. It acts as a cofactor for the enzymes[8] and enhances the activities of antioxidant enzymes, such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD)[9]. Vitamin C protects against oxidative damage[10] and enhances immunity by supporting the innate and adaptive immune system[11]. This study was performed to investigate the neuroprotective effects of vitamin C against cisplatin neurotoxicity via antioxidant and anti-inflammatory effects.

METHODS

Animals

In total, 24 adult female Wistar rats, weighing 200–210 g, were used in the study. Animals were housed in cages and maintained

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under standard conditions with 12-h light/dark cycles at room temperature (22±2°C). They were fed with a standard pellet diet and tap water *ad libitum* throughout the study. The protocol employed in the study was approved by the Institutional Animal Care and Ethical Committee of the University of Science (Ethical Number: 04210523). All chemicals were obtained from Sigma-Aldrich Inc. unless otherwise noted.

Experimental Procedure

In total, 24 rats were included in the study. Eight rats were considered as the control group, and they were not administered any drug. The remaining 16 rats were given cisplatin at a dose of 2.5 mg/kg/day twice a week for 4 weeks (total dose of 20 mg/kg) to induce neuropathy[12]. The rats administered cisplatin were divided into 2 groups. Group 1 rats (n=8) were given 1 mL/kg/day 0.9% NaCl (saline) intraperitoneally daily for 4 weeks, and Group 2 rats (n=8) were given 500 mg/kg/day vitamin C (Redox-C Ampul, 500 mg/5 mL, Bayer) intraperitoneally daily for 4 weeks[13]. Two of the rats receiving cisplatin+saline died during the study. No death was reported in rats receiving cisplatin+vitamin C. At the end of the study, all rats were tested for motor function and electromyography (EMG). Following EMG recordings, at the end of the study, all animals were sacrificed (cervical dislocation) with anesthesia (100 mg/kg, Ketasol, Richter Pharma AG, Austria)/xylazine (50 mg/kg, Rompun, Bayer, Germany), and their blood samples were collected by cardiac puncture for biochemical analysis. They were centrifuged at 3000 rpm for 10 min at room temperature and stored at -20°C until assay.

Measurement of lipid peroxidation

Lipid peroxidation was determined in plasma samples by measuring malondialdehyde (MDA) levels. Briefly, trichloroacetic acid and thiobarbituric acid reactive substances (TBARS) and reagents were added to the plasma samples and then mixed and incubated at 100°C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min, and the absorbance of the supernatant was read at 535 nm. MDA levels were expressed as nM, and tetraethoxypropane was used for calibration.

Measurement of tissue glutathione levels

Glutathione (GSH) content in plasma samples was measured spectrophotometrically according to Ellman's method. In this method, thiols interact with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and form a colored anion with a maximum peak at 412 nm. GSH levels were calculated from the standard calibration curve and expressed as μM.

Measurement of plasma tumor necrosis factor-α and interleukin 6 levels

Plasma tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Biosciences).

Sciatic nerve biochemical analysis

After decapitation, sciatic nerves were rapidly removed and stored at 20°C until biochemical analyses were performed. For tissue analysis, whole nerve tissues were homogenized with a glass homogenizer in phosphate-buffered saline (PBS) that was five times the volume of the obtained tissue (pH 7.4) and centrifuged for 15 min. The supernatant was collected, and the total protein concentration homogenates were determined according to Bradford's method using bovine serum albumin as the standard. The sciatic nerve levels of heat shock protein-70 (HSP-70) in the tissue supernatants were measured using the commercially available rat ELISA kits. All samples obtained from animals were measured in duplicate according to the manufacturer's guideline.

Electrophysiological recordings

Electrophysiological recordings were performed 3 days after cisplatin. EMG was recorded under anesthesia (100 mg/kg, Ketasol, Richter Pharma AG, Austria)/xylazine (10 mg/kg, Rompun, Bayer, Germany). EMG was obtained three times from the right sciatic nerve stimulated supramaximally (intensity 10 V, duration 0.05 ms, frequency 1 Hz, in the range of 0.5–5000 Hz, 40 kHz/s with a sampling rate) by a bipolar subcutaneous needle stimulation electrode (BIOPAC Systems, Santa Barbara, CA) from the sciatic notch. Compound muscle action potentials (CMAPs) were recorded from 2–3 interosseous muscles by unipolar platinum electrodes. Data were evaluated using 3.6.7 software (BIOPAC Systems, Inc) with distal latency and amplitude of CMAP as the parameters. During the EMG recordings, the rectal temperatures of the rats were monitored by a rectal probe (HP Viridia 24-C; Hewlett-Packard Company, Palo Alto, CA), and the temperature of each rat was kept at approximately 36–37°C by a heating pad.

Assessment of motor function

The motor performances of the rats were evaluated by an inclined-plane test according to the method described by Rivlin and Tator. Briefly, the rat was placed oblique to the long axis of an inclined plane. The initial angle of the inclined plane was 10°. The inclined angle slowly increased, and the maximum angle of the plate on which the rat preserved its position for 5 s without falling was recorded as motor score. The inclined plane angle was measured three times in each rat to find an average value.

Histology and nerve growth factor immunohistochemistry

Sciatic nerve specimens were embedded in paraffin, sectioned at 5-µm thicknesses, and stained with hematoxylin and eosin. All sections were photographed with an Olympus C-5050 digital camera mounted on an Olympus BX51 microscope. The diameter of the sciatic nerve axons was measured using Image-Pro Express 1.4.5 (Media Cybernetics, Inc). Six sections from each animal were used for the measurement of axon diameter. For each section, we measured 100 axons.

For quantitative immunohistochemical examination, six sections from each animal were used. Sections were incubated with 10% H_2O_2 for 30 min to eliminate endogenous peroxidase activity and then blocked with goat serum (Invitrogen) for 1 h at room temperature. Subsequently, sections were incubated with primary antibodies (Santacruz Biotechnology;1/100) against nerve growth factor (NGF). Antibody detection was carried out with the Histostain-Plus Bulk kit (Invitrogen) against rabbit immunoglobulin G, and 3,3´-diaminobenzidine (DAB) was used to visualize the final product. Two blinded observers counted the total immune-positive Schwann cells under a light microscope at 100× magnification. Data were expressed as mean ± standard error of the means (SEM).

Statistical analysis

Statistical evaluation was performed using SPSS version 15.0 for Windows. The groups of parametric variables were compared using the Student's t-test and analysis of variance. Also, the groups of nonparametric variables were compared using the Mann-Whitney U test. In addition, the Shapiro-Wilk test was used for parametric-non-parametric differentiation. Results are presented as mean±SEM. A $p<0.05$ was considered statistically significant.

RESULTS

Plasma MDA level as an indicator of lipid peroxidation was significantly higher in the cisplatin+saline group ($p<0.001$). TNF-α and IL-6 levels were also higher in the cisplatin+saline group when compared with the control group ($p<0.001$). But vitamin C significantly reduced MDA, TNF-α, and IL-6 levels ($p<0.001$). GSH levels were lower in both cisplatin+saline and cisplatin+vitamin C groups than control group, but vitamin C significantly ameliorated the GSH levels ($p<0.05$). Sciatic nerve HSP-70 levels were significantly higher in the cisplatin+vitamin C group than cisplatin+saline group ($p<0.001$). The effects of vitamin C on MDA, TNF-α, IL-6, GSH, and sciatic nerve HSP-70 levels are detailed in Table 1. CMAP amplitude and inclined plane score were significantly lower in the cisplatin+saline group than control group ($p<0.05$) (Figure 1). EMG and inclined plane scores were significantly improved by vitamin C ($p<0.05$) (Table 1). In the cisplatin+saline group, the myelin sheath was degenerated and the NGF expression was decreased (Figure 2). Axon diameter and NGF immunoexpression were ameliorated with vitamin C ($p<0.05$) (Table 1).

Table 1. The effects of vitamin C on plasma malondialdehyde, tumor necrosis factor-α, IL-6, glutathione, sciatic nerve heat shock protein-70 levels, electromyographic records, inclined-plane test, nerve growth factor expression levels, and axon diameters.

	Normal control	Cisplatin+saline	Cisplatin+500 mg/kg vitamin C
MDA (nM)	53.5±4.9	126.1±10.2**	92.9±8.1##
TNF-α (pg/mL)	19.4±1.1	81.5±4.3**	45.8±7.06#
IL-6 (pg/mL)	8.3±0.9	545.7±30.4**	165.1±28.2##
GSH (µM)	10.1±3.2	6.2±0.5*	8.1±9.7#
Sciatic nerve HSP-70 (µg/mg protein)	7.7±1.4	10.8±1.1*	36.7±5.9##
CMAP latency (ms)	2.18±0.04	2.79±0.08*	2.81±0.03
CMAP amplitude (mV)	13.6 ± 0.3	4.1 ± 0.4*	6.9±0.5#
Inclined plane score (°)	92.4±1.8	72.6±2.5*	90.1±3.1#
NGF expression (%)	71.8±9.2	36.3±12.5**	53.2±7.7##
Axon diameter, µm	3.28±0.32	2.04±0.11*	2.95±0.23#

Results were presented as mean±standard error of the means. Statistical analyses were performed by one-way ANOVA test. *p<0.05, **p<0.001 (different from control group); #p<0.05, ##p<0.001 (different from cisplatin and saline group).
MDA: malondialdehyde; TNF-α: tumor necrosis factor-α; GSH: glutathione; HSP-70: heat shock protein-70; CMAP: compound muscle action potential; NGF: nerve growth factor.

DISCUSSION

Cisplatin is one of the most effective drugs used in adult and pediatric cancer therapy. Cisplatin accumulates in the nucleus and forms adducts with nuclear DNA (nDNA), which leads to cytotoxicity in dividing cells[4]. Cisplatin also forms adducts with mitochondrial DNA (mtDNA), and after cisplatin therapy, intracellular reactive oxygen species (ROS) levels increase in normal cells[14]. In cisplatin-induced nephrotoxicity, ROS induce kidney damage by decreasing the antioxidant levels and intracellular GSH levels[15]. Excessive ROS production and deterioration of the oxidative/antioxidative balance are also important mechanisms in cisplatin-induced axonal injury. Several studies reported an association between cisplatin cytotoxicity and increased lipid peroxidation and decreased GSH levels. Vitamin C shows antioxidant effects by decreasing ROS and inhibiting lipid peroxidation[16]. Antioxidants such as resveratrol, curcumin, and vitamin E reduce toxicity by suppressing lipid peroxidation and anti-inflammatory effects[17-19].

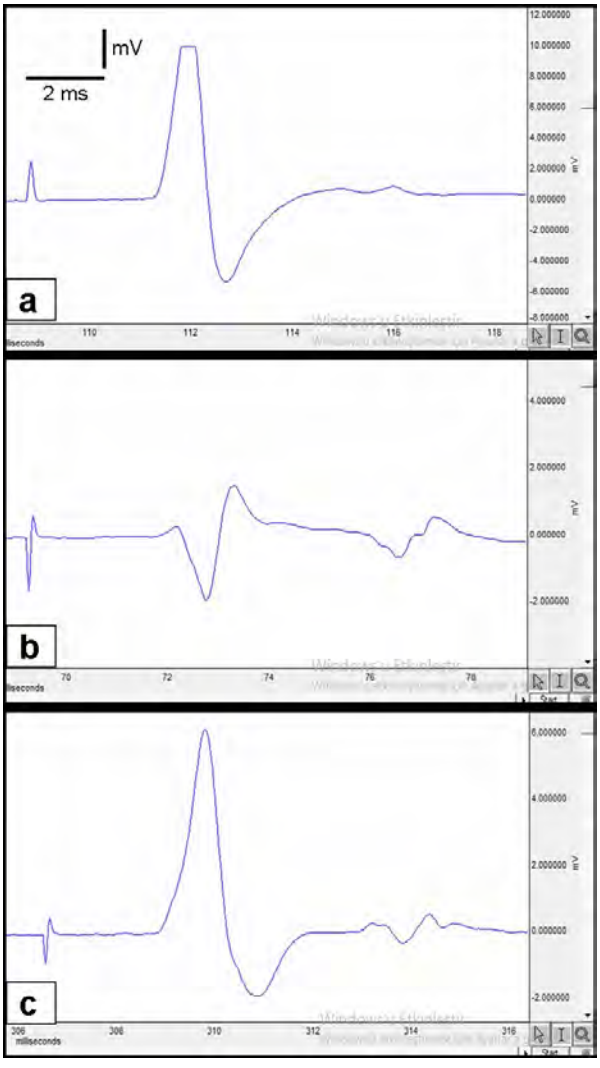

Figure 1. Compound muscle action potential recorded from: A) normal group, B) cisplatin+saline group, and C) cisplatin+vitamin C group

In our study, we measured plasma MDA levels as an indicator of oxidative stress. MDA formed as a result of polyunsaturated fatty acid peroxidation[20]. According to our results, plasma MDA levels increased due to cisplatin exposure, but vitamin C significantly reduced MDA probably by suppressing lipid peroxidation.

Akman et al. clearly demonstrated the protective effect of oxytocin (OT) in cisplatin-induced neurotoxicity[21]. The neuroprotective effect seems to be associated with antioxidant and anti-inflammatory (decrease in TNF-α levels) activities of OT. Vitamin C also decreased the levels of proinflammatory mediators. In our recent study, vitamin C decreased the levels of proinflammatory cytokines such as TNF-α and IL-6.

Heat shock proteins increased due to high temperature and severe stress. Albokhadaim et al reported that vitamin C shows antioxidant effects through HSPs in the liver and heart[22]. Silistre et al found the neuroprotective effects of ascorbic acid by increasing HSP-70 in rodent sepsis model[23]. In our study, sciatic nerve HSP-70 levels were statistically significantly higher in the cisplatin+vitamin C group than cisplatin +saline group ($p<0.001$). According to all of these findings, we may suggest that one of the neuroprotective mechanisms of vitamin C is an increase in HSP 70 levels.

The sensory neurons of the dorsal root ganglia (DRG) are the primary target of platinum-based chemotherapy. Mitochondrial dysfunction, oxidative stress, and DNA adducts lead to the apoptosis of DRG neurons and sensorial neuronopathy[23]. NGF is a member of the neurotrophin family. NGF is essential for the development and functional integrity of neurons[24]. NGF also acts as an antioxidant by inducing the expression of superoxide dismutase and catalase. NGF neutralizes the superoxide radicals and hydrogen peroxide[25]. Increased ROS levels induce the deficiency of nerve NGF levels. ROS production and related NGF decrease double the neurotoxic effects of cisplatin. NGF deficiency is also one of the major etiopathogenetic mechanisms of diabetic neuropathy. Obrosova et al. found a 1.5-fold decrease in the sciatic nerve NGF concentrations in diabetic rats[25]. In our study, neuronal NGF protein expression was statistically significantly lower in the cisplatin+saline group than control group ($p<0.01$). Also, the myelin sheath degenerated in the same group; 500 mg/kg of vitamin C ameliorated both axon diameter and NGF expressions in peripheral nervous tissues,

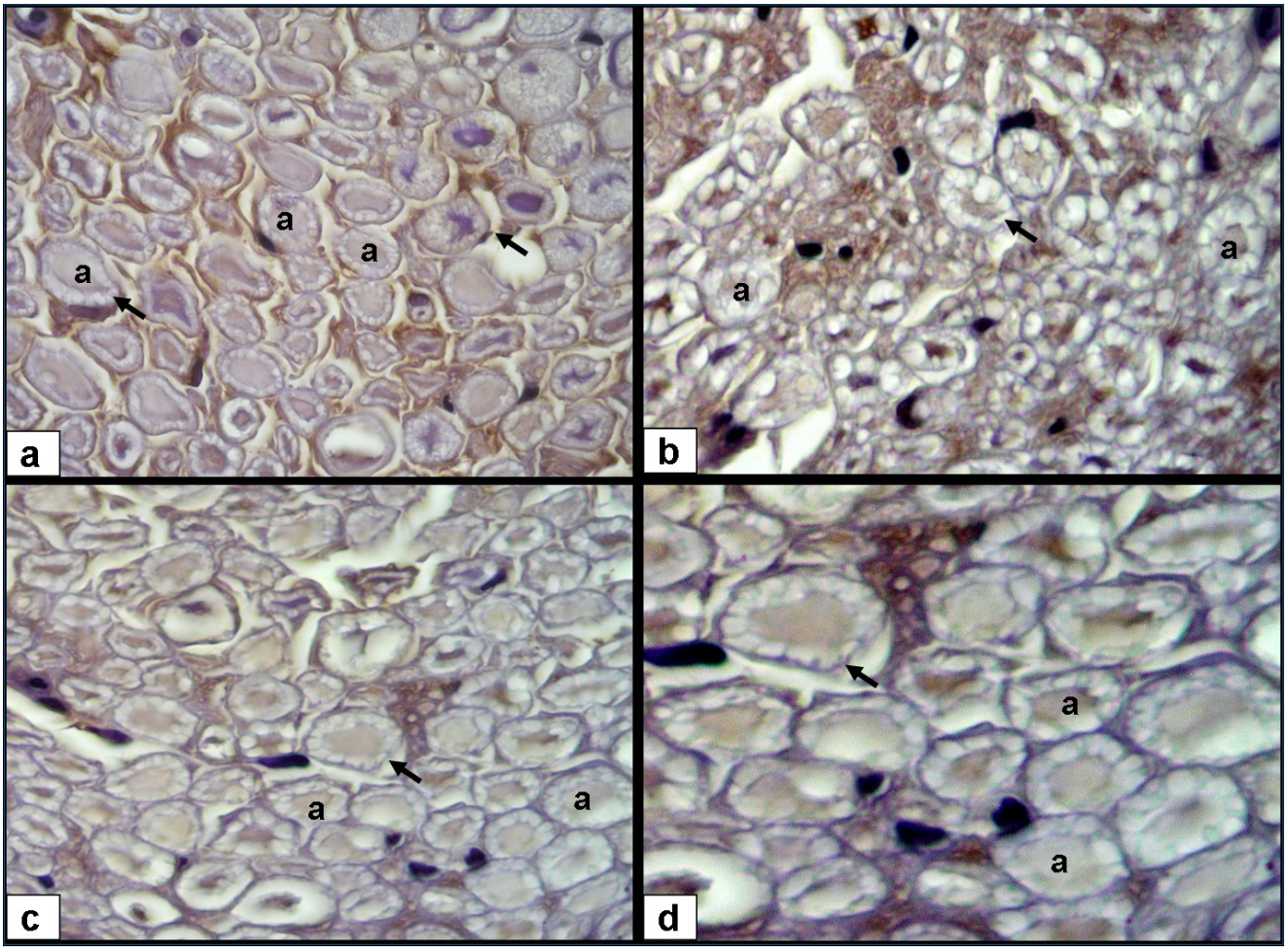

Figure 2. NGF immunohistochemistry; A) normal group; axon (a), myelin sheath (arrow) (3,3´-diaminobenzidine, 40×); B) cisplatin+saline group, decreased axon diameter and NGF expression, degenerated myelin sheath (3,3´-diaminobenzidine, 40×); C) cisplatin+vitamin C group, increased axon diameter and NGF expression (3,3´-diaminobenzidine, 40×); and D) improved myelin sheath (3,3´-diaminobenzidine, 100×)

including the sciatic nerve ($p<0.05$). Both oxidative stress and decreases in NGF concentration in the nerve were partially prevented by the antioxidant, i.e., vitamin C.

According to the efficacy of cisplatin in cancer treatment, peripheral neurotoxicity will continue to be one of the major dose-related side effects without any proven preventive treatment. Our experimental study demonstrated the ameliorated effects of vitamin C on cisplatin-induced neuropathy through increased HSP-70 and NGF levels and reduced inflammatory and oxidant effects. The results of this rat model study are promising to improve the adverse neurotoxic effects of cisplatin in cancer patients.

AUTHORS' CONTRIBUTION

EEP: Conceptualization, Formal analysis, Writing – original draft. **HGP**: Investigation, Supervision. **SE:** Investigation. **OE**: Writing – review & editing

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ORIGINAL ARTICLE

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The effects of thymoquinone on pancreatic cancer and immune cells

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SUMMARY

OBJECTIVES: Black cumin is widely used as a spice and as a traditional treatment. The active ingredient in black cumin seeds is thymoquinone. Thymoquinone has shown anticancer effects in some cancers. We planned to investigate its anticancer effect on pancreatic cancer cell lines.

METHODS: Thymoquinone chemical component in various doses was prepared and inoculated on pancreatic cancer cell culture, healthy mesenchymal stem cells, and peripheral blood mononuclear cell culture. IC50 values were calculated by absorbance data and measuring cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide staining of cells incubated with thymoquinone at 24, 48, and 72 h.

RESULTS: There was dose-related cytotoxicity. Maximal cytotoxicity was observed at 24 h and 100 μM thymoquinone concentrations in pancreatic cancer cell culture and mesenchymal stem cells. Any concentration of thymoquinone was not cytotoxic to peripheral blood mononuclear cell. Thymoquinone even caused proliferation at a concentration of 6.25 μM.

CONCLUSIONS: Since the cytotoxic concentration of thymoquinone on pancreatic cancer cell culture and mesenchymal stem cells is the same, it is not appropriate to use thymoquinone to achieve cytotoxicity in pancreatic cancer. However, since thymoquinone provides proliferation in peripheral blood mononuclear cell at a noncytotoxic dose, it may have an immune activator effect. Therefore, in vivo studies are needed to investigate the effect of thymoquinone on the immune system.

KEYWORDS: Cell survival. Mesenchymal stem cell. Pancreatic cancer. Peripheral Blood Mononuclear Cells. Thymoquinone.

INTRODUCTION

Black cumin (*Nigella sativa* L.) is a type of flower, which is common worldwide and in our country. Its seed is widely used as a spice and as a traditional treatment because it is believed to be beneficial for some diseases. The active ingredient in black cumin seeds is thymoquinone (TQ). A limited number of in vitro and in vivo studies suggest that TQ has many beneficial effects, such as anti-inflammatory, antimicrobial, and anticancer properties[1].

TQ is a natural phytochemical compound, and it has bioactivity in cancer cells[2]. TQ affects different molecular targets in various cancer cells, and many mechanisms have been proposed for its anticancer activity. Oxidative stress and inflammation are important mechanisms in cancer development. TQ reduces the oxidative stress with both antioxidant and anti-inflammatory effects and increases the expression and activity of antioxidant enzymes. It also prevents cancer formation by inducing apoptosis[3]. TQ can reduce the risk of cancer by preventing oxidative DNA damage induced by reactive oxygen radicals[4]. It also induces apoptosis by lowering the phosphorylation of NF-κB and IKKα/β. TQ inhibits its metastasis by increasing Janus kinase and p38 activity[5]. In a study, TQ showed anticancer activity in combination with gemcitabine on pancreatic cancer cell lines by suppression of Notch1, upregulation of PTEN, and inactivation of Akt/mTOR/S6 signaling pathways[6]. TQ noncytotoxic dose was found to boost the antiproliferative and apoptotic effects of some chemotherapeutics[7]. TQ has been shown to have immune-modulatory effects in some studies. In particular, it increases the number and activity of immune cells[8].

Black cumin is a well-known spice in our country and cancer patients often use it even without a doctor's recommendation. We observed that patients with end-stage metastatic pancreatic cancer used black cumin even though it was not recommended by us and benefited clinically. The cytotoxic effect of TQ on cancer cell lines has been demonstrated in

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previous studies. However, cytotoxicity on healthy cell lines has mostly not been studied in most of them[9]. Therefore, we planned to show the effects of TQ in pancreatic cancer cell culture (PANC-1), healthy mesenchymal stem cells (MSCs), and peripheral blood mononuclear cell (PBMC) culture. In this way, besides the effect of TQ on pancreatic cancer cells, its cytotoxic effect on healthy cells will be demonstrated. In addition, its effect on the immune cells will be investigated. We planned to determine the toxic effect and dose of different concentrations of TQ chemical components on PANC-1, MSC, and PBMC cells.

METHODS

In this study, after the TQ chemical component was dissolved with 100% DMSO, the concentrations forming the experimental groups were prepared with the complete medium.

Experimental groups

- Only cells (PANC-1, MSC, and PBMC);
- Only cell (medium containing ≤0.1% DMSO);
- 100, 50, 25, 12.5, 6.25, 3.125, 1, 0.1 µM TQ (separately) + cell.

IC50 values were calculated by measuring cell viability by MTT staining of cells incubated with TQ at 24, 48, and 72 h (the concentration-dependent curve was obtained by normalizing only the viability in the cell groups).

MTT-based cell viability analysis

TQ chemical component prepared at 100, 50, 25, 12.5, 6.25, 3.125, 1, and 0.1 µM doses was inoculated into 96-well plates at 10000 cells/well. Toxic effects of TQ at 24, 48, and 72 h were tested with MTT-based absorbance readings. Experimental groups were studied in five repetitions and their averages were taken.

Calculation of IC50 values

The IC50 values of TQ concentrations on PANC-1, MSC, and PBMC cells and the dose-response curve were calculated by entering logarithm values into the "non-linear regression" analysis data of the GraphPad Prism 8 program.

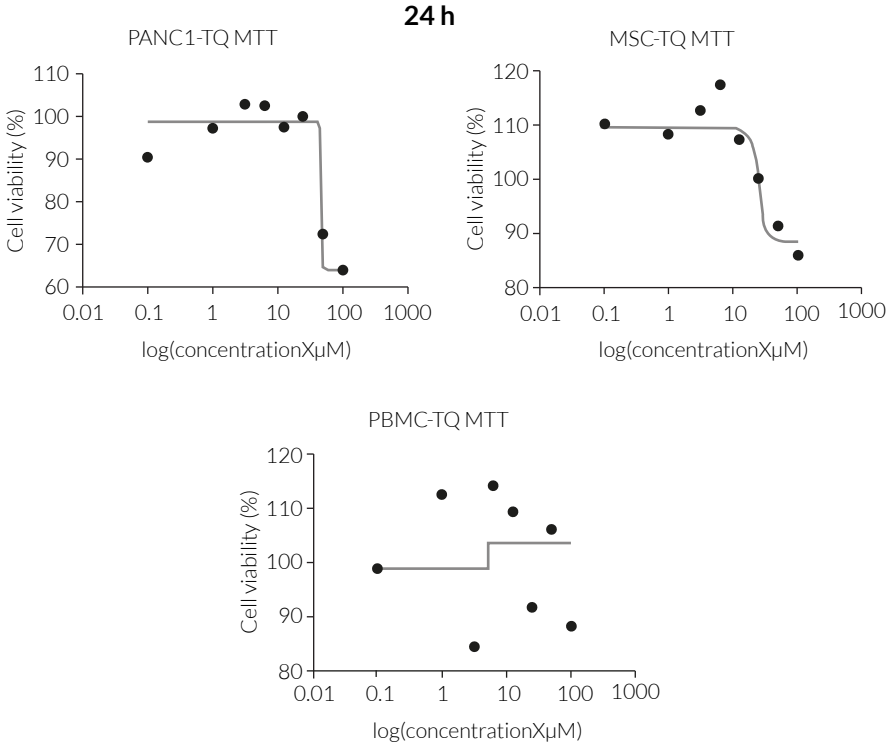
RESULTS

MTT-based cell viability analysis of 100, 50, 25, 12.5, 6.25, 3.125, 1, and 0.1 µM concentrations of TQ chemical components on PANC-1, MSC, and PBMC cells at 24, 48, and 72 h are given in Table 1. Effects of TQ chemical component on PANC-1, MSC, and PBMC cells at 24 and 72 h are given as dose-response curve in Figures 1 and 2. In PANC-1 and MSC, most cytotoxic doses of TQ were 100 µM; the cytotoxic effect decreased through lower doses. In both cell cultures, the maximal cytotoxicity was observed at 24 h, and it was decreased through 48 and 72 h. In PBMC culture, cytotoxicity was not observed. Even cell proliferation was observed at 6.25 µM TQ dose.

Table 1. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide-based cell viability analysis of mean values.

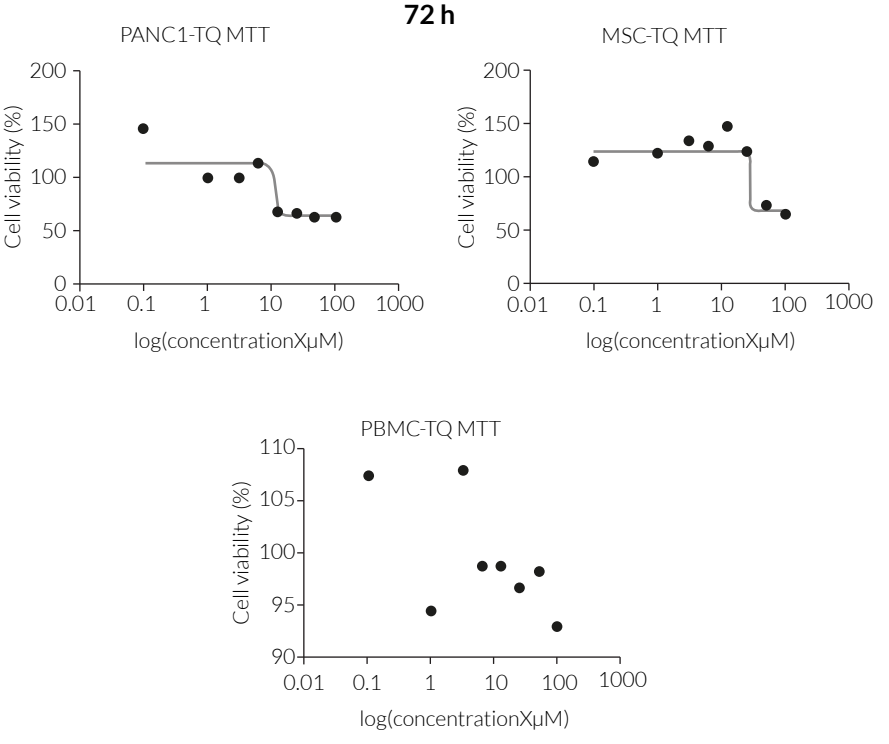
	PANC-1	PANC-1 (≤0.1% DMSO)	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	3.125 µM	1 µM	0.1 µM
24 h	0.3662	0.431	0.274	0.3102	0.4312	0.4198	0.4414	0.443	0.4192	0.3892
48 h	0.5416	0.5496	0.2776	0.3598	0.5504	0.5674	0.5678	0.566	0.5764	0.5224
72 h	0.5036	0.642	0.3242	0.3668	0.6202	0.7414	0.6478	0.6714	0.6122	0.573
	MSC	**MSC (≤0.1% DMSO)**	**100 µM**	**50 µM**	**25 µM**	**12.5 µM**	**6.25 µM**	**3.125 µM**	**1 µM**	**0.1 µM**
24 h	0.336	0.3282	0.2888	0.307	0.3366	0.361	0.395	0.3794	0.3646	0.3708
48 h	0.378	0.3596	0.2866	0.3006	0.3362	0.361	0.4162	0.4014	0.4006	0.429
72 h	0.5008	0.451	0.3124	0.3052	0.3262	0.3372	0.5644	0.4942	0.4974	0.7274
	PBMC	**PBMC (≤0.1% DMSO)**	**100 µM**	**50 µM**	**25 µM**	**12.5 µM**	**6.25 µM**	**3.125 µM**	**1 µM**	**0.1 µM**
24 h	0.2224	0.2136	0.1958	0.2356	0.2036	0.2432	0.254	0.187	0.2502	0.2196
48 h	0.249	0.303	0.3158	0.2716	0.2734	0.2676	0.2884	0.1872	0.3346	0.2274
72 h	0.2044	0.1864	0.19	0.2008	0.1976	0.2016	0.2018	0.2204	0.193	0.2194

DMSO: dimethyl sulfoxide; MSC: mesenchymal stem cells; PANC-1: pancreatic cancer cell culture; PBMC: peripheral blood mononuclear cell; µM: micromolar.



MSC: mesenchymal stem cells; PANC-1: pancreatic cancer cell culture; PBMC: peripheral blood mononuclear cell.

Figure 1. Dose-response curves at 24 h.



MSC: mesenchymal stem cells; PANC-1: pancreatic cancer cell culture; PBMC: peripheral blood mononuclear cell.

Figure 2. Dose-response curves at 72 h.

DISCUSSION

TQ has toxic effects on PANC-1. But cytotoxic doses are also found to be toxic to MSC. The nontoxic TQ dose to MSC had no cytotoxic effect on PANC-1. We concluded that it is not possible to provide a sufficient TQ cytotoxic dose in pancreatic cancer without damaging healthy cells. Mu et al.[6] showed that TQ has cytotoxicity on the PANC-1 cell line at 50 and 25 µmol/L doses. But they did not study the effect of TQ on healthy cells. Tan et al.[10] reported that TQ has a cytotoxic effect on the PANC-1 cell line in various doses but they also did not study on healthy cells. There are very few studies in the literature on TQ that has immunomodulatory and immunotherapeutic potential. Therefore, to investigate the cytotoxic effect of TQ on PANC-1 cells via immune cells, an experimental plan was established for PBMC cells. As a result of this experiment, it was found that no dose of TQ was cytotoxic to PBMC cells. It has even been found to proliferate PBMC cells. As a result of the analyses, we think if TQ has anticancer activity, this effect may not be occurred by direct cytotoxicity but by immune system activation. There are some studies about the immune activator effects of *N. sativa* protein or oil, but there is no any study with TQ. In a study, it was reported that *N. sativa* proteins can increase the production of TNF-alpha either by nonactivated or by mitogen-activated PBMC[11]. In another study, *N. sativa* proteins achieved the secretion of IL1-beta and IL-3 from PBMC[12]. At present, there is no any in vivo study to show the immune-activator effect of the TQ or *N. sativa*. Anticancer activity of *N. sativa* has been shown in some in vivo studies and this effect has been attributed to the anti-inflammatory and antioxidant properties of TQ[8].

To understand this immune activator mechanism of action, in vivo studies supported by different doses of TQ and control groups are needed. For this reason, we think in vivo animal studies are necessary to elucidate the anticancer mechanism of TQ.

CONCLUSIONS

Although black cumin is a plant that is frequently used by cancer patients without the knowledge of the doctor, its effect on cancer is not known exactly. In our study, there are some clues that it may have its main anticancer effects through the activation of the immune system rather than its direct cytotoxic effects. These results encouraged us to investigate the relationship between TQ and the immune system. More in vitro and animal experiments are needed to investigate the anticancer effects of TQ via immune cells.

AUTHORS' CONTRIBUTIONS

CA: Conceptualization, Data curation, Formal Analysis. **DDK:** Data curation, Formal Analysis. **GSK:** Data curation, Formal Analysis. **DÇ:** Data curation, Formal Analysis. **EO:** Data curation, Formal Analysis. **EK:** Data curation. **EY:** Data curation. **FÖ:** Data curation.

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ORIGINAL ARTICLE

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Cow's milk allergy immunoglobulin E-mediated: intake of proteins and amino acids

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SUMMARY

OBJECTIVE: Children with cow's milk allergy may be at nutritional risk due to the lower intake of nutrients, such as protein, calcium, and vitamin A, which are present in cow's milk. The objective was to evaluate children's diets with Children with cow's milk allergy compared with healthy controls as well as to compare the intake of proteins and amino acids from the diet followed by Children with cow's milk allergy who consume special infant formula or plant-based dairy alternatives with Children with cow's milk allergy who do not consume special infant formula or plant-based dairy alternatives.

METHODS: Through a cross-sectional controlled study, the dietary intake of 57 children (27 with immunoglobulin E-mediated Children with cow's milk allergy and 30 healthy controls) was evaluated. Using 24-h nutritional recalls, the total energy intake value, macronutrients, and amino acids were calculated.

RESULTS: No statistically significant difference was found between the Children with cow's milk allergy group and healthy controls for the intake of proteins and amino acids. However, the Children with cow's milk allergy do not consume special infant formula or plant-based dairy alternatives group had a lower protein (g/kg) and branched-chain amino acid (mg/kg) intake than the Children with cow's milk allergy consume special infant formula or plant-based dairy alternatives group.

CONCLUSIONS: The Children with cow's milk allergy group achieved the recommendations for the intake of proteins and amino acids compared to the healthy control group. However, the Children with cow's milk allergy do not consume special infant formula or plant-based dairy alternatives group had a lower intake of protein (g/kg) and branched-chain amino acid (mg/kg) than the Children with cow's milk allergy consume special infant formula or plant-based dairy alternatives group.

KEYWORDS: Amino acids. Children. Cow's milk allergy. Dietary intake. Food hypersensitivity.

INTRODUCTION

The literature well-documented that food allergy (FA) affects more children than adults[1], and cow's milk (CM) causes most food allergic reactions in childhood. The treatment for cow's milk allergy (CMA) is based on excluding CM and dairy products.

The impact of exclusion diets on children's growth has been emphasized in some studies which found lower height-for-age and weight-for-height than children without exclusion diets[2-6].

When evaluating the protein intake, it is crucial to go beyond the amount consumed as the composition of amino acids from the diet and protein digestibility are equally important factors[7].

Given the evidence of greater nutritional risk for children with CMA present in the literature, the lack of studies that assess the protein intake from a quantitative and qualitative point of view, considering the amino acids intake and protein digestibility, the present study was developed. The objective was to compare the dietary intake of proteins and amino acids of children with CMA with healthy controls as well as to compare intake of proteins and amino acids of children with CMA who consume special infant formula or plant-based dairy alternatives (CMA c-SIF/PBDA) with CMA who do not consume special infant formula or plant-based dairy alternatives (CMA dc-SIF/PBDA).

METHODS

Through a cross-sectional controlled study, 27 patients (aged from 0 to 8 years) with CMA mediated by immunoglobulin E (IgE) were evaluated according to their clinical history and history of sensitivity to CM or oral challenge test for positive CM, who visited the Allergy and Clinical Immunology Outpatient Clinic of the Federal University of São Paulo (UNIFESP) and the Menino Jesus Child Hospital, São Paulo, Brazil. Data were collected between July 2016 and January 2018. The control

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group consisted of 30 healthy volunteers regularly enrolled in a private school in the city of São Paulo, matched by sex and age.

Children with allergies to food other than milk, who were breastfed, used corticosteroids 3 months before data collection, and had disabsorptive diseases, such as celiac disease, cystic fibrosis, and inflammatory bowel diseases, were excluded from the survey. Those with chronic and acute diseases were excluded during data collection concerning the control group.

The Research Ethics Committee of Unifesp approved the study under protocol 2,621,736, and the Assent Form was applied to all participants and/or guardians before collecting data by a trained nutritionist.

Parents completed a nonconsecutive 3-day dietary recording to evaluate dietary intake, two during the week and one at the weekend[8]. Dietary intake was calculated using Microsoft Excel® with a national database[9].To calculate the intake of amino acids, the Food Processor® software was used with the United States Department of Agriculture (USDA) database.

To assess the adequacy of dietary protein intake, the mean values obtained were compared to the reference values proposed by the Institute of Medicine (IOM). To measure the adequacy of dietary intake of amino acids, the mean values obtained were compared to the reference values of FAO/WHO[7].

The Protein Digestibility Corrected Amino Acid Score (PDCAAS) was calculated based on the value of the most limiting essential amino acid chemical score of each protein source. PDCAAS was calculated by multiplying the lowest essential amino acid score by protein digestibility. Protein with PDCAAS ³1.0 was of good quality[7].

To estimate the number of portions of CM and dairy or CM replacements, the portions proposed by Tucunduva et al.[10] for CM and dairy by age group were used.

To compare the protein sources consumed between the groups, the average protein intake over 3 days was divided into vegetable proteins (all foods of vegetable protein source, except the foods here called CM replacements, namely, plant-based special infant formula and plant-based alternatives), animal proteins (all foods of animal-protein origin, except those here called CM and dairy, namely, dairy-protein-based infant formulas, growing-up milk, *in natura* CM, and all CM dairy products), and other protein sources (an assortment of infant formula, plant-based alternatives, growing-up milk, CM, and dairy products) in g/kg.

Statistical analysis

Data were entered and consolidated in an Excel spreadsheet (Office Microsoft®) and analyzed using the statistical package SPSS 19.0 (IBM®). Categorical variables were presented as absolute and percentage values. Continuous variables were analyzed for their normality using the Shapiro-Wilk test. For comparisons between groups, variables with parametric distribution were presented as mean and standard deviation and compared using the independent Student's t-test. Variables with nonparametric distribution were presented as median (minimum and maximum) and compared using the two-tailed Mann-Whitney U test. The statistical significance level of 5% ($p<0.05$) was adopted.

RESULTS

Table 1 describes the general characteristics of 27 children in the CMA group.

Regarding the CM substitute used by the CMA group, 51.8% of this group used some plant-based replacement. Furthermore, all these substitutes were soy-based (Table 1).

Regarding dietary intake (Table 2), it was observed that children in the CMA group had, compared to the healthy control group, a higher intake of vegetable protein in g/kg

Table 1. Characteristics of children with cow's milk allergy (n=27).

		Distribution
Sex (%)	Male	20 (74.1)[a]
Age	Years	4.0±1.9[b]
Ethnicity (%)	Caucasian	15 (55.5)[a]
	Black	6 (22.2)[a]
	Pardo	6 (22.2)[a]
Age group (%)	0–<2 years	4 (14.8)[a]
	2–<6 years	21 (77.8)[a]
	6–10 years	2 (7.4)[a]
Exclusive breastfeeding	Months	4.3 (0.0–7.0)[c]
Total breastfeeding	Months	13.0 (0.0–48.0)[c]
First allergic reaction age	Months	4 (1.0–10.0)[c]
First allergic reaction symptoms	Cutaneous	18 (66.7)[a]
	Systemic	5 (18.5)[a]
	Gastrointestinal	3 (11.1)[a]
	Respiratory	1 (3.7)[a]
CM replacement	SBR	14 (51.8)*
	NR	5 (18.5)[a]
	ISPF	5 (18.5)[a]
	EHF	3 (11.1)[a]
	FAA	1 (3.7)[a]

[a]n (%): Number (percentage); [b]Mean±standard deviation; [c]Median (minimum; maximum). FAA: free amino acid formula; ISPF: Isolated Soy Protein Formula; EHF: extensively hydrolyzed formula; SBR: soy-based replacement; NR: no replacement. *1 child was using these replacements combined with Isolated Soy Protein Formula.

Table 2. Comparison between children with cow's milk allergy and nonallergic controls of nutritional status, dietary intake, dietary intake of amino acid, and quality of protein consumed.

		Cow's milk allergy group (n=27)	Group control (n=30)	p
Anthropometry				
Age	Years	4.0±1.9[d]	4.0±1.8	0.958[a]
Body mass index	Z-score	-0.03 (-2.3–2.7)[5]	0.17 (-2.5–2.3)	0.867[b]
Body mass index classification (%)	Eutrophic	19 (70.1)	21 (70.0)	0.441[c]
	Thinness	1 (3.7)	4 (13.3)	
	Overweight	6 (22.2)	4 (13.3)	
	Obesity	1 (3.7)	1 (3.7)	
Height/age	Z-score	-0.24 (-2.1–0.5)	0.19 (-2.4–1.9)	0.004[b]
Stature classification (%)	Normal height	25 (92.6)	28 (93.3)	
	Short stature	2 (7.4)	2 (6.7)	0.653[c]
Dietary intake				
Total energy	Kcal/day	1615.3±427.3	1568.3±393.2	0.667[a]
Carbohydrate	%TCV	50.8 (39,2–66.7)	53.5 (42.2–85.9)	0.379[b]
Lipid	%TCV	33.1±4.8[d]	31.9±4.1	0.312[a]
Protein	%TCV	15.2 (10.2–27.3)	15.6 (12.6–20.8)	0.554[b]
Total protein	g/kg	3.8 (2.1–7.3)	3.9 (2,0–6.4)	0.701[b]
Vegetable protein*	g/kg	1.43 (0.62–3.90)	1.14 (0.47–1.98)	0.001[b]
Animal protein**	g/kg	1.66 (0.68–3.57)	1.46 (0.10–3.96)	0.035[b]
CM protein, dairy, or replacements	g/kg	0.67±0.42	1.32±0.69	<0.001[a]
Dietary intake per meal				
Breakfast and snacks				
Energy	Kcal	814.3±253.4	890.9±258.1	0.264[a]
Carbohydrate	%	30.2±7.3	32.4±6.0	0.224[a]
Lipid	%	14.4±3.8	17.9±4.6	0.002[a]
Protein	%	5.0±1.3	6.8±2.2	<0.001[a]
Protein	g/kg	1.30 (0.46–2.40)	1.64 (0,42–3.88)	0.059[b]
Lunch and dinner				
Energy	Kcal	822.1±240.8	678.2±239.8	0.028[a]
Carbohydrate	%	20.8±4.7	19.9±6.2	0.545[a]
Lipid	%	18.9±4.4	13.9±3.9	<0.001[a]
Protein	%	10.7±3.3	9.1±2.7	0.053[a]
Protein	g/kg	2.54 (1.39–5.70)	2.23 (0,68–4.09)	0.057[b]
number of CM portions or replacements		1.9±0.7	3.4±1.5	<0.001[a]
Amino acids				
Isoleucine	mg/kg	113.5 (32.2–237.7)	91.1 (24.7–284.4)	0.213[b]
Leucine	mg/kg	194.5 (61.9–379.2)	164.5 (45.3–485.2)	0.284[b]
Valine	mg/kg	122.8 (38.4–240.5)	107.6 (32.7–303.8)	0.330[b]
Aromatic amino acids	mg/kg	83.6±32.0	80.7±41.8	0.772[a]
Sulfur-containing amino acids	mg/kg	187.7 (70.6–387.3)	169.8 (53.7–508.4)	0.701[b]

Continue...

Table 2. Continuation.

		Cow's milk allergy group (n=27)	Group control (n=30)	p
Histidine	mg/kg	71.1±30.4	70.6±38.8	0.952[a]
Lysine	mg/kg	165.2 (48.8–322.7)	137.6 (33.9–389.3)	0.666[b]
Threonine	mg/kg	88.8 (29.2–173.5)	72.5 (21.6–202.3)	0.462[b]
Tryptophan	mg/kg	22.8 (8.5–52.3)	21.3 (6.8–57.3)	0.620[b]
BCAA	mg/kg	143.8 (44.2–285.8)	120.1 (34.2–357.8)	0.277[b]
Essential amino acids	mg	13970.0 (5283.3–25576.6)	11470.0 (3343.3–27953.3)	0.672[b]
Total amino acids	mg	35530.0 (13040.0–63630.0)	29936.6 (10083.3–71406.6)	0.632[b]
Protein quality				
PDCAAS		0.60±0.19	0.64±0.25	0.523[a]

[a]Student's t-test; [b]Mann-Whitney test; [c]χ^2; [d]Mean (standard deviation); Median (minimum and maximum). *Except proteins from special infant formula and plant-based alternatives. **Except proteins from CM and dairy products.
TCV: Total Caloric Value; CM: cow's milk; BCAA: branched-chain amino acid; PDCAAS: protein digestibility corrected amino acid score.
Bold indicates statistically significant values.

[1.43 g/kg (0.62–3.90) vs. 1.14 g/kg (0.47–1.98); p=0.001] and animal protein in g/kg [1.66 g/kg (0.68–3.57) vs. 1.46 g/kg (0.10–3.96); p=0.035]. When analyzing the consumption of proteins from CM and dairy products or CM replacements, it was observed that the CMA group, compared to the healthy control group, had a lower intake of proteins from CM replacements in g/kg (0.67±0.42 g/kg vs. 1.32±0.69 g/kg; p≤0.001). Also, the CMA dc-SIF/PBDA group had lower protein intake in g/kg (2.95±0.63 g/kg vs. 4.45±1.54 g/kg; p=0.044) than the CMA c-SIF/PBDA group.

When comparing the intake grouped by meals, it was observed that, at breakfast and snacks, the CMA group, compared to the control group, had a lower intake of protein in g/kg (5.0±1.3% vs. 6.8±2.2%; p≤0.001).

The CMA group, compared to the healthy control group, intake fewer portions of CM replacements (1.9±0.7) vs. CM and dairy group (3.4±1.5) p≤0.001.

No statistically significant difference was found regarding the intake of the amino acids in mg/kg, when the CMA group was compared with the healthy control group (Table 2). However, when the CMA dc-SIF/PBDA group (n=5) was compared with the CMA c-SIF/PBDA group (n=22) (Table 3), there was a statistically significant difference. There was lower intake of all branched-chain amino acids (BCAA) as follows: isoleucine (75.1±34.9 mg/kg vs. 129.9±48.4 mg/kg; p=0.025), leucine (126.3±57.9 mg/kg vs. 220.4±79.8 mg/kg; p=0.020), and valine (85.3±37.8 mg/kg vs. 140.3±50.3 mg/kg; p=0.031). There was no statistically significant difference in anthropometric indexes between the CMA dc-SIF/PBDA and the CM c-SIF/PBDA groups.

DISCUSSION

In this present study, no differences were found in the dietary intake of proteins and amino acids between the CMA and the healthy control groups, nor in the quality of protein consumed. However, the CMA dc-SIF/PBDA group had a lower protein (g/kg) and BCAA (mg/kg) intake than the CMA c-SIF/PBDA group.

Results similar to those observed in this study were previously reported by Rowicka et al.[11] in CMA group. The authors highlighted that the protein intake by children with CMA exceeded three times the recommendation. In contrast, other authors have mentioned lower protein intake by CMA children than those without dietary exclusion[12-14].

Regarding the analysis of amino acids from the diet of children with CMA, according to the best of our knowledge, there are no studies published to date.

Intake of amino acids is closely related to children's growth, as the amino acid recommendations for this age group should be considered[7]. In a study of 313 children from Malawi, who aged between 12 and 59 months (62% of them with low ZH), serum levels of amino acids were measured. The authors observed lower levels of all essential amino acids in the low ZH group than those with adequate growth[15].

A cross-sectional study with 5034 healthy Canadian children aged between 24 and 72 months verified the association between the intake of non-cow milk beverages and shorter stature in childhood. The authors observed that each cup of non-cow milk beverage consumed daily was associated with a 0.4 cm reduction in the children's height compared to those who consumed CM[16]. In our study, some CM replacements

Table 3. Evaluation of dietary intake of amino acids and proteins, protein quality, and anthropometric indexes between children with cow's milk allergy who do not consume special infant formula or plant-based dairy alternatives (n=5) and cow's milk allergy who consume special infant formula or plant-based dairy alternatives (n=22)

		CMA dc-SIF/PBDA (n=5)	CMA c-SIF/PBDA (n=22)	p
Amino acids (mg/kg)				
Histidine		48.6±23.7	76.3±29.8	0.064[a]
Isoleucine		75.1±34.9	129.9±48.4	0.025[a]
Leucine		126.3±57.9	220.4±79.8	0.020[a]
Valine		85.3±37.8	140.3±50.3	0,031[a]
Aromatic amino acids		64.4±27.6	87.9±31.9	0.141[a]
Sulfur-containing amino acids		146.9±65.2	217;3±83.5	0.091[a]
Lysine		115.4±63.7	180.5±69.4	0.067[a]
Threonine		65.5±31.5	100;4±36.7	0.061[a]
Tryptophan		18.7±8.6	27.7±11.8	0.119[a]
BCAA		95.6±43.4	163.5±59.4	0.024[a]
Protein/quality				
Protein (g/kg)		2.95±0.63[c]	4.45±1.54	0.044[a]
PDCAAS		0.61±0.32[c]	0.59±0.16	0.953[a]
Anthropometry				
Body mass index	Z-score	0.32±1.47	0.08±1.15	0.687[a]
Height-for-age	Z-score	-0.14 (-2.08–0.47)[d]	-0.27 (-2.05–0.46)	0.755[b]

[a]Student's-t-test; [b]Mann-Whitney test; [c]Mean (standard deviation); [d]Median (minimum and maximum).
CMA: cow's milk allergy; dc-SIF/PBDA: do not consume special infant formula or plant-based dairy alternatives; c-SIF/PBDA: consume special infant formula or plant-based dairy alternatives.
Bold indicates statistically significant values.

used by the CMA group may explain this group's lower stature, which in some cases was inadequate.

When evaluating protein intake, we must consider the origin of the protein, as it is generally agreed that animal-derived proteins play an important role in children's growth. It is known that the amino acids present in CM, especially the BCAA ones, influence the production of somatomedin C or IgF1, with a positive impact on growth[17]. When we assessed the dietary sources of proteins consumed by the groups studied, we observed that the CMA group had a higher intake of vegetable protein (from cereals and other foods, except from CM replacements) and animal protein (from meat and eggs) compared to the healthy control group. Opposite results were found by Maslin et al.[18], which compared the dietary intake between children with a CM elimination diet and children without elimination. It was verified that the group on the CM elimination diet had less animal protein intake than the group without the elimination diet.

In the Netherlands, a cohort evaluated the impact of the intake of different protein sources in 3564, 1-year-old children on BMI and height at 9 years. It concluded that the early high intake of animal protein was associated with higher BMI and height at 9 years old than those with predominant intake of vegetable protein[19].

In our study, the CMA group had lower protein at breakfast and snacks, which may be explained by the smallest number of portions of CM replacements intake consumed by these individuals. Similar results were found by Vassilopoulou et al.[20], who assessed the effects of FA on the eating habits of children aged 6–11 years. Children with FA had lower protein intake at breakfast and snacks than controls. In the CM replacement group, the absence of these meals was balanced by the intake of juice and dried fruits. Similarly, in our study, we observed that five children were not using any CM replacements in the CMA group. However, the recommendation in g/kg of protein consumed was achieved due to the higher intake of animal and vegetable proteins present in other meals.

When individuals in the CMA dc-SIF/PBDA group were compared with those in the CMA c-SIF/PBDA group, it was observed that children with CMA dc-SIF/PBDA had a lower intake of protein in g/kg and of BCAA in (mg/kg of weight body), with no differences in anthropometric indexes. This difference between the CMA groups must be interpreted carefully, considering that both achieved the amino acid

recommendations in mg/g of protein by intake of another protein source. However, it opens a possibility for future studies for evaluating the serum levels of these amino acids in children with CMA on an elimination diet.

The present study was a pioneer in describing the intake of amino acids in children with CMA compared with healthy controls. Still, it has the following limitations: cross-sectional design, limitations of the PDCAAS method, absence of a national database with amino acids present in foods, and sample size.

We conclude that the CMA group, under nutritional intervention, did not differ from the healthy control group in terms of intake of proteins and amino acids. However, the CMA dc-SIF/PBDA group had a lower intake of protein and BCAA than the CMA c-SIF/PBDA group.

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AUTHORS' CONTRIBUTIONS

ECAK: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **RBM:** Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. **TLNB:** Data curation, Formal Analysis, drafting the article, Writing – review & editing. **RMB:** Data curation, Writing – review & editing. **ROSS:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Does perinatal period pelvic floor muscle exercises affect sexuality and pelvic muscle strength? A systematic review and meta-analysis of randomized controlled trials

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SUMMARY

OBJECTIVE: The aim of this study was to systematically review the effect of pelvic floor exercises on female sexual function and pelvic floor strength in the prenatal and postnatal periods and to conduct a meta-analysis of available evidence.

METHODS: Published archives, including PubMed, Cochrane Library, Web of Science, and ULAKBİM databases, were scanned using keywords based on MeSH. Only randomized controlled trials were included. The data were analyzed using the Review Manager computer program (version 5.3).

RESULTS: Pooled standardized differences in means of sexual function in both pelvic floor exercise and control group were 6.33 (95%CI 5.27–7.40, p<0.00001) during pregnancy. The pooled standardized differences in means in sexual function after postpartum intervention was 1.19 (95%CI 0.08−−2.30, p=0.04).

CONCLUSION: Evidence has shown a little effect on the pelvic floor muscle training on sexual function in pregnancy and postpartum period in primipara women, and it is a safe strategy that can improve postpartum sexual function.

KEYWORDS: Pregnancy. Postpartum. Sexuality. Exercise. Meta-analysis.

INTRODUCTION

Sexuality is a natural and important part of human life[1,2]. Sexual dysfunction is defined as a disorder affecting sexual desire that can result in interpersonal difficulties, pronounced distress, and psychophysiological changes[3,4].

The etiology of female sexual dysfunction has a multifactorial structure[5-7]. Especially during pregnancy and after delivery, deterioration of pelvic muscle strength (PMS) is an important risk factor[3,7,8]. The literature has shown that pelvic floor muscle exercise (PFME) can improve sexual desire and orgasm capacity in the general population and in women with weak orgasm problems caused by poor pelvic muscle tone[8,9]. The literature on the effects of PFME on female sexual function (SF) has limited edition reviewed, especially for its efficacy during pregnancy and postpartum, and only two studies were meta-analyzed[6,7]. Therefore, the aim of this study was to systematically review the effect of PME on female SF and PMS in the prenatal and postnatal periods and to conduct a meta-analysis of available evidence.

METHODS

Systematic examination and meta-analysis of the studies evaluating the effect of PMFE on female SFs and pelvic floor strength in the prenatal and postnatal periods were performed. In the preparation of systematic review and meta-analysis, the criteria in the PRISMA and *Cochrane Experiments Systematic Reviews Handbook* were used.

Search strategy

A comprehensive, systematic search of PubMed, Web of Science, the Cochrane Library, and ULAKBİM databases was completed from the earliest date available until February 2020. The Web of Science Core Collection was searched using the following keywords: "pelvic floor exercise" OR "pelvic muscle strength" OR "sexual functions" AND "pregnancy" OR "postpartum." The search strategy was changed according to the characteristics of each database.

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Inclusion and Exclusion Criteria

The inclusion and exclusion criteria used were as follows:

(1) only randomized controlled trials (RCTs) were included in the study;
(2) in the intervention group, pelvic floor exercises to improve the pelvic floor were included if Kegel, Pilates, or yoga were used;
(3) studies that included effects of PFME on at least one SF variable including prenatal or postpartum desire, arousal, orgasm, pain, lubrication, and satisfaction; and
(4) studies published only in English and Turkish languages were included.

Study selection and data extraction

After the duplicate articles retrieved from the different databases were removed, two independent researchers (A.Y.K. and N.E.B.) screened titles and abstracts to identify which studies met the inclusion and exclusion criteria. If there was a contradiction between the researchers, the third researcher (N.G.) was assisted to reach an agreement. Data were obtained using standard data extraction forms including study characteristics (i.e., design, population, experiments, and result), PICOS (participant, experiment, comparison, outcomes, and study design) approach, age, gender, and follow-up time (Table 1).

Risk of bias assessment

The quality of the selected articles was evaluated by two researchers (A.Y.K. and N.E.B.) with the Quality Assessment Tool (EPHPP) checklist. The evaluation of the risk of bias of all selected articles was done by two authors (A.Y.K. and N.B.E.) independently using modified Cochrane tools for assessing risk of bias, following the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions*. The other author (N.G.) checked the results. Risk of bias was classified into seven domains. The bias risk for each area was classified as "low risk," "high risk," or "uncertain risk," according to the decision criteria in the "Risk of bias" assessment tool.

Quantitative data synthesis and analysis

Outcomes data including SF and PMS of the participants who had used PMEs were collected for analysis. Meta-analysis of study outcomes was performed using RevMan version 5.3.

For analysis of continuous data, mean differences (MD) or standardized mean differences (SMD) with 95% confidence intervals (CI) were used. SMD was used when the studies assess the same outcome but measure it in a variety of ways (e.g., all studies measure function, but they use different psychometric scales such as Female Sexual Function Index [FSFI] and Golombok-Rust Sexual Satisfaction Scale [GRISS]). Statistical heterogeneity was determined by I^2. A value of 0% indicates no observed heterogeneity, and larger values indicate increased heterogeneity. Coherence between researchers for independent article selection and bias scores was evaluated using the Cohen's kappa statistic. Only 62.5% (n=5) of the studies were graded 1 according to the EPHPP tool. Coherence between the observers was excellent both in the selection of articles and in the scoring of selected articles in terms of bias (Cohen's kappa was 0.95 for article selection and 0.97 for bias scoring; p=0.000).

RESULTS

Literature search

The PRISMA flowchart for searching and selecting literature is summarized in Figure 1.

The electronic database search and hand search yielded 369 potentially relevant studies. After removing duplicates, we screened 339 articles based on title or abstract. The remaining 33 full texts were assessed for eligibility. For the full-text screening, a third reviewer was needed to resolve disagreements, all regarding the blinding of the studies. Eight trials met all eligibility criteria and were included in qualitative synthesis (Figure 1).

Study characteristics

Eight trials (896 participants in total) were included in these reviews and meta-analysis[10-17]. The features of the studies are summarized in Table 1. All other studies started in the postpartum period except for the two studies (started during pregnancy)[10-13]. The duration of the experiments varies between 4 and 20 weeks. In most of the articles, women in the control group received routine postpartum care. However, in one study, the control group received conventional home-based training[17]. Women in the intervention group received the following treatments: those in Wang et al[17] received audio guidance training; those in Oakley et al[11] received biofeedback with pelvic floor exercise; those in Golmakoni et al.[16] received Kegel exercises; those in Haruna et al[14] received aerobic exercise; and those in the remaining four studies received PFME only. The entire patient population included primiparous women. In most studies, SF states were evaluated as the primary outcome. Two of the studies[10-13] evaluated SF in both pregnancy and postpartum period, while all other studies evaluated SF in the postpartum period. All studies included in the review have been reported on SF in the postpartum period.

Table 1. Study characteristics.

Author (reference)\ Publication date\ Country	Study type	population	Training protocol	Comparisons	Drop out	Outcome measures	Results
Wang et al.[17]	RCT	108 primiparous woman 1.Audio guidance group (n=54) 2-Control Group (n=54)	Participants in the intervention group received audio guidance training. – The pelvic floor muscle training practice guide is as follows: participants were directed to lie in the supine position bending the hips and knees and to relax the abdomen and hip muscles while selectively contracting and breathing the urethra, vagina and anus muscles. Meanwhile, the researchers were asked to evaluate on the abdomen, and on the other hand, by placing the participants on their hips and / and middle fingers in their vagina with postpartum sterile gloves to determine if the participants could properly contract and contract these muscles. – The general principles of training were: training at least two times per day and 15 minutes per time, or 150 contractions per day; keep training for at least 3 months. Researchers called the participants of the two groups once a month to answer their questions related to training or stress incontinence and encourage them to keep on training for at least 3 months.	The control group eceiving conventional home-based training through random looting.	– Interventions group (n=6) – Control group (n=4)	FSFI	Participants were not significant in 6 groups postpartum sexual function in both groups during the study (p=0.007)
Pourkhiz et al.[10] İran	RCT	84 primiparous woman 1-PFM training group (n=41) 2-usual care group (n=41)	PFM: at least twice a day, 8–12 contractions at each time, holding for 6–8 s. Start from 36 to 37 weeks and after giving birth as soon as one can FSFI SQOL-F: the mean total sexual function and sexual quality of life score was greater in the PFM training group; (p < 0.001) One in each group	Control: routine care	– PFM (n=1) – Control (n=1) One in each group	FSFI SQOL-F	the mean total sexual function and sexual quality of life score was greater in the PFM training group; (p<0.001)
Tennfjord et al.[15] Norway	RCT	175 primiparous woman 1-PFMT training group (n=87) 2-control group (n=88)	- The training group attended a weekly PFMT class for 4 months, starting 6 weeks postpartum. Also they did daily three sets of 8–12 PFM contractions at home. At 6 weeks (baseline) and 6 months postpartum women answered an electronic questionnaire.	-Control: routine care	– Interventions group (n=3) – Control groups (n=10)	ICIQ-FLUTSsex	No difference was seen between case and control groups in symptoms related to sexual dysfunction during 6 months of postpartum
Oakley et al.[11], Ohio	RCT	50 primiparous woman 1-PFPT group (n=27), 2-control group (n=23)	- The intervention group did PFPT with biofeedback and received Behavioral therapy. The intervention arm completed four 60-min PFPT sessions which beginning at week 6 after delivery. Both groups completed questionnaires as well at baseline (week 2) and weeks 12 postpartum.	Control: routine care	– Interventions group (n=2) – Control group (n=2)	FSFI, FIQOL, SF-12, UDI-6, IIQ-7, FISI	Both groups significantly improved in physical health (p<0.000) and sexual function (p<0.000) after 12 weeks of postpartum, but there was no difference between two groups.

Continue...

Table 1. Continuation.

Author (reference)\ Publication date\ Country	Study type	population	Training protocol	Comparisons	Drop out	Outcome measures	Results
Golmakani et al.[16], Meşhed, İran	RCT	79 primiparous woman 1-PFMT training group (n=40) 2-control group (n=39)	After 8 week of delivery women were trained to Contract their pelvic floor muscles for 5–10 s and relax for 5–10 s and repeating this exercise for 20 times (for 5 min). After 2 min of rest, they again had to perform this exercise for 3 times of 5 min. so that a total of 20 min of exercise is performed at each time. Twice daily, each time 15–20 times depending on their ability. 4 and 8 weeks after the beginning of the study compared in both group	Control: routine care	–Interventions group (n=12) – Control group (n=13)	Brink scale, Bailes sexual self-efficacy	Comparison of the two groups presented a significant difference in sexual self-efficacy after performing these exercises (p=0.001).
Haruna et al.[14], Tokyo	RCT	95 primiparous woman 1-Intervention group; (n=48), 2-control group; (n=47)	Exercise classes were held 4 times weekly for 4 weeks, 90 min each, at three months postpartum. The exercise class included: aerobic exercise (50–60 min) where the participant sits and bounces on an exercise ball. The outcome measures were assessed at two months postpartum (baseline) and at four months postpartum (outcome).	Control: routine care	– Interventions group (n=2) – Control group (n=4)	SF-36v2	The postpartum exercise class improved health-related QOL in the training group compared to the control group, although there were no significant differences in the Physical and Mental component of quality of life between the groups.
Çıtak et al.[12], Turkey	RCT	75 primiparous woman 1-PFM training group (n=37) 2-control group (n=38)	- In the 4th postpartum month women were trained to do PFM contraction. 2–3 s contraction and relaxation, ten times a day in the first 15 days. Thereafter, the duration of contraction and relaxation was changed to five seconds. Then increase the durations to 10seconds and the number of workouts to 15 sessions/day up to the end of the study. The results of both groups, obtained in the 4th and 7th postpartum months, were compared	Control: routine care	–Interventions group (n=21) – Control group (n=22)	FSFI	All domains except satisfaction were significantly higher in the training group compared with the controls. sexual arousal, lubrication, orgasm, and satisfaction scores were improved in the 7th month in the training group;(P < 0.001)
Reilly et al.13, UK	RCT	230 primiparous woman 1-PFE training group (n=120) 2-control group (n=110)	The exercises comprised three repetitions of eight contractions each held for six seconds, with two minutes rest between repetitions. These were repeated twice daily. At 34 weeks of gestation the number of contractions per repetition was increased to 12.	Control: routine care	– Interventions group (n=52) –Control group (n=47)	King's Health Questionnaire, SF-36	At 3 months, there was no difference between the intervention groups on any of the eight scales of the Kings Health Questionnaire. higher score for the general health measure in the Short Form-36 in those in the exercise group compared with the control group

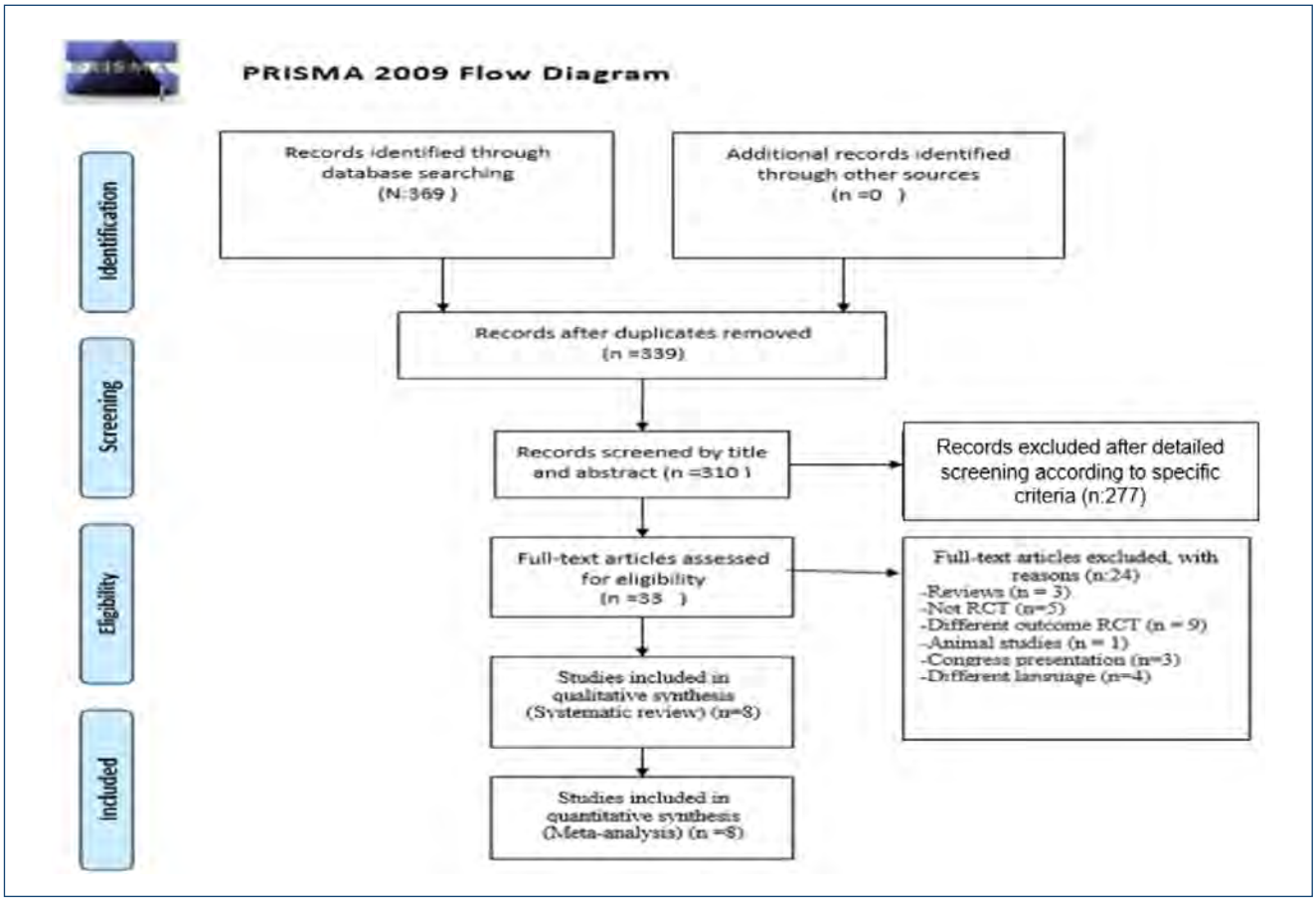

Figure 1. PRISMA flow diagram of selection of study process.

Outcome Measures

The forest graphic in Figure 2 shows us the meta-analysis of the effect of PMFE on SF. Four studies used the FSFI questionnaire[10-12,17] to evaluate SF.

Effect of exercises on SF

Six articles[10-12,15-17] reporting on SF were included in the meta-analysis. In the prenatal period, only one study reported sexual results. Figure 2 shows the effects of pelvic floor exercises on SF during pregnancy. The study[10], which included 82 participants in total (41 receiving PFME), examined the effects of PFME on SF. Pooled SMDs of SF in both PME and control groups were 6.33 (95%CI 5.27–7.40, p<0.00001).

When we evaluated PFME sexual status in the postpartum period, based on the random-effects model, SMDs of SF in both PME and control groups were 1.19 (95%CI 0.08–2.30, p=0.04). A meta-analysis of these studies revealed that PME can improve SF in the postpartum period. The included studies had high heterogeneity (I2=83.0%; p=0.02). The forest plot is shown in Figure 3.

Pelvic floor muscle strength

In the three studies,[10-12] the Oxford grading system, an accepted international method for determining the strength of the pelvic floor muscles (PFMs), was used. Other studies have evaluated PMS with different assessment tools. Eight articles have reported PMS-related results and are included in the meta-analysis[10-12,15-17]. The PMS SMDs in both groups were 1.06 (95%CI 0.12–1.99, p=0.03). Meta-analysis of these studies showed a significant relationship between PFME and PMS. The included studies had high heterogeneity (I^2=94.0%, p<0.00001). The forest plot of the meta-analysis is shown in Figure 3.

Risk of bias assessment

All studies have identified a sufficient method for random allocation of participants to exercise groups[10-17]. Six studies reported adequate allocation confidentiality using sequentially numbered and sealed opaque envelopes[10-12,14,15,17] and evaluated them at low risk of bias. In all studies included in the meta-analysis, it was not possible for the participants and researchers participating in the experiment to be blind to

the study.[10-12,14,15,17] Four studies are at low risk for blinding outcome evaluation.[10,11,15,17] Other studies have also been evaluated without blinding the outcome assessment and as at a high risk of bias.[12-14,16] In these six studies, the drop-outs were balanced between the intervention and control groups, or there were too few drop-outs to affect the study.[10,11,14-17] Apart from the study by Cıtak et al[12] (uncertainty bias risk), in all other methods of work, they were evaluated at the risk of reporting low bias, as they discussed important reported results, including negative results and match those reported in their records or protocols[10,11,15]. In particular, we expected a conflict of interest statement and a source of funding. None of the included studies reported other bias risk (Figure 4).

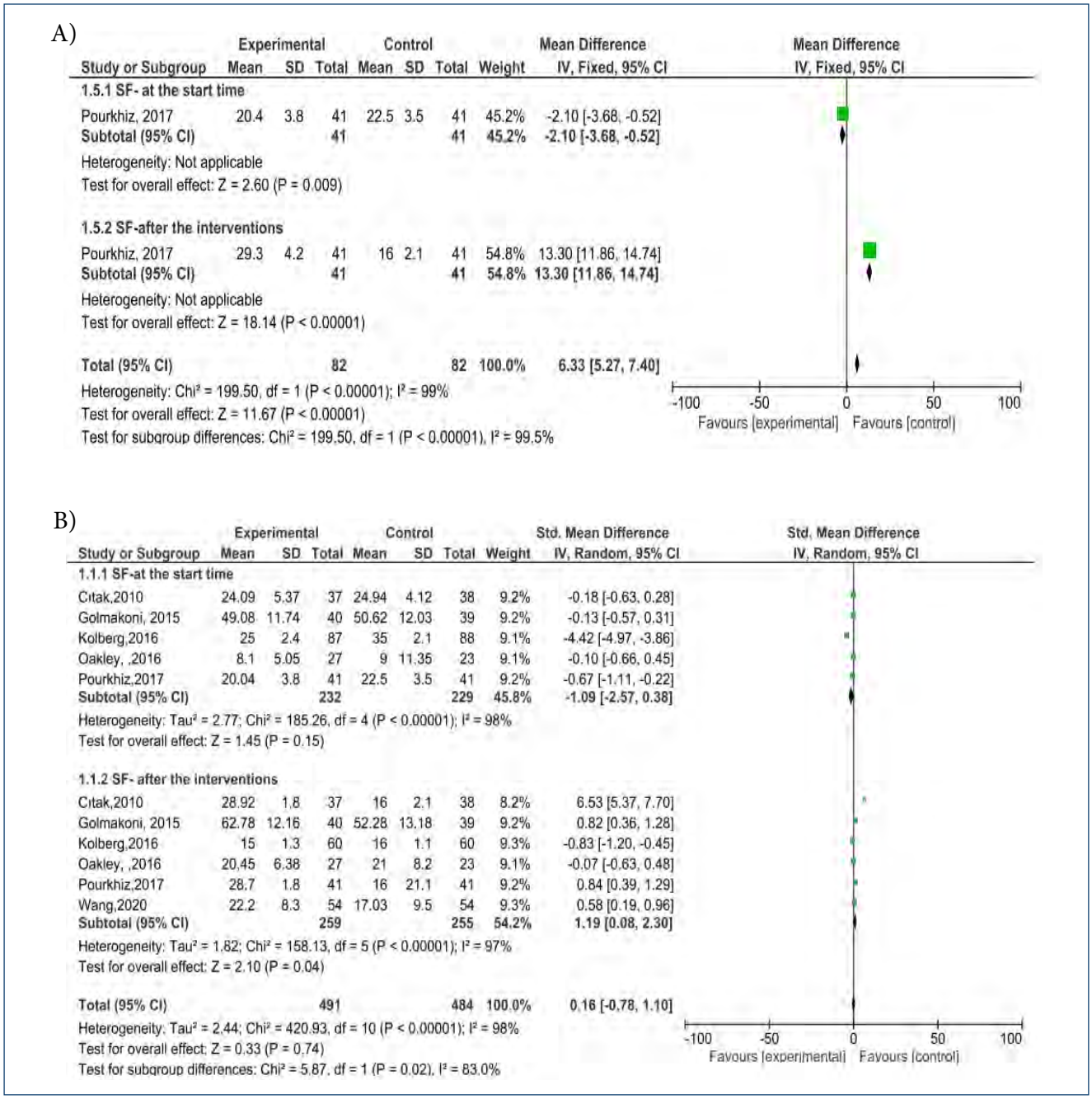

Figure 2. Effect of prenatal and postnatal pelvic floor muscle exercise on sexual function forest plot. A: Effect of prenatal pelvic floor muscle exercise on sexual function forest plot. B: Effect of postnatal pelvic floor muscle exercise on sexual function forest plot.

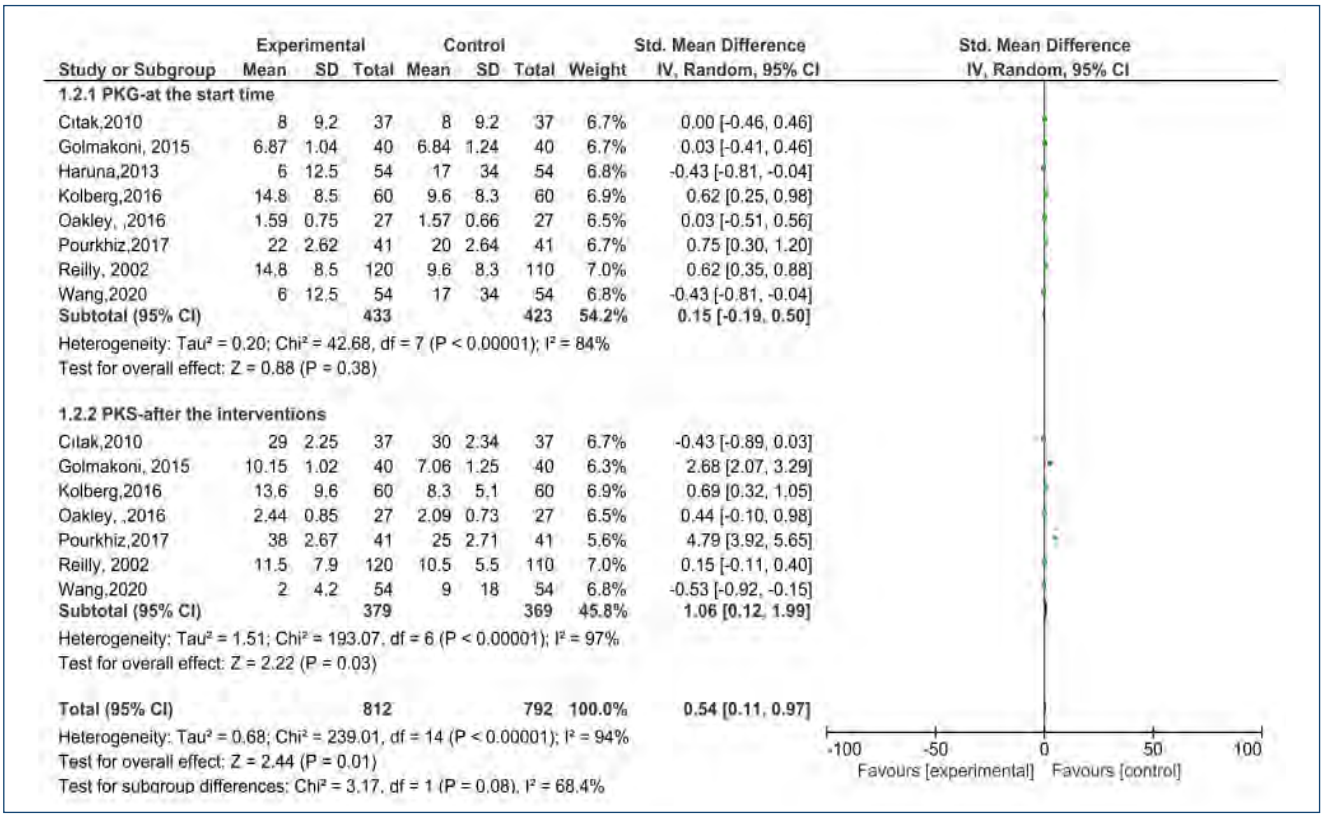

Figure 3. Effect of pelvic floor muscle exercise on pelvic floor strength forest plot.

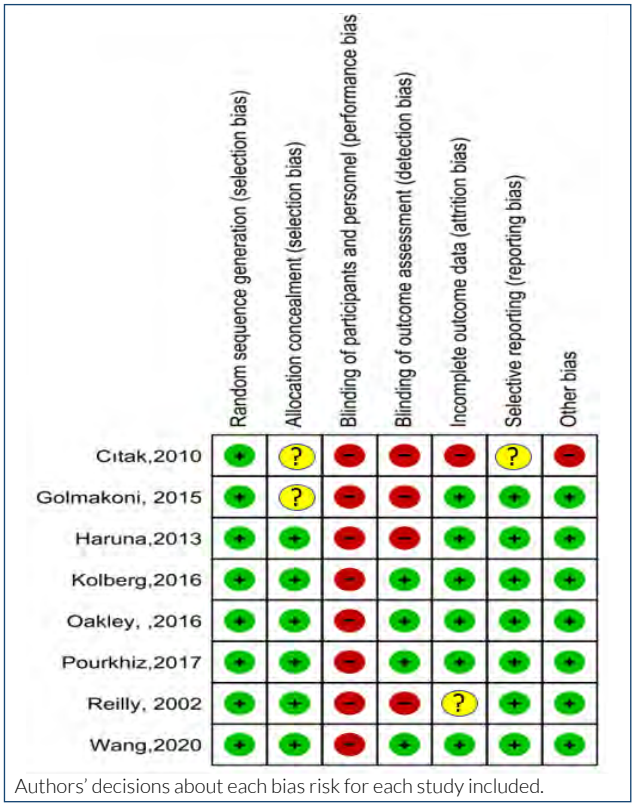

Figure 4. Risk of bias summary.

DISCUSSION

The purpose of this meta-analysis was to evaluate the effectiveness of PFMEs on SF in women during pregnancy and the postpartum period. Across the included studies, we examined whether there is evidence that PFMEs improve SF and PMS during pregnancy or in the postpartum period.

Although there was a significant increase in SF status as a result of using PFMEs, which we considered as the primary outcome in the examination, the evidence was generally of low quality. Therefore, we needed higher quality RCTs in this area to provide a more definitive answer. In addition to the study by Pourkhiz et al[10], eight studies on PFMEs do not provide sufficient data to evaluate the effect on SF during pregnancy, but according to the analysis of studies in postpartum period, the use of PFMEs resulted in a statistically significant increase in SF[11,12,15]. Although most studies show an improvement in SF, the results should be interpreted with caution due to the methodological limitations of some studies. Çıtak et al[12] had a high rate of attrition. However, high heterogeneity between studies is remarkable. Hadizadeh-Talasaz et al.[18] in their meta-analysis found that women who performed postpartum PFMEs showed a slight improvement in SF

problems. Wu et al.[19] reported a decrease in unsatisfactory SF in their meta-analysis.

According to some studies, in addition to sexual dysfunction during pregnancy and the prevalence of PMS, incontinence and quality of life are increasing[19,20]. The effect of PFME on PMS, incontinence, and quality of life during pregnancy is an important research area.[20,21] Our meta-analysis showed that after PFMEs, there was a significant improvement in PFM strength and quality of life, while a single study for incontinence showed no significant relationship with PFMEs. In addition, we determined that the quality of the evidence was from low to average, respectively. Studies on the pelvic floor during pregnancy revealed a clear link between pelvic floor disorder and low PMS[20]. Physical and hormonal changes caused by pregnancy are factors that can reduce PMS by affecting the pelvic floor. Although all studies have reported improvement in PMS, further studies are needed due to the low number of studies in this field and the low quality of evidence[21].

CONCLUSION

Evidence has shown a less effect on the PFM training on SF in pregnancy and postpartum period in primipara women, and it is a safe strategy that can improve postpartum SF.

PFME during pregnancy can prevent pelvic structure disorders and negative effects of sexuality in the later stages of pregnancy. However, study populations and quality of evidence are low. Although most studies and meta-analysis results show positive results, higher quality RCTs are needed in this area.

AUTHORS' CONTRIBUTIONS

AYK: Conceptualization; Data curation; Formal Analysis; Funding Acquisition; Investigation Methodology; Project administration; Resources; Software; Visualization; Writing – original draft. **NEB:** Data curation; Formal Analysis; Funding Acquisition; Investigation Methodology; Project administration; Resources. **NG:** Formal Analysis; Funding Acquisition; Project administration; Supervision; Validation; Writing – review & editing.

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ORIGINAL ARTICLE

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A prospective study of living kidney donors: 6 years follow-up from a cardiovascular disease risk perspective

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SUMMARY

OBJECTIVE: The purpose of this prospective study was to evaluate the clinical, laboratory, and donation-specific outcomes of living kidney donors 6 years after donation.

METHODS: We included a total of 93 kidney donors and 54 age- and sex-matched individuals as control group through a type 2 cohort consecutive recruitment. We detected kidney function abnormalities and the presence of hypertension, diabetes, and cardiovascular events during the 6 years follow-up period.

RESULTS: The mean serum creatinine levels were higher (p<0.001), and the estimated glomerular filtration rate levels were lower (p<0.001) in living kidney donors 6 years after donation when compared with controls. The protein/creatinine ratio of the study population was also higher (p=0.014). There was no difference in outcomes between the groups for end-stage kidney disease and cardiovascular mortality. A higher rate of new-onset hypertension (6.4 vs. 32.9%), diabetes mellitus (0.0 vs. 4.3%), chronic kidney disease (0.0 vs. 2.1%), and cardiovascular disease (0.0 vs. 2.1%) was demonstrated among donors 6 years after donation (p<0.001, respectively).

CONCLUSION: Our data have demonstrated that the reduction in Glomerular filtration rate induced by kidney donation might cause an increase in adverse renal and cardiovascular events.

KEYWORDS: Living donors. Hypertension. Cardiovascular Abnormalities. Diabetes Mellitus.

INTRODUCTION

Living donor kidney transplantation is the preferred treatment for end-stage kidney disease (ESKD), mainly because it improves graft and patient survival and quality of life when compared with the transplantation from a deceased donor and waiting list patients who remain on dialysis[1]. Turkey is among the countries with the most living donor transplants per million population. According to the 2020 Turkey Registry System Report, 2250 (90%) of 2500 kidney transplantations performed in 2020 are living donor transplantations[2]. Each year, over 27,000 people around the world become kidney donors, and this number is increasing in response to a shortage of kidneys for transplantation from deceased donors[3]. However, the mid- and long-term cardiovascular and metabolic risks among donors remain uncertain.

A number of studies suggest that the risk of developing ESKD in donors is similar to that of the general population[4] Some studies have suggested that there are small but measurable increases in the risk of HT, proteinuria, preeclampsia, gout, acute dialysis, and ESKD after donor nephrectomy, in addition to the risks of surgery[5,6]. These factors are associated with an increased risk for cardiovascular and all-cause mortality in the general population. Multiple studies have shown no evidence of reduced survival among living kidney donors as compared with the general population. In contrast, Mjoen et al. evaluated the long-term kidney function and cardiovascular and all-cause mortality over a 15-year follow-up period and found that all-cause death, cardiovascular death, and ESKD were significantly increased in donors after about 10 years[7].

In this study, we aimed to demonstrate the renal consequences of donation and the evidence of the effects of donation on the cardiovascular system in 93 living kidney donors after 6 years from donation and in 54 age- and sex-matched controls.

METHODS

Patients

We performed a type 2 cohort study to collect the data on the health status of kidney donors who had the transplantation operation in Kartal Training Hospital. Between January 2011

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and August 2014, a total of 157 living donor nephrectomy was performed in our transplantation center. We made phone calls to invite kidney donors to participate in the study. We could not make contact with 30 donors, and 93 of the remaining 127 donors accepted to participate in the study. The demographic characteristics of the study population at the time of donation were extracted from the medical records, which also included a detailed medical history. After 6 years of follow-up, demographic, clinical, and laboratory characteristics of kidney donors were updated. In addition, a control group was formed with 54 age- and sex-matched individuals who were selected based on self-reported medical history to fit the donor demographic and baseline characteristics. Clinical and laboratory characteristics of controls were also updated after 6 years from their baseline.

Definitions

Hypertension

Kidney donors and controls were defined as hypertensive if they had a previously known diagnosis of HT (treated or not) or if the office BP was measured >140/90 mmHg[8].

Diabetes

Diabetes was defined as fasting plasma glucose levels >126 mg/dL (7.0 mmol/L) or hemoglobin A1c (HbA1c) level >6.5% (48 mmol/mol) or in a patient with classic symptoms of hyperglycemia, a random plasma glucose ≥200 mg/dL (11.1 mmol/L)[9].

All analyses of the donors were performed at the time of donation and 6 years after donation, and all analyses of the control group were performed at baseline and after 6 years of follow-up in the biochemistry laboratory of Kartal Training Hospital. Chronic disease was defined as the presence of HT, DM, coronary artery disease (CAD) (defined as myocardial infarction, percutaneous coronary intervention, and coronary artery bypass surgery), CKD (GFR <60 mL/min/1.73 m^2), and ESKD.

Laboratory data

The blood examinations including serum creatinine, urea, glucose, HbA1c, lipid fractions, uric acid, ferritin, and parathormone (PTH) were conducted following overnight fasting. Microalbuminuria was defined as the presence of 30–300 mg/g of creatinine, and proteinuria was defined as the presence of >300 mg/g of creatinine[10]. Urine albumin and protein excretions were determined in the first-morning urine sample.

Statistical analysis

Descriptive data were presented as mean ± standard deviation (SD), median and interquartile range (IQR) for the continuous variables, and frequency and percentages (%) for the categorical variables. Continuous variables were evaluated for normality distribution using the Shapiro-Wilk test. T-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables were used in comparison with 2 independent groups. Categorical variables were compared by using the chi-square or Fisher's exact test for proportion. All significance tests were two-tailed, and p-values <0.05 were considered statistically significant. All statistical analyses were performed by the SPSS software version 21 (Chicago, IL). Reporting of the study conforms to the STROBE statement along with references to the STROBE statement and the broader EQUATOR guidelines[11].

Ethics

Ethics committee approval for the study was obtained from the Ethical Review Board of Kartal Training Hospital (514/193/5-01.13.2021). All procedures were performed in accordance with the ethical standards of the Declaration of Helsinki. All participants gave written informed consent for the study.

RESULTS

Demographic and clinical characteristics

The study population consisted of 93 kidney donors and 54 age- and sex-matched controls. Demographic and clinical characteristics of the patient and control groups are given in Table 1.

Table 1. General characteristics of the study population and controls at baseline.

	Kidney donors (n=93)	Controls (n=53)	p-value
Age, years	53.37 [27–68]	55.44 [28–68]	0.403
Gender (male/female), n (%)	34 (37)/58 (63)	26 (49)/27 (51)	0.278
Body mass index, kg/m^2	26.5 [17.9–34.4]	27.3 [17.1–38.7]	0.594
Waist circumference, cm	98 [73–131]	93 [77–143]	0.378
Hypertension (yes/no), n (%)	6 (6.4)/87 (93.5)	4(7.6)/49(92.4)	
Diabetes mellitus, n (%)	NA	NA	NA
Coronary artery disease	NA	NA	NA

NA: not applicable. Normally distributed data are presented as mean±standard deviation. Non-normally distributed data are presented as median and interquartile range (IQR).

Participants' mean age was 53.37 [27–68] years, with 58 female (63%) and 34 male (37%). Similar to the control group, kidney donors were generally female and middle-aged. In total, 39 (41.9%) donors were spouses, 33 (35.4%) donors were sons or daughters, 16 (17.3%) donors were parents or siblings, and the remaining 5 (5.4%) donors were other relatives. The mean duration following transplantation was 78.03±41.09 months. None of the participants developed major surgical complications after donor nephrectomy.

Primary outcomes

The biochemical parameters of the study population are shown in Table 2. In the donor population, the median eGFR 6 years after donation was 81.24 [67.36–88.62] mL/min/1.73 m^2 and was significantly lower in the donor group than that in the control group after 6 years of follow-up period (p<0.001). Serum creatinine was 0.93 [0.80–1.80] mg/dL 6 years after donation and significantly higher when compared with non-donors (p<0.001). While there was no significant difference between the two groups in the albumin–creatinine ratio in the spot urine (7.40 [3.70–15.50] vs. 7.22 [5.04–14.40] mg/g creatinine, p=0.431), the protein-creatinine ratio in the spot urine was found to be significantly higher in the donor group when compared with controls (85.52 [65.26–116.04] vs. 67.94 [56.12–87.80] mg/g creatinine, p=0.014).

Mean levels of ferritin were lower in the donor group 6 years after donation, when compared with controls (p<0.001) (Table 2). Mean levels of uric acid and parathyroid hormone (PTH) were significantly higher in the donor group, 6 years after donation, when compared with controls (p<0.001, and p=0.049, respectively) (Table 2). There was no significant difference between the two groups in terms of fasting blood glucose, HbA1c, low-density lipoprotein (LDL), albumin, and hemoglobin values.

Secondary outcomes

As shown in Table 3, 32.9% of the donors (n=31) have HT, 2.1% (n=2) of the donors have CAD, 2.1% (n=2) of the donors have CKD, and 4.3% (n=4) of the donors have diabetes 6 years after donation. Of the kidney donors, 6.4% (n=6) of the

Table 2. Laboratory parameters of the kidney donors and controls at baseline and after 6 years.

	Kidney donors at donation(n=93)	Kidney donors 6 years after donation(n=93)	Controls at baseline(n=54)	Controls after 6 years(n=54)	p-value
Urea (mg/dL)	27.50 [23.00–33.00]	35.00 [29.00–39.00]	28.00 [24.00–33.25]	27.00 [22.00–32.30]	**<0.001***
Creatinine (mg/dL)	0.75 [0.65–0.84]	0.93 [0.80–1.80]	0.67 [0.59–0.74]	0.70 [0.62–0.80]	**<0.001***
GFR (mL/min/1.73 m^2)	101.0 [92.58–109.36]	81.24 [67.36–88.62]	109.0 [101.75–117.0]	102.0 [94.9–109.1]	**<0.001***
Albumin/creatinine (mg/g creatinine)	5.50 [3.00–10.00]	7.40 [3.70–15.50]	7.05 [4.95–14.43]	7.22 [5.04–14.40]	0.431
Protein/creatinine (mg/g creatinine)	85.20 [66.85–109.90]	85.52 [65.26–116.04]	67.93 [56.29–87.79]	67.94 [56.12–87.80]	**0.014***
Hemoglobin (gr/dL)	13.45±1.70	13.61±1.57	13.60±1.48	13.87±1.51	0.325
Ferritin (ng/mL)	33.00 [14.25–59.79]	36.40 [19.90-64.90]	33.50 [14.65–81.95]	75.25 [31.40–112.70]	**<0.001***
Total cholesterol (mg/dL)	198.10±45.68	186.38±38.45	209.69±46.86	165.66±34.67	0.094
LDL cholesterol (mg/dL)	120.00±31.76	122.28±47.90	134.53±45.74	123.88±34.12	0.868
Albumin (mg/dL)	5.69±9.59	4.26±0.27	4.34±0.23	4.47±0.25	0.165
Glucose (mg/dL)	95.00 [89.6–101.0]	92.00 [85.00–106.00]	98.00 [89.50–105.25]	95.00 [86.00–108.00]	0.508
HbA1c (%)	5.50 [5.20–5.70]	5.72 [5.48–6.96]	5.40 [5.30–5.70]	5.70 [5.60–5.90]	0.717
Uric acid (mg/dL)	5.04±1.21	5.62±1.18	4.53±0.81	4.43±0.99	**<0.001***
PTH (pg/mL)	52.35 [40.7–67.72]	57.52 [40.70–76.55]	48.25 [35.58–68.63]	51.00 [36.80–65.30]	**0.049***

GFR: glomerular filtration rate; LDL: low-density lipoprotein; HbA1c: hemoglobin A1c; PTH: parathyroid hormone. Normally distributed data are presented as mean±standard deviation and non-normally distributed data are presented as median (IQR). Bold values indicate statistical significance at the p<0.05 level. *Kidney donors 6 years after donation vs. controls after 6 years.

Table 3. Target organ damage status of kidney donors 6 years after donation.

	Kidney donors at donation (n=93)	Kidney donors 6 years after donation (n=93)	Controls at baseline (n=54)	Controls after 6 years(n=54)	p-value
Hypertension (n, %)	6 (6.4)	31 (32.9)	4 (7.6)	6 (11.1)	**<0.001***
Diabetes mellitus (n, %)	NA	4 (4.3)	NA	1 (1.8)	**<0.001***
Cardiovascular morbidity (n, %)	NA	2 (2.1)	NA	1 (1.8)	**<0.001***
Cardiovascular mortality (n, %)	NA	NA	NA	NA	NA
Chronic kidney disease (n %)	NA	2 (2.1)	NA	NA	**<0.001***
End-stage kidney disease (n %)	NA	NA	NA	NA	NA

NA: not applicable. *Kidney donors at donation vs. 6 years after donation. Bold values indicate statistical significance at the p<0.05 level.

donors have HT at the time of donation. The rate of hypertensive kidney donor increased after 6 years when compared with the baseline (6.4 vs. 32.9%, p<0.001) (Table 3).

Similarly, 7.6% (n=4) of the control group have HT at baseline. As reported in the methods section, the control group was formed with age- and sex-matched individuals who were selected based on self-reported medical history to fit the donor demographic and baseline clinical characteristics. There were no other chronic diseases such as diabetes, CAD, CKD, and ESKD in the control subjects at baseline. When clinical characteristics of controls were updated after 6 years from their baseline, we found that 11.1% (n=6) of the control group have HT, 1.8% (n=1) have diabetes, and 1.8% (n=1) have CAD.

DISCUSSION

In this type 2 cohort study, we showed that not only the kidney function abnormalities were higher in the study population when compared with controls but also the incidence of new-onset HT was higher among kidney donors when compared with age- and sex-matched individuals. Furthermore, incidences of diabetes, CAD, and CKD were higher in the kidney donor group compared with their baseline.

According to our results, the mean eGFR was significantly lower in the donor group 6 years after donation compared with the control group (p<0.001) (Table 2). Also, the mean serum creatinine was significantly higher compared with non-donors (p<0.001). Our findings are in accordance with the current literature. Ibrahim et al reported that in an average of 12 years following donation, 15% of kidney donors had a GFR<60 mL/min/1.73 m^2 [12]. Similarly, Liboria et al reported that 29% of donors had an eGFR<60 mL/min/1.73 m^2 5 years after donation[13]. Karahan et al recently reported that 3 years after donation, 11% of the kidney donors had GFR<60 mL/min/1.73 m^2 [14]. We found that the mean eGFR was 81.2 mL/min/1.73 m^2 6 years after donation, and 2.1% (n=2) of donors had an eGFR<60 mL/min/1.73 m^2 in our study population. We interpreted that reduced renal function of kidney donors could be due to the reduction of kidney mass.

We also found that the mean levels of ferritin (36.40 [19.90–64.90] vs. 75.25 [31.40–112.70], p<0.001) were lower; the mean levels of uric acid (5.62±1.18 vs. 4.43±0.99, p<0.001) and PTH (57.52 [40.70–76.55] vs. 51.00 [36.80–65.30], p=0.049) were higher among kidney donors compared with controls. Kasiske et al reported that the GFR decreased 1.47±5.02 mL/min/1.73 m^2 per year in kidney donors between 6 and 36 months. The authors also reported that serum PTH, uric acid, homocysteine, and potassium levels were higher in kidney donors. The mean levels of PTH and uric acid in our study population were in line with Kasiske et al[15]. We found that kidney donors manifest several consequences of mild CKD in the long term. Yildirim et al reported that living kidney donors exhibit slightly reduced kidney function, increased oxidative stress, and decreased antioxidant activity[16]. It could be speculated that oxidant/antioxidant system imbalance may facilitate the development of kidney function abnormalities.

In addition, the protein–creatinine ratio of the kidney donors is significantly higher compared with controls (p=0.014). We also found that 6.4% (n=6) of donors have controlled HT at the time of donation and 32.9% (n=31) of donors have HT 6 years after donation. A meta-analysis of 48 studies showed a clinically insignificant increased risk for the development of HT or proteinuria in a long-term follow-up among kidney donors when compared with the age-matched controls[6]. Ibrahim et al reported that 7.5% of donors developed HT and 12% of donors developed albuminuria[12]. According to our results, 25% of donors developed new-onset HT, and none of the donors developed albuminuria and/or significant proteinuria 6 years after donation. Thiel et al showed that kidney donation triples the short-term risk of developing HT and that after nephrectomy, HT becomes the main risk factor for albuminuria. Thiel et al also reported that among the initially

normotensive donors, 43% of donors developed HT diagnosed by ambulatory blood pressure monitoring within the 10-year follow-up period[17]. We reported that hypertensives comprise 30% of our donor population within 6 years of donation. We could speculate that kidney donation leads to reduced kidney function and is associated with an increase in clinically insignificant proteinuria, as well as a rise in blood pressure greater than attributable to normal aging. Increased risk of developing HT may have important implications for the long-term cardiovascular health of kidney donors. We suggest that our data are critical for improving our understanding of the consequences of nephrectomy. Further prospective, controlled studies are needed to determine the incidence of HT, target organ damage, and possible complications of HT among donors.

Multiple studies have shown no evidence of reduced survival among living kidney donors as compared with the general population. Contrarily, Mjoen et al recently evaluated long-term cardiovascular and all-cause mortality among 1900 living kidney donors compared with a control group of 32,000 individuals who would have been eligible for donation over a 15-year follow-up period and found that the hazard ratios for all-cause death and cardiovascular death were significantly increased in donors after about 10 years. They also reported that living kidney donors have a 1.4-fold increased risk for cardiovascular morbidity compared with non-donor individuals eligible for donation[7]. According to our results, 4.30% (n=4) of kidney donors have new-onset diabetes and 2.1% (n=2) of donors have new-onset CAD 6 years after donation. Although there was no cardiovascular mortality in our study population; the incidence of HT, diabetes, and CAD is higher in the donor group compared with controls. It is still not possible to understand the pathophysiological effects of kidney donation on hemodynamic and vascular system among donors. We interpreted that it is impossible to exclude that donation may lead to an increase in adverse cardiovascular events. Potential donors should be informed of increased possible cardiovascular risk, at least new-onset HT and diabetes, associated with donation in the long term.

The findings of this study have to be seen in light of some limitations. First, in our follow-up, 25% of our donors could not be reached and their follow-up is not available. Second, our study was conducted with a relatively small population. Therefore, the study results may not reflect the general kidney donor population. Third, due to the design of the study, we could collect the data of the control group observationally. It would be interesting to assess the evolution of cardiovascular morbidity not only among kidney donors but also among age- and sex-matched individuals in a long-term follow-up study.

In conclusion, we detected a high incidence of HT, diabetes, CKD, and cardiovascular morbidity among kidney donors 6 years after donation. Further studies with larger populations are needed for the estimation of long-term risks associated with donation among living kidney donors.

AUTHORS' CONTRIBUTIONS

MM: Conceptualization, Data curation, Writing – original draft, Writing – review & editing.

EA: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Comparison between pain intensity, functionality, central sensitization, and self-efficacy in individuals with unilateral or bilateral knee osteoarthritis: a cross-sectional study

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SUMMARY

OBJECTIVE: This study aimed to compare pain intensity, stiffness, functionality, central sensitization, and self-efficacy, between individuals with bilateral knee osteoarthritis and unilateral knee osteoarthritis.

METHODS: We included sedentary participants with knee osteoarthritis. The diagnosis was defined by a specialist, in which there was a complaint of pain and/or altered function in the lower limbs (duration ≥3 months); morning stiffness; pain intensity ≥3; Kellgren-Lawrence 2–3° associated with X-ray; persistence of symptoms >3 months. We used the following tools: Western Ontario and McMaster Universities Arthritis Index, Numerical Pain Scale, Central Sensitization Inventory, and Pain Self-Efficacy Questionnaire. Intergroup comparisons were performed using the t-test.

RESULTS: The sample consisted of 118 adult individuals, divided into two groups: bilateral knee osteoarthritis (n=59) and unilateral knee osteoarthritis (n=59). We observed a significant difference ($p<0.05$) and a large effect size ($d\geq0.8$), in the comparisons between: stature, body mass index, physical function, central sensitization, and self-efficacy.

CONCLUSION: Individuals with bilateral knee osteoarthritis have higher levels of central sensitization, impaired functionality, and a lower level of self-efficacy.

KEYWORDS: Osteoarthritis. Chronic pain. Musculoskeletal diseases. Central nervous system sensitization. Chronic disease. Exercise.

INTRODUCTION

Osteoarthritis is a chronic degenerative disease that affects the joints, in particular, the knee is the most commonly affected. Pain, stiffness, and crepitus in joint movement are some of the symptoms that disable individuals. The treatment of this disease includes control of body mass (weight reduction), use of anti-inflammatory drugs, and exercise[1].

Recent studies have focused on understanding the risks of comorbidities associated with knee osteoarthritis[2], the biochemical and gait parameters after arthroplasty[2,3], and prognosis after therapies in bilateral knee osteoarthritis (B-KO) and unilateral knee osteoarthritis (U-KO)[4].

Asymmetry between the lower limbs seems to be more prevalent in individuals with B-KO[5], while the reduction in muscle strength and volume of the affected limb is more observed in individuals with U-KO[6], and both (B-KO and U-KO) have already been associated with primary and secondary hyperalgesia[7]. However, these clinical differences have been less investigated.

Marmon et al.[8] and Messier et al.[9] described that, regardless of the number of affected knees, individuals with knee osteoarthritis have similar functional capacity and biomechanical parameters. Riddle and Stratford[10] pointed out that people with U-KO have higher levels of pain; however, according to the authors themselves, the differences between the clinical variables of bilateral and unilateral involvement in knee osteoarthritis remain controversial and little known. In this perspective, this study aimed to compare pain intensity, stiffness, functionality, central sensitization, and self-efficacy between individuals

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with B-KO and U-KO. The hypothesis of the present study describes those individuals with B-KO, compared to those with U-KO, have higher levels of disability and central sensitization.

METHODS

Study design and ethical considerations

Quantitative cross-sectional study in accordance with STrengthening the Reporting of OBservational studies in Epidemiology[11]. The research was carried out at the integrated health clinic of Universidade Nove de Julho (Brazil); recruitment took place between February 2018 and December 2019. Recruitment was carried out through the waiting list of four basic health units in three regions of the city of São Paulo (SP, Brazil). Invitations to participate were made by telephone or personal contact. All participants signed a consent statement and an informed consent form. This study was approved by the Research Ethics Committee of Universidade Nove de Julho (number 24568013.0.0000.5511).

Participants and study size

One a priori sample calculation was performed, using the t-test for the difference between two independent means (two groups) through G*Power (version 3.1.9.7). We used the effect size of 0.60, alpha of 0.04, power of 0.88, and critical f of 3.25. As such, the total sample size was estimated at 118 volunteers to build 2 groups, namely, B-KO and U-KO, with the same number of participants[12]. We included participants aged between 18 and 70 years, sedentary, according to the International Physical Activity Questionnaire[13].

The diagnosis of knee osteoarthritis was defined by a specialist physician, who complained of pain and/or altered function in the lower limbs, lasting for 12 weeks or more; morning stiffness; pain intensity, verified by numerical pain scale ≥3; Kellgren-Lawrence[14] grade 2 or 3, associated with X-ray; persistence of knee symptoms lasting for >3 months[15].

Participants with the following health problems capable of affecting functional assessments were excluded: presence of severe comorbidities in the heart, liver, and/or kidney; presence of neoplasia, severe psychiatric, systemic, autoimmune or concomitant inflammatory diseases (lupus, intestinal); hypothyroidism; fibromyalgia; pregnancy and/or breastfeeding; and presence of therapeutic intervention in the last 6 months.

Measurement and bias

Three researchers participated in the research. Researcher 1 was responsible for recruiting, confirming the diagnosis, and allocating the volunteers. Researcher 2, responsible for administering the assessments, was blinded in relation to the distribution of the groups. Researcher 3 performed the data analysis. All researchers are specialists in the management of chronic musculoskeletal pain, with an average training time of 6 years. In addition, they underwent prior training to improve the evaluation procedures of the present study.

Assessment tools

Western Ontario and McMaster Universities Arthritis Index (WOMAC), having reliability (ICC>0.80) and internal consistency (Cronbach's α≥0.86), was validated by Fernandes on the Brazilian population[16]. The Brazilian Portuguese version has three domains: pain (items 1, 2, 3, 4, and 5), stiffness (items 6 and 7), and physical function (items 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24). However, Ferreira et al.[17] found that the Brazilian version of the WOMAC with two domains (pain, 4 items; physical function, 8 items) presents a more adequate structure. For each item, there are options of five responses (0–4); the score for each domain is calculated as the simple sum of the answered items: pain domain (0–20), stiffness (0–8), and physical function (0–68); the higher the score, the greater the impact of osteoarthritis on the domains.

Numerical pain scale (NPS) is an instrument validated by Ferreira-Valente et al. on Portuguese[18]. It consists of a sequence of numbers (from 0 to 10) in which the value 0 represents "no pain" and the numeral 10 represents "the worst pain imaginable." Thus, participants report pain based on these parameters.

Central sensitization inventory (CSI) was validated by Caumo et al.[19] on the Brazilian population. It has reliability (ICC>0.80) and internal consistency (Cronbach's α=0.91). It quantifies, through self-report, the degree of somatic and emotional complaints associated with central sensitization. It is divided into part A (25 items), in which each item can be scored on a Likert scale ranging from 0 to 4 points associated with the words "never" and "always"; and part B, a list of previous diagnoses related to central sensitization conditions. Severity levels are quantified in scores from 0 to 100; higher scores represent greater central sensitization.

Pain Self-Efficacy Questionnaire (PSEQ) has internal consistency (Cronbach's α=0.92) and reliability (0.93); it was validated by Nicholas et al.[20] on the Brazilian population It is a self-administered instrument capable of evaluating and expressing, in numbers, how confident the patient feels to express himself in the face of the situations presented in the 10 items. For each item, there are six options with their respective values in

ascending order, representing "not at all confident" to "completely confident." The final score ranges from 0 to 60 and is obtained by adding the values found. The higher the score, the greater the self-efficacy in pain conditions.

Statistical analysis

The distribution of variables was verified using the Kolmogorov-Smirnov test. We set the significance level at 5% for all statistical tests, which in turn were processed using the Statistical Package for the Social Sciences software, version 17.0 (Chicago, IL, USA). Comparisons between variables were performed using the t-test of independent samples and presented as follows: mean, standard deviation (SD), difference between means (MD), confidence interval (95%CI) of the difference between means and effect size, calculated using Cohen's d, with the following classification values: 0.2=small, 0.5=moderate, 0.8=large[21].

RESULTS

A total of 183 volunteers, with knee osteoarthritis, were recruited for the study, of which 65 were excluded, based on the eligibility criteria; thus, the final sample consisted of 118 adult participants, mostly female, divided into the following two groups: B-KO (n=59) and U-KO (n=59). Table 1 presents the characteristics of all participants.

In comparisons between groups, we observed a significant difference ($p<0.05$) and large effect size ($d\geq0.8$) in stature, body mass index (BMI), physical function, central sensitization, and participants' self-efficacy (Table 2).

DISCUSSION

Our results describe those individuals with B-KO have a greater impact on physical function, greater central sensitization, and lower self-efficacy. This analysis was performed on a number of individuals considered adequate for this study (according to the a priori sample calculation). The impact on physical function refers to the degree of difficulty in moving and performing self-care activities in the last 72 h; central sensitization refers to the presence of symptoms daily, or on most days, considering the last 3 months; and self-efficacy assesses how confident the individual was (considering the referred pain) at the time of this research consultation.

These findings should not be used as proof that B-KO individuals suffer more than U-KO individuals, as, according to the results, the variables "pain" and "stiffness" are not different ($p>0.05$) between the analyzed groups (B-KO vs U-KO). Still on the similarity of prognosis (B-KO and U-KO), Marmon et al.[8] described that the cases of B-KO and U-KO present clinical similarities in functional capacity, and Messier et al.[9] stated that the similarity in lower limb mechanics between individuals with B-KO and U-KO is sufficiently robust to consider the two subsets as a single sample.

Regarding pain intensity, our results differ from the study by Riddle and Stratford[10] (the authors reported that U-KO generates higher levels of pain). The existence of interpretive difficulties in outcomes that analyze pain in individuals with knee osteoarthritis has already been highlighted in previous studies. Cohort by Creaby et al.[22] indicates that the presence of unilateral pain seems to be associated with asymmetries in knee

Table 1. Clinical characteristics and all study participants (n=118) presented as a mean (standard deviation).

	B-KO (n=59)	U-KO (n=59)	t value	p-value
Sex (Female, %)[a]	56 (94.9)	51 (86.4)		0.11
Body mass (kg)[b]	72.16 (5.05)	70.28 (4.26)	2.18	0.03*
Stature (m)[b]	1.63 (0.07)	1.68 (0.05)	-3.87	<0.01*
BMI (kg/m^2)[b]	27.06 (2.88)	24.94 (2.17)	4.51	<0.01*
Age (years)[b]	65.54 (3.97)	68.25 (4.48)	-3.47	<0.01*
WOMAC (score)[b]				
Pain (0–20)	15.27 (2.36)	15.10 (1.43)	0.47	0.63
Stiffness (0–8)	6.13 (1.23)	5.93 (0.86)	1.03	0.30
Physical function (0–68)	51.81 (4.37)	48.25 (3.58)	4.83	<0.01*
NPS (0–10)[b]	5.57 (0.96)	5.52 (1.15)	0.26	0.79
CSI (0–100)[b]	29.71 (3.07)	22.42 (2.71)	13.64	<0.01*
PSEQ (0–60)[b]	21.96 (2.58)	28.16 (2.49)	-13.20	<0.01*

B-KO: bilateral knee osteoarthritis; U-KO: unilateral knee osteoarthritis; BMI: Body mass index; WOMAC: Western Ontario and McMaster Universities Arthritis Index; NPS: Numerical pain scale; CSI: Central sensitization inventory; PSEQ: Pain Self-Efficacy Questionnaire. [a]Values presented in absolute numbers (percentage), χ^2 test. [b]Values presented as average (standard deviation). *Significant difference (t-test of independent samples, p-value<0.05).

Table 2. Comparisons between groups (bilateral knee osteoarthritis; unilateral knee osteoarthritis) presented as mean, standard deviation, difference between means, confidence interval of this difference, and effect size (Cohen d).

Variables	Group	Mean	SD	MD	95%CI	d
Body mass (kg)	B-KO	72.16	5.05	1.88	0.17 to 3.58*	-0.40
	U-KO	70.28	4.26			
Stature (m)	B-KO	1.63	0.07	-0.44	-0.06 to -0.02*	0.82#
	U-KO	1.68	0.05			
BMI (kg/m²)	B-KO	27.06	2.88	2.12	1.19 to 3.06*	-0.83#
	U-KO	24.94	2.17			
Age (years)	B-KO	65.54	3.97	-2.71	-4.25 to -1.16*	0.64#
	U-KO	68.25	4.48			
WOMAC (score)						
Pain (0–20)	B-KO	15.27	2.36	0.16	-0.54 to 0.88	-0.08
	U-KO	15.10	1.43			
Stiffness (0–8)	B-KO	6.13	1.23	0.20	-0.18 to 0.59	-0.18
	U-KO	5.93	0.86			
Physical function (0–68)	B-KO	51.81	4.37	3.55	2.10 to 5.01*	-0.89#
	U-KO	48.25	3.58			
NPS (0–10)	B-KO	5.57	0.96	0.05	-0.33 to 0.43	-0.04
	U-KO	5.52	1.15			
CSI (0–100)	B-KO	29.71	3.07	7.28	6.23 to 8.34*	-2.51#
	U-KO	22.42	2.71			
PSEQ (0–68)	B-KO	21.96	2.58	-6.20	-7.13 to -5.27*	2.44#
	U-KO	28.16	2.49			

SD: standard deviation; MD: difference between means; B-KO: bilateral knee osteoarthritis; U-KO: unilateral knee osteoarthritis; BMI: Body mass index; WOMAC: Western Ontario and McMaster Universities Arthritis Index; NPS: Numerical pain scale; CSI: Central sensitization inventory; PSEQ: Pain Self-Efficacy Questionnaire. *Significant difference (t-test of independent samples, p-value<0.05). #Moderate effect size (Cohen's d≥0.5).

biomechanics, while bilateral pain is associated with symmetry (still needing further studies). Lange-Brokaar et al.[23] pointed out that the different patterns of synovitis, observed via magnetic resonance imaging with gadolinium chelate, were associated with different intensities of pain, but the mechanisms underlying these patterns of synovitis in individuals with knee osteoarthritis are still unknown.

When interpreting our data (Table 2), we observed a contradiction: self-efficacy was reported by individuals considering the level of perceived pain, however, when assessing pain in isolation (e.g., on a scale of 0–10), the groups were not different; thus, what explains individuals with B-KO having lower self-efficacy? The answer may be related to the significant difference (p<0.05) in the central sensitization variable (DM=7.28, d=2.51); however, this topic is still scarce in the literature.

These results also contribute to evidence-based clinical practice, as they show that pain intensity, although relevant in consultations with patients with knee osteoarthritis, should only be a variable parallel to other clinical observations from exams, tests, and questionnaires (e.g., central awareness, self-efficacy, and functionality); in addition, as pain intensity does not seem to be related to the level of knee involvement, patients with B-KO and U-KO should be equally evaluated.

The present study has limitations that must be addressed. The first is that we examined pain intensity and function severity, in both groups, through each patient's self-report (and it is known that self-report measures are influenced by pain intensity)[10]. The second refers to the design of this research (cross-sectional), which has no explanatory power on cause and effect. Thus, we suggest conducting a longitudinal study to provide additional information related to pain intensity, central sensitization, and severity of unilateral and bilateral function over time.

CONCLUSION

Individuals with B-KO have higher levels of central sensitization, impaired functionality, and a lower level of self-efficacy.

AUTHORS' CONTRIBUTIONS

LASO: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources,

Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **APS:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CAFPG:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **KLBD:** Validation, Visualization, Writing – original draft, Writing – review & editing. **GHSA:** Validation, Visualization, Writing – original draft, Writing – review & editing. **ARO:** Validation, Visualization, Writing – original draft, Writing – review & editing. **AVDF:** Validation, Visualization, Writing – original draft, Writing – review & editing. **MAA:** Validation, Visualization, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of statin response on cardiovascular outcomes in patients with ST-segment elevation myocardial infarction

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SUMMARY

OBJECTIVE: This study aimed to evaluate the effects of statin response on cardiovascular outcomes in patients with ST-segment elevation myocardial infarction.

METHODS: A total of 1029 ST-segment elevation myocardial infarction patients were enrolled in the study. The patients who failed to achieve >40% reduction in baseline low-density lipoprotein cholesterol levels within 30 days to 12 months after statin initiation were defined as suboptimal statin responders. The adjusted hazard ratios for cardiovascular outcomes for low-density lipoprotein cholesterol response to statins were estimated via the Cox proportional regression model. The relationship between the statin response and cardiovascular outcomes was also evaluated in a subgroup of on-treatment low-density lipoprotein cholesterol levels below 55 mg/dL.

RESULTS: Among the study population, 573 (55.6%) patients demonstrated suboptimal low-density lipoprotein cholesterol response to statin therapy. These patients showed a significantly higher incidence of the composite of major adverse cardiovascular events, including cardiovascular death, reinfarction, recurrent myocardial infarction, and target vessel revascularization during the follow-up compared with optimal responders (adjusted hazard ratios 3.99; 95%CI 2.66–6.01; p<0.001). In a subgroup of patients with on-treatment low-density lipoprotein cholesterol levels below 55 mg/dL, suboptimal statin responders also showed unfavorable cardiovascular outcomes (adjusted hazard ratios 8.73; 95%CI 2.81–27.1; p<0.001).

CONCLUSIONS: The present study showed that over half of the patients with ST-segment elevation myocardial infarction did not exhibit optimal low-density lipoprotein cholesterol response to statin. These patients have an increased risk of future major adverse cardiovascular events.

KEYWORDS: Cardiovascular disease. Low-density lipoprotein cholesterol. Myocardial infarction response. Prognosis. HMG-CoA reductase inhibitors.

INTRODUCTION

Lowering low-density lipoprotein cholesterol (LDL-C) with a 3-hydroxy-3-methylglutaryl-coenzyme reductase inhibitor (statin) has been recognized as a default strategy for preventing major adverse cardiovascular events (MACE) for both the primary and secondary care settings. Furthermore, it has been shown that more intensive LDL-C reduction with higher potency statin therapy further reduces MACE in individuals with acute coronary syndrome (ACS)[1,2]. Accordingly, the European Society of Cardiology guidelines suggested a greater reduction in LDL-C using high-intensity statin therapy in patients with ACS. However, due to the heterogeneity in individual response to statin therapy, effective LDL reduction is not achieved in most patients[3]. Numerous clinical, demographic, and genetic variables contributing to statin resistance have been reported[4,5]. However, there is limited evidence on the relationship between statin resistance and the risk of future cardiovascular (CV) events. A recent study involving primary care patients demonstrated an increased risk of future CVD in suboptimal statin responders compared to the group that responded optimally to statin therapy[6]. This study aimed to investigate the effects of statin response on CV outcomes in patients with ST-segment elevation myocardial infarction (STEMI).

METHODS

Patient collection data

The retrospective data of 3606 patients with STEMI were analyzed through the hospital database and the national electronic health information systems. The patients treated with fixed-dose high-intensity statin therapy for at least 12 months after the index hospitalization were selected. Patients receiving lipid-lowering treatment within 6 months before the index

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hospitalization were excluded from this study. Those who used additional lipid-modifying drugs other than statins were also excluded. A total of 1029 eligible patients were enrolled in the study. All patients underwent primary percutaneous coronary intervention and were discharged from the hospital with stable conditions. They received high-intensity statin therapy initiated within the first 24 h of admission and continued for at least 12 months. Statin adherence was evaluated through telephone calls and the patient's prescribing records. LDL-C values were obtained from hospital records and available data from the national electronic health information system. Patients had at least three LDL-C measurements (one measurement within 24 h of index hospitalization before statin administration and at least two measurements within 30 days to 12 months after statin administration). The lowest LDL level measured within 12 months after the statin therapy was used for the on-treatment response. The clinical and laboratory follow-up data were collected retrospectively from the hospital and national health database systems. Informed written consent was obtained from all patients, and the local ethical committee approved the protocol (Approval number: 71522473/050.01.04/408).

Definitions

High-intensity statin therapy was defined as atorvastatin used at 40–80 mg or rosuvastatin used at 20–40 mg. The National Institute for Health and Care Excellence guideline adopts >40% LDL reduction to define optimal statin response in high-risk patients[7]. A recent study by Akyea used this threshold in their primary analysis. Thus, in our study, patients who failed to achieve >40% reduction in baseline LDL-C levels within 30 days to 12 months after intensive statin initiation were defined as suboptimal statin responders. We compared the patients based on their statin responses to investigate the impact of statin response on CV adverse events.

Endpoints

The primary endpoint was the composite of MACE, such as CV death, reinfarction, recurrent myocardial infarction (MI), and target vessel revascularization (TVR) during the follow-up. The individual components of the primary endpoint were considered secondary endpoints.

Statistical analysis

Statistical analysis was performed by SPSS (Statistical Package for Social Sciences) 21.0 for the Windows program. The Kolmogorov-Smirnov test was performed to analyze the normality of the continuous variables. Continuous variables were expressed as means (SD) or median (interquartile range) according to whether they exhibited a Gaussian distribution, and categorical variables were expressed as proportions and percentages. The independent t test was used to compare normally distributed continuous data. Continuous data, which were not normally distributed, were analyzed using the Mann-Whitney U test. The chi-square test was used to compare the dichotomous data. The Cox proportional hazard analysis, adjusting for significant covariates predictive of cardiovascular events, was used to estimate the adjusted hazard ratio and 95% confidence interval (CI) to compare the incidence of the primary endpoint in suboptimal and optimal statin responders. We calculated MACE rates (per 1000 person-years) and 95%CIs for the primary endpoint for each group. Two-sided p-values of <0.05 were considered statistically significant.

RESULTS

Study population and baseline characteristics

The study population included 808 men and 221 women with a median age of 61 years. Among the study population, 573 (55.6%) patients demonstrated a suboptimal LDL-C response to statin therapy at 30 days to 12 months after starting treatment. The median follow-up time was 48 and 45 months for suboptimal and optimal statin responders, respectively. The demographic variables are summarized in Table 1.

Incidence of cardiovascular outcomes

During the follow-up, a primary endpoint event occurred in 339 patients. As shown in Table 1, the incidence of the composite MACE was significantly higher in patients with suboptimal LDL-C response to statin therapy compared to those with optimal LDL-C response (p<0.001). Among the individual components of the primary endpoint, there was a consistent pattern of favorable outcomes in patients with optimal LDL-C response to statin. CV death occurred in 29 patients (5.1%) in the suboptimal statin group and 6 patients (1.3%) in the optimal statin group (p=0.001). Patients with suboptimal statin response showed a significantly higher incidence of recurrent MI, TVR, and reinfarction than those with the optimal response (p<0.001). Outcomes for the selected secondary endpoints are also presented in Table 1. After adjustment of baseline covariates predictive of cardiovascular events (age, hypertension, prior MI, prior coronary revascularization, baseline LDL-C, baseline total cholesterol, baseline triglyceride, baseline HDL-C, on-treatment LDL-C, and high-sensitivity C-reactive protein (hs-CRP) levels), the risk of MACE remained significantly greater in patients

with suboptimal LDL-C response to statin compared with that in optimal responders (adjusted HR 3.99; 95%CI 2.66–6.01; p<0.001). The incidence rates for MACE were 17 and 102 per 1000 person-years for patients with optimal statin response and those with suboptimal statin response, respectively. We also showed that the suboptimal statin response was independently associated with reinfarction, recurrent MI, and TVR. However, no independent association was found between cardiovascular death and suboptimal statin response (adjusted HR 1.93; 95%CI 0.55–6.74; p=0.30) (Table 2).

A subgroup analysis of patients with on-treatment LDL-C levels below 55 mg/dl also showed unfavorable cardiovascular outcomes in suboptimal responders. The Cox regression analysis showed that suboptimal statin response is independently associated with the higher incidence of MACE (HR 8.73; 95%CI 2.81–27.1; p<0.001). The incidence rates for MACE were 16.9 and 61.2 per 1000 person-years for patients with optimal statin response and those with suboptimal statin response, respectively (Table 3).

DISCUSSION

This study revealed that 55.6% of STEMI patients treated with intensive statin therapy did not exhibit optimal statin responses. An increased risk of MACE was observed in these patients.

High variability in the percentage reduction of LDL-C may be encountered among subjects using the same dose of statin regimens. Some observational studies have investigated

Table 1. Baseline characteristics and outcomes by low-density lipoprotein cholesterol response to statin therapy.

		Total n (%) 1029 (100)	Suboptimal statin responders n (%) 573 (55.6)	Optimal statin responders n (%) 456 (44.3)	p-value
Age (median, IQR, mg/dL)		53/61/69	53/61/69	53/61/69	0.684
Gender (Female, n (%))		221 (21.5)	121 (21.1)	100 (21.9)	0.761
Hypertension, n (%)		655 (63.7)	379 (66.1)	276 (60.5)	0.068
Prior myocardial infarction, n (%)		56 (5.4)	46 (8)	10 (2.2)	**<0.001**
Prior coronary revascularization, n (%)		103 (10)	81 (14.1)	22 (4.8)	**<0.001**
Diabetes mellitus, n (%)		311 (30.2)	180 (31.4)	131 (28.7)	0.374
Smoking, n (%)		727 (70.7)	415 (72.4)	312 (68.4)	0.169
Baseline LDL-C (median, IQR, mg/dL)		111/133/159	103/124/150	120/145/171	**<0.001**
Baseline HDL-C (median, IQR, mg/dL)		34/40/46	33/38/45	36/41/47	**<0.001**
Baseline triglyceride (median, IQR, mg/dL)		72/112/176	74/115/179	71/109/171	0.290
Baseline total cholesterol (median, IQR, mg/dL)		169/198/227	162/189/218	178/208/241	**<0.001**
hs-CRP (median, IQR, mg/dL)		3/3.4/6.1	3/3.7/6.7	3/3/5.7	**0.003**
Admission glucose (median, IQR, mg/dL)		154.6±86	102/125/169	102/125/172	0.821
On-treatment LDL-C (median, IQR, mg/dL)		70/88/112	84/106/130	63/72/85	**<0.001**
Follow-up times (median, IQR, months)		31/48/64	31/48/65	30/45/64	0.105
Statin therapy, n	Atorvastatin 40 mg	562	301	220	>0.05
	Atorvastatin 80 mg	458	268	231	
	Rosuvastatin 20 mg	5	2	3	
	Rosuvastatin 40 mg	4	2	2	
MACE, n (%)		339 (32.9)	299 (52.2)	40 (8.8)	**<0.001**
Cardiovascular death, n (%)		35 (3.4)	29 (5.1)	6 (1.3)	**0.001**
Reinfarction, n (%)		89 (8.6)	85 (14.8)	4 (0.9)	**<0.001**
Recurrent myocardial infarction n (%)		260 (25.3)	235 (41)	25 (5.5)	**<0.001**
Target vessel revascularization, n (%)		321 (31.2)	283 (49.4)	38 (8.3)	**<0.001**

Data were shown as mean±standard deviation and n (%).
IQR: interquartile range; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; MACE: major adverse cardiovascular events. Bold values indicate statistical significance at the p<0.05 level.

individual variability in LDL-C response to statin therapy[6,8]. Recent research by Akyea et al. on the primary prevention population showed that 51.2% of patients had a suboptimal LDL-C response to statin therapy within 24 months. A cross-sectional study consisting of 22.063 patients receiving statins in both primary and secondary care demonstrated that up to half of patients (48.2%) did not achieve optimal lipid reduction goals[3]. In our study, only 45% of patients had an optimal LDL-C response to statin therapy, which is compatible with recent literature.

The exact mechanism underlying inadequate response to statins has not yet been well understood. Nevertheless, individual characteristics including age, sex, body weight, cigarette smoking, inflammatory stress, chronic kidney disease, diabetes mellitus, baseline lipid levels, and genetic variations have been identified as possible determinants of LDL-C response to

Table 2. Hazard ratios and incidence rates (per 1000 person-year) for the primary and secondary endpoints according to low-density lipoprotein cholesterol response to statin therapy

	Groups (n=1029)	CVD events (n)	Rate of CVD events (per 1000 person-years)	HR (adjusted)[a] (95%CI)	p-value
MACE	Optimal	40	17	**1**	**<0.001**
	Suboptimal	299	102	3.99 (2.66–6.01)	
Cardiovascular death	Optimal	6	2.6	1	**0.30**
	Suboptimal	29	9.9	1.93 (0.55–6.74)	
Reinfarction	Optimal	4	1.7	1	**<0.001**
	Suboptimal	89	30.5	18.17 (5.72–57.6)	
Recurrent myocardial infarction	Optimal	25	10.7	1	**<0.001**
	Suboptimal	235	80.7	5.44 (3.20–9.23)	
Target vessel revascularization, n (%)	Optimal	38	16.4	1	**<0.001**
	Suboptimal	283	97.2	4.04 (2.66–6.14)	

[a]The multivariable Cox regression models for MACE, cardiovascular death, reinfarction, recurrent myocardial infarction, and target vessel revascularization were adjusted for age, hypertension, prior myocardial infarction, prior coronary revascularization, baseline LDL-C, baseline total cholesterol, baseline triglyceride, baseline HDL-C, on-treatment LDL-C, and hs-CRP levels. LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; MACE: major adverse cardiovascular events. Bold values indicate statistical significance at the p<0.05 level.

Table 3. Estimates of hazard ratios and incidence rates (per 1000 person-year for the primary and secondary endpoints according to the statin responses in the subgroup of on-treatment low-density lipoprotein cholesterol ≤55 mg/dL).

	Group: LDL ≤55 mg/dL (n=287)	CVD events (n)	Rate of CVD events (per 1000 person-years)	HR (adjusted)[a] (95%CI)	p-value
MACE	Optimal	15	16.9	**1**	**<0.001**
	Suboptimal	19	61.2	8.73 (2.81–27.1)	
Cardiovascular death	Optimal	1	1.1	1	-
	Suboptimal	3	9.6	1	
Reinfarction	Optimal	2	2.2	1	0.01
	Suboptimal	6	19.2	14,7 (1.72–126.3)	
Recurrent myocardial infarction	Optimal	10	11.2	1	0.002
	Suboptimal	14	45.1	5.89 (1.89–18.3)	
Target vessel revascularization, n (%)	Optimal	15	16.9	1	0.001
	Suboptimal	16	51.5	7.93 (2.49–25.2)	

LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; MACE: major adverse cardiovascular events. [a]The multivariable Cox regression models for MACE, cardiovascular death, reinfarction, recurrent myocardial infarction, and target vessel revascularization were adjusted for prior myocardial infarction, prior coronary revascularization, baseline LDL-C, baseline total cholesterol, baseline triglyceride, baseline HDL-C, on-treatment LDL-C, and hs-CRP levels. Bold values indicate statistical significance at the p<0.05 level.

statin therapy[4,5]. Although our study did not primarily intend to determine predictors of suboptimal statin response, patients with suboptimal response were significantly more likely to have lower baseline LDL-C and higher hs-CRP levels compared with optimal response. Likewise, data from the EUROASPIRE V study showed that the percentage of LDL-C response was significantly lower in patients with lower baseline LDL-C levels[9].

Although statins reduce the risk of CV events, the impact of diminished response to statins on future CV adverse events has not been fully elucidated. A study by Kataoka et al.[10] evaluated the natural history of atheroma burden in hyporesponders to statin therapy. They revealed that statin hyporesponders exhibited more significant atheroma progression. Recently, Akyea et al. quantified the variability in LDC response to statins and its impact on future CVD events in the primary prevention population. They showed that the risk of incident CVD was significantly greater in suboptimal responders than in optimal responders. In our study, the risk of the composite of MACE, including CV mortality, reinfarction, recurrent MI, and TVR during the follow-up, was significantly higher in patients with suboptimal LDL-C response to a statin compared with optimal statin responders. Although our study was conducted on a secondary prevention population, the abovementioned previous studies support our findings.

In a subgroup analysis of our study, we found worse CV outcomes in patients with suboptimal response to statin therapy, even with on-treatment LDL-C levels below 55 mg/dl. The current ESC/EAS guideline recommends lowering the LDL level below 55 mg/dl and more than 50% LDL reduction in patients with ACS. In contrast, it is recommended that combination therapy with statins and ezetimibe or PCSK-9 inhibitors is only reasonable in patients with clinical CVD who are deemed to be at very high risk and have an LDL-C level of 55 mg/dl or higher despite maximally tolerated statin therapy. Thus, the management of the patients with history of MI failed to achieve a 40% reduction in baseline LDL-C, while their on-treatment LDL-C below 55 mg/dl remains controversial in regard to the benefit, side effects, and cost. A study by Ridker et al. showed that the magnitude percentage of the on-treatment LDL-C reduction directly related to the magnitude of cardiovascular risk reduction was observed[11]. The importance of reducing LDL-C by at least 50% was also validated by Waters et al.[12]. Their pooled analysis of several randomized trials from the secondary prevention population suggested that percent LDL-C reduction provides incremental prognostic value over attained LDL-C levels. However, due to the relatively small number of subgroup patients, it is not our intention to suggest that percent reduction targets for statin therapy are better than absolute treatment targets for statin therapy. Further studies are needed to evaluate the effectiveness of the nonstatin cholesterol-lowering drugs in patients with on-treatment LDL-C £55 mg/dl but not achieving percent reduction targets.

Several mechanisms could be considered to induce an increased risk of CV events in suboptimal statin responders. The effect of statins on cardiovascular risk reduction is mainly attributed to declines in LDL-C. A meta-analysis of individual participant data by the Cholesterol Treatment Trialists' Collaboration[13] reported that more intensive statin-mediated LDL-C lowering by 20 mg/dl resulted in a 15% further reduction in major CV events (including a 13% reduction in coronary death or nonfatal MI, a 19% reduction in coronary revascularization, and a 16% reduction in ischemic stroke). Therefore, in patients with a suboptimal LDL-C response to a statin, relatively high on-treatment LDL-C levels may have led to increased CV risk. Another mechanism related to high CV events in suboptimal statin responders might be their diminished pleiotropic effect. Some of the pleiotropic effects of statins may be mediated through their effects on hs-CRP. Previously, it was found that statin therapy decreased hs-CRP, an independent marker for future cardiovascular events[14,15]. This anti-inflammatory property of statins has been suggested to explain their beneficial effects on cardiovascular outcomes. In our study, suboptimal statin responders were more likely to have higher hs-CRP levels than optimal statin responders. However, the data on hs-CRP levels after statin therapy were lacking. Thus, suboptimal statin responders might have lower pleiotropic effects, potentially contributing to their high risk of MACE.

Limitations

Some limitations should be noted when interpreting our findings. First, the study sample comprised patients with statin therapy throughout the period of 12 months after the index hospitalization. After 12 months, the patterns of statin use were not taken into account in the analysis. Second, electronic drug prescribing data may not always correlate with the actual statin consumption. Although telephone visits have also checked statin adherence, the subject's self-reported adherence might be considered a limitation. Other limitations of the study include its retrospective nature, relatively short follow-up period for cardiovascular events, and the single-center patient cohort. The nonrandomized nature of the study could have resulted in selection bias.

CONCLUSIONS

The present study showed that patients with suboptimal statin response had an increased risk for future MACE, specifically CV death, reinfarction, recurrent MI, and TVR. Unfavorable

cardiovascular outcomes are also persistent in suboptimal responders; even their on-treatment LDL-C levels are reduced below 55 mg/dL. However, further randomized trials are needed to validate these findings.

AUTHORS' CONTRIBUTIONS

MBV: Methodology, Writing – original draft. **PV:** Resources, Validation. **SA:** Data curation, Formal Analysis. **HE:** Formal Analysis. **KC:** Writing – review & editing.

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ORIGINAL ARTICLE

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ICD indication in hypertrophic cardiomyopathy: which algorithm to use?

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SUMMARY

OBJECTIVE: This study aimed to evaluate the agreement in the indication of implantable cardioverter-defibrillators in patients with Hypertrophic cardiomyopathy, as per the 2014 European Society of Cardiology and 2020 American Heart Association recommendations, and evaluate fragmented QRS as a predictor of cardiovascular outcome.

METHODS: Retrospective cohort with 81 patients was evaluated between 2019 and 2021. Patients with hypertrophic cardiomyopathy ≥16 years old were included. Exclusion criteria include secondary myocardiopathy and follow-up < 1 year. Kappa coefficient was used to determine the agreement. Survival and incidence curves were determined by Kaplan-Meier method. A $p<0.05$ was considered significant.

RESULTS: The fragmented QRS was identified in 44.4% of patients. There were no differences between patients with and without fragmented QRS regarding clinical parameters, echocardiography, fibrosis, and sudden cardiac death risk. During follow-up of 4.8±3.4 years, there was no sudden cardiac death, but 20.6% patients with implantable cardioverter-defibrillator had at least one appropriate shock. Three of the seven appropriate shocks occurred in European Society of Cardiology low- to moderate-risk patients. Three shocks occurred in moderate-risk patients and four in American Heart Association high-risk patients. Overall recommendations agreement was 64% with a kappa of 0.270 ($p=0.007$). C-statistic showed no differences regarding the incidence of appropriate shock ($p=0.644$).

CONCLUSION: sudden cardiac death risk stratification algorithms present discrepancies in implantable cardioverter-defibrillator indication, both with low accuracy.

KEYWORDS: Sudden cardiac death. Hypertrophic cardiomyopathy. Implantable cardioverter-defibrillator. Cardiac arrhythmia.

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease, with an estimated prevalence of 1–167 individuals[1]. It is recognized as the main cause of sudden death (SD) in young people[2]. It is commonly asymptomatic. When present, the main symptoms are chest pain, dyspnea, palpitation, and syncope[3].

Through risk stratification strategies and prophylactic implantable cardioverter-defibrillator (ICD) indication, the mortality of patients with HCM has been reducing from 6 to <1% per year[1,4]. However, recommendations for implantation of ICD are divergent and tend to over- or underestimate the real risk of SD, increasing the risk of unnecessary intervention in low-risk patients or nonindication in high-risk patients[1,4,5].

Traditionally, the main risk factors for MS are age, report of syncope, family history of multiple sclerosis (MS), evidence of ventricular arrhythmia, and left ventricular hypertrophy (LVH) ≥30 mm[4,6]. Although widely validated, these parameters have low accuracy in predicting MS in low- and medium-risk patients, which correspond to the majority of patients with HCM[7].

Some studies have shown that fragmented QRS (fQRS) on electrocardiogram (ECG) correlates with myocardial fibrosis and represents a potential precursor of heart failure (HF) and arrhythmic events[7]. Despite this, the relevance of the fQRS in HCM is limited and its role in the prediction of SD is controversial[8].

The aim of this study was to evaluate the agreement in the indication of ICD as primary prophylaxis of SD in HCM patients, according to the 2014 European Society of Cardiology (ESC) and

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2020 American Heart Association (AHA) recommendations, and to evaluate the fQRS as a predictor of cardiovascular outcome.

METHODS

Type of study and population

This is a retrospective cohort study carried out in a university cardiology outpatient clinic specialized in HCM.

Inclusion criteria were diagnosis of HCM and age ≥16 years, while exclusion criteria were indication of ICD as secondary prophylaxis, follow-up time <1 year, and incomplete medical records.

The clinical variables collected were age, sex, clinical data (family history of SD, symptoms, ICD implantation), data from complementary examinations (ECG, transthoracic echocardiogram [ECOTT], cardiac magnetic resonance [CMR], 24 h Holter), clinical outcomes, and follow-up time.

The diagnosis of HCM was defined as LVH ≥15 mm in the largest segment (or ≥13 mm in those with a family history of HCM) in the absence of cardiac or systemic diseases that would justify ventricular overload[4].

The definition of SD was any sudden-witnessed death with or without documented ventricular fibrillation (VF), death within 1 h of the onset of new symptoms, or nocturnal deaths without prior history of worsening symptoms[9]. Time of follow-up was determined by the difference in years between the initial assessment and the last visit or outcome. The functional class was determined by the New York Heart Association (NYHA).

In the case of shock administration by the ICD, the electrograms recorded by the device were retrieved and analyzed. Shocks were considered appropriate in the event of sustained VT and VF[10].

The primary end point was the composite of SD or equivalent SD (SDE), namely, aborted MS and/or appropriate ICD shock. The secondary end point, acronym SEHS, was composed of SDE, hospitalization for decompensated HF, and fatal or nonfatal stroke.

The techniques used to perform the ECOTT and CMR were described previously[11].

Electrocardiographic analysis

The duration of QRS complex was manually determined in long lead II. In patients with narrow QRS (<120 ms), fQRS was defined as the presence of an additional R wave or notch in the R or S wave; in the case of wide QRS (≥120 ms), 2 notches or higher of R or S were considered[12].

Risk stratification for cdi implantation

The probability of MS in 5 years was calculated using the mathematical model validated by the ESC[4].

Each patient had its indication for ICD determined according to the recommendations of each guideline, grouped according to the level of clinical evidence:

- ESC 2014[4] – using estimated risk (ER) of SD in 5 years
  - Class IIa – ER ≥6%
  - Class IIb – ER <6 and ≥4%
  - Class III – ER <4%

- AHA 20201
  - Class IIa – at least one of the following: family history of MS, maximal LVH ≥30 mm, unexplained syncope; apical aneurysm; left ventricular ejection fraction (LVEF) ≤50%
  - Class IIb – nonsustained ventricular tachycardia (NSVT) or myocardial fibrosis on CMR ≥15%
  - Class III – absence of the aforementioned factors

Statistical analysis

Continuous variables were expressed as mean±standard deviation or as median and range of 25th and 75th quartiles, as appropriate, and categorical variables as absolute and proportional values. The Shapiro-Wilk test was used to determine normality.

Unpaired Student's t-test or Mann-Whitney U test was used in the analysis of continuous variables, while the χ^2 test or Fisher's exact test was used for categorical variables.

Survival curves, together with the p-value of the log-rank test, were calculated using the Kaplan-Meier method. The analysis was adjusted for age, sex, family history of MS, NYHA (I–II vs. III–IV), maximal LVH, gradient in left ventricular outflow tract (LVOT), syncope, and the presence of VT or NSVT on 24 h Holter. Receiver operating characteristics curve (ROC), area under the curve (AUC), and Harrell C-statistics analyses were used to assess the accuracy of the guidelines for predicting appropriate ICD shock. The linearly weighted kappa coefficient was calculated to determine the degree of agreement between the recommendations of the 2014 ESC and 2020 AHA 2020. For all the analyses, $p<0.05$ was considered significant.

Ethical aspects

The study was submitted and approved by the Research Ethics Committee. All Brazilian legal norms and Helsinki Declaration principles were observed. Since it was a retrospective study, the collection of the informed consent form was waived.

RESULTS

From March 2019 to February 2021, 96 patients with HCM were identified, of which 15 were excluded from the study

due to not meeting the established criteria; therefore, only 81 patients were included in the study.

The fQRS was diagnosed in 36 (44.4%) patients. There was no statistically significant difference between patients with and without fQRS regarding clinical, echocardiographic, fibrosis, and estimated risk of MS (Table 1).

During a mean follow-up of 4.8±3.4 years, no SD occurred, but 7 (20.6%) of 34 patients with ICD had at least one appropriate shock, 4 (4.9%) hospitalizations for decompensated HF, and 6 (7.4%) nonfatal cerebrovascular events. Three of the seven appropriate shocks occurred in patients considered to be at low or moderate risk by the 2014 ESC guidelines. In the case of the 2020 AHA guidelines, three of the appropriate shocks occurred in patients at moderate risk and four shocks in patients at high risk. The incidence of SDE was 10.2% and that of SEHS was 21.6%.

The agreement between the indications for ICD implantation according to the 2014 ESC and 2020 AHA guidelines was 64% (kappa 0.270; p=0.007) (Table 2).

The Kaplan-Meier curve showed a trend toward lower outcome-free survival in patients with fQRS (71.3 vs. 82.6%; p=0.515, Figure 1A). There was no statistical difference regarding the cumulative incidence of appropriate shock (10.5 vs. 16%, with and without fQRS; p=0.598, Figure 1B).

Considering the indications for ICD implantation as a binary outcome (implant [evidence IIa/IIb] or not implant [evidence III]), C-statistics analysis did not show differences regarding the incidence of appropriate shock (p=0.644). The AUC was 0.557 for the 2014 ESC recommendations (95%CI 0.406–0.707) and 0.548 for the 2020 AHA (95%CI 0.548–0.636) Figure 2.

Table 1. Basic characteristics of the study population.

	fragmented QRS (n=36)	No fragmented QRS (n=45)	p-value
Age, years	42.8±15.6	48.1±15.8	0.137[a]
Gender male	17 (47.2)	23 (51.1)	0.728[b]
SD family history	21 (58.3)	23 (51.1)	0.564[b]
NYHA III/IV	3 (8.3)	2 (4.4)	0.470[c]
Syncope history	13 (36.1)	19 (42.2)	0.168[b]
ICD implant	14 (38.9%)	20 (44.5)	0.615[b]
TTE			
LVEF (%)	67 (62–72)	69 (63–74)	0.284[d]
Max LVH (mm)	20 (16.5–27.6)	20 (17–24)	0.668[d]
LA (mm)	39 (34–46)	39 (38–44)	0.647[d]
LVOT (mmHg)	0 (0–31)	0 (0–30)	0.668[d]
Myocardial fibrosis (%)[π]	3.5 (2.3–7.5)	3.5 (1.7–13.8)	0.542[d]
ECG			
AFib	3 (8.3)	6 (13.3)	0.724[c]
LVH	18 (50.0)	17 (37.8)	0.399[d]
LAO	10 (27.8)	9 (20.0)	0.515[d]
Stroke/TIA	2 (5.6)	6 (13.3)	0.284[c]
SD risk in 5 years (%)	4.6 (2.7–7.3)	3.8 (2.1–6.1)	0.541[d]

Values expressed as n (%), mean±standard deviation, or median (p25–75%). SD: sudden death; NYHA: New York Heart Association functional class; ICD: implantable cardioverter-defibrillator; TTE: transthoracic echocardiogram; LVEF: left ventricular ejection fraction; Max LVH: maximal left ventricular hypertrophy; LA: left atrium; LVOT: left ventricular outflow tract; AF: atrial fibrillation; LVH: left ventricular hypertrophy; LAO: left atrial overload; Stroke/TIA: stroke or transient ischemic stroke.
[π]Only 55 (67.9%) patients underwent CMR; [a]Student's t-test; [b]χ^2 test; [c]Fisher's exact test; [d]Mann-Whitney U test.

DISCUSSION

In this study, we evaluated the agreement between the 2014 ESC and 2020 AHA guidelines in the indication of ICD as primary prophylaxis of MS in patients with HCM. Our results show significant divergence in the indication of ICD, with an overall agreement of 64%. The 2020 AHA algorithm indicated class IIa ICD in 69% of patients, compared to 40.7% by 2014 ESC. Two patients classified as low risk by ESC had appropriate shocks 1 and 5 years after ICD implantation. Of the 13 patients classified by the AHA as low risk, there was a divergence from the ESC in only one case.

The analysis of the agreement of indications resulted in a kappa of 0.270. Kappa coefficient between 0.21 and 0.39 represents minimal agreement, implying that only 4–15% of the indications analyzed between both guidelines are, in fact, reliable[13].

Mattos et al. demonstrated that in relation to the 2011 AHA, the ESC algorithm also had low agreement and would

Table 2. Implantable cardioverter-defibrillator indication agreement according to the degree of evidence.

	ESC 2014		
AHA 2020	III	IIb	IIa
III	12	0	1
IIb	w6	1	5
IIa	16	13	27

General agreement: 64.2%, kappa 0.270 (95%CI 0.118–0.422; p=0.003)
ESC: European Society of Cardiology; AHA: American Heart Association. IIa, IIb, III: degrees of scientific evidence for implantable cardioverter-defibrillator; implantation according to each guideline.

leave all patients (8/90) unprotected with appropriate shock[5]. Other studies have also demonstrated low sensitivity of the algorithm, especially in patients considered to be at low risk[14,15]. Our results showed low accuracy of the algorithm to predict MS, especially in the group considered low risk.

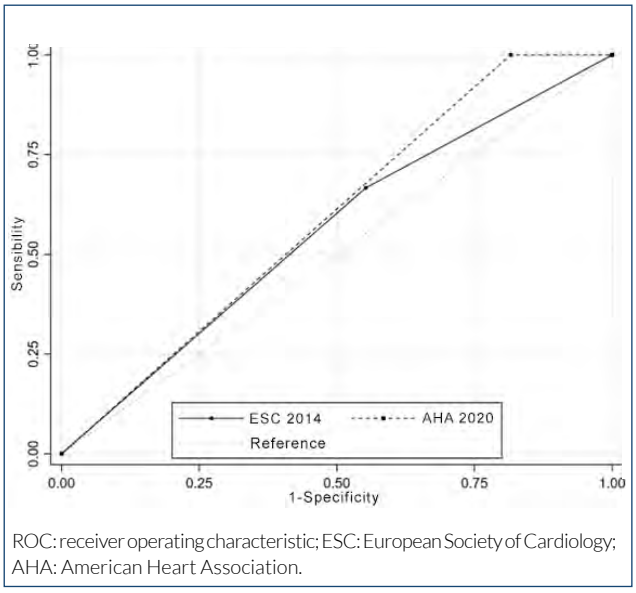
The 2020 AHA recommendations showed high sensitivity (100%), but their low specificity (17.6%) implies unnecessary indication of ICD in low-risk patients. ICD implantation is related to complications such as infection and inappropriate shocks, with an incidence of 2.1% per year[16]. In the C-statistics analysis, both guidelines showed similar discrimination in predicting appropriate shock.

In HCM studies, a good correlation has been shown between the presence of fQRS and fibrosis estimated by CMR and histology[17]. In our sample, it was not possible to demonstrate the association between fQRS and fibrosis. There was a trend toward a greater outcome-free survival in patients without fQRS, but this difference was not significant. There was no statistical difference between the cumulative incidence of appropriate shocks between patients with or without fQRS, despite a trend toward more shocks in the fQRS group (10 vs. 16%).

Few studies have evaluated the direct link between MS risk and appropriate ICD shock in patients with HCM and the presence of fQRS. One study evaluated the calculated risk of SD in 5 years of the 2014 ESC and showed that the presence of fQRS was related to a risk of SD >4%[18]. In the SHIFT study, the fQRS was included as a risk predictor with a hazard ratio of 3.6[7]. However, the study included only patients at low and moderate risk for MS, compromising its practical applicability.

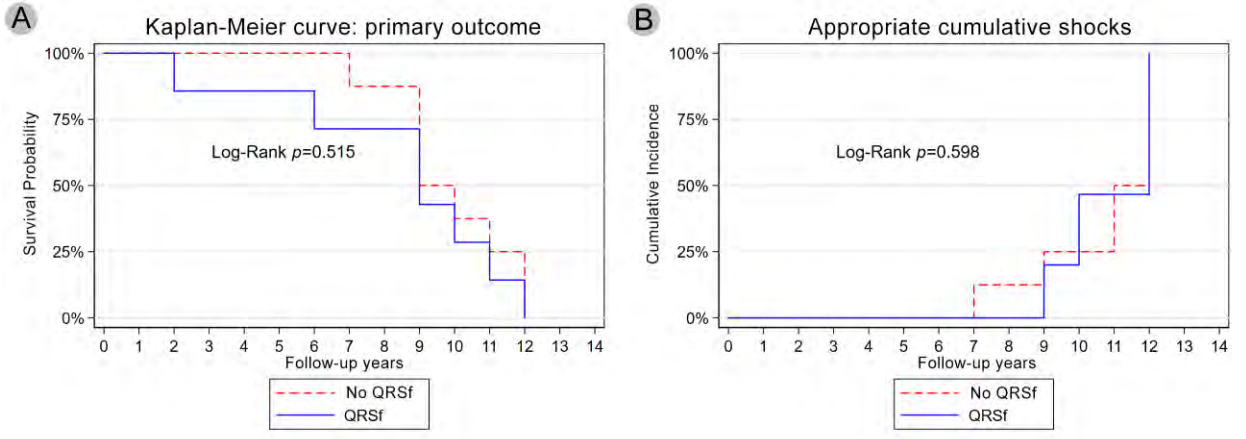
The spectrum of clinical presentation of HCM is quite heterogeneous. The mechanisms underlying the occurrence of fibrosis and arrhythmia are not fully understood and appear to be influenced by epigenetic factors[19]. Rigid predictor models are unable to represent the complexity of individual risk, which reinforces the role of specialist experience in risk stratification and individualized indication of primary ICD prophylaxis.

Some limitations of this study were the sample size, the retrospective design, and the factor of being unicentric. The occurrence of appropriate shocks would not necessarily represent



ROC: receiver operating characteristic; ESC: European Society of Cardiology; AHA: American Heart Association.

Figure 2. Receiver operating characteristic curve comparing the incidence of appropriate shocks according to European Society of Cardiology 2014 and American Heart Association 2020 guidelines implantable cardioverter-defibrillator indication.



A: Kaplan-Meier survival curve for the primary outcome. B: Incidence curve for appropriate shocks in patients with implantable cardioverter-defibrillator; implantation. fQRS: fragmented QRS.

Figure 1. Survival and incidence curves for the primary outcome and appropriate shocks.

life-threatening events and the patients treated tended to present later diagnoses and greater severity.

CONCLUSIONS

The 2014 ESC and 2020 AHA MS risk stratification algorithms for HCM patients present discrepancies in the indication of ICD implantation, both with low accuracy. The European guideline showed better specificity, while the American guideline showed excellent sensitivity, despite similar discrimination using C-statistics.

AUTHORS' CONTRIBUTIONS

MAOSV: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. **AVFC:** Data curation, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **EBC:** Investigation, Resources. **MM:** Investigation, Resources. **LSB:** Resources (Supporting), Writing – review & editing. **SGL:** Methodology, Writing – review & editing. **BMF:** Supervision. **DCO**: Methodology, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Effectiveness of peripheral nerve blockage on the symptoms of both diseases in patients with fibromyalgia and chronic migraine coexistence

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SUMMARY

OBJECTIVE: Peripheral nerve blockage treatments reduce central sensitization and are effective in patients with migraine. We wanted to evaluate the efficacy of peripheral nerve blockage in patients with fibromyalgia and migraine whose etiology may be responsible for central sensitization, and their associations are common.

METHODS: The files of patients with chronic migraine who had peripheral nerve blockage treatment in our clinic and had fibromyalgia were scanned. The patients underwent bilateral great occipital nerve, lesser occipital nerve, and supraorbital nerve blockage at baseline and in the second week. The revised Fibromyalgia Impact Questionnaire, Migraine Disability Assessment Scale, Visual Analog Scale scores, the number of days in pain, and the number of analgesics taken in the last month were recorded.

RESULTS: In the third month, Fibromyalgia Impact Questionnaire, Migraine Disability Assessment Scale, and Visual Analog Scale scores were significantly lower from baseline. While Fibromyalgia Impact Questionnaire scores in the third month were significantly lower than in the first month, no significant difference was observed between Visual Analog Scale scores. In the third month, the number of days in pain and the number of analgesics taken in the last month was significantly lower than the baseline but higher than the first month.

CONCLUSION: Peripheral nerve blockage has been found to be an effective treatment for the symptoms of both diseases in patients with migraine and fibromyalgia coexistence.

KEYWORDS: Migraine. Fibromyalgia. Nerve block. Chronic migraine.

INTRODUCTION

Fibromyalgia (FM) is a syndrome in which chronic and widespread musculoskeletal pain can be accompanied by medical conditions, such as migraine, irritable bowel syndrome, fatigue, visceral pains, depression, and sleep disorders[1]. Its prevalence in the general population is between 4 and 7%[2]. In Turkey, the prevalence was between 3 and 6%[3].

The prevalence of migraine was reported to be 17.6% in women and 5.7% in men in Europe and America[4]. The coexistence of FM and migraine draws attention in daily practice. In an epidemiology study with a large number of cases, the prevalence of migraine in patients with FM was 55.8%[5]. In a study evaluating the data of 1466 patients, FM was found in 24.3% of patients with migraine[6]. The frequency of FM was reported to be 17.4% in episodic patients with migraine[7].

The central sensitization hypothesis is most supported in FM etiology. Central sensitization may cause any pain of the nociceptive or neuropathic type. When the noxious stimulus stimulates the C and A- sensory fibers, and it causes a more intense perception of the painful stimulus in the stimulated area (hyperalgesia), and any stimulus applied to the affected area may cause painful perception (allodynia). Peripheral changes in the muscle and skin region in FM increase noxious inputs that may cause permanent changes in the nociceptive pathway and cause pain[8]. It is thought that inflammation, which starts with the activation of the trigeminovascular system in migraine, causes central and peripheral sensitization, resulting in the development of pain and allodynia[9].

In neurophysiological examinations, increased cortical response to painful stimuli was detected in patients with migraine and patients with FM in the attack phase[10]. It was thought that the inhibition of repetitive painful stimuli was impaired, and central sensitization developed accordingly[11]. Glutamate and substance P levels were high, serotonin and

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noradrenaline levels were low in CSF in patients with FM, and the imbalance between inhibitory and excitatory neurotransmitters during pain processing was thought to be related to central sensitization[12].

Migraine and FM are two diseases that often accompany each other and central sensitization is blamed for their etiology. There are also studies showing that prophylaxis drugs used for migraine have significant benefits on FM symptoms[13]. We thought that peripheral nerve blockage (PNB) treatments, which are known to be an effective treatment in migraine, can reduce peripheral stimulus and central sensitization, thus reducing the symptoms of FM disease, which is etiologically similar[14,15].

METHODS

In this retrospective study, the files of patients diagnosed with chronic migraine and FM and who underwent PNB in our clinic between 2020 and 2021 were scanned after obtaining approval from the ethics committee of Derince Training and Research Hospital (2021–126). The diagnosis of chronic migraine was made according to the criteria of the International Headache Classification Committee (ICHD-3)[16]. Among these patients, those diagnosed with FM according to the modified American College of Rheumatology (ACR) diagnostic criteria were included in the present study[17]. Patients with needle phobia, those who did not attend the second injection, and those with a history of drug allergy were excluded from this study. Sixty patients meeting these criteria were included in this study.

After the injection sites were wiped with an antiseptic solution, great occipital nerve (GON), lesser occipital nerve (LON), and supraorbital nerve (SON) blockage were applied to the patients. Then, 1.5 mL of 2% lidocaine was injected 2 cm lateral and 2 cm inferior to the occipital protuberant for GON blockage. LON blockage was performed by injecting 1.5 mL of 2% lidocaine from the 2/3 lateral point of the line between the occipital protuberance and mastoid. SON blockage was performed just above the supraorbital notch. All injections were made with a 27 G needle. Patients were followed up for 30 min after the procedure for early side effects. Two weeks later, the same protocol was repeated.

Migraine disability was determined by the Migraine Disability Assessment Scale (MIDAS) score. The MIDAS test is the most widely used test since 2001 to measure migraine disability[18]. The revised Fibromyalgia Impact Questionnaire (FIQR) test was used for functional assessment in patients with FM[19]. The number of days in pain, the number of analgesics taken in the last month, the Visual Analog Scale (VAS), MIDAS, and FIQR scores of the patients at admission, first month, and third month controls were noted.

The primary outcome was a decrease in MIDAS and FIQR scores, and the secondary outcome was a decrease in the number of analgesics taken and the number of days in pain.

Statistics

All data were analyzed using SPSS version 21.0 (IBM Corp.; Armonk, NY, USA). Data were expressed as the number of patients, mean, standard deviation, and median. Parametric statistical tests were used for normally distributed data, and nonparametric statistical tests were used for non-normally distributed data. Paired t-test was used in the analysis of dependent groups. The ANOVA test was used to evaluate repeated measures of normally distributed groups. A $p<0.05$ was considered statistically significant.

RESULTS

Data of 60 patients who met the inclusion criteria were evaluated. The mean age was 41.2±10.7 (range, 20–68), and the mean disease duration was 12.8±7.3 (range, 1–30). Notably, 53 female and 7 male patients were included in the present study (Table 1).

Pretreatment VAS scores were 8±0.7 (range, 6–9), MIDAS scores were 47.3±11 (range, 30–65), and FIQR scores were 52.4±5 (range, 40–59). The number of analgesics taken in the last month before the treatment was 21.9± 9.6 (range, 0–60), and the number of days in pain was 23.2±6.67 (range, 15–30).

MIDAS, VAS, FIQR scores at admission, first month, and third month, the number of analgesics taken in the last month, and the number of days in pain are given in Table 2.

It was observed that FIQR scores were significantly lower than baseline in the first month ($p<0.01$). When the FIQR scores of the first month and third month were compared, it was observed that the FIQR scores in the third month were significantly lower and the FM symptoms of the patients continued to regress ($p<0.01$).

It was observed that the VAS scores were significantly lower in the first and third months compared to the pretreatment ($p<0.01$), and there was no significant difference between the first month and third month VAS scores ($p=0.83$).

Table 1. Demographic data of patients.

Age (min–max)	41.2±10.7 (20–68)
Gender: male/female	7/53
Disease duration (min–max)	12.8±7.3 (1–30)

MIDAS scores were significantly lower than baseline in the third month (p<0.01).

The number of days in pain and the number of analgesics taken in the first and third months of the patients were significantly lower than the baseline (p<0.01). When the first month and third month data were compared, the number of days in pain and the number of analgesics taken were significantly higher in the third month (p<0.01) (Table 2).

DISCUSSION

FM and migraine are two diseases that are very common in society and their coexistence is quite common. Migraine was found in approximately half of the patients with FM and FM in one-quarter of patients with migraine[5,6].

Similar drugs are used in the medical treatment of both diseases. In the randomized controlled study conducted by Giamberardino et al., both migraine attack frequencies and FM flares were lower in the group, followed by the flunarizine treatment among patients with migraine and FM. FM flares were higher in patients with a higher frequency of migraine attacks[13]. Similarly, it has been reported that migraine headaches increase FM symptoms, and musculoskeletal pain increases headaches[20].

The central sensitization hypothesis is the most supported hypothesis in the etiology of both diseases. GON blockage in migraine is a proven treatment, which is thought to reduce the impulses from the upper cervical spinal cord to the trigeminal nucleus caudatus complex and make changes in the nociceptive pathway and inhibitory control mechanism, thus reducing central sensitization and affecting it[14,15,21].

We found one study in the literature investigating the effectiveness of PNB in patients with migraine and FM. Yilmaz et al. applied bilateral GON blockage to 20 patients with episodic migraine and FM diagnoses once a week for the first month, and then unilaterally once a month for 2 months. VAS, MIDAS, and FIQR scores of the patients decreased significantly in the first and third months compared to the pretreatment period. They also found that VAS, MIDAS, and FIQR scores were lower in the third month compared to the first month. They reported that the efficacy of GON blockage was more pronounced in the third month[22]. In our study, we applied PNB to patients with chronic migraine and FM coexistence. We applied bilateral GON, LON, and SON blockage in the beginning and in the second week. Then, we did not apply blockage again. In our study, the findings showed that VAS, MIDAS, and FIQR scores were lower in the third month controls compared to the pretreatment period. Although the third month FIQR score was similarly lower than the first month in our study, there was no significant difference between the first month and third month VAS scores. In addition, we found that the number of days in pain and the number of analgesics taken in the last month were higher in the third month than in the first month. Although we did not apply blockage again after the second week, in our study, the efficacy concerning FM symptoms was higher in the third month. However, the increase in the number of analgesics taken and the number of days in pain in the third month may suggest that the effectiveness of cranial nerve blockage for migraine has begun to decrease.

The limitations of our study are the absence of a control group, its retrospective nature, and the lack of follow-up in patients long enough to understand how long the efficacy of treatment continues.

CONCLUSION

In patients with chronic migraine and FM, PNB is an effective treatment that reduces symptoms and disability related to both diseases. Although the efficacy in patients with FM continued to increase in the third month, there were findings suggesting that the efficacy would begin to decline in the third month in patients with migraine. Randomized controlled studies on larger patient groups and longer follow-up of the patients will

Table 2. Data and comparison of clinical data of patients at baseline and follow-up.

	Baseline	1st mont	3rd month	0–1 mont	1–3 month
VAS	8±0.7	5.1±2.9	5.2±2.5	<0.01*	0.83*
FIQR	52.4±5	43.1±5.8	35.0±7.3	<0.01*	<0.01*
The number of analgesic taken	21.9±9.6	4.1±4.5	6.7±8.4	<0.01*	<0.01*
The number of days in pain	23.2±6.6	5.3±4.7	9.1±10	<0.01*	<0.01*
	Baseline	**3rd month**	**p**		
MIDAS	47.3±11	12.1±1.5	<0.01#		

*ANOVA test. #Paired sample t test.
VAS: Visual anolag scale; FIQR: Fibromiyalgia ımpact questionnaire; MIDAS: Migraine disability assessment.

be useful to reveal both the effectiveness of the treatment and how often the blockage should be applied. We also think that studies should be conducted to evaluate the effectiveness of GON blockage in patients with FM without migraine.

AUTHORS' CONTRIBUTIONS

GE: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **AY:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Complementary Ureterorenoscopy after extracorporeal Shock Wave Lithotripsy in proximal ureteral stones: success and complications

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SUMMARY

OBJECTIVE: The aim of this study was to demonstrate the effect of extracorporeal shock wave lithotripsy application on the success and complications of ureteroscopic lithotripsy in proximal ureter stones.

METHODS: The data of 87 patients who did not respond to shock wave lithotripsy and underwent ureteroscopic lithotripsy were retrospectively analyzed and classified as group I, and 99 patients who received ureteroscopic lithotripsy as primary treatment were classified as group II. Demographic features, response to treatment, and preoperative and postoperative complications were compared between the two groups.

RESULTS: There was no difference between the two groups in terms of gender, operation times, stone sizes, and ureteroscope diameters. (p>0.05). Infective complications such as postoperative fever, pyelonephritis, and urosepsis were similar in both groups (p=0.142, p=0.291, and p=0.948). Stone migration was observed in 10 (11.5%) and 6 (6.1%) patients in groups I and II, respectively (p=0.291). Impacted stone was seen in 47 (54%) patients in group I and in 15 (15.2%) patients in group II (p<0.0001). Mucosal laceration occurred in 11 (12.6%) and 3 (3%) patients in groups I and II, respectively (p=0.028). Ureteral perforation was detected in 3 (3.4%) patients in group I and 1 (1%) patient in group II, whereas ureteral avulsion was not observed in either group (p=0.524).

CONCLUSIONS: It was concluded that the application of shock wave lithotripsy before ureteroscopic lithotripsy in proximal ureter stones did not affect the success. Although the results are similar in terms of postoperative infection, shock wave lithotripsy application has been found to increase the risk of stone impaction into the mucosa and ureteral laceration.

KEYWORDS: Ureteral calculi. Extracorporeal shockwave lithotripsy. Ureteroscopy. Mucosal laceration.

INTRODUCTION

Ureteroscopic lithotripsy (URS) and extracorporeal shock wave lithotripsy (SWL) are the most common treatment modalities in the treatment of proximal ureteral stones[1]. According to the European Urology Association (EAU) guideline, the first-line treatment for proximal ureteral stones larger than 10 mm is URS, while SWL and ureteroscopy are recommended as the appropriate primary treatment options for proximal ureter stones smaller than 10 mm[2]. SWL is applied as an additional treatment in patients where URS fails; while URS is used as an additional treatment in patients where SWL fails[3]. The URS procedure performed after SWL is called "Salvage," "Secondary," or "Complementary" URS[4,5].

There are a number of studies in the literature comparing these two methods that are commonly used in the treatment of ureteral stones[3,6-8]. However, studies regarding the efficacy and complications of complementary URS in ureteral stones in patients unresponsive to SWL are scarce[4,5]. In this study, we aimed to investigate the effects of SWL treatment on the success and complications of URS in proximal ureteral stones.

METHODS

In the study, the medical files of patients who underwent URS for proximal ureteral stones between January 2017 and October 2019 in our clinic were retrospectively reviewed. The proximal ureter was defined as part of the ureter between the ureteropelvic junction and the upper border of the sacroiliac joint. In total, 186 patients whose data were completely recorded and who underwent SWL and URS were included in the study. The SWL failure was considered to be the absence of stone fracture after three sessions of SWL. The URS procedure that was performed after the failure of SWL was named as complementary URS as in the literature[5]. Regarding classification, 87 patients who underwent complementary URS were named as group I and 99 patients who underwent URS as primary treatment were named as group II.

The criteria for exclusion from the study included the patients who underwent previous URS, Percutaneous nephrolithotomy, open or laparoscopic ureterolithotomy, percutaneous nephrostomy or double-J stent placement before the

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procedure, patients who had any congenital kidney anomaly (urethrovesical junction stenosis, etc.) and underwent operation due to this anomaly, patients who did not want to continue SWL treatment, and those with pain and worsening hydronephrosis (Figure 1).

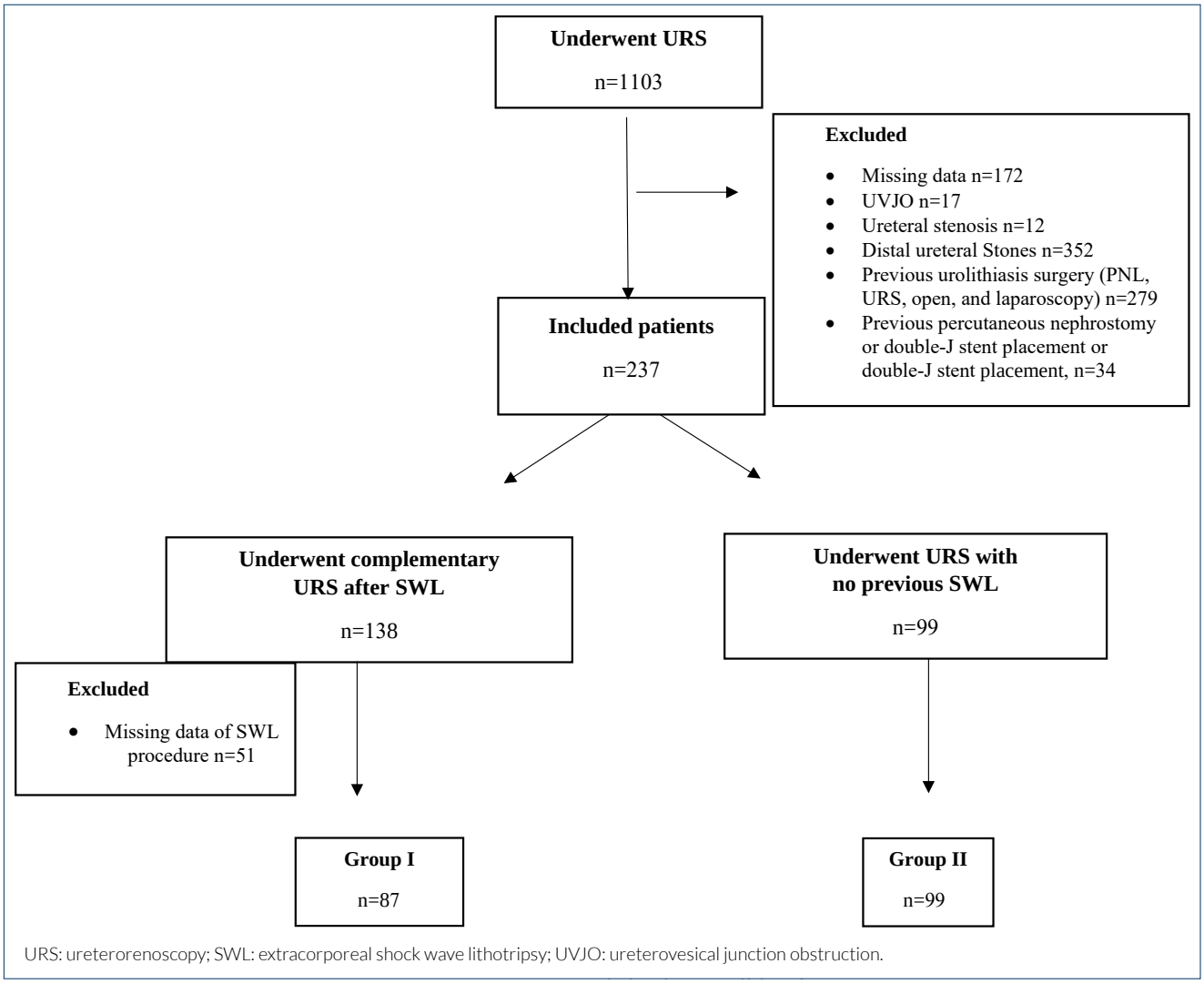
The SWL procedure was performed using electrohydraulic generators (Dornier HM-3) and at most three sessions at 1 week intervals. The shock wave per session was 80 impulses per minute and the maximum number of shock waves was 2000. The SWL failure was considered as the stone not breaking after three sessions of SWL application.

The URS procedure was performed under general and spinal anesthesia. Patients were administered 1 g of cefazolin sodium intravenously for prophylaxis. During the URS procedure, a 0.035-inch guidewire was introduced into the ureter and 7/9 Fr semirigid ureteroscopes (Karl Storz) were used. The Holmium YAG laser system was used in all patients as an energy source for stone crushing.

Intraoperative and postoperative complications were classified according to the modified Satava[9] and modified Clavien[10] systems, respectively. Body temperatures of 38°C and above were considered fever. Renal colic, which was severe enough to require analgesic use, was considered significant.

Patients with opaque stones were evaluated by plain abdominal X-ray and ultrasound (US) on the postoperative day 1 to evaluate the stone-free rates of the groups. Patients with residual stones and push back stones were evaluated by computed tomography (CT) scan in the first month and fragmented stones measuring >4 mm were considered residual.

Gender distribution, operation time, stone size, ureteroscope diameter, development of mucosal laceration, ureteral perforation, avulsion, postoperative fever, death due to pyelonephritis



URS: ureterorenoscopy; SWL: extracorporeal shock wave lithotripsy; UVJO: ureterovesical junction obstruction.

Figure 1. The flow chart.

and urosepsis, presence of impacted stone, stone migration, and stone-free rates were compared between the two groups.

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statistical analysis

The consistency of continuous variables to normal distribution was investigated with the Kolmogorov-Smirnov test. Variables with Gaussian distribution were presented as mean±SD, and variables with non-Gaussian distribution were shown as median (25–75th percentile). Student's t-test was used for comparison of variables with normal distribution, and the Mann-Whitney U test was used for comparison of variables with normal distribution. Pearson's χ^2 test or Yates' χ^2 test was used to compare group rates.

RESULTS

The mean age of the patients was 56.9±17.3 years in group I and 51.2±15.9 years in group II (p=0.021). There was no difference between the groups in terms of gender distribution, operation time, stone size, and ureteroscope diameter, but the frequency of the stone location on the right side was significantly higher in group II (p>0.05 and p=0.017) (Table 1).

The stone-free rates were 67.8 and 64.6% on the first postoperative day, and 85.1% and 78.8% in the first postoperative month in groups I and II, respectively. There was no statistically significant difference between the two groups in terms of stone-free rates both on the first postoperative day and in the first postoperative month (p=0.649 and p=0.270).

Infective complications such as postoperative fever, pyelonephritis, and urosepsis were similar in both groups (p=0.142, p=0.291, and p=0.948).

Stone migration was observed in 10 (11.5%) and 6 (6.1%) patients in groups I and II, respectively (p=0.291). Impacted stone was seen in 47 (54%) patients in group I and in 15 (15.2%) patients in group II (p<0.0001). Mucosal laceration occurred in 11 (12.6%) and 3 (3%) patients in groups I and II, respectively, and this difference was statistically significant (p=0.028). Ureteral perforation was detected in 3 (3.4%) patients in group I and 1 (1%) patient in group II, whereas ureteral avulsion was not observed in either group (p=0.524) (Table 2).

DISCUSSION

According to the EAU guidelines, the first-line treatment for proximal ureteral stones is URS or SWL[2]. The choice of ureteral treatment method depends on the localization of the stone, its size, presence and duration of renal colic, presence of dilation, patient selection, and available equipment[3,11,12]. Although SWL success rates for proximal ureteral stones vary between 40% and 82%, the URS success rate is higher than SWL[13-15]. According to our data, in patients with proximal ureter stones, the failure rate in primary cases was calculated as 21.2% and as 14.9% in secondary cases. In the literature, the URS failure rate for all stone localizations is 6.1–7.7% in primary cases and 3.5% in those who underwent complementary URS. The

Table 1. Demographic data, stone characteristics, ureteroscope diameters, and operation times in groups I and II.

	Group I (n=87)	Group II (n=99)	p-value
Age, year	56.9±17.3	51.2±15.9	**0.021**
Gender, M/F	55/32	67/32	0.523
Stone size, mm	11.1±3.8	11.9±4.7	0.210
Side			
Right (n)	40	63	**0.017**
Left (n)	44	36	
Bilateral (n)	3	0	
Diameter of ureteroscope			
7Fr (n)	63	62	0.156
9Fr (n)	24	37	
Operation time, min	33.3±18.2	34.3±12.6	0.681

M/F: Male/Female. Bold values indicate statistical significance at the p<0.05 level.

Table 2. The complications in groups I and II.

	Group I (n=87)	Group II (n=99)	p-value
Hydronephrosis (n)	73	71	**0.047**
Impacted stone (n)	47	15	**<0.0001**
Fever (n)	8	3	0.142
Pyelonephritis (n)	4	1	0.291
Stone migration (n)	10	6	0.291
Renal colic (n)	4	1	0.291
Mucosal laceration (n)	11	3	**0.028**
Ureteral perforation (n)	3	1	0.524
Avulsion (n)	0	0	–
Macroscopic hematuria (n)	5	2	0.344
Urinary tract infection (n)	6	2	0.203

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

URS success rate is lower in proximal ureter stones compared with distal ureter stones. The failure rate of the primary URS in distal ureteral stones is 3.3%, while it is 4.4% in the complementary URS, and there is no statistically significant difference between the primary and complementary URS success rates (p>0.2)[3,4,16]. In our study, all patients had proximal ureter stones, and the failure rate was 21.2% in primary cases and 14.9% in secondary cases.

The main reason for URS failure involves the problems in accessing it. In cases where successful access is achieved, URS failure is usually associated with stone size, localization, and the presence of hydronephrosis. The migration of the stone to the kidney, especially in the presence of hydronephrosis, is considered an important cause of URS failure in proximally located stones. Along with the fluid flow used during the URS procedure, the type of lithotriptor used to break the stone is also important in the migration of the stone. Stone migration is more common with pneumatic lithotriptors compared with laser lithotriptors. The migration of the stone to the kidney is seen in 6.7% of URS procedures[15]. In our study, the Holmium YAG laser was used in all patients. Although the stone retropulsion rate was higher in the complementary URS group (6.1 vs. 11.5%), it was not statistically significant (p=0.291).

Another cause of URS failure is impacted stones. Approximately, 4% of ureteral stones are impacted[17]. Prolonged stay of a ureteral stone in the same localization and/or SWL application facilitates the impaction of the stone by causing inflammation, edema, and hypertrophy in the ureteral wall[3,17]. Furthermore, patient age and stone size tend to be higher in impacted stones[18]. The incidence of impacted stone is higher in patients who undergo primary URS compared with complementary URS. In a study conducted by Tuğcu et al.[4], the rate of impacted stone was 35.1% in the complementary URS group and 9.85% in the primary URS group, whereas these rates were 38.4 and 17.4% in a study by Irer et al.[19]. In our study, the rate of impacted stone was significantly higher in the complementary URS group compared to the primary URS group (54 vs. 15.2%) (p<0.0001). In our study, we assume that the higher rate of impacted stone, especially in the complementary URS group, can be attributed to the fact that mean stone volume and average patient age were higher than in the literature. In addition, three sessions of SWL application may also play a role in higher impacted stone rate.

In a study comparing primary and complementary URS procedures, the success of URS in both primary and complementary URS groups was found to be 82.6%[19]. In another study conducted by Klinc et al.[5], success rates were 83 and 80.1% for stones smaller than 1 cm and 79.8 and 77.4% for stones larger than 1 cm in primary and complementary URS applications, respectively. There was no statistical difference between groups with regard to success rates in both stone sizes (p=0.35 and p=0.61). In these two studies, proximal ureteral stones were investigated. In our study, there was no statistically significant difference in terms of URS success rate between patients who underwent primary and complementary URS, and success rates were 78.8 and 85.1%, respectively (p=0.27).

Shock waves of SWL increase the edema and inflammation in urothelial mucosa and enhance the fragility of mucosal small vessels[20,21]. Theoretically, complications are expected to be high in interventions performed after SWL. However, in many studies, no significant difference was found between the complementary and primary URS in terms of total complication rates[4,5,22]. Yet, when complications are classified, it has been reported that Clavien grade I complications such as minimal mucosal laceration, mild bleeding, fever, and renal colic are seen more frequently in ureteral interventions after SWL[19,23,24]. Clavien grade I complication rate was 9.3% in the complementary URS group and 4.3% in the primary URS group, and the difference was statistically significant (p<0.001)[19]. Also, in this study, the ureteral perforation rate was 0.6% in the primary URS group and 1.2% in the complementary URS group (p<0.001). In our study, mucosal laceration rates were 3 and 12.6% for the primary and complementary URS, respectively. For the mucosal laceration, this difference was statistically significant (p=0.028), and there was no significant difference between fever, renal colic, ureter perforation, and urinary infection rates (p=0.142, p=0.291, p=0.524, and p=0.203).

CONCLUSIONS

It has been shown that SWL applied before the URS procedure does not affect URS success and has very low major complication rates similar to those in the primary URS. In light of this, it can be concluded that SWL increases the risk of the impacted stone in the ureter and elevates the risk of mucosal laceration during URS; however, it can also be said that the complementary URS procedure after SWL is as successful and safe as the primary URS procedure. The limitation of this study is that control of any residual stone was performed with a plain abdominal X-ray and US on postop day 1 only, and a CT scan was not carried out for all patients.

AUTHORS' CONTRIBUTIONS

ED: Conceptualization, Data curation, Formal Analysis, Project administration, Supervision, Writing – original

draft, Writing – review & editing. **EÖ:** Conceptualization, Project administration, Writing – original draft. **DST:** Conceptualization, Project administration, Supervision. **ÖD:** Data curation, Formal Analysis, Project administration, Writing – original draft. **MK:** Data curation, Formal Analysis, Project administration, Writing – original draft. **UO:** Conceptualization, Data curation, Formal Analysis, Project administration, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Relationship between frailty, according to three frail scores, and clinical and laboratory parameters of the geriatric patients with type 2 Diabetes Mellitus

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SUMMARY

OBJECTIVE: Type 2 diabetes mellitus is associated with significant morbidity and mortality. The term "frailty in the elderly" has become increasingly important with the increase of the elderly population, especially in diabetic subjects. Frailty is established by various scoring scales, such as Edmonton, Frail, and Prisma-7 scores. We aimed to evaluate the association between frailty and clinical and laboratory parameters of the geriatric type 2 diabetic patients.

METHODS: Diabetic patients over 65 years of age who presented to outpatient internal medicine clinics of our institution between June 2020 and January 2021 were enrolled to the study. Edmonton, Frail, and Prisma-7 scores were administered to the subjects. Study parameters were compared between well-controlled and poorly controlled diabetic groups according to diabetes control level and between frail and non-frail groups, according to each frailty scores.

RESULTS: Frailty according to Edmonton score was associated with increased risks of hospitalization (p=0.005) and mortality (p=0.02). Frailty according to frail score was associated with increased risk of hospitalization (p=0.009). Frailty according to Prisma-7 score was associated with increased risk of mortality (p<0.001).

CONCLUSION: We suggest that Edmonton frail score is superior to Frail and Prisma-7 scores in determining frailty in geriatric patients with type 2 diabetes mellitus, since it is associated with both increased risk of hospitalization and mortality within 6 months.

KEYWORDS: Morbidity. Mortality. Hospitalization.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) and related disorders are among the top 10 of the all-cause mortality worldwide. Frailty is a novel term that refers to decline in physical capacity and cognitive functions in the elderly, which could be accelerated by T2DM. Falls, dementia, delirium, increased hospitalization, and increased mortality are associated with frailty[1]. Even minor stress can cause serious morbidity and mortality in frail individuals[1]. Thus, many tools have been developed to assess frailty reliably, including Edmonton frail scale[2], Prisma-7 score[3], and Frail scoring system[4].

In present study, we aimed to assess possible association between frailty (according to each of Edmonton frail scale, Prisma-7 score, or Frail score) and clinical and laboratory parameters in geriatric subjects with T2DM.

METHODS

Design, setting, and population

Patients diagnosed with type 2 diabetes who presented to Bolu Abant İzzet Baysal University Hospital between June 2020 and January 2021 were included in the study. Subjects under 65 years of age or with active infection or inflammatory disease, who had trauma or surgery in the last 1 month, patients with malignancy, and those who did not want to participate were excluded from the study. By questioning the history of each patient (e.g., diabetes duration, medications used, and concomitant diseases), physical examination findings, blood pressure, and height-weight measurements were recorded. Body mass index (BMI), HbA1c, complete blood count, urea, creatinine, glomerular filtration rate (GFR), serum electrolytes, albumin, aspartate (AST) and alanine (ALT) aminotransferases, lipoprotein fractions, and spot urine albumin/creatinine values were recorded. The patients were grouped as well or poorly regulated diabetics according to their HbA1c levels (i.e., HbA1c≤7.5 well-regulated; HbA1c>7.5 poorly regulated).

Edmonton, Frail, and Prisma-7 frailty scales were applied to the patients face-to-face using a questionnaire. Patients were grouped according to whether or not they were frail for each scale (14–18). Those who scored 0–7 on the Edmonton vulnerability scale were not frail and those who scored 8–17 were considered frail. Those who scored 0–2 on the frail scale were

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considered not frail, while those who scored 3–5 were considered frail. Those who scored less than 3 on the Prisma-7 vulnerability scale were considered not frail, while those who scored 3 and more than 3 were considered frail. Laboratory parameters and anthropometric measurements were recorded. The patients or their relatives were contacted again 6 months after participating in the study, and the mortality and morbidity status requiring hospitalization during this period were recorded. General characteristics, laboratory values, other parameters, and frailty scores of the patients were compared between those well and poorly controlled T2DM groups as well as between the frail and non-frail patients according to each of Edmonton, Prisma-7, and Frail scores.

Statistical analyses

Study data were analyzed using the SPSS statistical software (SPSS 15.0, IBM Co., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine whether the data fit into the normal distribution between the study groups. Normally distributed data were analyzed by t-test and expressed as mean±standard deviation (SD). Data that did not fit the normal distribution were compared with the Mann-Whitney U test and expressed as the median (min–max). Intergroup comparison of categorical variability was performed with the chi-square test and expressed as n (%). The sensitivity and specificity of the study variables in predicting mortality or morbidity were evaluated by ROC analysis test. A $p<0.05$ value was accepted for statistical significance level.

RESULTS

A total of 100 diabetic subjects were enrolled to the study, of which 34 (34%) were women and 66 (66%) were men. Frailty scores, number of hospital admissions within 6 months, and mortality rates of the well and poorly controlled DM groups were not statistically different (p=0.754 and p=1, respectively). Notably, 28% of the study population was frail according to Edmonton scale, 44% according to Frail score, and 19% according to Prisma-7 score. There were no gender difference between frail and non-frail groups (p=0.17 for Prisma-7; p=0.09 for Frail; and p=0.49 for Edmonton scores). The association between frailty according to the Edmonton, Frail, and Prisma-7 scales and mortality and the number of mortality is shown in Table 1. The laboratory parameters of the frail and non-frail subjects according to the Edmonton scale, Frail score, or Prisma-7 score were summarized in Table 2.

Frail subjects according to either Edmonton scale (p=0.002), Frail score (p=0.004), or Prisma-7 score (p=0.004) were older than those who were not frail. Waist circumference of the frail group according to Edmonton scale was increased compared to the non-frail patients (p=0.04). BMI of the frail population according to Frail score was increased compared to the non-frail patients (p=0.04).

The sensitivity and specificity of the Edmonton, Frail, and Prisma-7 scales in predicting mortality were evaluated by ROC analysis. The Edmonton frailty scale (7 points and above) showed mortality with 88% sensitivity and 66% specificity (AUC=0.78, p=0.008, 95%CI 0.6–1.0). Frail scale (3 points and above) predicted mortality with 75% sensitivity and 59% specificity (AUC 0.67; p=0.1; 95%CI 0.5–0.9). Prisma-7 score (4 points and above) showed mortality with 75% sensitivity and 89% specificity (AUC 0.83; p=0.002; 95%CI 0.7–1.0) (Figure 1).

DISCUSSION

Main findings of present study were

1. diabetic regulation, either poor or well control, was not associated with hospitalization, mortality nor frailty in elderly,
2. frail subjects according to Edmonton score had increased mortality and hospitalization compared to non-frail subjects while frail subjects according to Frail scale showed association only with hospitalization and those frail subjects according to Prisma-7 score showed association only with mortality, and

Table 1. Relationship of frailty according to Edmonton, Frail, and Prisma-7 scales with 6-month mortality and number of hospitalizations.

	Mortality (%)	p	Number of hospitalizations**	p
Frail according to Edmonton scale (n=28) Non-frail according to Edmonton scale (n=72)	n=5 (18) n=3 (4)	0.02	1 (0–4) 0 (0–4)	0.005
Frail according to Frail score (n=44) Non-frail according to Frail score (n=56)	n=6 (14) n=2 (4)	0.07	1 (0–4) 0 (0–4)	0.009
Frail according to Prisma-7 score (n=19) Non-frail according to Prisma-7 score (n=81)	n=6 (32) n=2 (3)	<0.001	1 (0–3) 0 (0–4)	0.06

**Number of hospitalizations were expressed as median (min–max) (n=number of subjects).
Significant p-values were expressed as bold characters.

Table 2. Laboratory parameters of the frail and non-frail subjects according to Edmonton, Frail, and Prisma-7 scores.

	Frail according to Edmonton scale** Non-frail according to Edmonton scale**	p	Frail according to Prisma-7 score** Non-frail according to Prisma-7 score**	p	Frail according to Frail score Non-frail according to Frail score	p
Albumin (g/L)	3.9 (2.9–4.5) 4 (2.7–5)	**0.01**	3.7 (2.9–4.5) 4 (2.7–5)	**0.003**	3.9 (2.7–4.6) 4 (2.9–5)	**0.03**
Urea (mg/dL)	55 (28–128) 41 (17–146)	**0.02**	56 (28–126) 41 (17–146)	**0.01**	52 (28–146) 41 (17–118)	**0.02**
Creatinine (mg/dL)	1.2 (0.75–2.4) 1 (0.65–5.5)	**0.048**	1.2 (0.76–2.2) 1 (0.65–5.5)	**0.08**	1.1 (0.7–5.5) 1 (0.7–3)	**0.24**
GFR (mL/dL/1.73 m^2)	55 (21–94) 67 (10–99)	**0.03**	54 (21–94) 66 (10–99)	**0.04**	56±23 66±21	**0.03**
Hb (g/dL)	12.2±2.2 12.8±1.8	**0.21**	11.7±2.3 12.8±1.8	**0.02**	12±2 13.1±1.7	**0.004**
LDL (mg/dL)	83±29 101±44	**0.04**	82 (28–174) 90 (28–255)	**0.13**	91±42 100±41	**0.31**
Triglyceride (mg/dL)	133 (66–322) 146 (59–512)	**0.13**	115 (66–322) 155 (59–512)	**0.008**	127 (66–512) 166 (59–352)	**0.01**
Total cholesterol (mg/dL)	153 (67–236) 171 (65–326)	**0.03**	148 (67–236) 165 (65–326)	**0.03**	164±50 179±51	**0.14**

**Data with normal distribution were expressed as mean±SD and data that did not fit the normal distribution were expressed as the median (min–max). Significant p-values were expressed as bold characters.

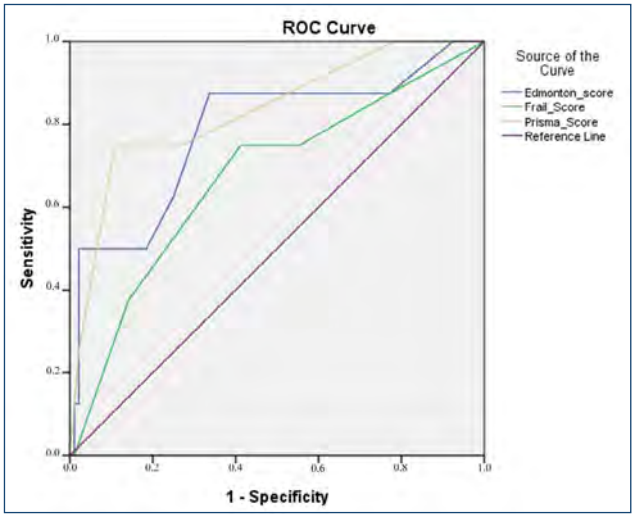

Figure 1. Receiver operative characteristics (ROC) curves of Edmonton, Frail, and Prisma-7 scales in predicting mortality.

3. Edmonton score greater than 6 points had the best sensitivity and Prisma-7 score over 3 points had the best specificity in predicting mortality.

We found that diabetic control level was not associated with frailty in diabetic subjects over 65 years. This was valid for determination of frailty with all three frailty scales. Being diabetic, well or poorly controlled, alone will not influence frailty. In the literature, there are studies revealing that poorly controlled diabetes leads to frailty by causing loss of functionality in many organs and systems with its accompanying complications[5]. HbA1c levels of the diabetic subjects were associated with frailty in Bilgin et al.'s study[6]. However, low HbA1c levels are also associated with increased mortality and risk of hospitalization in elderly patients with T2DM. In addition, Yanagita et al. reported that tight glycemic control was a risk factor for frailty in elderly patients[7].

Studies have shown that every 1 g/dL decrease in hemoglobin concentration increases the risk of frailty approximately 2 times according to the Frailty in Brazilian seniors (FIBRA) study conducted in Brazil[8]. Lower hemoglobin values in older participants were reported in our study, which is in line with the literature data. In addition, we found that participants with high frailty scores, according to all three scales, have more serious anemia.

Roshanravan et al reported the prevalence of frailty was 14% in subjects with stages 1–4 chronic kidney disease, which was almost twice of the prevalence of frailty in the control group without kidney disease[9]. Glomerular filtration rate (GFR) levels were lower and urea values were higher in frail subjects according to Edmonton scale, Frail score, or Prisma-7 score compared to non-frail subjects.

A decrease in lean body mass causes sarcopenia and frailty. Studies have shown that there was an inverse relationship between serum albumin level and frailty. Low serum albumin

level was suggested as an independent risk factor for frailty[10]. Similarly, we observed lower serum albumin levels in frail subjects according to all of three frailty scales compared to non-frail diabetics in present study.

Defining frailty and taking appropriate measures to prevent it become more important recently than before as the average life expectancy is getting longer for all populations[1,11]. Detection of the frail elderly enables to predict prolonged hospitalization and mortality, even when faced with moderate to mild stress situations[12]. In our study, unlike the Prisma-7 and Frail scales, frailty according to Edmonton score was found to be associated with both increased mortality and risk of hospitalization. Similarly, in a study from Vietnam, frailty according to Edmonton score was found to be associated with both prolonged hospitalization and 6-month mortality[13]. Frailty according to Frail score was reported to be associated with increased risk of hospitalization but mortality in present study. In accordance, Chong et al. found that frailty according to the Frail score predicted mortality in hospitalized patients successfully[14].

It is a fact that malnutrition and reduced muscle mass cause frailty. However, studies have also shown that excess weight also cause deterioration in metabolic balance and inactivity, paving the way for frailty[15]. Villareal et al. reported that physical exercise and weight loss can reduce frailty in older obese individuals[16]. A total of 4984 subjects older than 60 years were studied and higher body fat ratio and waist circumference measurements were found in frail subjects compared to non-frail age and sex-matched controls[17]. Moreover, Hubbard et al. suggested that increased waist circumference and abdominal fat were associated with frailty, even in low-weight individuals[18]. Consistently, we reported that abdominal obesity was more common in frail group compared to the non-frail diabetics in present study.

Increased 6- and 12-month mortality has been reported in frail subjects according to Prisma-7 score compared to non-frail elderly[3]. Similarly, in the present study, mortality was more common in frail group according to Prisma-7 score than the mortality in non-frail group.

We found lower AST and ALT values in patients who were frail according to Edmonton scale compared to non-frail group. The reduction in transaminase levels is thought to be associated with frailty as a result of malnutrition. Considering that pyridoxine (vitamin B6) is a cofactor for transaminases, a decrease in AST and ALT levels is expected in B6 deficiency[19]. Le Couteur et al. revealed that ALT may be a new biomarker of aging and is associated with frailty[20].

Frailty is associated not only with T2DM, as presented in our work, but also with other chronic conditions, such as cancer[21,22].

The fact that our study was a single-center study and carried out in a relatively small cohort limits the generalization of its results. However, to the best of our knowledge, it is the first study in which three separate frailty scales were evaluated in geriatric diabetic subjects and that observed the association of frailty scores with hospitalization and mortality.

CONCLUSION

We suggest that Edmonton frail score is superior to Frail and Prisma-7 scores in determining frailty in geriatric patients with T2DM, since it is associated with both increased risk of hospitalization and mortality within 6 months.

AUTHORS' CONTRIBUTION

GA: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **SA:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Relationship between contrast-induced nephropathy and long-term mortality after percutaneous coronary intervention in patients with chronic coronary total occlusion

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SUMMARY

OBJECTIVE: Intervention in chronic total occlusion lesions involves long procedure time, a serious contrast load, and complex procedures. In this study, we aimed to investigate mortality rate of patients who had procedural coronary angiography done for chronic total occlusion lesions in coronary angiography series and who developed contrast-induced nephropathy.

METHODS: A total of 218 patients with chronic total occlusion lesion in at least one coronary artery, from three different medical centers, who underwent procedural coronary angiography were recruited for the study. Patient population was divided into two groups: those who developed contrast-induced nephropathy and those who did not. Mortality due to all causes was investigated between both groups throughout a 100-month follow-up.

RESULTS: Mean age of patients with incidence of contrast-induced nephropathy was 66.7±11.8, and 23.8% of them were comprised by female. We found a significantly higher mortality in long-term follow-up in the patient group with contrast-induced nephropathy (42.9 vs. 57.1%, p=<0.001). According to Kaplan-Meier analysis performed additionally, survival during follow-up was significantly shorter in this group and, in logistic regression analysis, it was an independent predictor of mortality (OR 11.78; 95%CI 3.38–40.9).

CONCLUSION: We identified that the development of contrast-induced nephropathy is associated with long-term mortality. It might be possible to reduce adverse events with prophylactic approaches before the procedure and close follow-up of such patients after the procedure.

KEYWORDS: Coronary occlusion. Acute renal injury. Mortality. Atherosclerosis.

INTRODUCTION

Atherosclerosis forms the basis of coronary artery disease, and it is a progressive process accompanied by inflammation, showing systemic involvement[1]. Chronic total occlusion (CTO) of coronary arteries is described as complete occlusion of vein lumen for a minimum of 3 months (TIMI 0 flow)[2]. CTO is commonly seen in the range of 18–52% of patients undergoing coronary angiography (CAG)[3-5]. Revascularization of CTO lesions by percutaneous coronary intervention (PCI) leads to improved left ventricular functions and positive contributions to mortality. Many studies have demonstrated clinical benefits of revascularization of CTO lesions[6-8]. Recently, progress has been made in PCI treatment methods in CTO lesions[9,10]. With increased PCI experience in CTO lesions, antegrade and retrograde methods have increased the success rate of procedures[10]. Nevertheless, success rates have decreased due to complex CTO lesions, long-term exposure to radiation, and the use of large volumes of contrast agent[11]. Contrast-induced nephropathy (CIN) is described as an increase of 0.5 mg/dL or ≥25% in baseline serum creatinine levels after 48–72 h of exposure to contrast agent. Its incidence is expected to be in the range of 1–6% in general population and it is even higher relative to protein kinase G (PKG)[12]. However, frequency of CIN has increased in patients with impaired kidney function, and this percentage was more than 50% in high-risk patients[13].

Patients with CTO who undergo revascularization are usually older and more likely to have diabetes, multiple coronary artery disease, low left ventricle ejection fraction (LVEF), and poor renal function[14]. Accordingly, such patients are at higher risk for CIN, one of the major complications after the procedure[15].

Previous studies reported an incidence of CIN after PCI of about 6–7% in CTO lesions[11]. CIN accounts for about 11% of acute renal failure (ARF) cases and is the third leading cause of hospital-acquired ARF. Hospital mortality rate has been reported to be 22% of the patients developing CIN and 1.4% of those who does not[16].

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Studies in the literature mainly focus on scoring systems estimating development of contrast nephropathy and predictors; in contrast, in this study, we aimed to investigate long-term mortality rate in patients with CTO lesions who had procedural CAG and developed CIN.

METHODS

A total of 218 patients with CTO lesion in at least one coronary artery, from three different medical centers, who underwent procedural CAG between February 2010 and April 2012 were recruited for this study. It was planned as a multicenter, retrospective, and cross-sectional study. CAG images of the patients were examined and chosen by three different cardiologists. Demographic characteristics, laboratory findings, echocardiography findings, and follow-up data of the patients were obtained from the hospital's database. Mortality throughout 100-month follow-up process was considered primary end point. Study consent was obtained from local ethics committee in accordance with 2013 Declaration of Helsinki. Inclusion criteria were patients who had CTO in at least one coronary artery and underwent procedural CAG, EF≥40, and age between 18 and 90 years. Exclusion criteria were EF≤40, end-stage kidney failure, history of renal transplantation, pregnancy, hypotension, the use of intra-aortic balloon pump, rheumatic and connective tissue diseases, malignancy, and active infection. A non-ionic, low-osmolality contrast agent was used in all patients included in the study. Angioplasty technique and the amount of contrast agent used were at the discretion of the physician. All patients included in the study were given hydration with a liquid containing 1200 mg of *N*-acetyl cysteine in a minimum 1000 cm^3 of 0.9% isotonic sodium chloride solution before and after the procedure.

Definitions

Blood samples were collected from anterior facet of anterior arm of all study patients while they were in supine position after their admission to cardiology clinic. Blood parameters were measured in the serum separated by centrifuging at 3000×g cycles at room temperature. Patients with blood pressure above 140/90 were considered hypertensive, and those below 90/60 were considered hypotensive. Patients with low-density lipoprotein (LDL) value of 160 mg/dL were considered to have hyperlipidemia. In echocardiography laboratory, patients' LVEF were measured using transthoracic 2D echocardiography (Vivid S6, GE Medical Systems, USA). CIN was described as an increase of 0.5 mg/dL or ≥25% in serum creatinine concentration in the first 48–72 h after CAG. A glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2, determined by Cockcroft-Gault formula, was considered renal failure.

Statistical analysis

The IBM SPSS version 24.0 software package was utilized for analysis. Baseline continuous variables were presented as means (standard deviation [SD]) or median with the first and third quartiles (Q1–Q3) depending on the distribution of data. Categorical variables were described as frequency and percentage. Normal distribution of variables was analyzed through Kolmogorov-Smirnov and Shapiro-Wilk tests. Logarithmic transformation was made since the variables showed abnormal wide distributions for blood parameters, namely, platelets, glucose, neutrophils, and lymphocytes. Kaplan-Meier test was used to analyze the correlation between development of CIN and survival during a 100-month follow-up period. Continuous variables were compared through Student's t-test or Mann-Whitney U test, as appropriate. Univariable analysis was applied for continuous variables, while chi-square or Fisher's exact test for categorical variables. Both univariable and multivariable logistic regression analyses were performed to evaluate the parameters affecting the development of CIN. Univariable and multivariable Cox regression analyses were used to identify predictors of mortality associated with all reasons. Only parameters with p≤0.1 were included in the evaluation during multivariate regression analyses. A p<0.05 was considered statistically significant for all tests.

RESULTS

Demographic data and accompanying diseases of all the study patients are summarized in Table 1. Mean age of patients who developed CIN was significantly higher than those who did not (66.7±11.8 vs. 62.4±11.1, p=0.027). Development of CIN was significantly higher in peripheral artery patients and in those with low EF (14.3 vs. 3.4%, p=0.005 and 43.7±11.8 vs. 49±10.6, p=0.003) (Table 1).

We found a significantly higher mortality rate in the CIN patient group (57.1 vs. 42.9%, p=<0.001) (Table 1).

The patients are compared for laboratory data in Table 2. C-reactive protein (CRP) and LDL values were significantly higher in the CIN group [0.62 (0.30–6.8) vs. 0.38 (0.10–2.5), p=0.029 and 117±33.8 vs. 103±38, p=0.035] (Table 2).

Regression analyses of CIN development during a 100-month follow-up period by demographic characteristics, clinical properties, and laboratory parameters are summarized in Table 3. Multivariable analysis was applied to parameters with a p≤0.1 after univariable analysis (Table 3).

Table 1. Demographic, clinical characteristics, and laboratory parameters of groups.

Parameters	Patients developing CIN (n=42)	Patients not developing CIN (n=176)	p-value
Age, years	66.7±11.8	62.4±11.1	**0.027**
Female, n (%)	10 (23.8)	49 (27.8)	0.597
Hypertension, n (%)	19 (45.2)	59 (33.5)	0.155
Hyperlipidemia, n (%)	3 (7.1)	8 (4.5)	0.490
Diabetes mellitus, n (%)	17 (40.5)	52 (29.5)	0.171
Chronic kidney disease, n (%)	4 (9.5)	6 (3.4)	0.089
Smoking, n (%)	15 (35.7)	43 (24.4)	0.137
Cerebrovascular disease, n (%)	3 (7.1)	3 (1.7)	0.053
Peripheral arterial disease, n (%)	6 (14.3)	6 (3.4)	**0.005**
Left ventricle ejection fraction, n (%)	43.7±11.8	49±10.6	**0.003**
Mortality, n (%)	24 (57.1)	31 (42.9)	**<0.001**
Follow-up period (IQR)	42 (21.2-65)	33 (21-57.7)	0.539

CIN: contrast-induced nephropathy; IQR: interquartile range. Bold indicates significant value.

Table 2. Logistic regression analysis of development of contrast-induced nephropathy in 100-month follow-up.

Parameters	Patients developing CIN (n=42)	Patients not developing CIN (n=176)	p-value
White blood cell, $10^3/\mu L$	10.0±3.5	9.6±3.5	0.540
Hemoglobin, gr/dL	13.4±1.9	13.6±1.8	0.462
Neutrophile, $10^9/L$	7.1±3.4	6.6±3.5	0.409
Lymphocyte, $10^9/L$	1.9±0.86	2.1±0.89	0.087
Platelet, $10^3/\mu L$	251±91	245±70	0.641
Glucose, mg/dL	134 (100-254)	118 (99-187)	0.389
Creatinine, mg/dL	1.09±0.32	1.00±0.46	0.202
Glomerular filtration rate, mL/min	74.8±25.0	82.9±24.5	0.058
Sodium, mEq/L	136.4±3.5	135.5±10.5	0.576
Potassium, mEq/L	4.3±0.65	4.6±2.6	0.459
Albumin, mg/dL	3.5 (3-3.9)	3.7 (3.3-3.9)	0.182
C-reactive protein, mg/dL	0.62 (0.30-6.8)	0.38 (0.10-2.5)	**0.029**
Total cholesterol, mg/dL	188±41	179±50	0.281
HDL, mg/dL	37.5±7.4	40±11	0.151
LDL, mg/dL	117±33.8	103±38	**0.035**
Triglyceride, mg/dL	137 (96-198)	145 (98-213)	0.851

CIN: contrast-induced nephropathy; HDL: high-density lipoprotein; LDL: low-density lipoprotein. Bold indicates significant value.

Table 3. Regression analyses of development of CIN during a 100-month follow-up period by demographic characteristics, clinical properties, and laboratory parameters.

Parameters	Univariable analysis		Multivariable analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.03 (1.00–1.06)	**0.029**	1.04 (1.0–1.08)	**0.048**
Gender	0.81 (0.37–1.77)	0.598	–	
Hypertension	0.61 (0.30–1.20)	0.157	–	
Diabetes mellitus	0.61 (0.30–1.23)	0,173	–	
Chronic kidney disease	0.33 (0.09–1.24)	0.103	0.37 (0.06–2.15)	0.274
Smoking	0.58 (0.28–1.19)	0.140	–	
Peripheral arterial disease	4.72 (1.44–15.48)	**0.010**	0.13 (0.02–0.75)	**0.023**
Left ventricle ejection fraction	0.95 (0.92–0.98)	**0.004**	0.96 (0.92–1.0)	0.054
Hemoglobin, gr/dL	0.93 (0.77–1.12)	0.461	–	
Platelet, $10^3/\mu L$	1.30 (0.09–17.3)	0.839	–	
Glucose, mg/dL	3.65 (0.74–17.8)	0.109	1.64 (0.21–12.4)	0.629
C-reactive protein	1.13 (1.03–1.25)	**0.007**	1.10 (1.0–1.22)	**0.048**
Total cholesterol	1.0 (0.99–1.01)	0.281	–	
Number of sick vessels (multiple total vessels are present/not present)	0.49 (0.21–1.12)	0.094	0.53 (0.18–1.52)	0.240
Rentrop (good/poor)	1.31 (0.66–2.60)	0.436	–	

Bold indicates significant value.

Cox regression analysis performed among all factors affecting mortality in CTO patient population revealed that age, diseases such as hypertension (HT), diabetes mellitus (DM), and CIN, EF and hemoglobin results were significant in univariable analysis. In multivariable analysis (parameters with p≤0.1 were included), it was concluded that CIN incidence in CTO patient population is an important and independent predictor of mortality among all parameters (OR 3.02; 95%CI 1.41–6.45; p=0.004) (Table 4).

Kaplan-Meier analysis conducted to investigate the relationship between incidence of CIN and survival during follow-up period showed that mortality increased significantly with increasing follow-up period in patients developing CIN (log-rank p<0.001) (Figure 1).

DISCUSSION

In this study, we identified that mortality significantly increased in long-term follow-up of patient groups requiring complex procedures such as CTO with incidence of CIN after the procedure. Development of CIN with increased procedure time in CTO lesions is an important problem. Previous studies reported its frequency in approximately 13% after coronary procedures. It has been accompanied by prolonged hospital stays, increased death rates, and costs[17]. In our study, patients who developed

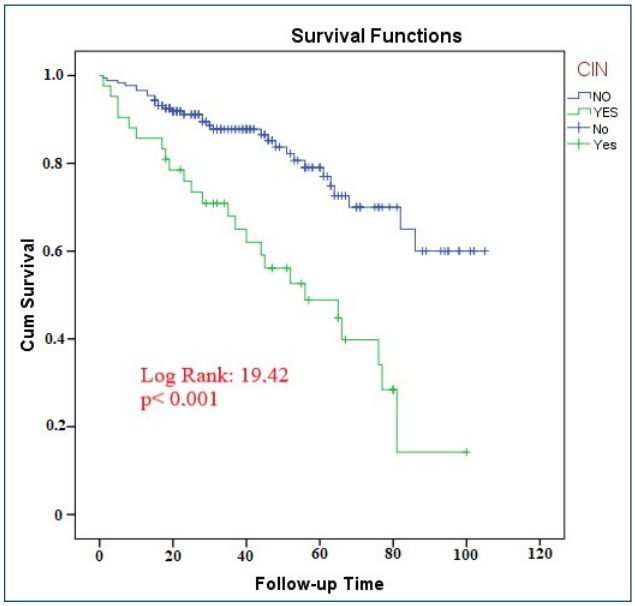

Figure 1. Correlation Between Development of CIN and Survival During a 100-Month Follow-Up

Table 4. Cox proportional hazard regression analysis of risk of death regression model during 100-month follow-up in the study population.

Parameters	Univariable analysis		Multivariable analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.04 (1.01–1.06)	**0.001**	1.00 (0.97–1.04)	0.629
Gender	0.89 (0.50–1.58)	0.707	–	
Hypertension	1.71 (1.01–2.91)	**0.046**	1.59 (0.78–3.23)	0.197
Diabetes mellitus	1.83 (1.07–3.11)	**0.025**	1.18 (0.40–3.48)	0.753
Chronic kidney disease	2.26 (0.90–5.69)	0.083	0.80 (0.15–4.12)	0.792
Smoking	1.04 (0.59–1.85)	0.876	–	
Peripheral arterial disease	1.88 (0.80–4.40)	0.145	–	
Left ventricle ejection fraction	0.95 (0.93–0.97)	**<0.001**	0.99 (0.95–1.02)	0.545
CIN development	3.14 (1.83–5.37)	**<0.001**	3.02 (1.41–6.45)	**0.004**
Hemoglobin	0.81 (0.70–0.93)	**0.003**	0.80 (0.65–0.98)	**0.031**
Neutrophile	2.80 (0.82–9.55)	0.100	–	
Lymphocyte	0.16 (0.04–0.65)	**0.010**	0.35 (0.05–2.44)	0.292
Platelet	0.44 (0.06–3.29)	0.426	–	
Glucose	4.97 (1.51–16.38)	**0.008**	2.22 (0.23–21.41)	0.489
C-reactive protein	1.09 (1.02–1.16)	**0.005**	1.02 (0.94–1.10)	0.561
Total cholesterol	0.45 (0.03–5.96)	0.552	–	
Number of sick vessels	0.60 (0.30–1.19)	0.147	–	
Rentrop (good/poor)	0.96 (0.56–1.64)	0.891	–	

OR: odds ratio; CI: confidence interval; CIN: contrast-induced nephropathy.
Bold indicates significant value.

CIN after CTO procedures were from an older age group with lower EF rates. Though the number of patients with peripheral artery disease was relatively lower, we observed significantly more development of CIN in that group. We reported relatively more CIN incidence, albeit not significant, for patients with a history of HT, DM, chronic kidney disease (CKD), and smoking. These results are in good agreement with previous studies reported in the literature. CTO procedures are longer and more complex, requiring the use of larger volumes of contrast agent. One of the most important factors establishing the procedure time and the amount of contrast agent is the operator's experience. Our medical centers are experienced and have been applying the procedure to CTO lesions for a long time. Hydration with normal saline before PCI continues to be the most effective approach to prevent CIN. It abates direct toxic effects of the contrast agent on epithelial cells, decreasing the concentration and viscosity of the contrast agent in tubular lumen[18]. Hydration is usually applied to patients in all risk categories; however, it is considered a requirement in the management of patients with an eGFR <60 mL/min/1.73 m^2. Larger volume may accelerate the elimination of contrast agent, directly reduce renal toxicity, and decrease secretion of vasoconstrictors and reactive oxygen species. Many studies have demonstrated that hemoglobin, hyperglycemia, and high-sensitivity CRP are independent risk factors[19]. In our study, CRP value was significantly higher (p=0.029), and also fasting glucose values were partially higher, though not significantly, in patients with CIN. Contrast medium may enhance oxygen affinity of hemoglobin and impair oxygen transmission to peripheral tissues. Hyperglycemia may result in increased production of free oxygen radicals with increasing oxidative stress[20,21]. Further, in Mehran scoring, risk factors for mortality include DM, congestive heart failure, volume of contrast agent, age >75 years, and the use of intra-aortic balloon pump[22]. Shacham et al. conducted a study on myocardial infarction patients with preserved EF≥50 and elevated ST segment. They strongly demonstrated that older patients with poorer kidney function and history of heart failure have higher rates of mortality associated with developing acute kidney damage, hospitalization, and all causes, compared to partially younger patients with better kidney function and no heart failure[23]. Any acute reduction in kidney perfusion due to low cardiac output may result in ischemia and hypoxia in kidneys. Moreover, direct kidney damage, reactive oxygen species, and activation of sympathetic nervous system play a critical role in CIN development[24]. In our study, mortality rate in patients who developed CIN (57.1%) was higher than those who did not (42.9%). The results of our study are consistent with previous findings in the literature showing a strong association between CIN and mortality[25]. Therefore, renal functions of high-risk patients exposed to higher contrast load who have undergone a more complex and longer procedure due to conditions such as CTO must be monitored at admission and discharge. The most commonly accepted strategies in preventing CIN include exercising care in selecting patient profile and contrast volume and applying hydration.

There are limitations in this study. First, it was designed as retrospective, so bias was inevitable, and in addition, the sample size was not large enough.

CONCLUSION

During intervention in lesions with a more complex procedure, requiring more contrast load, such as CTO, the rate of developing CIN increases in the presence of risk factors. CIN development is associated with hospital and long-term mortality. It might be possible to reduce adverse events with prophylactic approaches before the procedure and close follow-up of such patients after the procedure.

AUTHORS' CONTRIBUTIONS

TG: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **AA:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **MD:** Formal analysis, Writing – original draft. **MÖ:** Conceptualization, Data curation, Writing – original draft. **BA:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Urethral monopolar cauterization: alternative infravesical obstruction model in male rats

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SUMMARY

OBJECTIVE: We aimed to determine which method gives the most consistent results between urethral monopolar cauterization and standard urethral partial ligation methods for the urethral obstruction model.

METHODS: Thirty male rats were randomly divided into control, partial ligation, and monopolar cauterization groups. Six weeks after experimental procedures, the experimental groups were evaluated cystometrically, biochemically, and histologically.

RESULTS: According to the cystometric results, bladder capacity, baseline bladder pressure, and compliance data of the monopolar cauterization group were higher than those of the partial ligation and monopolar cauterization groups ($p<0.05$ and $p<0.01$, respectively). As a biochemical evaluation, malondialdehyde levels in bladder tissues of group control were higher than partial ligation and monopolar cauterization groups ($p<0.05$ and $p<0.01$, respectively). The collagen type I level of the control group was higher than the partial ligation and monopolar cauterization groups ($p<0.01$ and $p<0.05$, respectively). Collagen type III levels of the monopolar cauterization group were higher than those of the control group ($p<0.01$), but the Collagen type I/Collagen type III and transforming growth factor-β levels of the monopolar cauterization group were significantly lower than those of the control group ($p<0.001$). As a histological evaluation (hematoxylin and eosin), fibrosis in the lamina propria was more prominent in the monopolar cauterization group than in the control group ($p<0.05$). In addition, the muscular thickness was higher in the monopolar cauterization group compared with control and partial ligation groups ($p<0.001$ and $p<0.01$, respectively).

CONCLUSION: The needle-tipped monopolar cauterization of the posterior urethra may be the method of choice for creating a chronic infravesical obstruction model of infravesical obstruction in male rats.

KEYWORDS: Bladder. Cauterization. Fibrosis. Ligation. Urethral obstruction.

INTRODUCTION

The use of animal models mimicking infravesical obstruction-related urinary symptoms and physiological alterations in males has a critical role in evaluating the potential therapeutic methods[1]. Several methods in animal models have been reported since 1984 and female rats have been more frequently used because of their simpler anatomy and the straightforwardness of the procedures due to absence of the accessory sex organs. In the studies with male rats, midprostatic urethral obstruction with retropubic approach was more common[2-4]. However, probable interaction with the intra-abdominal organs increased the morbidity in the retropubic approach.

Melman et al.'s study, in which urethral partial ligation (PL) with perineal approach and known midprostatic obstruction methods were compared in animal models, was the first study including cystometric and histological examinations. As a result of this study, it was reported that the PL method was superior causing less morbidity[5]. The PL method is the standard infravesical obstruction model in male rats and used for many years. However, this may not be an effortless and speedy method because it requires master suturing skills.

A new, simpler, and faster obstruction model has recently been reported as an alternative to the standard PL model[6]. In this study, the obstruction model was defined by the urethral monopolar cauterization (MC) method and partial obstruction was proven by imaging (retrograde urethrography) performed at the end of the second week after the application. However, this novel model has not been compared with the standard PL method yet.

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We compared the standard PL procedure with the promising and applicable new model to determine which one of the two methods produced the most consistent outcome and demonstrate the efficacy and adverse effects of both procedures regarding physiological, histological, and molecular attitudes.

METHODS

Animal model for infravesical obstruction

All experimental protocols were performed according to the University of Health Sciences Animal Care and Use Committee Guidelines (protocol number 2019-07/01). A total of 30 male Sprague-Dawley rats (350–400 g) were randomly divided into 3 groups with 10 rats in each group as follows: control (C), standard urethral PL, and urethral MC. We administered 100 mg/kg ketamine hydrochloride and 30 mg/kg chlorpromazine intraperitoneally for anesthesia induction and used a 23-gauge catheter sheath for transurethral catheterization[7]. Then, we exposed the posterior part of the urethra through a penoscrotal midline incision. Standard urethral PL procedure, which was previously described by Melman and colleagues, was performed in the PL group[5]. In this procedure, a midline vertical incision of 1 cm was made from the penoscrotal junction to the midscrotum to gain access to the bulbous urethra. The urethra was then isolated from the cavernous bodies, and a sterile metal bar of 0.91 mm in diameter was placed on the prostatic urethral surface. The 3-0 polypropylene suture was secured, and the bar was removed leaving the prostatic urethra partially obstructed. A 4-0 silk suture was used to reapproximate the muscle layer, and a 4-0 nylon suture was used to close the skin.

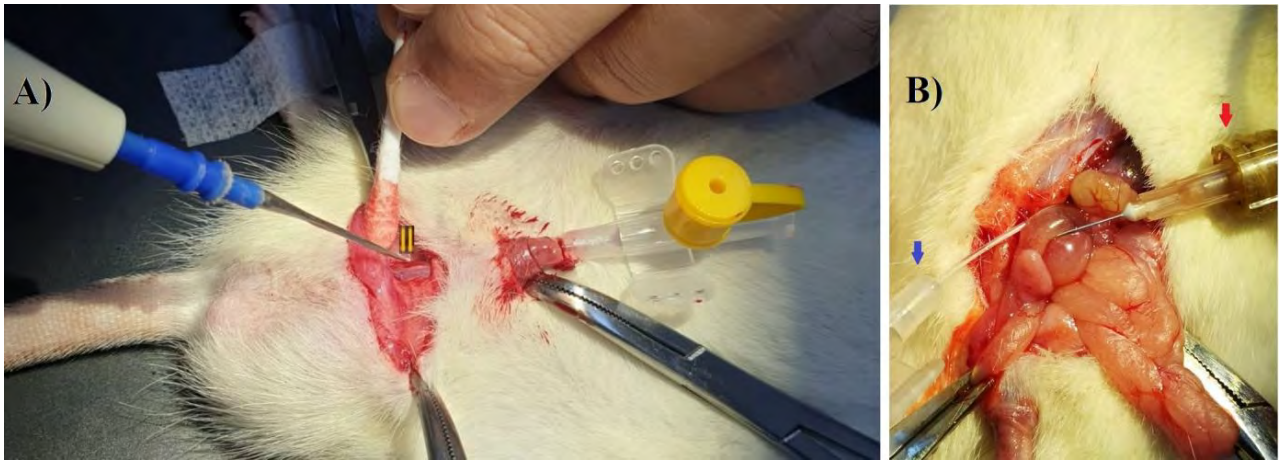
In the MC group, we carried out the monopolar urethral cauterization procedure as described by Tavukcu and colleagues in 2017. In this model,

1. transurethral catheterization with a 23-gauge catheter was performed in the same manner following the anesthetic induction with an intraperitoneal injection of 100 mg/kg ketamine hydrochloride and 30 mg/kg chlorpromazine,
2. the posterior urethra was exposed through a penoscrotal incision of approximately 15 cm,
3. a coagulation current with a level of 10 W was applied for 1 second with the guidance of the catheter at two locations in 2 mm distance, and
4. the procedure was completed after removal of the urethral catheter (Figure 1A).

In both procedures, we closed the skin incision with interrupted monofilament sutures. The C group had only a sham operation.

Cystometric analysis

After 6 weeks, cystometric analysis was performed on all rats. Under general anesthesia by using an intraperitoneal injection of 100 mg/kg ketamine hydrochloride and a 30 mg/kg chlorpromazine, two angiographic catheters were inserted percutaneously into the bladder (24 gauge, 1–2 cm, Baxter Healthcare AS)[8]. One of the catheters was for infusion and the other was for a pressure transducer and an amplifier unit (COMMAT Pharmacology and Physiology Instruments, Ankara, Turkey) (Figure 1B). The amplifier was connected to a data acquisition module (MP35 data acquisition system, Ankara, Turkey).



(A) The procedure of urethral monopolar cauterization. A coagulation current was applied at two locations, each at a 2-mm distance on the posterior urethra (yellow arrows point these locations). (B) The cystometric evaluation by placing the suprapubic catheter and the transducer. Blue arrow: transducer, red arrow: filling catheter.

Figure 1. Catheter applications to experimental animals.

The basal bladder volume was calculated by evacuating the urine from the bladder manually with a syringe. While infusion to the bladder began manually, the other catheter allowed the pressure to be recorded on a computer with the Biopac Student Lab PRO recording software (Biopac Systems Inc., CA, USA). Basal bladder pressure and the maximum capacity bladder pressure were calculated from the records in mmHg and the results were transformed to cm H_2O. The maximum bladder capacity (BC) was noted and the procedure was completed. The bladder compliance was calculated as the bladder pressure per 1 mL

Malondialdehyde levels

We determined the levels of MDA, the end product of lipid peroxidation, in bladder tissue homogenates using the Ohkawa et al.'s method9. The findings were expressed in nanomoles per milligram of protein.

Quantitative Real-Time Polymerase Chain Reaction analysis

Total RNAs were isolated using the RNAzol RT solution (MRC, USA) according to the manufacturer's instructions for quantifying mRNA expression in bladder tissues. After completion of RNA isolation, RNA concentration and purity were calculated using NanoDrop 2000 (Thermo Scientific, USA). For this purpose, 1 µL RNA samples were pipetted in the device for the determination of 260/280 and 260/230 ratios. Concentrations of all RNA samples were equalized before reverse transcription. RNAs were reverse transcribed into cDNA using Script cDNA Synthesis Kit (Jena Bioscience, Germany). The resulting cDNA was amplified by qRT-PCR using qPCR EvaGreenMaster (Solis BioDyne, Estonia). The real-time conditions were carried out on the CFX-96 RT-PCR System (Bio-Rad, USA) as follows: 95°C for 12 min and then 35 cycles of 95°C for 15 s; 55°C for 20 s, and 72°C for 20 s. Relative mRNA transcripts levels were calculated according to the $2^{-\Delta\Delta CT}$ method, and the relative expression of each gene was normalized to that of glyceraldehide-3-phosphate dehydrogenase (GAPDH).

Histological analysis

After the experimental protocol was complete, the animals were sacrificed and the bladder tissues from each of the groups were fixed in 10% formalin for 48 h, paraffin-embedded, and cut into 4 µm sections, and the samples were stained with H&E and Masson's trichrome (MT) for morphological examination. Bladder tissue fragments were investigated for muscular thickness in µm and a semi-quantitative scoring was performed by the pathologist in order to determine the presence of fibrosis and congestion as follows: absent "0," low "1," and high "2".

Statistical analysis

The Graphpad Prism 7 software program (CA, USA) was used for statistical analysis. For normally distributed data, the Tukey's test was used, and for data that was not normally distributed, the Dunn's multiple comparison test was used. The mean±SD and median (interquartile range [IQR]; 25–75th percentile) were used to express the results.

RESULTS

There was not any mortality in the groups in the preoperative, operative, and postoperative periods. The mean duration of the surgical procedures was 14 and 10 min in PL and MC groups. The duration of the procedure was approximately 4 min shorter in the MC group.

The complete cystometric data evaluation was performed to all animals. The maximum BC and the compliance values were significantly higher in the PL and MC groups than the C group. The highest values were in the MC group ($p<0.01$), but the baseline bladder pressure (BBP) was the lowest in the MC group ($p<0.01$) (Table 1).

The PL and MC groups exhibited a significant increase in MDA compared to the C group ($p<0.05$ and $p<0.01$, respectively) (Table 1).

In the bladder tissues, COL1A1 expression was found to be significantly higher in PL and MC groups compared with the C group using qRT-PCR test ($p<0.01$ and $p<0.05$, respectively). When the COL3A1 expression was examined, it was found to be significantly higher only in the MC group compared with the C group in the bladder tissues utilizing the qRT-PCR analysis ($p<0.01$). Transforming growth factor-β (TGF-β) expression was found to be significantly lower only in the MC group compared with the C group ($p<0.001$) (Table 1).

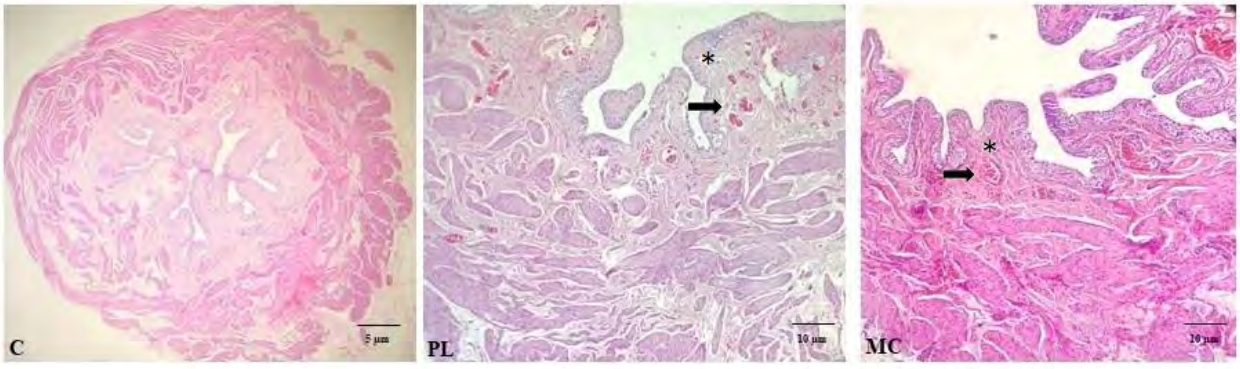
In the prostate tissues, COL1A1/COL3A1 expression was found to be significantly higher only in the MC group compared with the C group, while TGF-β expressions were found to be significantly lower only in the MC group compared with the C group using qRT-PCR test ($p<0.05$ and $p<0.001$, respectively) (Table 1).

In the histological examination of the bladder, the C group demonstrated a regular morphology of the lamina propria and the muscular layer and the PL group had a similar morphology with the C group having slightly increased congestion and fibrosis. Fibrosis in the lamina propria was more prominent in the MC group than in the C group ($p<0.05$). In the MC group, the muscular thickness was higher compared with C and PL groups ($p<0.001$ and $p<0.01$, respectively) (Figure 2).

Table 1. Results of the cystometric and biochemical evaluations of the bladder tissues and results of the biochemical evaluations of the prostate tissues.

Bladder tissues				
Group → cystometric variables ↓	**C**	**PL**	**MC**	**p**
BC	0.25 (0.17–0.3)	0.47 (0.415–0.515)	0.5775 (0.445–0.6825)	C–PL *p<0.05 C–MC **p<0.01
BBP	19.03 (16.65–22.09)	15.63 (13.93–17.33)	15.29 (13.59–18.01)	C–PL *p<0.05 C–MC **p<0.01
MAXBCP	22.09 (20.39–24.13)	20.05 (16.99–22.77)	20.73 (17.67–25.49)	ns
MAXBCP/BBP	3.06 (1.7–4.08)	4.42 (1.7–6.8)	5.44 (3.06–7.82)	ns
Compliance	0.0116 (0.007–0.014)	0.0238 (0.020–0.028)	0.0286 (0.0216–0.0374)	C–PL *p<0.05 C–MC **p<0.01
Biochemical variables ↓				
MDA (nmoL/mg protein)	7.327 (3.368–12.91)	25 (19.55–34.54)	28.31 (22.25–36.32)	C–PL *p<0.05 C–MC **p<0.01
COL1A1/GAPDH	1±0	0.725±0.08385	0.7917±0.1118	C–PL **p<0.01 C–MC *p<0.05
COL3A1/GAPDH	1±0	1.063±0.5301	1.345±0.1674	C–MC **p<0.01
COL1A1/COL3A1	1±0	0.8272±0.3797	0.5917±0.07429	C–MC ***p<0.001
TGF-β/GAPDH	1±0	0.7729±0.2835	0.4829±0.07761	C–MC ***p<0.001
Prostate tissues				
Group → biochemical variables ↓	C	PL	MC	Post-hoc p
COL1A1/GAPDH	1±0	1.733±0.5523	1.403±0.7068	ns
COL3A1/GAPDH	1±0	1.243±0.8879	0.3417±0.1579	ns
COL1A1/COL3A1	1±0	2.788 (0.7591–4.098)	4.235 (3.478–5.169)	C–MC *p<0.05
TGF-β/GAPDH	1±0	1.155±0.8878	0.2767±0.14	C–MC ***p<0.001

C: control; PL: urethral partial ligation; MC: urethral monopolar cauterization; BC: bladder capacity; BBP: baseline bladder pressure; MAXBCP: maximum bladder capacity pressure; MDA: malondialdehyde; COL1A1: collagen type I; COL3A1: collagen type III; TGF-β: transforming growth factor-β; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. Values are given as a mean±SD or median (IQR 25–75th percentile), *p<0.05, **p<0.01, ***p<0.001, ns: no significant.



Group → Variables ↓	C	PL	MC	p
Congestion	0.0 (0.0-0.25)	0.0 (0.0-1.0)	0.0 (0.0-1.0)	ns
Fibrosis	0.0 (0.0-0.0)	0.0 (0.0-0.25)	0.5 (0.0-1.0)	C-MC * p<0.05
Muscular thickness	732.2±44.73	762.4±37.43	837.8±63.33	C-MC *** p<0.001 PL-MC ** p<0.01

C: Control group demonstrated regular morphology of the lamina propria and the muscle tissue, original magnification 5×; PL: Urethral partial ligation. Slightly increased congestion (→) and collagen density. MC: urethral monopolar cauterization, group had the similar morphology with urethral partial ligation group. The bladder wall thickness was greater in the urethral injury group than the others, original magnification 10×.
*In the urethral partial ligation group, original magnification 10×.

Figure 2. Histological sections of the bladders with staining of hematoxylin and eosin

DISCUSSION

This study shows that the needle-tipped MC of the posterior urethra is a simple and reproducible method for creating an effective infravesical obstruction model in male rats. We carried out the MC method in a shorter time and more easily in our study. The PL method, which we have utilized frequently in the previous years, requires experience to dissect posterior urethra and a learning period to suture the rat urethra. Operative mortality and morbidity did not differ between the techniques.

The fibrosis of the bladder involves several molecular mechanisms. Regardless of the etiology, the initial injury is followed by a common morphological pattern including the replacement of the specialized cells by fibroblasts and the collagen deposition which result in alterations in the bladder function[10]. It is well established that the inflammation is usually followed by the tissue fibrosis[11]. It was reported that the production of COL1A1 was increased and COL3A1 was decreased[12]. In our study, COL1A1 expression in the prostate was significantly higher in PL and MC groups compared to the C group. However, the COL1A1-to-COL3A1 ratio, which is a strong indicator of fibrosis, was significantly higher in only MC group according to our QRT-PCR analysis in prostate tissues. Moreover, COL1A1 expression levels in the bladder of the control group were higher than those of the PL and MC groups, while COL3A1 expression levels were lower. COL1A1-to-COL3A1 ratio and TGF-β expression levels in the bladder of the control group were higher than the MC group. In addition, histological analyses of the bladder demonstrated more apparent fibrosis and congestion in the lamina propria and a slightly higher muscular thickness in the MC group than in the other groups. These results in the MC group can be interpreted to be consistent with the detrusor hypertrophy in the compensatory response to the infravesical obstruction[13].

In the development of the fibrosis, TGF-β is shown to be a key factor[14]. Lower TGF-β expression in the first few hours after the infravesical obstruction with a concomitant increase after the onset of chronicity of the injury and tissue fibrosis was reported in studies[13,15,16]. In our study, TGF-β expression in the prostate and bladder tissues was lower in only the MC group compared with the C group. These results are compatible with results of bladder tissues in our previous study as urethral injury rat model[6]. Although in that study COL1A1-to-COL3A1 ratio was significantly higher than C group on the 14th day of injury while in our recent study we investigated tissues in the sixth week of surgery. In our recent study, COL1A1-to-COL3A1 ratio and TGF-β expression were significantly lower than C group which might be interpreted as chronic stage of fibrosis.

The probable role of the oxidative stress on the physiopathology of the bladder dysfunction remains controversial. Previous studies showed that an ischemic period in the bladder, which is subjected to an acute distention, was followed by a reperfusion period resulting in free radical production and these studies speculated about a probable association of the reperfusion period with the chronic infravesical obstruction[17,18]. In these reports, MDA, which is the end product of the lipid peroxidation caused by free radicals, was used as a marker of cell membrane damage and disruption. The levels of MDA in the bladder tissues were significantly higher in the PL and MC groups than in the C group in our study. The highest level of MDA in the MC group demonstrates that this procedure is more thriving than the PL to create a chronic obstruction model. Bisogni et al. investigated total antioxidant status (TAS) in bladder tissue was high in infravesical obstruction model of rats[8]. The levels of the biomarkers of the oxidative stress were higher in the MC group compared with the C group.

When the cystometric measurements were examined, BC was significantly higher in both infravesical obstruction models than in the C group. This result was consistent with the results of Melman and colleagues' study, which compared the PL group with the C group and found that the BC was significantly higher in the former[5]. In Bisogni and colleagues' study, in which the infravesical obstruction model was created by placing a 2-mm silver ring around the bladder neck, the BC did not differ between groups[8]. Another important finding in our study was that the lowest mean BBP was found in the MC group.

Technique of the cystometric analysis is another important issue. Conscious rats are more preferred for cystometric analysis and suprapubic catheters are used[19]. However, for this purpose, bladder tissue is needed to be perforated and sutured thereafter. This might make histological and physiological results vague by impairing bladder function and causing inflammation[19]. If cystometric analysis under general anesthesia is preferred as in our study, transurethral catheterization is performed. Therefore, perforation or saturation of the bladder is not carried out. However, anesthetic agents can also have effects on the bladder function[20].

Among few studies including cystometric analyses in infravesical obstruction models, Melman and colleagues did not assess the bladder compliance in their study. The infravesical obstruction model group was reported to be significantly hypo compliant comparing with the control group in the study of Bisogni and colleagues, in which the cystometric evaluation was performed in the fourth week. In our study, both groups, especially the MC group, were significantly hyper compliant compared with the C group. These results

were consistent with the histological findings of the chronic infravesical obstruction.

According to all results from our cystometric, histological, and molecular analyses, the MC method results in stronger obstruction findings and emerges as an expeditious and simpler alternative to the PL method.

CONCLUSION

Compared with the standard urethral PL method, MC method provides higher rates of fibrosis in the prostate tissue, while bladder tissue results were considered chronic stage of fibrosis. The needle-tipped MC of the posterior urethra may be the method of choice for creating a chronic infravesical obstruction model of infravesical obstruction in male rats.

AUTHORS' CONTRIBUTION

SA: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **HHT:** Conceptualization, Formal Analysis, Writing – original draft. **IS:** Formal Analysis, Writing – original draft. AGS: Formal Analysis. **YEK:** Data curation, Formal Analysis, Formal Analysis. **CE:** Data curation, Formal Analysis. **ÖY:** Formal Analysis, Writing – review & editing. **HK:** Conceptualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Obesity in cases undergoing the surgical procedure of lung lobectomy: risk or benefit?

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SUMMARY

OBJECTIVE: The aim of the study was to evaluate the effect of body mass index on patients' short-term results following lung lobectomy.

METHODS: In this retrospective study, we compared the perioperative and short-term postoperative results of obese (BMI≥30 kg/m^2) versus non-obese patients (BMI<30 kg/m^2) who underwent anatomical lung resection for cancer. The two groups had the same distribution of input risk factors and the same ratio of surgical approaches (thoracoscopy vs. thoracotomy).

RESULTS: The study included a total of 144 patients: 48 obese and 96 non-obese patients. Both groups had the same ratio of thoracoscopic vs. thoracotomy approach (50/50%), and were comparable in terms of demographics and clinical data. The g roups did not significantly differ in the frequency of perioperative or postoperative complications. Postoperative morbidity was higher among non-obese patients (34.4 vs. 27.1%), but this difference was not statistically significant (p=0.053). Hospital stay was similar in both study groups (p=0.100). Surgery time was significantly longer among obese patients (p=0.133). Postoperative mortality was comparable between the study groups (p=0.167).

CONCLUSIONS: Obesity does not increase the frequency of perioperative and postoperative complications in patients after lung lobectomy. The slightly better results in obese patients suggest that obesity may have some protective role.

KEYWORDS: Obesity. Lung lobectomy. Risk factor. Obesity paradox. Thoracoscopy.

INTRODUCTION

Lung cancer is the most common cause of cancer-related death worldwide, resulting in 1.8 million deaths per year, and is the most common malignancy in men, with an annual incidence of 2.2 million new cases[1]. Reflecting this global trend, in the Czech Republic in 2021, the incidence reached 67 per 100,000, and mortality was 56 per 100,000. Over 5,000 people, mostly men, die from lung cancer each year in the Czech Republic[1]. Surgical resection is the primary treatment modality for patients with non-small cell lung cancer.

Obesity, which has become the largest pandemic of the 21st century, directly endangers 18.5% of the population in the Czech Republic. Being overweight is a proven risk factor for several surgical complications, including intra-abdominal perioperative complications, surgical site infections, and incisional hernias after abdominal surgery[2-4]. Obesity also significantly increases the overall risk of postoperative complications, such as myocardial infarction and respiratory or urinary tract infections[3]. It is generally acknowledged that body mass index (BMI) is associated with postoperative complications in abdominal surgery. However, the relationship between BMI and thoracic surgery has not yet been sufficiently studied, particularly in terms of lung resection. Interestingly, the authors of several recent studies have suggested that the risk of postoperative complications after cardiothoracic surgery in obese patients may be similar to or even lower than in nonobese[5-7]. One recent meta-analysis refers to obesity as a possible protective factor in patients after lung resection[8].

To the best of our knowledge, the available literature includes insufficient data regarding the relationship between BMI and

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short-term lung resection results. Therefore, in this study, it is purposed to investigate the effect of BMI on early postoperative outcomes in patients undergoing lung lobectomy.

METHODS

We conducted a retrospective analysis of data from the cases who had undergone surgery at the University Hospital Ostrava in 2016–2018. This study included patients who received anatomical lung resection (lobectomy) for primary lung cancer. The analysis excluded patients with postoperative histologically unproven primary lung cancer (inflammatory tumors or metastases); patients with incomplete data with the emergency surgery, due to their different entry risks.

BMI was used to assess the degree of obesity, by dividing the cases into two groups: obese (BMI≥30) and nonobese (BMI<30). The obesity threshold was set at a BMI of 30 in accordance with the official WHO classification. To maintain proportionality and to reduce the impact of unequal surgical burden, patients operated on via thoracotomy and by thoracoscopic approach were included in both groups in the same proportion.

Demographic and clinical data were extracted from medical records. The following parameters were analyzed: age, sex, BMI, risk of anesthesia according to the American Society of Anesthesiologists (ASA), tumor size and histopathology, perioperative outcomes (i.e., duration of surgery and blood loss), and short-term postoperative outcomes (i.e., length of hospital stay, 30-day postoperative morbidity, and mortality). Postoperative complications were evaluated according to the Clavien-Dindo classification adapted for thoracic surgery, which was introduced by Seely et al.[9] in 2010 (Table 1).

The extracted data were analyzed by the methods of descriptive statistics, utilizing the mean, standard deviation, and t-test for the quantitative values. The chi-square test was used for categorical values. A p-value of <0.05 was considered to indicate statistical significance.

RESULTS

A total of 144 cases with lung lobectomy had been incorporated in this study for which Table 2 lists their demographic and clinical data. This study included 52 (36.1%) women and 92 (63.9%) men, with a mean patient age of 63.9±8.9 years (range, 25–83 years). Preoperatively, 82 (56.9%) patients were classified as ASA II and 55 (38.2%) as ASA III. The mean tumor size was 3.4±1.6 cm. The most common tumor types were revealed as adenocarcinoma and squamous cell carcinoma.

The mean BMI of the study group was 27.8±5.0 kg/m^2 (range, 18–39 kg/m^2). A total of 48 (33.3%) cases were classified as obese (BMI≥30 kg/m^2), and 96 (66.7%) as nonobese (BMI<30 kg/m^2). The vast majority of obese ones had mild-to-moderate obesity, with a mean BMI of 33.4±2.7 kg/m^2. The obese versus nonobese groups exhibited no statistically significant differences in age, gender, ASA classification, tumor size, or histopathological findings (Table 2).

Table 3 lists the patients' short-term postoperative outcomes. Of the 144 lung lobectomies, 72 (50%) were performed using a thoracoscopic approach, and 72 (50%) via thoracotomy. The mean operation time was 101.2±32.2 min (range, 45–190 min). The time interval for surgery was significantly longer in the obese group than in the nonobese (p=0.133). The bleeding was evaluated as a perioperative complication, and significant blood loss (≥300 ml) was reported in 3 (2.1%) patients. No significant difference in the frequency of intraoperative complications between the study groups has been revealed (p=1.000).

Table 1. Classification of morbidity and mortality after thoracic surgery.

Grade	Complication	Necessary conditions
I.	Pneumothorax	Without chest tube reinsertion
	Delirium	<3 days
	Prolonged secretion from the chest tube	
II.	Prolonged air leak	>7 days
	Delirium	>3 days
	Pneumonia	
	Anemia	
	Urinal infection	
IIIa.	Pneumothorax	Requiring chest tube reinsertion
	Emphysema	Requiring chest tube reinsertion
	Prolonged air leak	Requiring chest tube reinsertion
IIIb.	Postoperative bleeding	Requiring surgical revision
	Pneumothorax	Requiring surgical revision
	Emphysema	Requiring surgical revision
	Prolonged air leak	Requiring surgical revision
IV.	Respiratory failure	
	Renal failure	
	Cardiac failure	
V.	Death	

The mean length of hospital stay was 10.9±6.1 days (range, 4–35 days), without significance (p=0.100). The average 30-days postoperative morbidity rate was 31.9% and the incidence of postoperative complications was higher in nonobese (34.4% vs. 27.1%), without significance (p=0.053). According to the Clavien-Dindo classification adapted for thoracic surgery, the mild complications (Grades 1–2) were described in 34 (23.6%) cases while more serious ones (Grades 3–4) in 12 (8.3%). The nonobese patients exhibited a higher incidence of mild and severe complications without significance. The 30-day postoperative mortality rate was 2.8% without significance (p=0.167).

Table 2. Demographics and clinical data of study patients.

	BMI<30 (n=96)	BMI≥30 (n=48)	p-value	Total (n=144)
Age in years, mean±SD	63.5±9.4	64.7±7.9	0.400	63.9±8.9
Gender, n (%)			0.668	
Female	33 (34.4)	19 (39.6)		52 (36.1)
Male	63 (65.6)	29 (60.4)		92 (63.9)
BMI, kg/m², mean±SD	25.1±3.1	33.4±2.7	<0.001	27.8±5.0
ASA, n (%)			0.245	
I	2 (2.1)	1 (2.1)		3 (2.1)
II	58 (60.4)	24 (50.0)		82 (56.9)
III	32 (33.3)	23 (57.9)		55 (38.2)
IV	4 (4.2)	0 (0)		4 (2.8)
Tumor size, cm, mean±SD	3.5±1.7	3.3±1.7	0.334	3.4±1.6
Histopathology, n (%)			0.291	
Adenocarcinoma	55 (57.3)	22 (45.8)		77 (53.5)
Spinocellular carcinoma	32 (33.3)	19 (39.6)		51 (35.4)
Neuroendocrine carcinoma	5 (5.2)	6 (12.5)		11 (7.6)
Parvocellular carcinoma	4 (4.2)	1 (2.1)		5 (3.5)

Table 3. Intraoperative and postoperative outcomes of study patients.

	BMI<30 (n=96)	BMI≥30 (n=48)	p-value	Total (n=144)
Surgery approach, n (%)			1.000	
Thoracoscopy	48 (50)	24 (50)		72 (50)
Thoracotomy	48 (50)	24 (50)		72 (50)
Surgery time, min, mean±SD	98.3±31.2	107.1±33.5	0.133	101.2±32.2
Operative blood loss, n (%)			1.000	
<300 mL	2 (2.1)	1 (2.1)		3 (2.1)
>300 mL	94 (97.9)	47 (97.9)		141 (97.9)
Hospital stay in days, mean±SD	11.5±6.4	9.8±5.3	0.100	10.9±6.1
30-Day postoperative morbidity, n (%)	33 (34.4)	13 (27.1)	0.249	46 (31.9)
Postoperative complications, n (%)			0.053	
1	6 (6.3)	7 (14.6)		13 (9.0)
2	17 (17.7)	4 (8.3)		21 (14.6)
3	10 (10.4)	2 (4.2)		12 (8.3)
4	0 (0)	0 (0)		0 (0)
5 (mortality)	4 (4.2)	0 (0)	0.167	4 (2.8)

DISCUSSION

Obese persons represent a high-risk group of surgical patients, particularly concerning early and septic complications. Association between obesity and short-term thoracic surgery outcomes remains controversial though BMI is a significant risk factor for perioperative and postoperative complications after intra-abdominal surgery [5-7,10,11].

Many studies have repeatedly demonstrated varying relationships between some risk factors and treatment outcomes, e.g., better dialysis outcomes in patients with obesity, hypertension, or high cholesterol[12] which findings contrast with the general observations in the otherwise healthy population, where obesity often appears to be a risk factor. This situation is described by the term "reverse epidemiology," and the observation that obesity may play a protective role in some patients is called the "obesity paradox."

The possible protective effect of obesity was first described in 1999 among obese cases undergoing hemodialysis[13] and has subsequently been studied and evaluated by many researchers, especially in cardiology[14,15]. In 2002, Gruberg et al.[16] described significantly better outcomes after percutaneous coronary intervention in moderately obese patients with ischemic heart disease. Several meta-analyses have demonstrated that the protective effect of obesity is a viable phenomenon even in patients with heart failure and heart attack[15,17].

The perioperative results in our cohort suggest that obese patients do not have an increased risk of perioperative complications during lung lobectomy. The obese and nonobese groups showed comparable rates of significant perioperative blood loss. The operative time was significantly longer for obese patients, but higher BMI did not affect the chosen surgical approach (thoracoscopic approach versus thoracotomy). Other authors have previously described the relationship between obesity and prolonged thoracic surgery. Julien et al. analyzed data from 19,337 patients, and found that obesity was associated with longer operation time but not with higher 30-day mortality[18].

In our study, the obese and nonobese patient groups did not exhibit different outcomes when stratified according to the chosen surgical approach. This finding leads us to conclude that obesity does not affect the feasibility and safety of thoracoscopic lung lobectomy. The thoracoscopic approach is currently considered the standard method of lung lobectomy, as confirmed by several studies[19,20].

The postoperative results in our cohort suggest that obesity is not a risk factor associated with increased postoperative morbidity and mortality in patients after lung lobectomy. It even appears that obese patients exhibited lower numbers of both mild and severe postoperative complications. Although our data do not demonstrate that obesity was a significant protective factor, the p-value at the cutoff point may seem to support this paradox.

The available literature includes insufficient data regarding the effects of obesity on patients' postoperative outcomes after lung resection. Smith et al. evaluated 499 patients after anatomical lung resections. However, their study included a wide variety of cases and did not focus only on lung lobectomies, but rather included all procedures from segmentectomy to pneumonectomy. The authors did not describe any differences in the incidence of perioperative and postoperative complications between the groups of obese and nonobese patients. Interestingly, they observed that obesity appeared to have a protective effect against postoperative respiratory complications[21]. Petrella et al.[22] studied a group of 154 patients after standard pneumonectomy. In contrast to our outcomes and the conclusions of Smith et al.[21], Petrella et al.[22] revealed that postoperative respiratory complications were five times more common among obese cases. The authors concluded that obesity should be considered as another risk factor in patients requiring pneumonectomy.

Launer et al.[23] conducted a study based on the largest inpatient care database in the United States. They analyzed a total of 1238 obese and 31,983 nonobese lung cancer cases after lung lobectomy and reported that postoperative morbidity and mortality did not significantly differ between these two patient groups. Therefore, the authors concluded that obesity should not be considered a risk factor for lung resection.

Importantly, the theory of obesity as a possible protective factor has not yet been sufficiently clarified or explained. Childers and Allison[24] used mathematical modeling methods to describe the main phenomena of reverse epidemiology. They found that mortality was highest in patients with extreme BMI: either severely underweight or morbidly obese. Patients with a moderate BMI (overweight, mild obesity, or moderate obesity) exhibited the lowest mortality: less than patients with a BMI within the normal range. In this study, the vast majority of obese patients had mild-to-moderate obesity, and no patient suffered from morbid obesity. We assume that a population with a higher proportion of morbidly obese patients would exhibit a higher frequency and severity of postoperative complications.

The chosen surgical approach has a significant effect on the postoperative course. Lung lobectomy performed via thoracotomy is associated with longer postoperative recovery and a higher risk of postoperative complications, particularly pneumonia, compared to lung lobectomy performed via the thoracoscopic approach[25]. To eliminate this unequal risk, we included patients operated on via thoracotomy and the thoracoscopic approach

in the same proportions in both the obese and nonobese study groups. The main limitation of our study is its retrospective and nonrandomized design. However, it was a single-institution study including a sufficient number of patients, and with targeted elimination of adverse factors affecting the outcome.

CONCLUSIONS

Obesity does not increase the occurrence or severity of perioperative and postoperative complications in patients after lung lobectomy. Outcomes of this study might support theories that obesity is a benefit in patients undergoing lung resection. However, further larger studies are required to reliably confirm the mentioned claim.

AUTHORS' CONTRIBUTIONS

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

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The effect of nutritional scores on mortality in COVID-19 patients

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SUMMARY

OBJECTIVES: While studies on the treatment for the coronavirus disease 2019 (COVID-19) pandemic continue all over the world, factors that increase the risk of severe disease have also been the subject of research. Malnutrition has been considered an independent risk factor. Therefore, we aimed to investigate the clinical effect of dietary habits and evaluate the prognostic value of the Controlling Nutritional Status score in the COVID-19 patients we followed up.

METHODS: A total of 2760 patients hospitalized for COVID-19 were examined. Patients were retrospectively screened from three different centers between September 1 and November 30, 2020. A total of 1488 (53.9%) patients who met the criteria were included in the study. Risk classifications were made according to the calculation methods of prognostic nutritional index and Controlling Nutritional Status scores and total scores. The primary outcome of the study was in-hospital mortality.

RESULTS: The groups with severe Controlling Nutritional Status and prognostic nutritional index scores had a significantly higher mortality rate than those with mild scores. In the multivariable regression analysis performed to determine in-hospital mortality, the parameters, such as age (OR 1.04; 95%CI 1.02–1.06, p<0.001), admission oxygen saturation value (SaO_2) (OR 0.85; 95%CI 0.83–0.87, p<0.001), and Controlling Nutritional Status score (OR 1.34; 95%CI 1.23–1.45, p<0.001), were independent predictors. The patient groups with a low Controlling Nutritional Status score had a higher rate of discharge with recovery (p<0.001).

CONCLUSIONS: Higher Controlling Nutritional Status scores may be effective in determining in-hospital mortality in patients with COVID-19. Nutrition scores can be used as a useful and effective parameter to determine prognosis in patients with COVID-19.

KEYWORDS: Malnutrition. Prognostic nutritional index. COVID-19. Nutrition status. Pandemic.

INTRODUCTION

The pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which emerged in Wuhan, China, in December 2019, was defined as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) in February 2020[1,2]. The SARS-CoV-2 infection can present with a variety of clinical manifestations from asymptomatic to mild upper respiratory tract disease, but it can also cause respiratory failure due to viral pneumonia[3]. This has caused COVID-19 to become an important public health issue worldwide. While studies on the treatment for the coronavirus pandemic continue all over the world, factors that increase the risk of having a severe disease have been the subject of research. Since the nutritional status of the host plays a crucial role in the defense system against infection, individuals with malnutrition are more susceptible to a number of infectious diseases that can lead to harmful consequences[4,5]. Malnutrition has been considered an independent risk factor[6]. Especially, the dietary habits of elderly patients with chronic diseases are often poor, which makes them a risk group for a possible infection[3]. Therefore, nutritional assessment is important to determine the prognosis of patients. The Controlling Nutritional Status (CONUT) score is a new and comprehensive index calculated using lymphocyte count, total cholesterol, and serum albumin levels[7]. The prognostic nutritional index (PNI), calculated from serum albumin concentration and total lymphocyte count, was used to estimate the risk of complications after gastrointestinal surgery. The CONUT and PNI scores, a simple, cost-effective, and efficient screening tool to determine the nutritional status of inpatients, have been used for assessing the prognosis of many tumors[8-10]. Recently, the CONUT and PNI scores have been reported to be independently associated with poor prognosis in many cardiovascular diseases[11-13].

Therefore, whether there is a relationship between the CONUT and PNI scores with COVID-19 should be investigated. Many studies on COVID-19 have focused on the epidemiology of COVID-19 patients, their clinical characteristics, and secondary events that develop during the follow-up period. However, the number of

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studies to evaluate the nutritional status of these patients is limited. Therefore, we aimed to investigate the clinical effect of dietary habits and evaluate the prognostic value of the CONUT and PNI scores in COVID-19 patients we followed up.

METHODS

A total of 2760 patients hospitalized for COVID-19 were examined. Patients were retrospectively screened from three different centers between September 1 and November 30, 2020. All patients with positive PCR test results of combined oral and nasopharyngeal swab samples were included in the study. A total of 850 patients whose computed tomography (CT) and clinical findings were compatible with COVID-19, but whose two PCR tests performed on two consecutive days were negative, were excluded from the study. Moreover, hematological diseases, chronic liver diseases, and malign cancers were excluded. In addition, patients with missing albumin, lymphocyte, and total cholesterol data were excluded from the study. A total of 1488 patients who met these criteria were included in the study.

Venous blood samples were obtained from all patients on admission due to COVID-19. The CONUT and PNI scores were calculated after the diagnosis of COVID-19 from on-admission blood samples. Information on the drugs used by patients was obtained from the database of the Ministry of Health of the Republic of Turkey. Demographic characteristics, laboratory results, physical examination, and follow-up data of patients were obtained from the hospital database. The treatment and follow-up of patients diagnosed with COVID-19 infection were performed in line with the COVID-19 guideline recommendations of the Ministry of Health of the Republic of Turkey. The diagnosis of acute respiratory distress syndrome (ARDS) was based on the WHO interim guidelines. The primary endpoint of the study was in-hospital mortality. ARDS, intensive care follow-up, ventilator support, and discharge with recovery were the secondary endpoint. The data of this study were obtained from three different centers operating as a third-level health institution (university hospital, training, and research hospital) in Turkey. This study was conducted according to the guidelines laid down in the Declaration of Helsinki. Our clinical study received ethics committee approval on June, 11, 2021 from the Health Sciences University Diyarbakır Gazi Yaşargil Training and Research Hospital/Turkey, with number 781.

Definitions

The CONUT score is calculated based on three parameters, namely, serum albumin level, total cholesterol level, and total lymphocyte count. After the CONUT score was calculated, those with a score of <2 in the normal group without malnutrition, those with a score between 2 and 4 in the mild malnutrition group, those with a score between 5 and 8 in the moderate malnutrition group, and those with a score of ≥9 in the severe malnutrition group were included.

The PNI was calculated using the following formula; 10 × serum albumin value (g/dL) + 0.005 × total lymphocyte count in peripheral blood (per mm^3). After calculating the PNI score, those with a score of <35 in the severe malnutrition group, those with a score between 35 and 38 in the moderate malnutrition group, and those with a score of >38 in the normal group without malnutrition were included.

Nutrition scores are not routinely used in clinical practice in the institutions where the data were obtained.

Statistical analysis

The IBM SPSS version 24.0 software package was used for analyses. Normal distribution of data was analyzed using the Kolmogorov-Smirnov test. Categorical variables were expressed as percentages (%) and were compared using the chi-square test or Fisher's exact test. Continuous variables with normal distribution were expressed as mean±standard deviation (SD) and were compared using the Student's t-test. Continuous variables with non-normal distribution were expressed as median (25–75th percentile) and were compared using the Mann-Whitney U test. Logarithmic transformation was used as blood parameters and showed abnormally wide distributions for CRP, D-dimer, and ferritin. The Kaplan-Meier analysis with log-rank test was performed according to their CONUT scores to determine a 60-day survival. Receiver operating characteristic (ROC) analysis was used to evaluate the sensitivity and specificity of CONUT and PNI scores in predicting mortality in COVID-19 patients. To determine confounding independent predictors of mortality in patients with COVID-19, univariate and multivariate logistic regression analyses were performed. The variables resulting from the univariate model with a p-value<0.05 were entered as covariates in the multivariate model. All values were given as odds ratio (OR) and 95% confidence interval (CI), both in univariate and multivariate logistic regression models. A p-value <0.05 was considered statistically significant for all tests.

RESULTS

A total of 1488 patients, 814 women (54.7%) and 674 men (45.3%), were included in the study. The patients were divided into four groups according to the CONUT score. Risk classifications were made according to the calculation methods and total scores of PNI and CONUT scores. Patients were compared in Table 1 in terms of primary outcome according

Table 1. Demographic, clinical, laboratory characteristics of groups, and predictive power of controlling nutritional status and prognostic nutritional index scores for mortality.

Parameters	All patients(n=1488)	Survival(n=1163)	Death(n=325)	p-value
Age, years	64.5±14.4	62.3±14.4	72.3±11.4	**<0.001**
In-hospital stay, days	8 (6–12)	8 (6–11)	11 (7.5–16)	**<0.001**
Gender, female, n %	814 (54.7)	661 (56.8)	153 (47.1)	**0.002**
Body mass index, kg/m^2, mean (±SD)	26.1±3.3	26.2±3.2	25.8±3.5	0.106
Hypertension, n (%)	885 (59.5)	666 (57.3)	219 (67.4)	**0.001**
Coronary artery disease, n (%)	322 (21.6)	229 (19.7)	93 (28.6)	**0.001**
Heart failure, n (%)	84 (5.6)	60 (5.2)	24 (7.4)	0.124
Diabetes mellitus, n (%)	511 (34.3)	387 (33.3)	124 (38.2)	0.102
Chronic renal failure, n (%)	83 (5.6)	57 (4.9)	26 (8)	**0.031**
Chronic obstructive pulmonary disease, n (%)	109 (7.3)	74 (6.4)	35 (10.8)	**0.007**
Cerebrovascular disease, n (%)	84 (5.6)	56 (4.8)	28 (8.6)	**0.009**
Atrial fibrillation, n (%)	64 (4.3)	35 (3)	29 (8.9)	**<0.001**
Unilateral lesions, n (%)	57 (3.8)	54 (4.6)	3 (0.9)	**0.002**
Bilateral lesions, n (%)	1395 (93.8)	1077 (92.6)	318 (97.8)	**0.001**
Acute respiratory distress syndrome, n (%)	259 (17.4)	44 (3.8)	215 (66.2)	**<0.001**
Nasal O_2, n (%)	1007 (67.7)	764 (65.7)	243 (74.8)	**0.002**
Mechanical ventilator, n (%)	282 (19)	27 (2.3)	255 (78.5)	**<0.001**
High flow nasal oxygen, n (%)	125 (8.4)	45 (3.9)	80 (24.6)	**<0.001**
Admission SaO_2 (oxygen saturation), %	89 (84–92)	90 (87–93)	80 (70.5–85)	**<0.001**
Systolic blood pressure, mmHg	120 (110–130)	120 (110–125)	120 (110–130)	**<0.001**
Diastolic blood pressure, mmHg	70 (60–80)	70 (65–80)	70 (60–80)	**0.001**
White blood cell, 10^3/μL	7.1 (5.3–9.9)	6.9 (5.2–9.1)	8.7 (5.8–12.4)	**<0.001**
Hemoglobin, g/dL	13.1 (11.9–14.3)	13.3 (12–14.4)	12.8 (11.4–14)	**0.001**
Neutrophile, 10^9/L	5.3 (3.7–7.9)	5 (3.6–7.1)	7.3 (4.5–10.7)	**<0.001**
Lymphocyte, 10^9/L	1.10 (0.78–1.51)	1.2 (0.87–1.6)	0.79 (0.58–1.1)	**<0.001**
Platelet, 10^3/μL	209 (169–265)	212 (171–266)	203 (159–260)	0.118
Glomerular filtration rate, mL/min	72 (46–89)	76 (54–90)	49 (31–73)	**<0.001**
Albumin, g/dL	3.3 (2.9–3.7)	3.4 (3.1–3.7)	2.9 (2.6–3.2)	**<0.001**
Total cholesterol, mg/dL	183 (156–213)	186 (157–216)	176 (147–200)	**0.002**
Ferritin, ng/mL	384 (192–729.5)	343 (173–653)	530 (291–1096)	**<0.001**
C-reactive protein, mg/dL	76 (32.6–125)	65.9 (27.9–109.5)	115 (67.9–167)	**<0.001**
Procalcitonin, ng/mL	0.11 (0.06–0.27)	0.09 (0.05–0.18)	0.32 (0.12–1.2)	**<0.001**
D-dimer, ng/mL	274 (178–477)	249 (168–402)	418 (258–892)	**<0.001**
CONUT, n (%)				
Normal	263 (17.7)	254 (21.8)	9 (2.8)	**<0.001**
Mild	641 (43.1)	565 (48.6)	76 (23.6)	
Moderate	511 (34.3)	317 (27.3)	194 (59.7)	
Severe	73 (4.9)	27 (2.3)	46 (14.2)	
PNI, n (%)				
Normal	771 (51.8)	730 (62.8)	41 (12.6)	**<0.001**
Moderate	282 (19)	208 (17.9)	74 (22.8)	
Severe	435 (29.2)	225 (19.3)	210 (64.6)	

CONUT: controlling nutritional status; PNI: prognostic nutritional index.
Bold values indicate statistical significance at the p<0.05 level.

to clinical and demographic characteristics and laboratory parameters. The mortality rate was significantly higher in the groups with severe CONUT and PNI scores than that in the mild groups (p<0.001, Table 1). As the CONUT score increased, follow-up with noninvasive mechanical ventilator, mechanical ventilator, high-flow nasal oxygen, progression to ARDS, mortality rate, and intensive care follow-up period were found to be significantly higher. In the patient groups with low CONUT scores, the rate of cure and discharge was higher (p<0.001, Appendix 1).

In the univariable analysis, age, gender, chronic renal failure, hypertension, hemoglobin, neutrophil, C-reactive protein, D-dimer, ferritin, admission oxygen saturation, systolic blood pressure, and CONUT scores were found to be significantly more effective on in-hospital mortality. In multivariable regression analysis, advanced age, admission oxygen saturation, and CONUT scores were independent predictors of in-hospital mortality (Table 2).

In ROC analysis, the CONUT score predicted mortality with 75% sensitivity and 91% specificity. The area under the ROC curve was 0.786 (95%CI 0.758–0.814, p<0.001). The PNI score predicted mortality with 64% sensitivity and 97% specificity. The area under the ROC curve was 0.806 (95%CI 0.779–0.833, p<0.001).

In the Kaplan-Meier analysis, as the CONUT score increased, the in-hospital follow-up time and mortality increased. At the end of the 60-day follow-up, the number of patients in the patient group with a normal CONUT score increased from 263 to 254, from 641 to 565 in the mild group, and from 511 to 317 in the middle group; in the severe patient group, the number of patients decreased from 73–27 (Figure 1).

In the classification made according to the CONUT score, the effect of the groups on mortality was examined. Mortality rate was significantly higher in groups with high CONUT scores than in groups with low CONUT scores (p<0.001, Figure 1).

Table 2. Univariable and multivariable regression analysis for determine predictor of in-hospital mortality: Effect of controlling nutritional status score on clinical prognosis in patients with COVID-19.

Parameters	Univariable analysis		Multivariable analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	**1.06 (1.04–1.07)**	**<0.001**	1.04 (1.02–1.06)	**<0.001**
Gender, male	0.67 (0.52–0.86)	**0.002**	0.72 (0.49–1.04)	0.084
Body mass index	0.96 (0.93–1.00)	0.107	*	
Heart failure	1.4 (0.89–2.39)	0.126	*	
Cerebrovascular disease	0.53 (0.33–0.86)	0.010	1.04 (0.53–2.03)	0.904
Chronic renal failure	0.59 (0.36–0.95)	**0.033**	0.57 (0.29–1.12)	0.105
Hypertension	1.54 (1.19–1.99)	**0.001**	1.09 (0.73–1.61)	0.658
Chronic obstructive pulmonary disease	0.56 (0.36–0.85)	0.008	1.30 (0.71–2.36)	0.384
Diabetes mellitus	0.80 (0.62–1.04)	0.102	*	
Coronary artery disease	0.61 (0.46–0.81)	0.001	0.90 (0.60–1.35)	0.620
Hemoglobin	0.88 (0.83–0.94)	**<0.001**	0.96 (0.87–1.06)	0.446
Neutrophil	1.12 (1.09–1.16)	**<0.001**	1.01 (0.97–1.05)	0.418
Procalcitonin	1.02 (1.00–1.03)	0.007	0.99 (0.98–1.01)	0.677
C-reactive protein	4.48 (3.18–6.33)	**<0.001**	0.91 (0.58–1.43)	0.699
D-dimer	4.14 (3.09–5.56)	**<0.001**	1.04 (0.68–1.59)	0.833
Ferritin	3.32 (2.42–4.55)	**<0.001**	1.65 (1.03–2.64)	**0.034**
Admission SaO_2	0.82 (0.80–0.84)	**<0.001**	0.85 (0.83–0.87)	**<0.001**
Systolic blood pressure	1.01 (1.005–1.021)	**<0.001**	1.00 (0.98–1.01)	0.959
CONUT	1.58 (1.49–1.68)	**<0.001**	1.34 (1.23–1.45)	**<0.001**

CONUT: controlling nutritional status.
Bold values indicate statistical significance at the p<0.05 level.

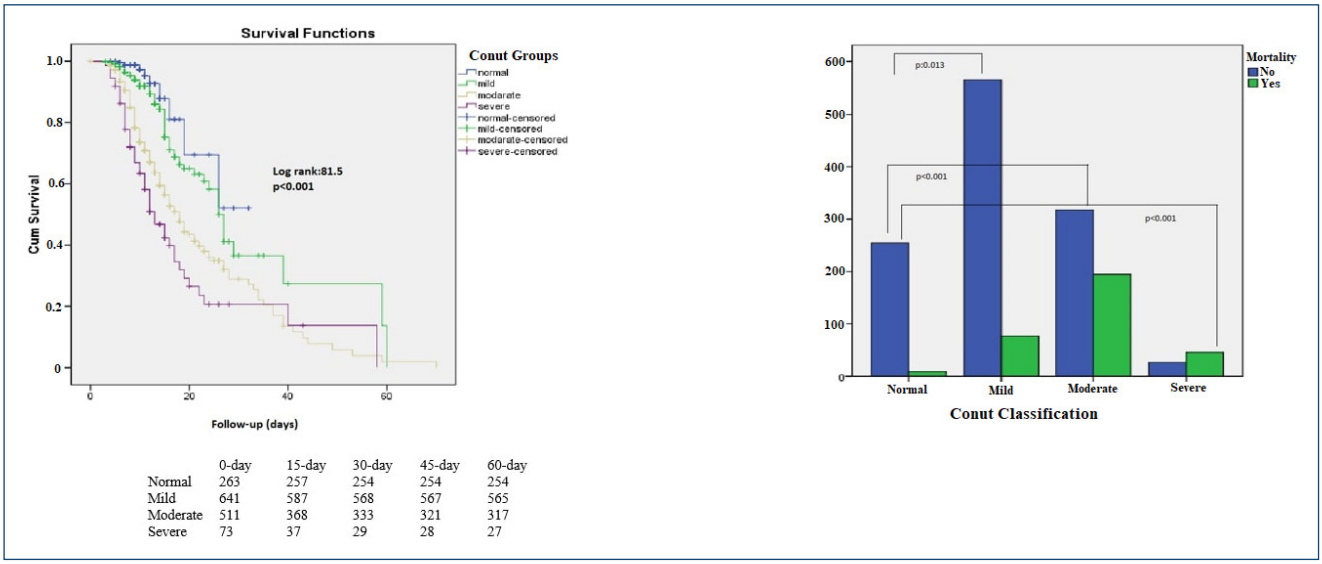

Figure 1. Correlation between controlling nutritional status score and 60-day survival by Kaplan-Meier analysis and comparison of mortality between controlling nutritional status groups.

DISCUSSION

Undernourished patients with COVID-19 infection had weaker immune functions, especially higher inflammatory responses. Nutritional status can influence viral genome mutations from a mildly pathogenic virus to a highly virulent virus and its spread to hosts[5]. Inflammation and malnutrition always exist concomitantly, as malnutrition can enhance the susceptibility to infections; meanwhile, infections further promote malnutrition via increased demand for nutrients and decreased appetite. Therefore, a thorough assessment of the nutritional status of patients helps clinicians determine the prognosis of the disease and treatment strategy. Virus invasion is able to result in the changes of white blood cells in peripheral blood, induce a cytokine storm, and thereby generate a series of immune response[14]. White blood cell and neutrophil counts were related to cytokine storms caused by viral invasion. 2019-nCoV infection can cause an exuberant inflammatory response, and uncontrolled pulmonary inflammation may be the major cause of fatality in COVID-19[15]. COVID-19 enters the cell via the ACE2 receptor. However, the virus may enter the bloodstream and accumulate in organs such as the gastrointestinal tract, heart, and kidneys, causing further damage. Serum protein is an important factor of the three standards of CONUT and is also a reliable systematic index of inflammation[16]. Pro-inflammatory cytokines, such as IL-6 and TNF-α, and CRP can also decrease the concentration of serum albumin and regulate albumin synthesis by liver cells[17]. Albumin is a good serum protein that determines the nutritional status of the patient. It makes up the majority of serum total protein and is mainly responsible for serum osmotic pressure. In addition to its oncotic properties, albumin also has antioxidant and anti-inflammatory properties in scavenging reactive oxygen radicals and limiting their production. Lymphocytes are an important part of the immune system, and the prognostic role of lymphocyte count has been investigated in many studies in cardiovascular diseases. As these three indicators can comprehensively assess the general condition of patients, we used the CONUT score as an indicator to assess the nutritional status of patients. As expected in the beginning of the study, the groups with moderate and severe CONUT scores had significantly higher values of length of intensive care stay, progression to ARDS, mechanical ventilation support, and mortality rate. Therefore, malnutrition may cause an increased incidence of death in COVID-19 patients. Wei et al. showed that the CONUT score has a prognostic effect in patients with COVID-19 in a previous single-center study. They found that malnutrition was associated with a poor prognosis. This result supported the results of our study[18].

Inflammation and malnutrition always coexist because malnutrition can increase susceptibility to infections. In the meantime, infections further increase malnutrition through increased demand for nutrients and decreased appetite[19]. A study by Eckart et al. on 2465 patients showed that a high serum CRP concentration was associated with low albumin levels, suggesting increased inflammatory parameters[20]. Moreover, in their study on 416 patients with COVID-19, Shi et al. reported that 82 (19.7%) of them had a cardiac injury, with a higher in-hospital mortality rate compared to those without cardiac injury (51.2 vs. 4.5%)[21]. They stated that cardiac injury was

common among COVID-19 patients and was associated with a higher risk of mortality[21].

While defense system cells play a central role in the effective host response against various pathogens, deficiency of immune cells disrupts immune homeostasis, causing pathological conditions. Recently, Qin et al. reported an association between the pathological process of COVID-19 disease and the immune system[22]. Patients with weaker immune functions were more likely to be infected with COVID-19[3]. Since there is a strong relationship between nutrition and immunity, malnutrition may result in weakened immune functions[5]. In clinical practice, the serum albumin level and lymphocyte count can be combined and used for PNI. The PNI was originally designed to assess immune nutritional status. This risk index has been widely used to assess surgical risk, particularly in patients with cancer, malnutrition, and systemic inflammation, and in gastrointestinal operations. Many studies have reported that a lower PNI score is associated with higher mortality in patients with cardiovascular diseases[23]. A low PNI level may reflect the patient's malnutrition status, resulting in a deterioration in intravascular osmotic pressure, which is mainly generated by albumin. In addition, low PNI levels may indicate a decrease in the body's immune response to acute illness, manifested by disruption of intravascular osmotic pressure, when a systemic infection occurs. For these two reasons, mortality may be higher in patients with COVID-19 infection with a low PNI score. In our study, we found higher in-hospital mortality in patients with low PNI scores.

Study limitations

It was a retrospective study with a relatively small sample size. CONUT and PNI scores were not evaluated after hospital discharge. Therefore, the effect of changes in CONUT and PNI scores on clinical outcomes during the post-discharge follow-up period could not be evaluated. Malnutrition was assessed using only the CONUT and PNI scores. Other nutritional indicators such as Mini Nutritional Assessment (MNA), Subjective Global Assessment (SGA), and Geriatric Nutritional Risk Index (GNRI) were not used. In addition, the CONUT and PNI scores may be affected by hormonal changes such as serum catecholamine and cortisol, but we could not measure these hormone levels in our study. In addition, the CONUT and PNI scores may have yielded subjective results due to drugs or the presence of some undetected conditions.

CONCLUSIONS

Factors such as CONUT score, advanced age, and low admission oxygen saturation can be used as useful and effective parameters to predict the prognosis of COVID-19 patients. In particular, the CONUT score can help physicians pre-clarify patients with a poor prognosis and offer individualized treatment to improve their survival.

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AUTHORS' CONTRIBUTION

AA: Conceptualization, data curation, formal analysis, writing – original draft, and writing – review & editing. **TG:** Conceptualization, data curation, formal analysis, writing – original draft, and writing – review & editing. **MD:** Formal analysis and writing – original draft. **MÖ:** Conceptualization, data curation, and writing – original draft.

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Appendix 1. Effect of controlling nutritional status score on clinical prognosis in patients with COVID-19.

Clinical Prognosis	CONUT GROUPS					p-value
	Normal (n=263)	Mild (n=641)	Moderate (n=511)	Severe (n=73)	Total (n=1488)	
Discharge with recovery	250 (95)	546 (85.2)	338 (66.1)	31 (42.5)	1165 (78.3)	**<0.001**
Non-Invasive Mechanical Ventilator	16 (6)	76 (11.9)	140(27.4)	28 (38.4)	260 (17.5)	**<0.001**
Mechanical Ventilator	12 (4.5)	82 (12.8)	154(30.1)	34 (46.6)	282 (19)	**<0.001**
High Flow Nasal Oxygen	5 (1.9)	45 (7)	58(11.4)	17 (23.3)	125 (8.4)	**<0.001**
Acute Respiratory Disstress Syndrome	12 (4.5)	75 (11.7)	148(29)	24 (32.9)	259 (17.4)	**<0.001**
Intensive Care Follow-Up	24 (9.1)	136 (21.2)	236(46.2)	52 (71.2)	448 (30.1)	**<0.001**
Mortality	9 (3.4)	76 (11.9)	194 (38)	46 (63)	325 (21.8)	**<0.001**

REVIEW ARTICLE

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An ancient examination in the face of a modern pandemic: systematic review of major clinicopathological autopsy findings

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INTRODUCTION

Autopsy consists of the examination of a corpse to determine the time and cause of death, as well as to evaluate any disease or injury that may have been present. Initially, autopsies functioned primarily as an anatomical analysis, but in the eighteenth century, they have taken on an investigative function through the study of pathological findings[1].

Due to new imaging methods, the reluctance of families, and new regulations, the performance of autopsy examinations started to decline in the 1980's. However, autopsies still play an important role in learning and reducing the rate of diagnostic errors[2]. Autopsies are fundamental to modern medicine, as in the evaluation of clinical procedures and medical education. Furthermore, in view of the recent circumstances of the coronavirus disease 2019 (COVID-19) pandemic, they are gaining more prominence for the purposes of studying this disease responsible for great socioeconomic and world health damage, whose pathophysiology is still poorly understood[3].

Thus, the purpose of this review was to clarify the role of autopsy in the context of the COVID-19 pandemic, as well as to present the main findings in autopsy examination of patients diagnosed with COVID-19.

METHODS

This review was conducted by two independent researchers, using the SciELO, PubMed, LiLacs, and Scopus databases. The following descriptors were chosen: "Coronavirus," "SARS-CoV-2," and "Autopsy." The filters used were as follows: Language English; article type: Classical Article, Clinical Study, Journal Article, Multicenter Study; within the past 1 year; and in English or Portuguese. Inclusion criteria were as follows: articles containing information on findings in autopsy examination of patients with a confirmed diagnosis of COVID-19. Exclusion criteria were as follows: articles not published in English or Portuguese; review articles, case report studies, and case series.

The selection occurred in three stages (Figure 1). First, studies were identified from the PubMed search string ((((Covid-19[Title/Abstract]) OR (Sars-Cov-2[Title/Abstract])) AND (Autopsy[Title/Abstract])) AND (English[Language])); and in Scopus, -(-TITLE-ABS-KEY-(-covid-19) -OR -TITLE-ABS-KEY (sars-cov-2) AND TITLE-ABS-KEY (autopsy)) -AND.- DOCTYPE (-ar -) -AND. PUBYEAR. -> 2018. -AND. -(-LIMIT-TO- (-LANGUAGE -, - "English"-) -). In the second stage, articles were excluded according to the type of study and language. In the last stage, the titles and abstracts of the remaining studies were read and those with nonconsistent themes were excluded. Finally, the remaining studies were included in this review (Figure 2).

DISCUSSION

Autopsy in the history of medicine

In prehistoric times, Inuit and Australian Aborigines studied mammalian anatomy by hunting large animals. However, it was noted in Greece history that autopsy was initiated to be applied in the medical sciences. This legacy was almost lost during the Middle Ages due to positions contrary to autopsies[1].

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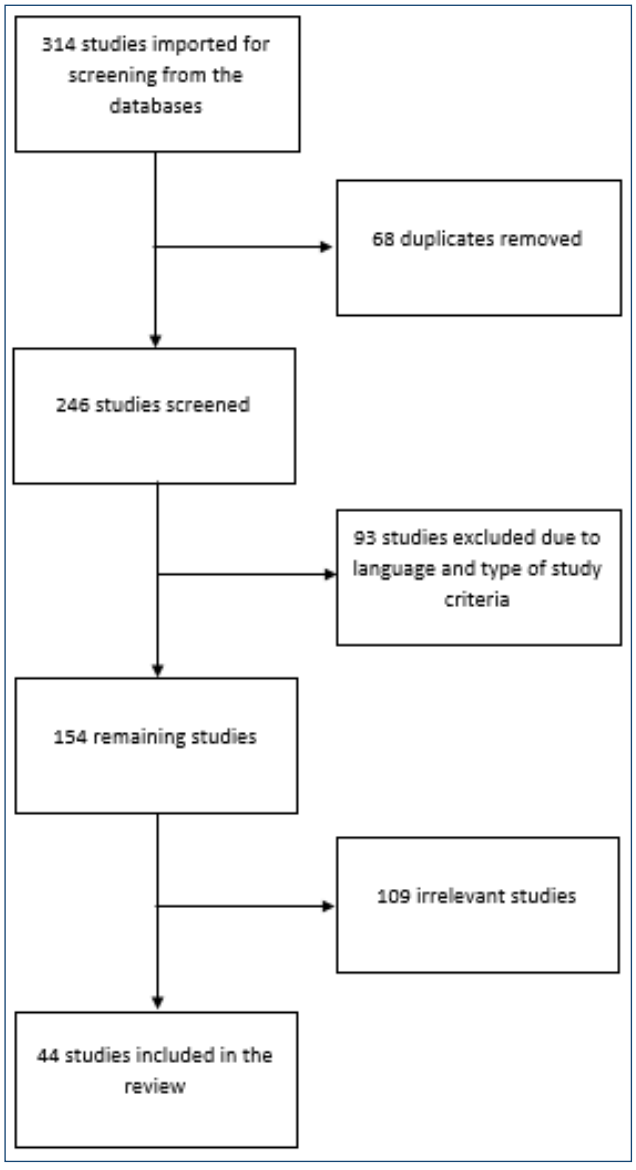

Figure 1. Linear flowchart of the screening and selection of studies.

The educational and scientific changes that occurred with the industrial revolution led to the appearance of dissecting rooms in most large hospitals, where anatomopathology was introduced as the basis for diagnosis and nosology. The performance of autopsies increased considerably when Cabot proved that they could detect misdiagnoses and the Flexner Report criticized the state of medical education in the United States. However, the occurrence of autopsies decreased again from the 1980's[1,2].

In contemporary times, many medical specialties are related to autopsy, such as forensic medicine and cardiology. In addition, autopsy has developed into several specialties, such as radiological, microbiological, and molecular autopsy[2].

The role of autopsy in COVID-19

Respiratory changes

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in the respiratory tract is caused by the surface protein S, which binds to angiotensin-converting enzyme 2 (ACE2), expressed in cells of the nasal epithelium and in large quantities in type 2 pneumocytes in the lower respiratory tract. Through immunohistochemical examination, the presence of ACE2 was confirmed in alveolar cells damaged by SARS-CoV-2 infection[4].

At the macroscopic level, several studies have described congested and heavy lungs, with their surface exhibiting pleuritis and a distinctive irregular mosaic pattern of pale areas alternating with purplish and dark hypercapillary areas, which are slightly protuberant, such that the pattern is visible on the cut surfaces[3,5-9]. In addition, the lung tissue is both firm and friable.

Microscopy frequently indicates the presence of diffuse alveolar damage, both exudative and proliferative, which is a nonspecific finding. There is also alveolar inflammation, with the presence of hyaline membranes, hyperplasia of type 2 pneumocytes, microvascular thromboembolism, capillary congestion, interstitial edema, intra-alveolar fibrin deposition, interstitial fibroblasts, and squamous metaplasia in the more advanced cases[5,6]. According to Fox et al., the inflammatory infiltrate was composed of a mixture of CD4+ and CD8+ T lymphocytes, located predominantly in the interstitium and around the bronchioles and blood vessels[9].

Borczuk et al. verified the presence of focal white spots on the mucosa of the upper and middle airways, hyperemic pharyngeal mucosa, and mixed inflammatory infiltrate, predominantly lymphocytic, with the presence of fibrin and ulcerations. However, they highlighted that there was no significant evidence correlating these findings with intubation and bacterial or fungal pneumonia[5].

Cardiovascular alterations

In some autopsies, the presence of elevated cardiac enzymes (troponin T and/or B-type natriuretic propeptide amino terminal fraction) was observed. The increase in troponin three days before the death of some patients corroborates a possible association between troponin elevation and mortality. This increase may have several causes, such as thrombosis of the microvasculature and cardiac veins[9-11].

A significant finding was right ventricular dilatation from elevated brain natriuretic peptide, resulting from pulmonary hypertension due to damage generated in the pulmonary vessels by the disease[9,10].

Figure 2. Main Systemic findings of autopsy studies.

Amendments	Microscopic Findings	Macroscopic Findings	Authors
Respiratory/lungs	Histopathology: Diffuse alveolar damage, with hyaline membranes; necrosis of alveolar lining cells; pulmonary fibrosis from diffuse alveolar damage; interstitial fibrosis; desquamation of alveolar cells; microvascular thromboembolism; thrombosis of pulmonary vessels with microangiopathy; severe endothelial injury; capillary congestion; interstitial edema; extensive granulocytic infiltrates with lymphocytes, macrophages, and monocytes; hyperplasia of type 2 pneumocytes with cytomegaly and large nuclei; bacterial pneumonia associated with SARS-CoV-2 infection Immunohistochemistry: Lymphocytic mononuclear infiltrates with predominance of $CD4^+$ over $CD8^+$ cells; presence of ECA2 and TMPRSS2 in the affected alveolar cells; increased expression of Ki-67; CD68+ macrophages mainly in the pulmonary interstitium; CD60+ giant cells	Pneumonia; pulmonary congestion and enlarged, heavy lungs; firm parenchymal edema; pleuritis; venous thrombosis; pulmonary embolism; superficial pleuritis; hemorrhagic foci; pulmonary hemorrhage	Witchamann et al. (5) Fox et al. (13) Schaefer et al. (36) Borczuk et al. (9) Kommoss et al. (16) Rapikiewicz (17) Lax et al. (28) Bösmüller et al. (23) Hanley et. al (31) Youd et al. (14) Elsoukkary et al. (24) Grosse et al. (18) Skok et al. (33) Valdivia-Mazeyra et al. (26) Wang et al. (21) Li et al. (35) Damiani et al. (7) Remmelink et al. (19) Nunes-Duarte-Neto et al. (32) Tian et al. (20) Yang et al. (34) Ackermann et al. (10) Edler et al. (11)
Respiratory/trachea and bronchial source	Histopathology: mucosal ulcers with mixed inflammatory infiltrate of neutrophils and fibrin	Mucosa with whitish areas	Borczuk et al. (9)
Cardiovascular	Hypertrophy of cardiomyocytes; interstitial fibrosis; coronary thrombosis; thrombosis of small myocardial vessels; myocarditis; pericarditis	Venous thrombosis; myocardial fibrosis; myocardial hypertrophy; cardiomegaly; coronary artery arteriosclerosis; cardiac amyloidosis	Fox et al. (13) Rapikiewicz (17) Lax et al. (28) Hanley et al. (31) Elsoukkary et al. (24) Grosse et al. (18) Wang et al. (21) Basso et al. (38) Nunes-Duarte-Neto et al. (32)
Hematological	Deep vein thrombosis; medullary hypercellularity with increased number of megakaryocytes; systemic thrombosis; splenic and lymph node autolysis; bone marrow embolism; lymphadenitis; splenic lymphoid hypoplasia; splenic pulp atrophy	Deep venous thrombosis of lower extremities; splenitis	Schaefer et al. (36) Rapikiewicz (17) Valdivia-Mazeyra et al. (26) Elsoukkary et al. (24) Grosse et al. (18) Wang et al. (21) Nunes-Duarte-Neto et al. (32) Roncati et al. (27) Brook et al. (29)
Renal	Virions in proximal tubule cells; acute tubular necrosis; benign nephrosclerosis; dilatation of peritubular capillaries; renal arteriosclerosis; interstitial inflammation; microthrombi in glomeruli		Rapikiewicz (17) Lax et al. (28) Santoriello et al. (37) Hanley et al. (31) Elsoukkary et al. (24) Grosse et al. (18) Nunes-Duarte-Neto et al. (32) Su et al. (40)
Reproductive	Thrombosis in testicles; orchitis	Prostatic vein thrombosis	Witchamann et al. (5) Nunes-Duarte-Neto et al. (32)
Hepatic	Macrovesicular steatosis; microthrombosis of sinusoidal capillaries; lymphocytic lobular infiltrates; platelet aggregates in portal veins; signs of hemophagocytosis by activation of macrophages; lobular hepatitis; hepatic fibrosis; abnormal periportal vessels; hepatomegaly	Cirrhosis; chronic passive congestion	Rapikiewicz (17) Lax et al. (28) Bösmüller et al. (23) Elsoukkary et al. (24) Wang et al. (21) Sonzogni et al. (39) Remmelink et al. (19)
Endocrinological	Pancreatitis; areas of adrenocortical necrosis; adrenal microinfarction	Pancreatitis	Hanley et al. (31)
Nervous	Cerebral hemorrhage; focal ischemic necrosis; cerebral edema and/or vascular congestion	Acute cerebral infarction; cerebral atrophy	Grosse et al. (18) Remmelink et al. (19)

Tian et al. and Grosse et al. found important cardiovascular changes, such as myocardial hypertrophy, acute myocardial infarction, focal myocardial fibrosis, and coronary atherosclerosis, which may be related to preexisting diseases. Also, we observed lymphocytic inflammatory infiltrate, although not significant, associated with damage to cardiomyocytes, with no evidence of viral myocarditis and no characteristics of viral cytopathic effect observed. These changes, therefore, may be secondary or related to underlying diseases[9-13].

As the polymerase chain reaction only detects the residual viral genome, it is unknown whether viral particles in the cardiac cells correspond to active viral replication or a previous infection without clinical relevance[12]. In contrast, there are other potential mechanisms of myocardial injury, such as severe respiratory infection with hypoxia, sepsis, systemic inflammation, pulmonary thrombosis and thromboembolism, cardiac adrenergic hyperstimulation during cytokine release syndrome, and myocarditis[11,13].

A numerically significant finding of COVID-19-positive individuals was massive cardiac amyloidosis, assuming that these patients died from cardiac decompensation[14].

There are indications that fulminant myocarditis can occur in SARS-CoV-2 infection, likely contributing to the morbidity of COVID-19. However, there are cases of "acute cardiac injury" in patients that do not necessarily translate into myocarditis or acute myocardial ischemia and no significant lymphocytic inflammatory infiltrates were found, highlighting the need for further studies on the cardiac impacts of SARS-CoV-2. These cardiomyopathies were also associated with metabolic disorders, such as severe metabolic and respiratory acidosis, recurrent in patients with COVID-19, which is another variable that should be analyzed in their pathogenesis[9-13].

Megakaryocytes were also found, with higher levels in the thrombi and vascular beds, cardiac tissue, and bone marrow. The morphology of these cells suggests active platelet production. This could contribute significantly to thrombosis, which is related to multiple organ failure, severe hypoxia, and death in COVID-19 patients[9].

Hematological alterations

Hematological alterations are closely related to the pathogenesis of COVID-19. There is evidence that the presence of microthrombi is associated with lesions present in several organs besides the lungs. Studies point out that under certain inflammatory conditions, there is an attempt to contain pathogens through the aggregation of platelets, neutrophils, and the coagulation cascade, a process called immunothrombosis. Nicolai et al. confirmed the presence of neutrophils embedded in fibrin clots in the microthrombi formed in this process, in addition to the existence of an increased number of thrombi containing granulocytes in autopsies of COVID-19 patients when compared with the lungs of patients who died of nonpulmonary diseases[15]. Elevation in fibrin degradation product (D-dimer) corroborates the association between a procoagulant state and disease severity, as does the histopathological evidence of microvascular thrombosis in the affected organs[3,15,16].

Several studies have demonstrated the presence of platelet-rich thrombi in the pulmonary, renal, cardiac, and hepatic microvasculature, which was also observed in the presence of megakaryocytes, bone marrow, microvasculature of the heart, and glomeruli, which was higher than usual in the lungs[3,10,17,18]. This elevation is possibly due to the state of hypercoagulability caused by severe cases of the disease[17]. Thrombosis has been found in several organs at different stages of the disease course, even with complete anticoagulation treatment, suggesting its great relevance in the disease process[10,15].

In macroscopic analysis of the lymph nodes, an increase in their structure was noted, with lymphocyte depletion and the absence of germinal centers[20]. The splenic white pulp was atrophied due to lymphocyte depletion[20,21]. Brook et al. also observed an increase in the red pulp and the presence of irregular necrosis in the spleen or large areas of infarction, which they related to be possibly due to shock[21].

Renal changes

SARS-CoV-2 viral RNA at high titers was detected in the kidneys of some patients who died from COVID-19[3]. On histopathological examination, renal signs of shock were found in most autopsies, such as diffuse acute tubular necrosis with enlarged tubular lumen, flattened tubular epithelium, and interstitial edema. In addition, small fibrin thrombi were found in the glomerular capillaries[8,20,22].

Similar to the pulmonary tissue, chronic inflammatory infiltrate was observed in areas with interstitial fibrosis and tubular atrophy. In transmission electron microscopy, podocytes with prominent activation containing several vesicles with virus-like particles in the cytoplasm were visualized, relating to SARS-CoV-2 replication[8,10,22]. Acute tubular necrosis was the main renal lesion found in autopsies, since these cells express the ACE2 receptor[10,16,20,23]. This direct infection of renal cells was proposed as a mechanism of acute renal damage, given the characteristics of acute renal lesions found, such as extensive tubular epithelial vacuolization[10,22].

Other changes

The studies also evidenced other alterations. In the liver tissue, the main findings were as follows: fibrosis, steatosis, centrilobular congestion, hepatomegaly, and coagulative necrosis mainly around

the central veins, a condition associated with lobular hepatitis triggered by some drugs. However, these findings could be due to the patient's past pathological history, as no specific histopathological correlation has been demonstrated for direct lesions caused by SARS-CoV-2, although viral particles have been detected[13]. These events are due to mechanisms such as cytokine storm, hypoxia, hypovolemia, and aggravation of chronic lesions of preexisting conditions. Dominic et al. speculated that ischemic liver lesions may indicate the presence of hepatic vascular thrombosis[14,16,20].

In the central nervous system, a mild inflammatory infiltrate of T lymphocytes was observed around the vessels, and ischemic alterations were also found in neurons of the cortex and white matter. Moderate to intense activation of microglia was observed as the most prominent pathological feature[11,14,16].

Digestive and pancreatic alterations were rarely noted. However, mild lymphocytic inflammatory infiltrate was observed in the digestive system and hemorrhagic pancreatitis[11,23].

In the seminiferous tubules, especially in the Sertoli cells, vacuolization and cytoplasmic rarefaction were observed[19]. Another alteration found was the loss and desquamation of the intratubular cells in the lumens of the seminiferous tubules. Edema and inflammatory lymphocytic infiltrates were found in the interstitium.

CONCLUSIONS

Autopsy plays an enormous role in the study of the pathogenesis of COVID-19 and contributes to the design of therapeutic plans as well as to the prognostic definition. There are still many limitations in the existing studies, both in relation to design and sample size. Thus, this review represents a stimulus for future studies that confirm the relationship between the infection by COVID-19 and possible systemic findings. We also highlighted the association between thrombotic events evidenced in various studies and infection by SARS-CoV-2, which is consistent with the published literature.

AUTHORS' CONTRIBUTIONS

MAMP: Conceptualization, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **LNLS**: Supervision, Investigation, Data curation, Writing – original draft, Writing – review & editing. **ACSS**: Supervision, Writing – review & editing. **LP**: Supervision, Writing – review & editing. **JC**: Investigation, Data curation. **MPAL**: Writing – original draft.

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REVIEW ARTICLE

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Opioid-free postoperative analgesia compared to traditional analgesia after thoracic surgery: scoping review

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INTRODUCTION

Patients receiving opioids for pain treatment can paradoxically become more sensitive to pain stimuli[1]. This condition is known as opioid-induced hyperalgesia[1]. It can explain why some patients require more opioid agents to treat their pain[1]. Some factors in animal studies have been identified as a potential explanation: activation of neuroexcitatory mechanisms, long-term potentiation, and descending pain facilitation[2]. Nociception process can be increased by neuroinflammation due to the activation of microglia and astrocytes[2]. It also identified other factors that can be a potentiate nociception, such as toll-like receptor 4, excitatory molecules, and the anti-opioid systems[2].

Opioid prescription is common after surgical procedures, but it can be excessive for many patients with different pain intensities[3,4]. Although opioids have great benefits for postoperative analgesia, adverse events and nonmedical uses can occur, and the experience can be catastrophic[5,6].The routine use of opioids cannot be justified for all types of surgeries, because alternative drugs can be used and low-risk surgical procedures are included in the treatment with opioids[5,6]. Researchers have already demonstrated persistent opioid use after surgical procedures[5-7].

Some strategies have been developed in thoracic surgery before this manuscript[8]. The guideline has been associated with reduced opioid usage, but it seems to be more appropriate to avoid opioid usage in postoperative thoracic surgery. Strategies for this purpose have to be explored further in research and disclosed in anesthesiology journals.

So, it is reasonable to identify drugs or protocol treatments to avoid the use of opioid drugs. Randomized controlled trials were published testing the effectiveness of thoracic surgery, but until this moment, these findings have not been proven effective to be disseminated in clinical practice. The objective of this scoping review was to identify and describe the effectiveness of opioid-free postoperative analgesia when compared to opioid analgesia after thoracic surgery.

METHODS

A scoping systematic review was done to examine the available research on opioid-free postoperative analgesia after thoracic surgery. A review protocol was developed before the research and is available at https://tinyurl.com/protocol001.

We used the methodology proposed by Arksey and O'Malley[9]. Our protocol followed the Preferred Reporting Items for Systematic Review and Meta-Analysis Extension for Scoping Review Protocols (PRISMA-ScR) guidelines[10]. The method followed five consecutive stages: identifying the research question; identifying relevant studies; study selection; charting the data; and collating, summarizing, and reporting the results[9].

The authors defined opioid-free analgesia as any postoperative pain management regimen that does not involve action on opioid receptors. It can be multimodal or unimodal, and pharmacological or nonpharmacological.

Stage 1: Identifying the research question

We planned to answer the following specific questions:

1. What drugs or combinations of them are being used effectively for opioid-free postoperative analgesia after thoracic surgery to avoid the use of opioid drugs?
2. What is the duration of postoperative analgesia?

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Stage 2: Identifying relevant studies

This review was planned to use data from systematic reviews, randomized controlled trials, and cohort studies to compare two techniques of postoperative analgesia (analgesia with opioid versus analgesia without opioid) for thoracic surgery. The following databases were searched: MEDLINE (Medical Analysis and Retrieval System Online) via PubMed (1966 to May 2021), LILACS (Literatura Latino-Americana e do Caribe em Ciências da Saúde – 1982 to May 2021), and Scopus. We used terms to scan PubMed and adapted them for other databases. The search strategy for PubMed was: ((“thoracic surgical procedures”[MeSH Terms] OR “thoracic surgery”[MeSH Terms] OR thoracic surgery[Text Word]) and (“analgesics, opioid”[All Fields] OR “analgesics, opioid”[MeSH Terms] OR opioid[Text Word]) and (“analgesics”[All Fields] OR “analgesics”[MeSH Terms] OR analgesic agents[Text Word]) AND (“Pain, Postoperative”[Mesh])).

There were no restrictions on any language, date, or document format. The references of the studies included studies that were analyzed to identify other relevant studies.

Stage 3: Study selection

Titles, abstracts, and keywords were scanned to identify studies through the search strategy for all databases. Two reviewers identified studies independently. The papers identified as a possibility to answer our research questions were obtained and read in full. Discordances were settled through consensus meetings. This stage followed the PRISMA statement[11].

Stage 4: Charting the data

Studies identified and read in full were charted into an Excel spreadsheet. A standardized form was developed by the review team to collect data. We contacted the authors of the relevant studies when data were not clear or understandable.

Outcomes considered important for this review were as follows:

1. Primary outcomes: length of analgesia or pain scores, length of hospital stay, length of stay in the intensive care unit (ICU), frequency of complications in the postanesthesia care unit (PACU), frequency of complications during hospitalization, and degree of satisfaction.
2. Secondary outcomes: therapeutic schemes of analgesic drugs.
3. Complementary data: characteristics of the studies including design, country, year, surgical procedures, and interventions.

Outcomes in this review were defined as follows:

1. Length of analgesia is time without pain. When these data were absent, we used data from pain scores.
2. Length of hospital stay is the time elapsed between hospital admission and discharge. It was considered in days.
3. Length of stay in the ICU is the time elapsed between ICU admission and discharge. It was considered in hours.
4. Frequency of complications in the PACU is the number of adverse events that occurred in the PACU. It was described in percentages.
5. Frequency of complications during hospitalization is the number of adverse events that occurred during the time elapsed between ICU discharge and discharge from the hospital. It was considered in percentages.
6. Degree of satisfaction is the level of satisfaction with respect to the hospital stay, surgical procedures, and anesthesia received. It was considered as described by the authors of the included studies.
7. Therapeutic schemes are the combinations of drugs used and their mode of administration.

Stage 5: Data summary and synthesis of results

The characteristics of the included studies were summarized. Studies were classified according to the outcomes reported. According to some guidelines, the risk of bias is not necessary for scoping reviews[10,12]. The authors of this review believe that general information can provide a general idea of quality assessment. The statistical analysis of the included studies was evaluated to identify statistical sources of flaws[13].

RESULT

In total, 1847 articles were identified from the search strategy, and 20 articles were identified as relevant to this scoping review. In the selection process, eight articles were excluded due to inadequate comparators or incomplete data. Thus, 12 articles were included in this scoping review[14-25]. The reference list of the included articles was analyzed to identify relevant articles, but no new articles were included in this process (Figure 1). The therapeutic regimens can be seen in Table 1, and the characteristic of included studies and critical appraisal are listed in Table 1.

Length of analgesia was evaluated in all included articles[14-25]. The results were favorable to the group without an opioid in six articles[15,18,20-24]. Kaiser et al.[15] reported the effectiveness of the group without opioids [intercostal nerve infusion of 0.5% bupivacaine (20 mL) + continuous infusion of 0.5% bupivacaine

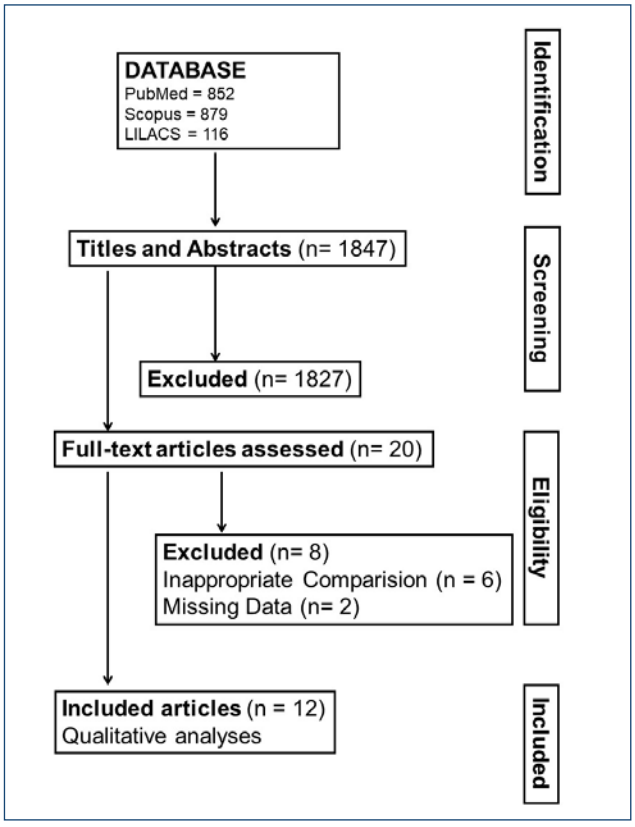

Figure 1. Flowchart of the selection process of the included articles.

(0.1 mL/kg/h) + ornipressin (0.05 U/mL) for 5 days] on the second day after surgery. El-Dawlatly et al.[18] reported no difference at rest, but ketoprofen (100 mg IM) associated with bupivacaine 0.5% interpleural (0.4 mL/kg) showed effectiveness at inspiration and coughing up to 24 h. Li et al.[22] reported that effectiveness of the group without opioids [flurbiprofen (50 mg intravenously)] occurred up to 8 h after surgery. Biçer et al.[23] reported greater effectiveness in the group where there was a combination of 0.5% bupivacaine (20 mL) + dexmedetomidine (100 µg) for up to 24 h. Deng et al.[21] reported effectiveness for up to 48 h with ultrasound-guided continuous serratus plane block with patient-controlled nerve analgesia (continuous infusion of 0.2% (30 mL) and 0.3% (for maintenance) ropivacaine). Dastan et al.[24] reported superior analgesia with ketorolac (90 mg/24 h) and paracetamol (3 g/24 h) in the anesthetic recovery room and at other times up to 24 h. There was no difference between the groups when considering rest, but the result was favorable to the group without opioids during times of coughing.

Length of hospital stay was evaluated in two studies[20,21]. Bauer et al.[20] reported no difference between groups. Deng et al.[21] reported a shorter time in the opioid-free group.

Complications during hospitalization were evaluated in five studies[17,19,20,22,25]. Yoshioka et al.[17] reported more nausea and

Table 1. Characteristics of the included studies and therapeutic regimens.

Author (year)	Participants	n	Intervention	Comparison	Type of surgery	Country	Critical appraisal
Dauphin et al.[14]	Inappropriate description	72	Intercostal infusion of 0.5% bupivacaine (0.3 mL/kg bolus + 0.1 mL/kg/h infusion) for 3 days	Epidural infusion of morphine (70 g/kg bolus + 7 g/kg infusion) for 3 days	Thoracotomy	Canada	Missing data
Kaiser et al.[15]	Adults scheduled for anterolateral thoracotomy (lobectomy or bilobectomy without pleural resection)	30	Intercostal nerve infusion of 0.5% bupivacaine (20 mL) + continuous infusion of 0.5% bupivacaine (0.1 mL/kg/h) + ornipressin (0.05 U/mL) for 5 days	Meflumenaminic acid (500 mg 6/6 h) + continuous infusion into the epidural space of 0.25–0.375% bupivacaine (4–6 mL/h) with 2 mg/ml fentanyl	Thoracotomy (fourth intercostal space)	Switzerland	Possible failure in pain analysis
Tuncel et al.[16]	ASA I and II, lung tumor, one patient with hydatid cyst in each group	60	ropivacaine 0.2% dose (mL)=height (cm) – 100/10 Infusion continues for 3 days	Ropivacaine 0.2% + sufentanil 0.75 mcg/mL dose (mL)=height (cm) – 100/10 Infusion continues for 3 days	Toracotomia posterolateral	Turkey	Postoperative infusion regimen was not clearly described
Yoshioka et al.[17]	Adults, lobectomy, or partial lung resection	46	There was no detailed description of the drugs	Epidural anesthesia with 0.25% bupivacaine (5 mL in initial dose + continuous infusion associated with 1 mg fentanyl)	Video-assisted thoracic surgery	Japan	No indication that hypothesis test was used in some analysis

Continue...

Table 1. Continuation

Author (year)	Participants	n	Intervention	Comparison	Type of surgery	Country	Critical appraisal
El-Dawlatly et al.[18]	ASA I and II	40	Three groups: 1 – ketoprofen (100 mg intramuscular), 2 –bupivacaine 0.5% interpleural (0.4 mL/kg), 3 – Ketoprofen (100 mg intramuscular) + bupivacaine 0.5% interpleural (0.4 mL/kg)	Pethidine 1.5 mg/kg (intramuscular)	Thoracoscopic sympathectomy	Saudi Arabia	Repeated measures analysis was not performed
Dabir et al.[19]	ASA I and II	36	Intercostal space infusion of 0.25% bupivacaine (30 mL) + 10 ml of saline solution. Repeating the same solution every hour for 24 h	Intercostal space infusion of 0.2 mg/kg morphine in 40 ml of saline solution. Repeating the same solution every hour for 24 h	Posterolateral thoracotomy	Iran	Repeated measures analysis was not performed
Bauer et al.[20]	18 years of age and scheduled for planned video-assisted thoracic surgery under general anesthesia with thoracic paravertebral block	70	Continuous paravertebral thoracic infusion of ropivacaine (2 mg/mL)	Continuous paravertebral thoracic infusion sufentanil (0.25 mcg/mL) and ropivacaine (2 mg/mL)	Video-assisted thoracic surgery	France	Final analysis contains patients who received morphine in both groups
Deng et al.[21]	Inappropriate description	60	Ultrasound-guided continuous serratus plane block with patient-controlled nerve analgesia – continuous infusion of 0.2% (30 mL) and 0.3% (for maintenance) ropivacaine	Patient-controlled intravenous analgesia with continuous infusion of sufentanil (sufentanil 1.5 μg/kg) + tropisetron (5 mg – diluted with normal saline to 100 mL) at a flow rate of 2.0 mL/h	Single-port thoracoscopic surgery	China	Repeated measures were analyzed with inadequate testing
Li et al.[22]	Miastenia Gravis	200	Flurbiprofen (50 mg intravenously)	Tramadol (100 mg intramuscular)	Thymectomy via mediastinal	China	Tramadol was administered intramuscularly
Biçer et al.[23]	ASA I and II, aged between 18 and 65 years old	93	Two groups of paravertebral block: 0.5% bupivacaine (20 mL) and 0.5% bupivacaine (20 mL) + dexmedetomidine (100 μg)	Intravenous morphine via patient-controlled analgesia	Thoracotomy	Turkey	Repeated measures were analyzed with inadequate testing
Dastan et al.[24]	ASA I and II in a referral hospital	101	Two groups: ketorolac (90 mg/24 h) and paracetamol (3 g/24 h)	Morphine 10 mg in intravenous bolus and infusion within 24 h (0.5 mg/h with a total of 10 mg)	Video-assisted thoracoscopy	Iran	Sample size may overestimate the results
Miao et al.[25]	ASA I and II, 18–65 years old, BMI less than 30, and lung cancer	54	Dexmedetomidine and patient-controlled postoperative intravenous analgesia with 0.1 μg/kg/h dexmedetomidine + 3 mg/kg ketorolac	Postoperative patient-controlled intravenous analgesia with 1.5 μg/kg sufentanil + 3 mg/kg ketorolac	Thoracoscopic surgery	China	Sample size cannot be verified

ASA: American Society of Anesthesiologists; BMI: Body mass index in kg/m^2.

vomiting in the opioid group. Dabir et al.[19] reported no serious complications in both groups. Bauer et al.[20] and Miao et al.[25] reported no differences between groups. Li et al.[22] reported more complications in the opioid group.

Degree of satisfaction was evaluated in one study[24]. The authors reported no statistical difference between groups.

Length of ICU stay and frequency of complications in the PACU were not analyzed in the included articles.

DISCUSSION

This scope review demonstrated that some drugs are being used to promote opioid-free analgesia after thoracic surgery. The duration of analgesia was up to 48 h. Statistical analysis of primary studies demonstrated the presence of flaws in the choice of statistical tests. These flaws can lead to inconclusive results when considering protocols tested in the included studies. More studies are needed to clarify the controversy identified in this scope review.

Effectiveness of analgesia was seen as length of analgesia. Six studies reported favorable results[15,18,20,22-24]; however, the time of analgesia was different between studies. Some authors of the included studies reported effectiveness in rest position and others in the cough effort. The authors of this review believe that the difference in pathologies and in surgical techniques led to different intensities of pain. It can justify differences between studies.

We identified the following therapeutic schemes as effective: intercostal nerve infusion of bupivacaine followed by continuous infusion of bupivacaine and ornipressin[15], ketoprofen via intramuscular and interpleural bupivacaine[18], continuous paravertebral thoracic infusion of ropivacaine[20], flurbiprofen via intravenously[22], paravertebral block of bupivacaine and dexmedetomidine[23], and ketorolac or paracetamol via intramuscular[24].

Length of hospital stay analysis demonstrated that the difference between groups was only significant for a few hours, so it does not contribute to decision-making in clinical practice.

Five studies evaluated the complications[17,19,20,22,25]. The authors considered that there was no difference between the groups or there were reports of complications that were already expected for the group with opioids, such as nausea and vomiting. The authors of this review did not link complications to mortality.

The contribution to clinical practice from this scope review was the identification of potential drugs that can be used in daily clinical practice. The statistical flaws prevented the choice of one protocol to guide physicians. Physicians must first consider the similarity between the populations assessed in the included studies and drugs used in their daily clinical practice. The choice of analgesic protocols has to be individualized.

The contribution to future research lies in the identification of statistical flaws and the absence of data for some variables. The main statistical flaw is the lack of sample size calculation description. The description of the sample size allows the analysis of statistical power[13]. The other statistical flaw was the inappropriate choice of statistical tests that could lead to false-positive results[13].

The main limitation of scope reviews is the absence of risk of bias analysis; however, the conclusions of this review took into account the statistical analysis used in the included studies. The other limitation of this research was considering data from different surgeries, but the purpose of the scope review is to provide a broad description of the findings for further systematic reviews and randomized clinical trials.

The opioid-free analgesia may be more effective after 2–48 h in postoperative thoracic surgery. However, there are still controversies and good quality future studies are needed to assess the effectiveness of drugs in this clinical setting. We suggest some outcomes in future studies to test effectiveness: mortality, degree of satisfaction, quality of life, and complications in patient follow-up.

AUTHORS' CONTRIBUTIONS

RRA: Conceptualization, Supervision, Writing – original draft. **NOL**: Supervision, Writing – review & editing. **MVMRR**: Writing – original draft, Writing – review & editing. **FWSR**: Data curation, Formal Analysis. **CFSR**: Data curation, Formal Analysis. **FTB**: Conceptualization, Writing – original draft, Writing – review & editing.

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REVIEW ARTICLE

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Research progress of platelet-rich fibrin in alveolar ridge preservation

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INTRODUCTION

The alteration of the hard- and soft-tissue contour after tooth extraction has been studied extensively. Research has found that significant changes in the alveolar bone and surrounding tissues occur after the extraction of natural teeth[1]. Particularly in the upper jaw, a large amount of bone loss occurs during the natural healing process after tooth extraction, resulting in changes in the three-dimensional shape of the alveolar bone[2]. These changes bring difficulties to later implant restoration, and complex soft- and hard-tissue incremental surgery is often required to compensate for tissue defects[3]. Even though different treatments have been attempted to reduce or avoid surrounding tissue defects caused by tooth loss, studies have failed to identify a technique that compensates for that issue[4]. Therefore, finding a safe and inexpensive biological material for alveolar ridge preservation is a common goal in current research.

Biological additives, such as platelet concentrates, have also been proposed as adjunctive therapies for bone regeneration. As a second-generation blood concentrate, platelet-rich fibrin (PRF) is rich in platelets and various cytokines (which can effectively promote the regeneration of soft and hard tissues) and has the advantages of low cost and easy preparation[5]. In addition, the preparation of PRF does not require the addition of anticoagulants, and all components originate from the body, which eliminates ethical controversy. At the same time, its three-dimensional structural features increase its stability, making it suitable for long-term use[6]. Randomized controlled studies have shown that the use of L-PRF as a socket filling material to achieve the preservation of horizontal and vertical ridge dimensions 3 months after a tooth extraction is beneficial[7].

This article aimed to review the development history, application prospects, and research results of soft tissue and alveolar bone PRF in order to provide theoretical support for the clinical application of PRF.

METHODS

We searched the PubMed database for English-language articles in peer-reviewed journals that were published between April 1995 and December 2020 using the terms "platelet concentrates," "platelet-rich plasma," and "platelet-rich fibrin." We checked the relevance of the titles and abstracts of the 4,025 articles searched. We reviewed and presented the development of platelet concentrates. Then, the PubMed database was searched using the terms "platelet-rich fibrin," "alveolar bone," and "application." Randomized, double-blind, placebo-controlled trials (RCTs) with results reported as intention-to-treat analyses were considered the highest quality data. Large prospective cohort studies, meta-analyses, and systematic literature reviews were also included as appropriate for supplementing the RCT results. There were no specific inclusion or exclusion criteria. The reference lists of retrieved reviews were searched for additional articles, and the relevant references from retrieved articles were also evaluated. The effects of PRF on alveolar ridge preservation and soft-tissue protection are discussed below with reference to these documents (Figure 1).

DEVELOPMENT HISTORY OF PLATELET CONCENTRATES

The development history of platelet concentrates can be traced back to the 1980s and 1990s. They first appeared in the form of platelet-rich plasma (PRP). However, the preparation process is complicated, and it requires the addition of heterologous thrombin and anticoagulant; this increases the risk of immune rejection and cross infection and hinders the formation of blood coagulation and fibrin clots, thus limiting its application in the treatment of tooth extraction sockets[8].

In 2000, Dr. Choukroun[9] developed a new preparation technology using a 100% natural method (without anticoagulants or thrombin) to formulate a new generation of platelet-concentrate PRF. A comparison of the most important characteristics of PRP and PRF is presented in Table 1.

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Comparing the performances of PRP and PRF, we can see that PRF is more efficient with better characteristics than PRP. PRF has broad application prospects for the treatment of tooth extraction sockets.

PRESERVATION EFFECT OF PLATELET-RICH FIBRIN ON THE ALVEOLAR BONE IN SITE PRESERVATION

After a natural tooth is extracted, due to the loss of blood supply to the periodontal ligament, the alveolar bone will undergo rapid absorption and reconstruction[10,11]. In 2013, Professor Hauser and his team reported that PRF could induce the formation of new bone in the extraction socket. A micro-computed tomography (CT) analysis showed that in the PRF group, the alveolar socket had higher bone density and more bone trabeculae with better trabecular spacing; the healed bone formed a better microstructure with a more ideal bone quality, and the width of the alveolar ridge was well preserved[12].

Yüce and Kömerik selected 40 patients with alveolar osteitis following third molar extractions to observe the soft-tissue healing rate using the modified index of Landry, Turnbull,

Table 1. Overview of important characteristics of two blood products (platelet-rich fibrin and platelet-rich plasma).

Blood products	PRF (2000)	PRP (1998)
Protocol	Easy	complex
Speed rate	Fast	Slow
Reproducibility	No bias	Possible bias
Use of anticoagulants	No	Yes
Amount obtainable	Good	Enough
Costs of the protocol	Low	High
Amount of fibrin obtainable	High	Low
Speed of fibrin formation	Physiological	High
Fibrin morphology	Trimolecular	Tetramolecular
Leukocytes amount (%)	65	0–50
Immunomodulatory properties	Yes	Poor
Neo-angiogenic potential	+++++	+
Osteoconductive potential (scaffolding)	High	Poor
Mechanical properties (sol-gel-membrane)	Good	Enough
Presence of MSCs	Yes	Yes

PRF: platelet-rich fibrin; PRP: platelet-rich plasma; MSCs: mesenchymal stem cells.

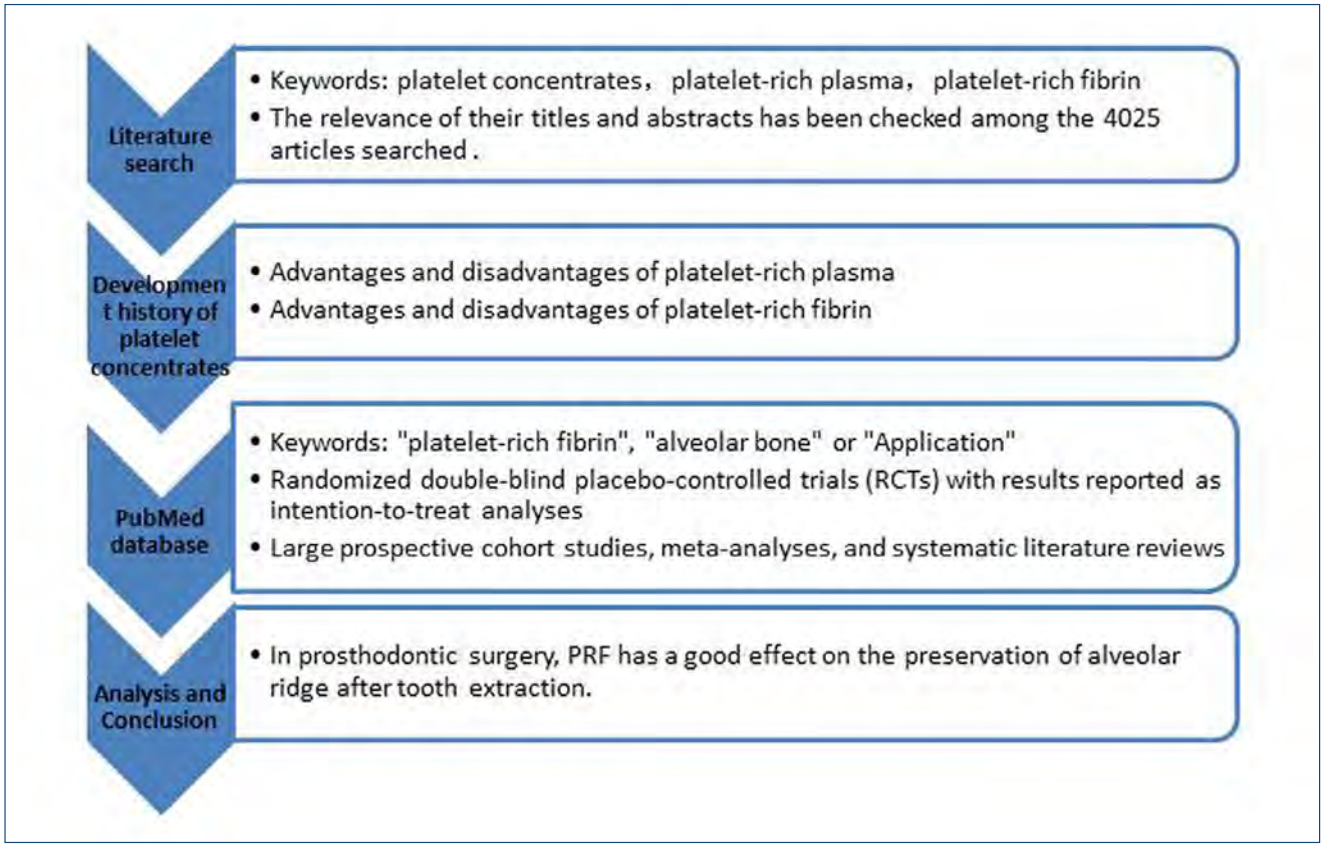

Figure 1. Platelet-rich fibrin related information.

and Howley. The results revealed that, after PRF treatment, the healing rate of epithelial tissue was significantly faster than in patients who had not received PRF treatment[13].

A clinical trial conducted by Professor Das compared PRF and β-tricalcium phosphate collagen (β-TCP-Cl) as a single-root alveolar socket transplantation material for the treatment of extraction sockets. Both materials had a fast replacement rate, but histologically, β-TCP-Cl showed higher bone density and tissue maturity; at the same time, it had less medullary space. The study showed that PRF offers comparable alveolar ridge preservation and reduction of alveolar bone resorption, especially for buccal bone plates (PRF: 1.5-mm bone loss; β-TCP-Cl: 0.99-mm bone loss)[14].

In 2016, Andwandter reported the healing of the extraction socket after filling with PRF. Immediately after tooth extraction and again after 4 months, personalized acrylic scaffolds were used for bone detection, and cone-beam CT (CBCT) was used to obtain imaging measurements. It was clinically observed that the alveolar ridge top absorbed (1.18±2.4 mm) horizontally, and there was a bone loss of 1.25±2.0 and 0.83±2.0) mm at 2 and 4 mm of the root of the ridge, respectively. The vertical bone resorption of the buccal bone plate was 0.44±3.5 mm. The imaging analysis showed that the buccal bone plate lost 0.27±2.5 mm perpendicular to the bone and 0.03±1.6 mm to the tongue. The width of the alveolar ridge was reduced by 1.33±1.43 mm. These results were similar to those of a systematic review in which buccal bone plate resorption was 0.5–1 mm after the application of bone-graft materials[15].

Temmerman et al. studied the effect of PRF as a filling material for alveolar ridge preservation. This study included patients with single-jaw bilateral symmetrical tooth extraction, and CBCT examinations were performed on day zero and again after 3 months. At the time node, the average alveolar ridge width difference was measured at three levels below the top of the buccal lingual alveolar ridge (1 mm below the top of the ridge [main observation variable] and 3 and 5 mm below the top of the ridge). At 1 mm below the ridge, the reduction in the alveolar bone width between experimental group (-22.84%) and control group (-51.92%) was statistically different ($p<0.005$). There was a statistically significant difference in the amount of filling in the extraction socket (visible mineralized bone) between experimental group (94.7%) and control group (63.3%)[7].

Wang Binping et al. took 30 patients with extracted posterior teeth as research sample. Immediately after extraction, the alveolar socket was filled with PRF for site preservation. CBCT was performed for 4–6 months to observe the changes in the height and width of the alveolar bone. The conclusion was that PRF site preservation technology could well preserve the height and width of the alveolar bone in the posterior tooth area, which was conducive to later implant restoration[16]. At the same time, the application of PRF in the preservation of tooth extraction sites could accelerate wound healing, inhibit the absorption of the patient's alveolar ridge, and lay the foundation for the implementation of subsequent dental implants[17].

The fibrin network of PRF can maintain red blood cells to slowly release growth factors, including transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor, and insulin-like growth factor-I (IGF-I)[18]. Among these, TGF-β1 can promote the rapid proliferation of oral cells, and PDGF can regulate the migration, proliferation, and survival of mesenchymal cells. VEGF plays an important role in angiogenesis and the reconstruction of damaged tissue. Finally, IGF-I is a regulatory factor for a variety of cell proliferation and differentiation processes (Table 2).

Thus, after a natural tooth is extracted, the application of PRF can maintain the slow release of growth factors from red blood cells through the formed fibrin network, inhibit the resorption of the patient's alveolar ridge, and induce the

Table 2. Platelet therapy growth factor functions.

Platelet-derived growth factor (PDGFaa, PDGFbb, PDGFab)	Triggers the activities of neutrophils, fibroblasts, and macrophages Chemoattractant/cell proliferator Stimulates mesenchymal cell lineages
Transforming growth factor (TGF-β1, TGF-β2, TGF-β3)	Promotes cellular differentiation and replication Stimulates matrix and collagen synthesis Stimulates fibroblast activity and collagen production
Vascular endothelial growth factor (VEGF)	… Angiogenesis … Stimulates synthesis of basal lamina
Fibroblastic growth factor (FGF)	Angiogenesis Fibroblast production
Epithelial cell growth factor (ECGF)	Stimulates epithelial cell replication
Insulin-like growth factor (IGF-1)	Promotes cellular growth and proliferation

formation of new bone in the extraction socket. At the same time, PRF can accelerate the healing of epithelial tissue. This lays the foundation for the implementation of subsequent dental implants.

PRESERVATION EFFECT OF PLATELET-RICH FIBRIN ON SOFT TISSUE IN SITE PRESERVATION

PRF has been proven to continuously stimulate the proliferation of gingival fibroblasts, osteoblasts, and periodontal ligament cells[19]. At the same time, PRF can promote soft-tissue regeneration by releasing PDGF and TGF-β[20]. Therefore, PRF has great potential for promoting the healing of soft tissues around extraction sockets and enhancing aesthetic repair.

Sybil et al. recruited 25 patients with bilateral surgical disimpaction of the mandibular third molar and placed them in PRF and no-PRF groups on the left and right sides of extraction wounds. The results showed that PRF has a significant effect on soft-tissue healing[21]. In recent years, Lv Min and other researchers in China have also confirmed the ability of PRF to promote soft-tissue healing. By placing PRF in the extraction wounds of 140 affected mandibular third molars and comparing the results with conventional blood-filled clots, it was found that 3 months after the operation, there were only three cases of adjacent tooth periodontal attachment loss in the PRF group, while there were 25 cases in the blood clot group, of which 3 cases were larger than 2 mm with infection. Experiments have proven that PRF can promote the restoration of adjacent teeth periodontal tissue[22].

In vivo research on the anterior soft-tissue defect model of a rabbit hard palate showed that PRF could accelerate soft-tissue wound healing and reduce scar formation[23]. PRF has a significant promoting effect on the proliferation of dermal fibroblasts via the mitosis and migration of dermal fibroblasts and soft-tissue reconstruction. At the same time, studies have found that PRF can promote the proliferation of fibroblasts and keratinocytes in the gums[24]. In addition, He et al.[25] used mass tissue culture to isolate and culture human gingival fibroblasts (HGFs). Using flow cytometry, the results showed that after the PRF treatment of HGFs, the proportion of HGFs in the S phase was significantly prolonged.

Liu et al. placed double PRF membranes in 46 and 47 implant surgery cases. A 5-month follow-up revealed that the width of the keratinized gingiva was significantly increased, and the colour and shape of the mucosa were clearly abnormal[26]. At present, PRF has also played an important role in clinical treatments in other medical fields. For example, when chronic venous ulcers of the lower extremities were covered with porous PRF film, in the subsequent 16 weeks of wound closure speed testing, the ulcers on 66.7% of patients had essentially healed[27].

In summary, the mechanism of PRF on soft-tissue repair may have the following causes. First, a large number of PDGF receptors can be found in periodontal tissue. PDGF can act as a chemokine for fibroblasts in the gingival periodontal ligament to promote soft-tissue regeneration. Second, TGF-β1 (produced by macrophages, fibroblasts, keratinocytes, and platelets) can promote the healing of skin wounds. In addition, PRF contains a large amount of VEGF, which promotes vascularization to support the regeneration of soft tissues, activate mitosis, and the migration of vascular endothelial cells, thereby activating the early vascularization of tissue repair and promoting the formation of soft tissues[28].

SUMMARY AND PROSPECTS

At present, denture implants are widely used in oral restorations, so it is very important to maintain a good three-dimensional shape of the alveolar socket after tooth extraction[29,30]. From a clinical application perspective, PRF has a significant alveolar ridge preservation effect and a good protective effect on the surrounding soft tissue. At the same time, since the material is derived from the recipient itself, it has good biocompatibility and no problems with immune rejection, and it can be considered a safer autologous biological material.

Despite the lack of strong evidence in the reviewed articles, the favourable effect of PRF on alveolar ridge preservation during natural tooth extraction and implantation procedures is evident. Given its ease of preparation, low cost, and biological properties, PRF can be considered a reliable treatment option. However, standardization of the protocol is required to obtain reproducible results. The use of enough PRF clots or membranes seems to be crucial to obtaining an optimal effect. Due to the lack of standardization in the study's design and variables analysed, further RCTs with long-term follow-up are needed to assess the beneficial effect of PRF on alveolar ridge preservation.

AUTHORS' CONTRIBUTION

SLH: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **HGZ:** Conceptualization, Data curation. **NL:** Formal Analysis, Writing – original draft. **XHZ:** Formal Analysis, Writing – review & editing. **HHC:** Formal Analysis, Writing – review & editing.

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REVIEW ARTICLE

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The role of gut dysbiosis-associated inflammation in heart failure

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INTRODUCTION

Heart failure (HF) is an important public health problem, with high mortality and morbidity. Its prevalence has increased due to the aging of the population, once the disease affects approximately 1–2% of the adult population in developed countries, rising to more than 10% among people over 70 years of age. In Brazil, according to DATA-SUS, an organ of the Ministry of Health, more than 26000 patients died due to HF in 2012[1].

HF patients are recognized by a progressive increase in congestion that is associated with an elevation of circulating biomarkers of inflammation, a condition that is associated with impairment in functional capacity and predicts poor clinical outcomes. Inflammation in HF patients is a frequent condition, contributing to the pathogenesis and progression of the disease through diverse mechanistic pathways that culminate with increased levels of pro-inflammatory cytokines, especially interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α)[2].

Although inflammation is a common condition in HF patients, it is still poorly understood what the origin of the inflammatory process in these patients is[3]. Recent evidence suggested that gut microbiome plays a major role in both health maintenance and disease. The imbalance of microbial communities in the gut, named gut dysbiosis, seems to be a potential contributor to HF progression by activating inflammatory pathways[4].

Thus, the possible cross talk between gut dysbiosis and HF severity is intriguing and has the potential to identify new pathways and treatment strategies for HF. So, the aim of this revision was to clarify the possible association of gut dysbiosis, inflammation, and HF, and possible diagnosis, prevention, and treatment strategies.

Gut microbiota

The human gut microbiota is a complex ecological community that has likely coevolved with humans for millions of years, resulting in reciprocal physiological changes. The colonization of gut bacteria begins at birth and gradually becomes more diverse by 2–3 years of age, when it begins to resemble the adult gut microbiota[4]. It has been established that $>10^{14}$ (>100 trillion) microorganisms (e.g., bacteria, archaea, yeast, and viruses) inhabit the human intestine, with differences in numbers of microbes and microbiota composition along the digestive tract[5].

At the moment, four main bacterial phyla have been identified in the human gut: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and P*roteobacteria*, with the phyla *Firmicutes* and *Bacteroidetes* being the most characteristic in the healthy gut (>90%)[5]. However, the composition of human microbiota is subjected to a number of changes during health and disease, being influenced by stress, diet, exercise, disease, and medications, and becoming less diverse again toward extreme old age[4,6].

Thus, the effect of gut microbiota on host physiology is not limited to processing food nutrients otherwise indigestible, but promotes the host's health in a number of other ways, which include a local protective function regulating mucosal barriers and the immune system preventing the proliferation of pathogens[5]. Therefore, the effects of gut flora on host metabolism and immunity might be considered a key mechanism in human physiology.

Gut dysbiosis and HF severity

Gut dysbiosis has generally been described as a significant deviation from the functional microbiome[4]. Each of the following three conditions can be considered as dysbiosis:

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(A) loss of valuable microbial organisms,
(B) expression of pathobionts of possibly beneficial microorganisms, and
(C) loss of general microbial variet1y[7].

The literature already describes the possible association between gut dysbiosis and the manifestation or worsening of several diseases, e.g., HF[4,8].

HF is a disease characterized by a state of chronic inflammation with elevated circulating levels of pro-inflammatory cytokines, such as TNF-α, as originally described by Levine et al. in 1990[9]. These circulating cytokines act as cardiosuppressors via different pathways that include alterations in myocardial intracellular calcium homeostasis, reduction in mitochondrial activity, and alterations in matrix metalloproteinase expression, resulting in an adverse response from myocardial, which includes negative inotropism, cardiomyocyte hypertrophy, and apoptosis[10]. However, the origin of inflammation in patients with HF is still controversial and includes different hypotheses, highlighting a decrease in intestinal perfusion and mucosal ischemia, resulting in gut disruption with increased gut permeability, and subsequently enhancing the translocation of bacteria and bacterial toxins in the blood, which can contribute to systemic inflammation and then to HF exacerbations[10,11].

The intestinal epithelium acts as an impervious barrier to prevent lipopolysaccharide (LPS) translocation. However, in a dysbiosis condition, the intestinal barrier increases in permeability as a result of a disruption to the regulation of the epithelial cell-to-cell tight junction protein network. A compromised intestinal barrier can be associated with bacterial translocation from the gut into the systemic circulation increasing the risk of inflammation and metabolic endotoxemia (ME), and may represent an important mediator of low-grade systemic inflammation[7,12]. Figure 1 summarizes the possible relationship between gut dysbiosis and HF.

LPS is the major component of the outer membrane of Gram-negative bacteria. Under septic circumstances, circulating LPS acts as a pathogen-associated molecular pattern, being able to stimulate the innate immune system, mediating a local or systemic inflammatory response. LPS can also stimulate nonimmune cells and initiate the inflammatory process. The literature reports that an innate LPS-pattern recognition receptor, the Toll-like receptor-4 (TLR-4) is widely expressed in the body, including cardiac tissue[13]. Thus, the innate inflammatory response can be induced in cardiomyocytes by LPS independently of the immune cell involvement[14].

Biomarkers of intestinal dysbiosis

Given the relevance of gut-associated inflammation in HF patients, the early identification of this condition is fundamental for the treatment and aggravation of this disease[15]. Thus, in the face of dysbiosis, some metabolites, including N-oxide-trimethylamine (TMAO), short-chain fatty acids (SCFAs), circulating LPS, and zonulin primary and secondary bile acid, are generated and may act as biomarkers of intestinal dysbiosis, predicting inflammation in HF[16].

TMAO is a urine toxin stimulated by choline, phosphatidylcholine, and L-carnitine fermentation that occurs biologically in the intestinal microbiota[17]. However, in conditions of gut dysbiosis, the levels of TMAO are elevated in the circulation, which can contribute to the severity of heart disease, especially by stimulating chronic inflammation[7]. The literature reports that increased levels of TMAO contribute to overexpression of pro-inflammatory cytokines, such as TNF-α and Il-1β, and also the attenuation of anti-inflammatory cytokines such as IL-10[18]. Recent evidence has suggested the TMAO level as a biomarker to assess gut barrier permeability[19].

Zonulin is a family peptide produced in the intestinal and hepatic cells that regulate a protein complex named tight junctions. The literature has reported that high levels of zonulin are associated with increased intestinal permeability[20], a condition that allows the translocation of LPS from the intestinal lumen into circulation, resulting in endotoxemia and a low-grade chronic inflammation through the activation of Toll-like receptors[21].

The SCFAs acetate, propionate, and butyrate are the main metabolites produced in the colon by bacterial fermentation of dietary fibers and resistant starch, exerting effects on the colon as energy supply and trophic factors[22]. SCFAs improve gut health through a number of local effects, ranging from maintenance of intestinal barrier integrity, mucus production, to protection against inflammation[22]. Higher fecal SCFAs are also associated with central obesity, hypertension, and subclinical measures of cardiometabolic disease (e.g., inflammation, glycemia, and dyslipidemia)[23].

LPS is the major component of the outer membrane of Gram-negative bacteria. Increased gut permeability enhances the penetration of gut microbiota-derived LPS from the intestine into the bloodstream[24]. High levels of serum LPS have been associated with pathological processes, including diabetes, the progression of kidney disease, obesity, and inflammation. LPS induces inflammation via a cascade of inflammatory responses following the recognition of lipid A by immune cells. Lipid A is the toxic component of LPS and serves as the microbe-specific molecular signal that binds to the surface receptor complexes of immune cells, which comprise TLR-4[25].

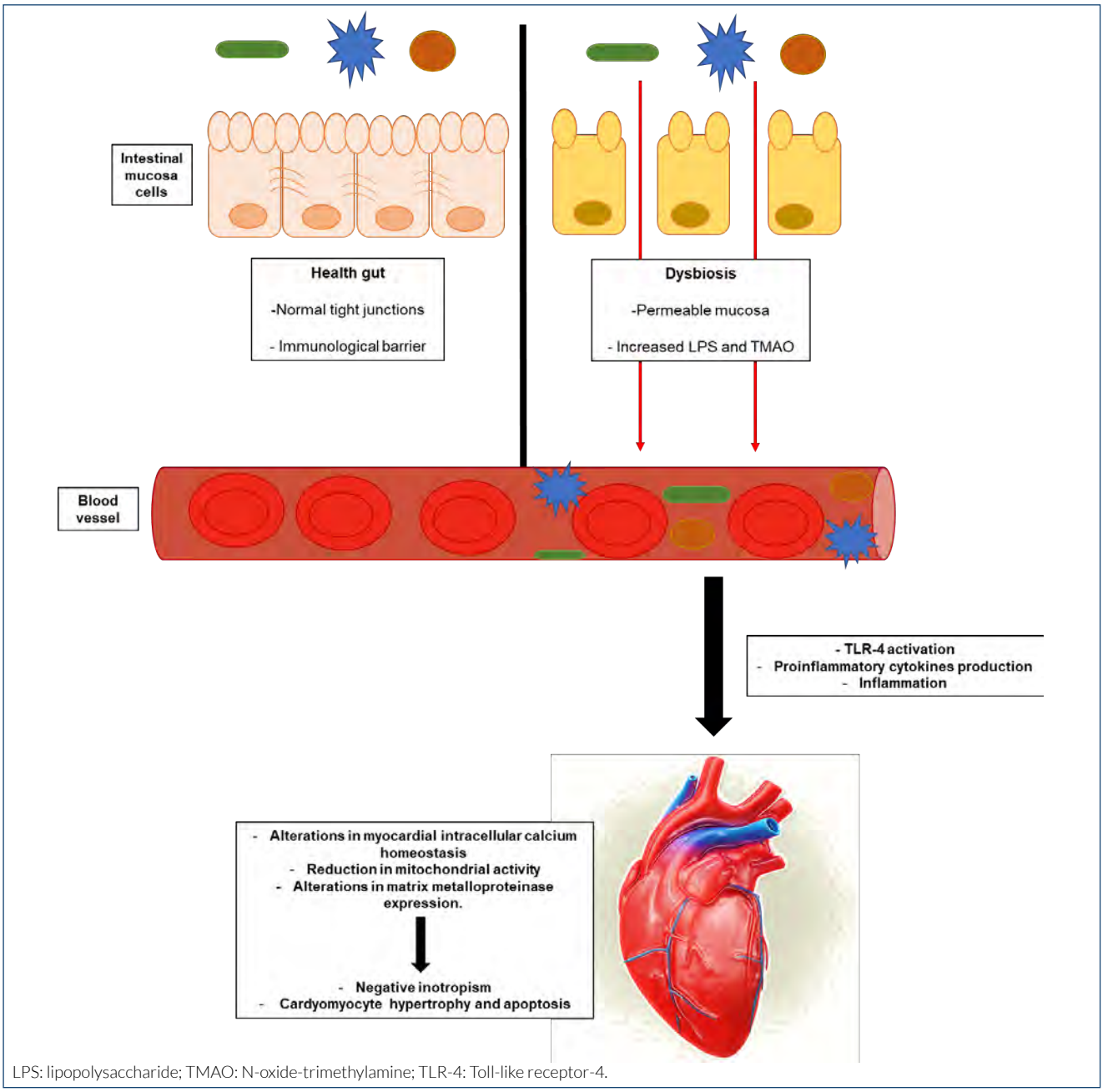

Figure 1. Relationship between gut dysbiosis and heart failure. Under health conditions, gut mucosa has normal tight junctions and works as an immunological barrier. In dysbiosis, mucosa becomes permeable and the levels of lipopolysaccharide and N-oxide-trimethylamine increase. This condition induces an inflammatory response by Toll-like receptor-4 activation, resulting in negative inotropism and cardiomyocyte hypertrophy and apoptosis.

Modulation of dysbiosis as a potential target in heart failure

Once dysbiosis may contribute to the pathogenesis and progression of HF, modulation of this condition could be an effective therapeutic target. Among the main interventions, the literature reports diet modification, including high intake of fruits and vegetables and low consumption of red meat and simple carbohydrate, is well-documented[26].

Probiotics are live beneficial bacteria that re-establish an appropriate intestinal balance by different mechanisms, including pH modulation, antibacterial compound production, and competition with pathogens. Probiotics mainly include bifidobacteria, yeasts, and lactic acid bacteria[26,27]. Prebiotics are non-digestible carbohydrates used as fermentation substrates and stimulate the proliferation and activity of beneficial intestinal bacteria. It includes oligofructose administered

by supplements or consumed in foods, such as asparagus, sugar beet, garlic, chicory, onion, banana, etc[28].

Fecal microbiota transplantation (FMT) is a method of treating intestinal microecological imbalance and reconstructing normal intestinal function by introducing bacteria or metabolites from donor feces into diseased receptors. It is used to treat *Clostridium difficile*. There are no clinical studies that evaluate FMT in HF patients[27].

Antibiotic treatment destroys the balance of intestinal flora, leading to a decrease in flora abundance and changes in composition[27]. A study conducted by Zhou et al. has shown that antibiotics injected to eliminate intestinal bacterial translocation are able to alleviate systemic inflammation and myocardial cell damage in mice with myocardial infarction[29]. It is important to emphasize that improper use of antibiotics can kill beneficial bacteria in the body, making pathogens resistant and causing various adverse reactions. Thus, the positive and negative effects of the use of antibiotics have to be considered.

FINAL CONSIDERATIONS

Gut dysbiosis can be both a cause and consequence of inflammation in HF and plays a central role in disease pathogenesis and progression. Although some studies have suggested the association among gut dysbiosis, inflammation, and HF, more studies are necessary to elucidate the involved mechanisms. Additionally, the modulation of gut dysbiosis is an important strategy to be tested in clinical studies as a possible intervention to reduce the inflammation and HF severity.

AUTHOR'S CONTRIBUTIONS

FVFF: Conceptualization, Writing – original draft. **ETNM:** Conceptualization, Writing – original draft. **JSS:** Conceptualization, Writing – original draft. **AJTF:** Conceptualization, Writing – original draft **TAV:** Conceptualization, Writing – original draft. **SGZB:** Conceptualization, Writing – original draft. **CRC:** Conceptualization, Writing – original draft.

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COMMENTARY

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Comment on "Prediction of impacts on liver enzymes from the exposure of low-dose medical radiations through artificial intelligence algorithms"

Song Zhang[1]*

Dear Editor,

We have recently read an article entitled "Prediction of impacts on liver enzymes from the exposure of low-dose medical radiations through artificial intelligence algorithms[1]." In this study, artificial intelligence-based predicting model random forest was proved accurate in prediagnosing alterations in liver enzymes.

However, we still have some suggestions to discuss with researchers.

First, the goal of this study was to explore liver function damage. The increase in transaminase is only one of the laboratory indicators of liver function damage, which still needs to be confirmed by follow-up, clinical symptoms, or imaging.

Second, the population samples collected in the training set and test set of this study should exclude exposure to other factors, such as alcohol, drugs, and basic diseases, causing liver function damage.

Third, supervised learning is a "negative or positive" model based on standard setting. There is a complex process of progression and recovery when it is in the early liver function damage and the subclinical liver function before the damage reaching irreversible state. Therefore, the criteria set by the model used in the study, i.e., whether it can fully define the pathological state of the real world need to be considered carefully.

In fact, many of these deficiencies are due to the limitations of research conditions. Therefore, we look forward to further studies by researchers.

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