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The compliance's role in the mitigation of judicial demands

Fábio Roberto Cabar[1*], Gustavo Sant Anna Bento Domingues[2]

The Constitution of the Federative Republic of Brazil in 1988 established health as a fundamental right, a right of all citizens and a duty of the state to guarantee it. In addition, it brought the creation of the Unified Health System (SUS)[1].

Guaranteed in Article 196 of the Federal Constitution, Brazil's Unified Health System (SUS) is the only public health system in the world that serves more than 190 million people, 80% of whom depend exclusively on public services for any healthcare[2].

The Federal Constitution attributed to the state the guarantee of health, which, when interpreted singly, leads to the belief that the state must provide health in an unrestricted way (physical, mental, and social well-being, at all levels of care), that is to say, not just the absence of disease; thus, public health users have sought this constitutional guarantee through judicial means, which has substantially increased the number of lawsuits, pleading in court the most varied objects, including treatments that are not provided by the SUS and that are available in the private network, medications, appointments, and procedures[3].

When the claim is taken to the court, the judge will freely form his or her conviction, judging each claim individually, often without considering the social consequences collectively. Spending on lawsuits in health consumed 1.3 billion BRL in 2016; the ten most expensive drugs were responsible for 90% of this value[4]. The money ends up coming out of people's health programs and from several segments of the budget.

The public service has a general character, as it must ensure the supply of the basic needs of society without distinction. With the multitude of clinical protocols and the incursion of new technologies in health, there is consequently an impact on the budget of public entities, leading managers to equalize health resources for the collective good to the detriment of individual situations.

Faced with this scenario, it is essential to analyze the causes and seek a solution to the litigation relief.

JUDICIAL CLAIMS AGAINST THE CLINICS HOSPITAL OF THE FACULTY OF MEDICINE OF THE UNIVERSITY OF SÃO PAULO (HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO, HCFMUSP)

In the year 2021, 10,371 lawsuits were filed against the State of São Paulo requesting non-standardized medicines by the Unified Health System (SUS); from January to October of the following year, there were 9,170 actions dealing with the same issue[5-7].

Specifically, against the HCFMUSP, 111 civil lawsuits were filed from January 2017 to August 24, 2022, with 104 discussing civil liability and only seven for supplying medication. Of these lawsuits, only two had unfavorable final court decisions, and the rest of the actions are in progress or had decisions favorable to the Autarchy. Considering the thousands of outpatient visits, hospitalizations, and surgical procedures performed every year in this institution, the number of lawsuits against the State of São Paulo is considered low when compared to other public institutions. Thus, this relevant fact must be analyzed with care and attention.

COMPLIANCE'S ROLE IN THE PREVENTION OF LEGAL DISPUTES

The word compliance is derived from the English verb ''to comply", which means to act according to an order, a set of rules, or a request. In the corporate environment, compliance is related to conformity or even corporate integrity, aligned with the company's rules, which must be carefully observed and complied with.

Since 2018, the HCFMUSP has a Compliance Board, having been the first public hospital in Brazil to have this type of care. Its purpose was to serve as an information disseminator

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in order to raise awareness of all professionals through training, educational guidance, and support related to issues involving the behavior within ethical and bioethical principles, conflicts of interest, and possible legal and administrative consequences derived from actions in the performance of its voluntary functions, thus for the maintenance of quality in the attendance to the users, fair application of the medical resources, and, at the same time, the observance of the principles of public administration[8].

Also, as a complement to its functions, the HCFMUSP Compliance Board, together with the institution's Law Center, provides support in the validation and preparation of documents and conducts protocols.

For all the above, it should be noted that, through this action, the Compliance Board plays an important role in mitigating individual and institution risks and in reducing lawsuits filed against public entities, precisely what has been observed in recent years. For this reason, it is desirable that other health institutions adopt this policy, which would certainly greatly benefit the entire population that depends on public resources for their well-being.

AUTHORS' CONTRIBUTIONS

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

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Brazilian guidelines for allergen immunotherapy in the treatment of allergic rhinitis

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INTRODUCTION

Epidemiological studies show that allergic rhinitis (AR) is observed in 10–40% of the world's population. This disease significantly compromises the quality of life, impairing development in children and professional activities in adults. AR is also frequently associated with allergic asthma (AA)[1,2]. It has been observed that 15–38% of patients with AR develop concomitant AA. This relationship between AR and AA is based on robust pathophysiological mechanisms, which are consistent with the united airways theory. This model states that environmental exposure to allergenic molecules in genetically predisposed individuals directs the production of specific cytokines responsible for the development of the allergic inflammatory process in the nasal mucosa and lungs[1,3].

The association between AR and AA or atopic dermatitis (AD) is very common, usually developing since childhood, representing a phenomenon called the atopic march. Therefore, patients with AR should be evaluated in a broad and systemic way due to the implications and interactions of this disease that is part of a broad allergic process that can affect the upper airways, lower airways, skin, and mucous membranes. These diseases, classified as atopic diseases, are characterized by the presence of a specific, genetically directed immune response after exposure to allergens[1,2,4,5]. In Brazil, the components derived from the house dust mites *Dermatophagoides farinae* (Df), *Dermatophagoides pteronyssinus* (Dp), and *Blomia tropicalis* (Bt) are the main allergens associated with the etiology of AR. Particularly in southern Brazil and in rural areas, pollens are also allergens associated with the etiology of AR[6].

Knowledge of the pathophysiology of AR is important for understanding the diagnostic strategies and therapeutic possibilities. Sensitization in the nasal mucosa starts with the presentation of allergens by antigen-presenting cells, such as dendritic cells, macrophages, and Langerhans cells, to naive $CD4^+$ T lymphocytes, which at the level of innate immunity may present themselves as dysfunctional, and individuals with genetic predisposition in the presence of allergens have a tendency to differentiate naive $CD4^+$ T cells into Th2 cells, which are characterized by producing interleukin (IL)-4, IL-5, and IL-13. In addition, other important cytokines in this allergen-specific response or even in nonspecific triggers (irritants, pollutants, virus infection, etc.) are IL-25, IL-33, and thymic stromal lymphopoietin produced by respiratory mucosal epithelial cells. These cytokines (alarmins) can contribute to induce immunoglobulin E (IgE) production and the recruitment of eosinophils to the site of the inflammatory allergic process by stimulating, respectively, IL-4- and IL-5-producing Th2 and ILC2 cells. This entire process is currently referred to as type 2 inflammation, characterizing the pathophysiological mechanisms of AR and AA[5,6].

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The Allergic Rhinitis and its Impact on Asthma (ARIA) guideline was an initiative during a World Health Organization workshop in 1999 that established guidelines for the treatment of AR based on allergy testing and therapeutic approach using evidence-based medicine strategies (Grading of Recommendations, Assessment, Development and Evaluation [GRADE] Approach). The ARIA recommendations state that allergen immunotherapy (AIT) represents one of the cornerstones in the treatment of AR with a level of evidence of A. The guidelines of the European Academy of Allergy and Clinical Immunology (EAACI), World Allergy Organization (WAO), and the American Academy of Allergy, Asthma and Immunology (AAAAI) until 2022 represented the main official documents establishing guidelines for the use of AIT. Recently, the "position paper" of the Brazilian Association of Allergy and Immunology (ASBAI)[6] was published, establishing recommendations for good AIT practices in Brazil. Most of the consensus in the field considers AIT to be the unique treatment capable of modifying the allergen-specific immune response by promoting desensitization and a state of tolerance. The control of AR symptoms remains satisfactory in the long term even after the end of the AIT, reducing or even abolishing the use of drugs. Therefore, we can consider this therapy potentially able to promote total remission of the disease[1,5,6,7,8,9].

The present study aimed to contribute to the Guidelines Project, an initiative of the Brazilian Medical Association. Through evidence-based medicine strategies, we conducted a systematic review in order to guide and standardize management and procedures on the use of AIT in the treatment of AR. Clinical issues on the selection of patients eligible for treatment with AIT through clinical history, allergy testing and/or serum-specific IgE, information on safety and efficacy, indications and contraindications, monitoring treatment, routes of application, and considerations on adequate professional preparation were addressed and discussed.

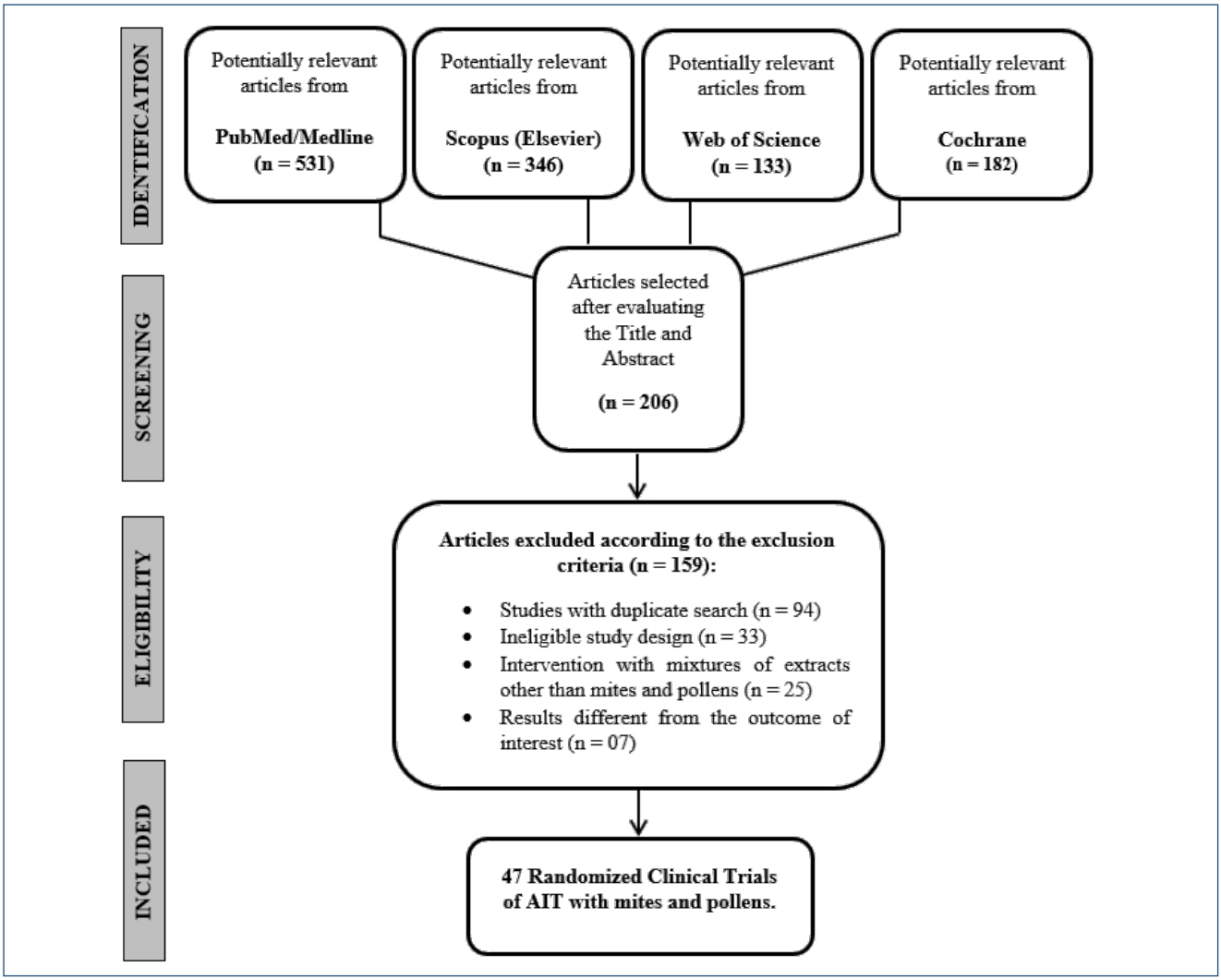

Figure 1. Flow diagram of the randomized clinical trial selection process by Preferred Reporting Items for Systematic Reviews and Meta-Analyses

METHODS

Members of the Scientific Department of Immunotherapy of the ASBAI conducted a systematic review of randomized clinical trials (RCTs) for the construction of medical guidelines on the use of sublingual and subcutaneous immunotherapy with dust mites and pollens in AR. Figure 1 shows flow diagram of the RCT selection process by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

The research methods and criteria are available in the International Prospective Register of Systematic Reviews (PROSPERO) protocol with registration number CRD42022383864; the data from the studies were qualitatively evaluated following the PRISMA guidelines.

ELIGIBILITY CRITERIA

Inclusion criteria were defined following the P.I.C.O.S. framework. Studies that met these criteria were eligible.

1. Population: patients diagnosed with persistent and/or moderate-to-severe AR (ARIA criteria) aged >2 years.
2. Intervention: standard treatment (ARIA) with AIT with dust mites or pollens or standard treatment without AIT.
3. Comparator: standard treatment with AIT and without AIT.
4. Outcomes: for the primary endpoint, we evaluated symptom reduction with clinical improvement of rhinitis.
5. Study type: RCTs published in the past 30 years until November 2022, in English, Portuguese, and Spanish languages.

SEARCH STRATEGY AND STUDY SELECTION

Searches were performed in MEDLINE/PubMed, Web of Science, Scopus, and Cochrane Library databases for articles published until November 30, 2022, using the following descriptors, through the Medical Subject Headings tool, in the same search protocol: for subcutaneous immunotherapy with dust mites: "allergic rhinitis" AND "allergen immunotherapy" AND "house dust mite extracts" AND "subcutaneous"; for sublingual immunotherapy with dust mites: "allergic rhinitis" AND "allergen immunotherapy" AND "house dust mite extracts" AND "sublingual"; for subcutaneous immunotherapy with pollens: "allergic rhinitis" AND "allergen immunotherapy" AND "pollens extracts" AND "subcutaneous"; and for sublingual immunotherapy with pollens: "allergic rhinitis" AND "allergen immunotherapy" AND "pollens extracts" AND "sublingual."

DATA EXTRACTION AND SYNTHESIS

Quality assessment was obtained using the GRADE approach to assign levels of evidence and rate the strength of recommendation of the results. The quality of evidence was classified into four levels: high, moderate, low, and very low. The following factors were considered to determine the level of evidence: study design, methodological limitations (risk of bias), inconsistency, imprecision, and magnitude of effect. After this analysis, the strength of the recommendation was identified as weak or strong, and an evaluation of the clinical trials was performed together.

For risk of bias assessment, the revised Cochrane Risk of Bias (RoB2) tool was used for selected randomized trials. RoB2 was judged as low, moderate, high, or unclear for each domain: randomization process, deviations from intended interventions, lack of outcome data, outcome measurement, selection of reported outcomes, and overall bias. The domains included in this tool were divided according to the phase of the intervention: pre-intervention (bias due to confounding, bias in selection of participants for the study), intervention (bias in classification of interventions), and post-intervention (bias due to deviations from intended interventions, bias due to lack of data, bias in measurement of outcomes, and bias in selection of reported outcomes).

CLINICAL QUESTIONS: EVIDENCE ANALYSIS

Tables 1, 2, and 3 present the data analysis of the risk of bias and grading of the value of evidence by the GRADE approach. In each clinical question answered below, these analyses were taken into account to establish the conclusions and recommendations. The GRADE analysis was performed using the set of articles analyzed specifically for house dust mites and pollens.

Question 1: Is subcutaneous allergen immunotherapy effective in allergic rhinitis in children and adults?

The clinical picture of AR may present in seasonal or perennial clinical form, caused respectively by pollen/fungi and house dust containing predominantly components derived from house dust mites, animal epithelia, and fungi[2,6,7,10-17].

In cases of moderate-to-severe persistent AR, AIT, administered by sublingual (SLIT) or subcutaneous (SCIT) route, is a therapeutic modality considered one of the pillars of the professional practice of the specialist in allergy and immunology. AIT has shown to be effective, contributing significantly to clinical improvement by reducing symptom scores

Table 1. RoB2 analysis to house dust mite allergen immunotherapy.

Intention-to-treat	Unique ID	Study ID	D1	D2	D3	D4	D5	Overall
	1	Bahçeciler - 2001	+	+	+	-	!	
	2	Bergmann - 2014	+	+	+	+	-	
	3	Bernstein - 2018	+	+	+	+	+	
	4	Bozek - 2013	+	+	+	+	+	
	5	Chen - 2020	+	!	+	+	+	
	6	De Bot - 2012	+	!	+	+	!	
	7	Demoly - 2021	+	+	+	+	+	
	8	Di Gioacchino - 2012	+	+	+	+	!	
	9	Didier - 2015	!	+	+	+	!	
	10	Dokic - 2005	+	+	!	+	!	
	11	Guez - 2000	+	!	+	-	+	
	12	Karakoc-Aydiner - 2015	+	!	+	+	+	
	13	Masuyama - 2019	+	!	+	+	!	
	14	Mosbech - 2015	+	+	+	+	+	
	15	Okamoto - 2017	+	+	+	+	!	
	16	Okamoto - 2019	+	+	+	!	!	
	17	Riechelmann - 2010	+	!	+	+	+	
	18	Tonnel - 2004	+	!	+	+	!	
	19	Tseng - 2008	+	!	+	!	+	
	20	Valero - 2022	!	+	+	+	!	
	21	Varney - 2003	+	+	+	+	!	
	22	Vesna - 2016	+	+	+	+	+	
	23	Xian - 2019	+	+	+	+	+	
	24	Yu Guo - 2017	+	+	+	+	+	
	25	Yukselen - 2013	+	+	+	+	!	

+	Low risk
!	Some concerns
-	High risk

D1	Randomisation process
D2	Deviations from the intended interventions
D3	Missing outcome data
D4	Measurement of the outcome
D5	Selection of the reported result

and medication use, whose effects may persist for several years after discontinuation (termination). Thus, the etiologic diagnosis of AR responsible for IgE antibody-mediated sensitization, determining its clinical relevance, is crucial for the allergist with RQE (specialty qualification record) doctor in allergy and immunology and/or pediatric allergy practice area to carry out the selection (formulation) of allergenic extract components and their use in different dilutions in an appropriate manner for the proper choice of route of administration, whether subcutaneous or sublingual, and its application scheme (protocol). Also, it is of fundamental importance to know the properties of the allergens so that the specialist can

Table 2. RoB2 analysis to pollens allergen immunotherapy.

Intention-to-treat	Unique ID	Study ID	D1	D2	D3	D4	D5	Overall
	1	Ahmadiafshar - 2012	+	!	+	+	!	
	2	Bowen - 2004	+	!	+	+	!	
	3	Bozek - 2020	+	+	+	+	!	
	4	Bufe - 2004	+	+	+	+	!	
	5	Clavel - 1998	+	!	+	-	+	
	6	Couroux - 2019	+	+	+	+	+	
	7	De Blay - 2007	+	!	+	+	!	
	8	Durham - 2012	+	+	+	+	+	
	9	Gotoh - 2019	+	+	+	+	+	
	10	Lou - 2020	+	+	+	+	+	
	11	Nolte - 2020	+	+	+	+	+	
	12	Nolte - 2021	+	+	+	+	!	
	13	Okamoto - 2015	+	!	+	+	!	
	14	Pfaar - 2008	+	!	+	+	-	
	15	Pfaar - 2010	+	+	+	+	!	
	16	Pfaar - 2019	+	+	+	+	+	
	17	Sharif - 2019	+	+	+	+	!	
	18	Ünal - 2020	-	+	+	+	+	
	19	Wahn - 2012	+	+	+	!	+	
	20	Worm - 2019	+	+	+	+	!	
	21	Yang - 2022	+	+	+	+	+	
	22	Yonekura - 2021	+	+	+	+	+	

+	Low risk
!	Some concerns
-	High risk

D1	Randomisation process
D2	Deviations from the intended interventions
D3	Missing outcome data
D4	Measurement of the outcome
D5	Selection of the reported result

choose whether or not to mix certain allergens in cases of polysensitized patients[6,18-26].

This systematic review included 25 double-blind, placebo-controlled (DBPC) RCTs with a total of 4,518 patients with perennial AR with or without asthma who underwent immunotherapy with house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, in a 1:1 ratio) and 3,887 placebo-treated control patients, and when analyzed by the GRADE approach (Table 3; classification of recommendations, assessment, development, and evaluation) showed a level of CERTAINTY considered HIGH, with no seriousness detected in the parameters of risk of bias, inconsistency, indirect evidence, and imprecision, as shown in Table 3 (GRADE for RCTs involving AIT with dust mites). Among the total of these 25 RCTs, 3 studies involved three comparative groups: SCIT, SLIT, and placebo/control (pharmacotherapy only, in the study conducted by Karakoc-Aydiner)[15], and the rest employed only one active treatment modality. Thus, in all seven trials (four trials with SCIT active group and placebo; three trials with SCIT and SLIT active groups versus control) that employed ITSC, they demonstrated clinical efficacy in the treatment of AR by reducing symptom and/or medication scores compared to the placebo group, as shown in Table 1 (RoB2, AIT with dust mite allergens).

Table 3. GRADE analysis.

Question: Dp and Df mite extracts compared to Placebo with the same organoleptic characteristics for persistent and/or moderate-to-severe allergic rhinitis (ARIA criteria)									
Context: To evaluate the reduction of symptoms with clinical improvement in allergic rhinitis.									
Certainty assessment							Number of patients		Certainty
Number of studies	Study design	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Dp and Df mite extracts	Placebo with the same organoleptic characteristics	
25	Randomized clinical trials	Nonsevere	Nonsevere	Nonsevere	Nonsevere	None	4.518	3.887	⊕⊕⊕⊕ High

Question: Grass pollen extract compared to placebo for perennial or seasonal allergic rhinitis									
Context: To evaluate the reduction of symptoms with clinical improvement in allergic rhinitis.									
Certainty assessment							Number of patients		Certainty
Number of studies	Study design	Risk of bias	Inconsistency	Indirect evidence	Imprecision	Other considerations	Grass pollen extracts	Placebo with the same organoleptic characteristics	
22	Randomized clinical trials	Severe	Nonsevere	Nonsevere	Nonsevere	None	2.945	2.248	⊕⊕⊕◯ Moderate

For AIT with grass or tree pollen allergens, 22 DBPC RCTs were included, with a total sample size of 2,945 patients with seasonal AR receiving grass or tree pollen immunotherapy and 2,248 patients in the placebo group. Analyzing these trials, because they are the most heterogeneous trials, the joint analysis of these trials by GRADE (Table 3) showed a level of CERTAINTY considered MODERATE, although it was not detected any severity in the parameters of inconsistency, indirect evidence, and imprecision, but showed serious risk of bias, as can be seen in Table 3 (GRADE of RCTs with pollens). Of the total of these 22 RCTs, 5 employed SCIT, and 17 SLIT, as shown in Table 2 (RoB2, AIT with pollen allergens).

Conclusions

1. SCIT with house dust mites is effective in AR in children and adults (GRADE: high; GRADE OF RECOMMENDATION: strong).
2. SCIT with pollens is effective in AR in children and adults (GRADE: moderate; RECOMMENDATION: strong).

Question 2: Is subcutaneous immunotherapy safe in allergic rhinitis in children and adults?

Despite the evidence of beneficial clinical effect of SCIT, this therapeutic modality presents risks of developing adverse effects, either in children or adults, especially local reactions such as discomfort, erythema, edema, pain, and pruritus at the application site, usually of mild intensity. Local treatment can be given for these local reactions with cold/iced compresses and/or topical corticosteroids or oral antihistamines. However, patients with frequent and extensive local reactions should be treated with caution, as they may be at greater risk of systemic reactions. In this context, systemic adverse effects may occur, mostly mild, including sneezing, pruritus, nasal congestion, and/or urticaria, which are easily controlled and are not troublesome for the continuation of immunotherapy. In patients with AR and concomitant asthma, it is always recommended to evaluate the acute exacerbation of asthma and measure the peak flow before the application of SCIT, and it should be suspended in the presence of acute asthmatic symptoms. In addition, the greatest concern should be directed toward the serious systemic adverse effects, which, although rare, can occasionally present anaphylaxis and even death has been reported in the literature. Thus, for SCIT, applications require a location with appropriate infrastructure[18], according to the Annex of Resolution CFM 2.215/2018 (Federal Medical Council), and immediate medical care. In cases of anaphylaxis, the treatment of choice is intramuscular application of millesimal epinephrine/adrenaline. Antihistamines and systemic corticosteroids are considered secondary medications. It is recommended that the site of the SCIT should be at the prescribing physician's facility[2,6,9,14].

In addition, Purkey et al.[21] in their evidence-based review recommended the use of SCIT for patients with AR, whether seasonal or perennial, especially for those who are not responsive to usual pharmacological therapy and whose symptoms

significantly impact their quality of life. These authors stated that SCIT is safe when administered carefully to specific patients and applied in settings capable of providing appropriate medical care in the event of systemic adverse reactions.

Conclusions

1. SCIT with house dust mites is safe in AR in children and adults (GRADE: high; GRADE OF RECOMMENDATION: strong).
2. SCIT with pollens is safe in AR in children and adults (GRADE: moderate; GRADE OF RECOMMENDATION: strong).
3. It is recommended that SCIT should be performed at the prescribing physician's facility. The application must always be performed under medical supervision in a place with adequate infrastructure to attend eventual systemic adverse reactions[2,6].

Question 3: Is sublingual immunotherapy effective in allergic rhinitis in children and adults?

Due to its clinical efficacy and high safety, SLIT, initially approved by European health surveillance agencies, particularly in Italy, has spread its use all over the world, including countries in the East, such as Japan, China, and Australia; North America, such as the United States and Canada; and several countries in South America, especially Brazil.

Among the 25 RCTs employing AIT with dust mite allergens used in this systematic review shown in Table 1 (RoB2, AIT with dust mite allergens), 21 clinical trials used SLIT containing a proportional mixture of the dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, all of which showed clinical efficacy by reducing symptom and/or medication scores when compared to the placebo group, except in a study by Karokoc-Aydiner[15] which was found to have reduced symptoms in both the intervention group and the placebo group. Interestingly, 12 trials employed SLIT in the form of sublingual drops, and 9 studies used SLIT in the form of tablets. Thus, the data from most well-controlled clinical trials have demonstrated that SLIT is indeed effective in treating AR in both children and adults, not only in its short-term use (12 months), but also in its long-term use (up to 3 years in the active group). Therefore, it has been well documented through controlled double-blind trials that SLIT is capable of inducing modifying effects on the natural course of the disease, particularly when SLIT is employed with grass pollens, since the duration of its effects lasts for at least 2 years after a 3-year treatment period[24]. Its preventive effect should also be taken into consideration, since children and adolescents with AR treated with SLIT are found to have less chance of developing asthma later, that is, this intervention has altered the atopic march. Due to its beneficial effects, SLIT with house dust mites has been registered and authorized as a drug/medication by health surveillance agencies[25].

Among the 22 RCTs using grass or tree pollens presented in Table 2, 17 trials used SLIT, 10 of which were in the form of sublingual drops, 6 in tablet form, and 1 in spray form. Also, SLIT with grass and tree pollens has been shown to be effective, whether employing a continuous or noncontinuous regimen. In the latter type, the period of SLIT administration can be on a pre-seasonal, pre-co-seasonal, or seasonal regimen. Meta-analysis studies, where a set of patients are analyzed by different investigators, have shown that SLIT with grass extract in pre-co-seasonal regimens has progressively reduced the combined symptom and medication scores over the course of treatment, a reduction from 29% in the 1st year to 45% in the 3rd year of treatment. It has also been noted that the clinical efficacy of using SLIT with pollens can be seen from the first month of treatment[25].

Conclusions

1. SLIT with house dust mites is effective in AR in children and adults (GRADE: high; GRADE OF RECOMMENDATION: strong).
2. SLIT with pollens is effective for AR in children and adults (GRADE: moderate; RECOMMENDATION: strong).

Question 4: Is sublingual immunotherapy safe in allergic rhinitis in children and adults?

SLIT is generally well tolerated, even at high doses, with good clinical safety[27-45]. In the vast majority of patients undergoing SLIT, the predominant adverse effects are mild or moderate oral reactions, such as itching, and mouth and throat irritation. Many of these effects are observed early in the course of treatment (in the induction phase). Tingling sensation (oral paresthesia), lip edema, tongue edema, glossodynia, dysgeusia, abdominal pain, diarrhea, and headache have also been reported. Coughing and dyspnea are likely to occur in patients who have AR concomitant with asthma[26,36,40,42]. It is important to know that mild adverse effects are relatively frequent, with studies showing that 46–69% of the patients treated with SLIT with grasses have reported that the adverse effects were directly linked to the treatment. In this regard, 5% of patients have discontinued treatment due to adverse effects secondary to SLIT.

Radulovic et al.[3] performed a meta-analysis of 60 clinical trials of SLIT in patients with AR with or without asthma, and the overall interpretation was that SLIT was shown to be quite safe, showing predominantly mild-to-moderate local reactions with no need for treatment in numerous studies, but there were no serious adverse reactions, and no patients required the use of adrenaline. Thus, the authors considered that analyses of adverse events were crucial, giving the advantage of SLIT as an alternative to SCIT for its low incidence of systemic adverse effects. Local reactions are common in SLIT with seasonal or perennial allergens compared to the placebo group, and these effects are unavoidable but are generally seen as an inconvenience that cause little distress and have no lasting effect, although some effects may be distressing enough to abandon treatment. Systemic reactions are largely confined to the upper respiratory tract and associated organs (rhinitis, conjunctivitis, or rhinoconjunctivitis), with these occurring more frequently in the SLIT group than in the placebo group. Gastrointestinal effects occur predominantly in pediatric patients, but no reactions were considered serious. Importantly, no serious systemic reaction, anaphylaxis, or death was observed in this meta-analysis.

Di Bona et al.[27], in their systematic review and meta-analysis, found the occurrence of adverse events in 1,384 (61.3%) of 2,259 adult and child patients who received SLIT with grass pollen allergens and in 477 (20.9%) of 2,279 patients in the placebo group. In addition, seven patients in the SLIT group were reported to have had adverse events related to immunotherapy that required the application of epinephrine. The authors concluded that the findings showed little benefit of SLIT with grass pollen tablets for reducing symptom and medication (antihistamines and corticosteroids) scores in patients with seasonal allergic rhinoconjunctivitis, and thus, due to the small benefit, these authors opined that convenience and ease in its administration do not seem to be sufficient reasons for choosing this route.

It should be noted that the EAACI guidelines recommend both routes of administration, subcutaneous or sublingual, for the treatment of AR or allergic rhinoconjunctivitis, perennial, or seasonal, in children or adults. The allergic disease should necessarily be mediated by IgE antibodies to clinically relevant allergens in one or more allergen groups, especially in patients with moderate or severe allergy, whose symptoms affect the quality of life or nighttime sleep[27-29]. It is crucial to know and keep in mind that the recommendations for good clinical practice in AIT from the ASBAI are in agreement with these EAACI guidelines[6,9]. However, the data needed to determine which route of administration is more effective, subcutaneous or sublingual, are currently insufficient[29]. Therefore, each specialist in Allergy and Immunology should carefully analyze each case individually, using their technical and scientific knowledge, and, together with the patient or caregiver, choose and decide on the best route of administration of AIT.

Conclusions

1. SLIT with house dust mites is safe for AR in children and adults (GRADE: high; RECOMMENDATION GRADE: strong).
2. SLIT with pollens is safe for AR in children and adults (GRADE: moderate; RECOMMENDATION: strong).

Question 5: What are the criteria for indicating allergen immunotherapy in allergic rhinitis?

AR can be classified in terms of frequency into intermittent and persistent, and in terms of intensity into mild and moderate-to-severe, according to the ARIA guidelines[1]. The so-called seasonal form, whose main characteristic is intermittence, is caused by a mechanism of immediate hypersensitivity to allergens that are predominantly external to the home (mainly pollens and fungi); on the contrary, persistent (perennial) rhinitis is characterized by sensitization to in-home allergens, such as dust mites, fungi, cockroaches, and animals.

The main criterion for AIT indication is that the rhinitis should be moderate-to-severe, caused by an identified allergen responsible for the induction of specific IgE antibodies, either perennial or seasonal, that is related to the patient's symptoms, and whose drug therapy, together with specific environmental control measures, has not been sufficient for symptom control. This criterion was used in all DBPC RCT studies analyzed in this current systematic review. A few comments will follow.

All these studies referred to *Dermatophagoides pteronyssinus* and *D. farinae*, as shown in Table 1 (RoB2 AIT with mites)[15,19,30-52] or regional pollens, according to Table 2 (RoB2 AIT with pollens)[12,16,24,26,53-70], requiring more consistent studies on other common mites in our environment, such as *Blomia tropicalis*, and even controlled studies with other aeroallergens, such as fungi and epithelium from domestic animals. Nevertheless, Aria[1] as well as guidelines from AAAAI[2], EAACI[5], and ASBAI[6] recognized the AIT as valid when performed with other extracts, as long as they are of good quality, preferably standardized, and with the correct mixture of allergens/antigens, since some allergens may have proteolytic enzymes that inactivate other components of the mixture.

Besides the diagnosis of allergic sensitization, the correlation between allergic sensitization and the onset of symptoms is essential for the indication of AIT. In this context, several

authors have performed nasal provocation tests[15,19,33,42,45,47,66] and ocular provocation tests[44,64,67] to better characterize this association.

Regarding age, DBPC studies in young children are scarce. The minimum age reported was 4 years for SLIT[34,67] and 5 years for SCIT[15]. Considering that SLIT is safe and easily accepted by children, the Brazilian consensus suggests an age of 2 years as the lower limit of indication for this treatment[6]. There is no maximum age beyond which AIT cannot be used, and the contraindications are much more due to comorbidities in this age group than the age itself. Gotoh et al.[59] used SLIT in a large number of patients between 5 and 64 years of age. Bozek et al.[33,55] studied elderly patients up to 75 years old, attesting to the efficacy and safety of AIT, since these contraindications are respected.

Most studies and consensus suggest the age of 65 years as the limit for AIT indication, since the immune response decreases and the risks increase with senescence[2,5,6,24,49,65].

Conclusions

The indications for AIT in patients with AR or allergic Rhinoconjunctivitis are as follows:

1. Moderate-to-severe disease not controlled despite environmental and medication measures or when the patient desires control without the use of medications.
2. Accurate diagnosis of IgE-mediated allergic sensitization through allergy testing (prick test) and/or serum-specific IgE.
3. Correlation between allergic sensitization and triggering of symptoms. In practice, this correlation is clinical and, if possible, nasal and/or ocular provocation tests can be added; however, these procedures are more often reserved for studies.
4. Patients with minimum and maximum age and clinical condition compatible with the chosen treatment (SLIT or SCIT), namely from 2 to 4 years for sublingual treatment and above 5 years for subcutaneous treatment, up to approximately 65 years old for both therapies.

Question 6: What are the absolute and relative contraindications of allergen immunotherapy in allergic rhinitis?

SLIT has a higher safety profile than SCIT since the latter can develop systemic reactions and even anaphylaxis, which is extremely rare in the sublingual route[46-48]. Therefore, contraindications are less restrictive in SLIT. However, in general, the diseases listed below constitute relative or absolute impediments to indicating both.

Severe and poorly controlled asthma

This is an absolute contraindication in all studies and consensus statements[2,4-6,8,9,12,14-17,19,20,24,26,29-70].

AR is often associated with asthma, and it is mandatory that asthma be controlled before AIT can be indicated. Individuals with FEV1 whose value is less than 70–80% of baseline are not included in research protocols[15,30,32,33,38]. However, mild or moderate asthma, since it is controlled, is not an absolute contraindication but a relative one because the risks versus benefits of the procedure have to be controlled, particularly in AIT-SC[15,16], although the sublingual route is more indicated for these patients[15,26,30,33,44,45,52,56,57,64-67].

Underlying diseases

Diseases cited as contraindications to AIT are severe diseases of the immune system, such as autoimmunities; active infectious diseases, such as tuberculosis; heart disease, especially coronary heart disease; and any other disease that contraindicates the use of adrenaline: severe hypertension, even if controlled; severe kidney disease; systemic use of corticosteroids; use of beta-blockers and angiotensin-converting enzyme (ACE) inhibitors; use of immunosuppressants; severe AD; neoplasms; psychiatric diseases that prevent the individual from being fully conscious; lack of adherence to treatment; and drug abuse[26,37,44,47,49,51,54,64,67].

However, according to the main consensus[1,2,6,29], the stage of the disease and its severity must be considered, since controlled immunological diseases, use of ACE inhibitors, beta-blockers, and diseases in general, where the risk of AIT is lower than its benefits, are relative contraindications.

Some studies report anatomical alterations of the upper airways and/or previous otorhinolaryngological surgery as exclusion factors for AIT[35,46], but these are not absolute contraindications, and the cost/benefit ratio and the correct diagnosis of rhinitis should always be considered in these cases.

Nolte et al.[61] excluded patients with eosinophilic esophagitis for using SLIT.

Pregnancy and lactation

There is consensus among researchers that for pregnant and nursing women, AIT should not be prescribed[19,24,26,32,37-39,41,45,47-49,51,54,57,63-66]. In this context, Guo et al.[51] have even required that patients be on contraceptives to enter in their research protocol. However, if the patient becomes pregnant during treatment, the consensuses recommend that treatment does not need to be discontinued, but that the allergen concentration should not be increased if the AIT is still in induction phase[1,2,4-6,29]. This is in agreement with Mosbech et al.[41] who reported pregnancy

during the course of the study without mentioning that such patients were excluded from the study.

Conclusions

1. Poorly controlled asthma and severe active diseases (especially immunological, infectious, and neoplastic) are absolute contraindications for using AIT.
2. Eosinophilic esophagitis is an absolute contraindication for the use of SLIT.
3. Controlled cardiovascular diseases, use of ACE inhibitors, beta-blockers, chronic diseases under control, and mild psychiatric diseases are relative contraindications where risk versus benefit must be evaluated individually.
4. Pregnancy and lactation are conditions that absolutely contraindicate the beginning of treatment, but not in its continuity, when increasing the AIT concentration is contraindicated if it is in the induction phase.
5. Lack of compliance should be considered as a factor to contraindicate the initiation or continuation of the AIT.

Question 7: What are the criteria for monitoring the effectiveness of allergen immunotherapy in allergic rhinitis?

There are simple questionnaires, where a score is assigned according to the intensity of symptoms and need for medication, in diaries requested to the patient or caregivers, and at regular intervals these scores are analyzed[15,19,24,26,30-32,35,39,40-46,48,49,51-54,56,57,63-67]. Several authors use the visual analog scale (VAS) standardized by ARIA[15,33,35,37,47], in which rhinitis symptoms, such as obstruction, itching, sneezing, rhinorrhea, and ocular symptoms, as well as the general perception of such symptoms in the quality of life, are jointly measured on a ruler with figures, and the patient is asked to mark his or her situation along this ruler, which ranges from 0 (totally asymptomatic) to 10 (very bad symptoms, totally uncontrolled)[71,72]. Some authors use their own VAS, with different scores for symptoms[42,44,51,64].

In addition, some researchers ask for an overall score for the AIT to be given at each year of treatment where zero is where there was worsening of rhinitis after 1 year with therapy and the maximum score where there was marked improvement[31,49,51]. Studies also emphasize the need to have questionnaires for specific scoring regarding adverse effects[32,35,37,38,40]. Quality of life questionnaires have been added in several trials[16,35,40,41].

Currently, studies with immunological biomarkers such as IgG4 and specific IgE still show conflicting results, and they are not used in clinical practice for monitoring efficacy or even for treatment discontinuation, remaining restricted to the research field. It is also important to note that the decrease in papule size in skin tests is controversial, with some authors reporting a decrease[19,30,53], but others not[39,47,53]. Therefore, this is not a good parameter for monitoring or for the efficacy of the AIT.

Conclusions

1. Currently, the criteria for monitoring AIT are clinical, evaluating the symptom and medication scores, preferably through the various scales provided in the consensuses. This evaluation can be complemented with quality of life questionnaires.
2. Assessment of side effects should also be monitored.
3. There are currently no clinically available immunological biomarkers for monitoring AIT.
4. Skin testing should not be performed as a means of monitoring the efficacy or duration of the AIT.

Question 8: What are the recommendations for discontinuation of allergen immunotherapy in allergic rhinitis?

All consensus statements[1,2,4-6,29] suggest a minimum of 3 years of duration of AIT, at least for perennial allergens, which is necessary to have a sustained response to treatment. In fact, Durham et al.[24] continued to evaluate patients treated or not treated (control group) after the end of SLIT during 3 years for pollens and found a significant improvement in the active group regarding clinical scores even 2 years after the end of treatment. Chen et al.[34] observed children for three more years after 3 years of treatment with SLIT for dust mites and likewise found sustained efficacy in the group that received active treatment. Gotoh et al.[59] likewise obtained positive results even after 2 years of the termination of SLIT for pollen, maintained for 3 years in the pollen seasons.

Conclusions

1. The optimal duration time for AIT is 3–5 years after the beginning of the maintenance phase. AIT should be maintained for at least 3 years to achieve lasting efficacy.
2. In case of pollinosis, AIT can be performed only for a few months before and during the pollen season (pre-co-seasonal regimen), although in most Brazilian regional, allergens are perennial and not seasonal, except in the southern states.
3. As previously mentioned, the skin test is not a good parameter for discontinuation of AIT, and at present, there are no laboratorial biomarkers to guide the duration of the treatment.

4. Clinical evaluation is always the best parameter to assess the efficacy of AIT. In case of lack of clinical results after reaching the maintenance dose, AIT can be discontinued.

CONCLUDING REMARKS

The main purpose of this systematic review was to establish best practice guidelines for the use of AIT in the treatment of AR. Evidence-based medicine strategies were used to answer relevant clinical questions. The primary endpoints investigated in each study included in this systematic review showed a high degree of evidence for the efficacy and safety of AIT in the treatment of AR in patients sensitized to house dust mites, which correspond to the major allergens associated with the etiopathogenesis of AR in Brazil. We emphasize that recognition of allergic sensitization through appropriate allergy testing and careful clinical evaluation of patients is critical to recognize patients with indications for allergy treatment. Since AR is one of the diseases that is part of the atopic march, a systematic evaluation of patients should be performed, taking into consideration the diagnosis and treatment of other atopic diseases such as AA and AD.

The appropriate choice and management of allergenic extracts to be used in the personalized vaccine used in the AIT is a fundamental condition for achieving the expected results in clinical practice. In Brazil, CFM Resolution No. 2215/2018 regulates the use of allergenic extracts for diagnostic and therapeutic purposes in allergic diseases[18]. The technical responsibility of allergy and immunology services must be exercised by a physician with a RQE in Allergy and Immunology, in the CRM of their jurisdiction, according to Chapter III, article 9, paragraph 1 of the Annex of CFM Resolution No. 2147/2016. In services with exclusive care of pediatric patients, the technical responsibility must be exercised by a physician with an RQE in Allergy and Immunology or RQE of qualification in Pediatric Allergy and Immunology.

Taken together, the data presented here allow us to make a strong recommendation for the use of AIT, either subcutaneously (SCIT) or sublingually (SLIT) in the treatment of AR.

AIT induces changes in the immune response and promotes symptom control in AR through immunomodulation of the allergen-specific response. In this way, AIT allows for clinical remission of AR for prolonged periods without the use of drugs, even after administration has ceased. This therapeutic strategy is currently the only known way to modify the natural history of allergic diseases. Due to the immunomodulation promoted by AIT, patients with AR, besides benefiting from the control of symptoms through this allergen-specific treatment, can also be preventively protected against the development of other atopic diseases such as AA and AD.

AUTHORS' CONTRIBUTIONS

FMA: Investigation, Project administration, Methodology, Writing – original draft, Writing – review & editing. GVAGL: Investigation, Methodology, Writing – original draft, Writing – review & editing. EAT: Writing – original draft, Writing – review & editing. EG: Writing – original draft, Writing – review & editing. NARF: Investigation, Methodology. MCR: Project administration. DS: Investigation, Project administration, Methodology, Writing – review & editing. NPMR: Investigation, Project administration, Methodology, Writing – review & editing. ESCS: Project administration, Writing – review & editing. WMB: Project administration, Writing – review & editing.

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Preoperative pulmonary artery hypertension as a risk factor: the tip of the iceberg

Mesut Engin[1*], Ufuk Aydın[1], Yusuf Ata[1], Senol Yavuz[1]

Dear Editor,

We have read the article by Velioglu et al.[1], entitled "Does pulmonary hypertension affect early-term outcomes of off-pump coronary artery bypass surgery?" with great interest. We congratulate the authors for their valuable contributions and successful off-pump coronary surgeries. However, I would like to discuss some points about preoperative pulmonary hypertension in patients scheduled to undergo off-pump coronary artery bypass graft (OPCABG) surgery.

In this current study, the authors included a total of 1,107 patients undergoing elective first-time OPCABG surgery in this retrospective observational cohort study. The patients were categorized into two groups according to their preoperative systolic pulmonary artery pressure (SPAP) values. The PHT group (n=104) consisted of patients with an SPAP value >30 mmHg, while the non-PHT group (n=1003) consisted of patients with an SPAP value ≤30 mmHg. The authors concluded that both patient groups had similar postoperative outcomes[1]. Could this be due to the large number of patients with an SPAP value between 30 and 50 mmHg? How many patients with an SPAP value above 50 mmHg were in the PHT patient group? We would like to receive your valuable comments on this matter.

Chronic obstructive pulmonary disease (COPD) is an important lung disease[2]. In these patients, PHT may develop according to the severity of obstruction and inflammation[3]. In this study, in which pulmonary hypertension was investigated as a risk factor, we think that the frequency of COPD is a very important parameter. In this study, the frequency of COPD was found to be 22.1% in the PHT group and 7.4% in the non-PHT group, with a p-value of 0.019[1]. Considering the number of patients in the patient groups, we think that the p-value may be inaccurate with the current rates. We believe that it would be useful to check it statistically. This valuable study may be misleading, as it can be a good resource for further systematic review studies.

The European System for Cardiac Operative Risk Evaluation II (EuroSCORE II) is an invaluable risk-scoring system in open-heart surgery operations[4]. Has EuroSCORE II been calculated for your patient groups? Could this be the reason for the similar postoperative results between the groups?

Finally, we would like to address the postoperative atrial fibrillation (PoAF) condition, which is an important problem that occurs after coronary bypass operations. The incidence of PoAF was 41.3% in the PHT group and 22.1% in the non-PHT group. The p-value is specified as 0.033. First, we think that the p-value should be recalculated because the significance seems stronger. Many factors may affect this significant difference in the frequency of PoAF between the groups. Perioperative medical treatment preferences are also important for PoAF[5-7]. What were your perioeprative beta-blocker statin and hypertensive treatment protocols in your patient group?

AUTHORS' CONTRIBUTIONS

ME: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **UA:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **YA:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **SY:** Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Comment on "Serum prealbumin: a potential predictor of right ventricular dysfunction in patients receiving programmed hemodialysis"

Longcai Fang[1], Xiaofang Wang[2*]

Dear Editor,

We read with great interest a recent study1 investigating the relationship between serum prealbumin concentration and right ventricular dysfunction (RVD) in patients undergoing programmed hemodialysis. In this study1, the results found that patients with RVD had lower prealbumin concentration compared with patients without RVD (23.83±8.50 mg/dL versus 31.38±6.81 mg/dL, p=0.001), and prealbumin concentration had an important role in predicting RVD. However, after reading this study carefully, we think that the following questions deserve further clarification.

First, as described in Table 1 of that study1, C-reactive protein (CRP) was higher in patients with RVD compared with patients without RVD [1.45 (0.95–3.30) versus 0.80 (0.40–1.60), p=0.023], suggesting that both prealbumin and CRP concentrations had a significant difference in patients with and without RVD. In this case, either lower prealbumin or elevated CRP concentrations may be associated with poorer right ventricular function, and it cannot be roughly assumed that lower right ventricular function is only associated with lower prealbumin, ignoring the role of CRP. Previous studies have shown that elevated CRP is associated with the prognosis of various diseases, including RVD2 and programmed hemodialysis3. Therefore, it is necessary to adjust for the potential influence of CRP when exploring the relationship between prealbumin and right ventricular function.

Second, the treatment strategy for RVD patients is unclear. The study followed all participants for up to 3 years and compared the difference in mortality between patients with and without RVD. However, detailed treatment strategies for RVD are indistinct. The treatment strategy is undoubtedly closely related to the prognosis of RVD patients. In the absence of treatment strategies, a possible hypothesis is that the higher mortality might be due to inappropriate treatment strategies independent of lower prealbumin concentrations. Therefore, it is necessary to provide treatment strategies between groups.

Third, brain natriuretic peptide (BNP) is currently well-recognized as a useful serological marker that is closely related to the prognosis of heart failure patients and is widely used in clinical practice. Notably, approximately one-third (18/57) of the subjects in this study were patients with RVD. However, information about BNP is unknown. In addition, a previous study4 demonstrated a significant negative correlation between BNP and prealbumin in hemodialysis patients (r=-0.46, p=0.001). Therefore, it may be possible to obtain an accurate relationship between prealbumin and RV function after excluding the potential influence of BNP.

In general, the conclusions of this study provide novel evidence and references for clinical practice, but further clarification of the above concerns will undoubtedly increase the accuracy and reliability of the conclusions.

AUTHORS' CONTRIBUTIONS

LF: Conceptualization, Investigation, Supervision, Writing – original draft. **XW:** Conceptualization, Investigation, Supervision, Writing – review & editing.

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Comments on "Effect of coolant spray on rib fracture pain of geriatric blunt thoracic trauma patients: a randomized controlled trial"

André Pontes-Silva[1*], Karolayne dos Santos Lima[2], Cassius Iury Anselmo-e-Silva[1], André Luiz Lopes[3], Aldair Darlan Santos-de-Araújo[1]

This paper[1] aimed to evaluate the effectiveness of cryotherapy in elderly patients with rib fractures due to blunt thoracic trauma via a prospective randomized controlled study (coolant spray group, n=51; placebo spray group, n=50). The authors concluded that coolant spray therapy can be used as a component of multimodal therapy to provide adequate analgesia due to rib fractures in geriatric patients; however, some points of this research do not support this conclusion.

First of all, the authors did not describe the study hypothesis (this weakens the results). Second, they did not present the minimal clinically important change of the VAS on the patients' pain level (making it impossible to analyze clinical relevance). Comparisons of outcomes in clinical trials should consider the minimal clinically important change of the mean differences because the p-value only shows statistical significance, which interpretation translates just a hypothesis test governed by a probability of previously defined error (α)[2,3]. Third, they did not present the assess the effect size (e.g., Cohen's d=[M1–M2]/S_{pooled})[4,5] of the comparisons between the times and groups (making it impossible [again] to analyze clinical relevance). Fourth, they should have assessed the patients' disability for pre- and post-intervention comparisons.

As such, the new conclusion is as follows: although this study has shown significant differences between coolant spray therapy and placebo (used as a component of multimodal therapy to provide adequate analgesia due to rib fractures in geriatric patients), its clinical relevance is yet unknown.

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AUTHORS' CONTRIBUTIONS

APS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **KSL:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **CIAS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ALP:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ADSA:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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SHORT COMMUNICATION

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A cross-sectional study on the Nesfatin-1 serum levels of Vietnamese patients with pre-diabetes

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Over the past few years, the number of diabetes patients in the world has increased rapidly, with many serious complications, making it one of the most pressing concerns in society. According to the International Diabetes Federation (IDF), there were 537 million people worldwide (aged 20–79) suffering from diabetes in 2021. This number is expected to rise to 783 million in 2045[1]. Vietnam is among the top 10 countries with the highest increase rate of diabetes cases, at 5.5% per year. As reported by the Vietnamese Association of Diabetes and Endocrinology (VADE), there are currently 5 million Vietnamese people living with diabetes, accounting for 6% of the population. This number is predicted to increase to 7 or 8 million by 2025[2]. Diabetes is estimated to be the cause of death for at least 80 people each day. The percentage of undiagnosed diabetes cases in Vietnam is nearly 62.6%[3]. If left undetected and untreated in the early stages, pre-diabetes can increase the risk of dangerous complications and severely affect the patient's health.

In recent years, Nesfatin-1 has been identified as one of the cytokines associated with diabetes[4,5]. Peripheral Nesfatin-1 was linked to several clinical laboratory parameters that influenced nutrition and metabolism[6]. Therefore, determining the concentration of Nesfatin-1 serum is important for assessing the disease progression, predicting the damage to target organs, and evaluating the impact of treatment measures.

A cross-sectional study was carried out on 524 patients diagnosed with prediabetes and 205 healthy people serving as the control group. These participants were taken from periodic health check-up groups at general hospitals in northern Vietnam. Pre-diabetes was diagnosed for those with fasting blood glucose (FBG) ≥126 mg/dL, hemoglobin A1c (HbA1c) ≥6.5%, or with classic symptoms of hyperglycemia[7]. The concentration of Nesfatin-1 serum, anthropometry, and clinical parameters associated with the cardiovascular, hepatic, and renal organs were determined and analyzed.

Nesfatin-1 has the effects of suppressing appetite, reducing gastric motility, reducing cholesterol, triglycerides, and white adipose mass, as well as lowering lipid production and glucose in the blood. The Nesfatin-1 serum level of pre-diabetes patients was 1.5 times lower than in the control group (0.66 vs. 1.12 ng/mL) (Table 1). Blood glucose-related indices of pre-diabetes, such as HbA1c and

Table 1. Demographic, anthropometric, and metabolic characteristics of pre-diabetes.

Group	Control	Patients
Numbers (male/ female)	205 (100/105)	524 (272/252)
Age (years)	52.02±17.89	52.21±18.18
Duration time (years)	–	1.98±0.73
Body mass index (kg/m^2)	21.59±1.21	22.05±1.23
Waist hip ratio	0.90±0.11	0.86±0.10
Hemoglobin A1c (%)	5.40±0.65	6.41±1.35
Fasting blood glucose (mmol/L)	5.65±0.78	7.76±1.71
Total cholesterol (mmol/L)	4.84±1.01	6.02±1.11
Triglycerides (mmol/L)	1.71±0.77	2.72±1.59
High-density lipoprotein cholesterol (mmol/L)	1.41±0.38	1.30±0.47
Low-density lipoprotein cholesterol (mmol/L)	2.65±0.72	2.88±1.18
Alanine aminotransferase (UI/L)	29.97±3.54	38.09±4.64
Aspartate aminotransferase (UI/L)	26.77±2.81	37.34±6.36
Creatinine serum (umol/L)	88.92±9.42	108.12±12.87
Creatinine urine (umol/L)	107.89±8.83	118.01±7.24
Nesfatin-1 serum (ng/mL)	1.12±0.38	0.66±0.37

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FBG, were also higher than in the control group (6.41% and 7.76 mmol/L vs. 5.52% and 4.83 mmol/L). The parameters associated with cardiovascular disease in the pre-diabetes group were also significantly elevated, such as total cholesterol (6.02 mmol/L) and triglycerides (9.72 mmol/L). The data relating to cholesterol density, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) varied between study groups. Although they did not reach the warning or prudent level (HDL-C<1.0 mmol/L and LDL-C>3.3 mmol/L), they were still worse compared to the control group. Therefore, special monitoring and early treatment are essential.

The results of liver and kidney tests showed that the condition of the liver was heading in a bad direction, as indicated by the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values (38.09 and 37.34 UI/L, respectively). Additionally, the creatinine serum and creatinine urine results for the pre-diabetes group were also not positive (108.12 and 118.01 µmol/L, respectively).

The study aimed to evaluate the correlation between Nesfatin-1 serum levels and the test parameters in pre-diabetes patients (Table 2). The results indicated that these values were lower in Vietnamese pre-diabetes patients compared to other Asian patients but higher than those in Europeans[8-10]. Furthermore, there was a positive correlation between the Nesfatin-1 serum levels and ALT, AST, and creatinine (r=0.113, p=0.009; r=0.133, p=0.002; and r=0.091, r=0.094, p<0.05). Conversely, a negative correlation was observed between Nesfatin-1 serum levels and body mass index (BMI), HbA1c, cholesterol, and triglycerides (p<0.05). Some previous studies have reported similar correlations but without statistically significant results (p>0.05)[10-12]. The variability in sample size, patient selection, experimental methods, and ethnographic factors may explain these differing results.

This study represents the first published on Nesfatin-1 serum levels in Vietnamese pre-diabetes patients and reveals differences in concentration compared to other countries worldwide. The correlation between Nesfatin-1 serum concentration and clinical parameters associated with cardiovascular, liver, and kidney conditions was recorded for the first time in Vietnamese pre-diabetic patients, with statistically significant correlations observed between Nesfatin-1 serum levels and creatinine, aminotransferase, triglycerides, lipoprotein, cholesterol, hemoglobin A1c, and BMI.

Table 2. The correlation analysis results between Nesfatin-1 serum levels and diabetes.

Parameters	Pre-diabetes	
	r	p-value
Age	-0.064	0.143
Body mass index	-0.105	0.015
Waist hip ratio	-0.037	0.394
Hemoglobin A1c	-0.108	0.014
Fasting blood glucose	0.040	0.365
Total cholesterol	-0.117	0.007
Triglycerides	-0.102	0.020
High-density lipoprotein cholesterol	0.094	0.031
Low-density lipoprotein cholesterol	-0.132	0.002
Alanine aminotransferase	0.113	0.009
Aspartate aminotransferase	0.133	0.002
Creatinine serum	0.091	0.037
Creatinine urine	0.094	0.032

AUTHORS' CONTRIBUTIONS

NMD: Formal Analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **MNN:** Conceptualization, Supervision, Review & editing. **TTBV:** Formal Analysis. **MTN:** Data curation. **STD:** Data curation.

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SHORT COMMUNICATION

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Troponin elevation on admission and mortality after hospital discharge among patients with COVID-19

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INTRODUCTION

According to the World Health Organization, coronavirus disease 2019 (COVID-19) has resulted in over 6.8 million deaths worldwide as of January 2023[1]. However, variable rates of in-hospital and post-discharge morbidity and mortality have been described since the beginning of the pandemic. Regional differences in medical care, patient characteristics, access to vaccination, and effective interventions are important factors that contribute to these discrepancies. Nonetheless, several biomarkers have demonstrated prognostic value in this scenario primarily during the hospitalization period[2].

High-sensitivity troponin I (hs-TnI) has been widely studied in the context of COVID-19, and elevated levels on admission are predictive of short-term outcomes[3]. However, the long-term prognostic significance of myocardial injury during the acute phase of COVID-19 remains unknown. The objective of this study was to determine the association between hs-TnI elevation above the 99th percentile upper reference limit on admission in hospitalized patients with COVID-19 and long-term survival among those who were successfully discharged.

METHODS

Medical records from consecutive patients with confirmed COVID-19 admitted to a single institution between March and July 2020 were retrospectively analyzed. Only those with a positive polymerase chain reaction result for severe acute respiratory syndrome coronavirus 2 were screened for inclusion. The analysis was further restricted to patients with an initial hs-TnI measurement who were successfully discharged from the hospital. Clinical data, including age, comorbidities, oxygen saturation, and tomographic findings on admission, were collected in addition to further laboratory information (leukocyte count, C-reactive protein, D-dimer, and creatinine). Myocardial injury was defined by hs-TnI levels above the 99th percentile upper reference limit (URL) of the assay (>34 pg/mL). Long-term survival was determined by consulting a public, governmental online database of births and deaths from the state's justice court. Previously collected clinical and laboratory data were then correlated with mortality after the index hospitalization.

The Stata® 11.0 software was used for statistical analysis. Categorical variables were analyzed with[2] and Fisher's exact test. Continuous variables were expressed by the median and 25th to 75th percentile interquartile range. Such data were evaluated by the Wilcoxon-Mann-Whitney and Kruskal-Wallis tests. Variables with significance in the univariate analysis were included in a multivariate logistic regression model to determine independent predictors of death. Survival curves were constructed from Kaplan-Meier estimates, and differences were analyzed using the log-rank test. A p-value<0.05 was considered significant. The study conforms to the guidelines of the Declaration of Helsinki and obtained appropriate Institutional Review Board approval on July 6, 2021, under project number 19101573. Informed consent was not required due to the retrospective nature of the study.

RESULTS

Among the 230 patients admitted with a positive RT-PCR during the study period, 194 survived until hospital discharge, of which 149 had hs-TnI values on admission and were included in the analysis. The median age was 65 years (52–78), and 56.4% were males. Troponin elevation occurred in 21 patients (14.1%), and 36.2% were treated in the intensive care unit

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(ICU). Patients with myocardial injury were older, had greater prevalence of hypertension and previous cardiovascular disease, and presented with greater pulmonary compromise on admission chest tomography. Additional baseline characteristics are described in Table 1 according to the troponin values.

After a median follow-up of 631 days (612–643), 9 (6%) deaths occurred subsequently to hospital discharge, of which 6 were recorded within 1 year. In the univariate analysis, age (OR 1.08; 95%CI 1.02–1.14, p=0.008), hypertension (OR 8.47; 95%CI 1.03–69.53, p<0.047), and hs-TnI elevation (OR 16.7; 95%CI 3.8–73.6, p<0.001) were associated with mortality. In the multivariate model, hs-TnI elevation remained predictive of subsequent death (OR 7.45; 95%CI 1.4–39.4, p=0.018), after adjusting for age, hypertension, ICU admission, C-reactive protein, and creatinine values. Figure 1 represents the Kaplan–Meier survival estimates after hospital discharge according to hs-TnI elevation.

DISCUSSION

Hospitalization due to COVID-19 often imposes significant short-term morbidity and an increased risk of death, especially in the presence of chronic comorbidities. However, even among those who are successfully discharged, long-term sequelae may persist. Among 1,733 patients who survived an initial hospitalization period in China, Huang et al. reported persistent symptoms in 76% of the cohort after 6 months of follow-up. Manifestations included fatigue, muscle weakness, chest pain, psychiatric illnesses, and respiratory impairment[4]. Similarly, in a meta-analysis published by Leon-Lopez et al., more than 50 long-term effects were associated with the disease among almost 48,000 patients from multiple studies[5].

Readmission and death rates are also relevant, with a 27% risk of either outcome within 60 days post-discharge[6]. Most importantly, in a study involving 153,760 patients with

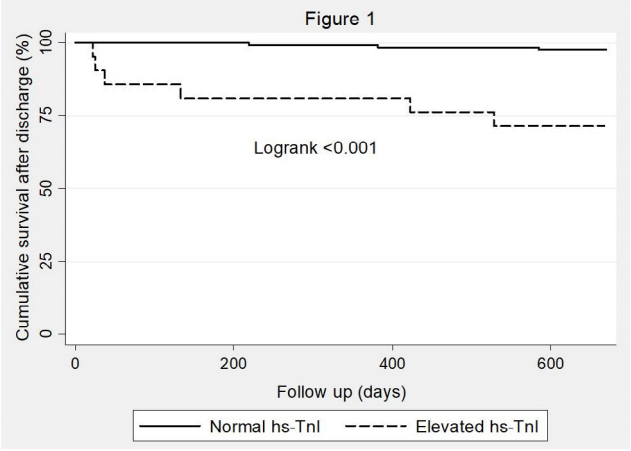

Figure 1. Kaplan-Meier survival estimates of patients discharged after hospitalization for COVID-19. High-sensitivity troponin I elevation on admission was associated with decreased long-term survival. hs-TnI: high-sensitivity troponin I.

Table 1. Baseline characteristics of COVID-19 patients on admission, according to high-sensitivity troponin I elevation.

Characteristics	hs-TnI >99th percentile URL		p-value[a]
	No (n=128)	Yes (n=21)	
Age, median (IQR), years	61 (51–74)	84 (78–89)	<0.001
Male, No. (%)	73 (57.0)	11 (52.4)	0.69
BMI, median (IQR), kg/m^2	26.8 (24.2–30.1)	27 (24.4–28.3)	0.52
Medical history, No. (%)			
Hypertension	60 (46.9)	16 (76.2)	0.02
Diabetes	36 (28.1)	8 (38.1)	0.35
Coronary artery disease	12 (9.4)	6 (28.6)	0.01
Oxygen saturation[b], median (IQR), %	93 (91–96)	94 (86–95)	0.12
Total lung opacities[c], median (IQR), %	15 (6.1–27.5)	26.8 (11.4–49.7)	0.05
Laboratory values, median (IQR)			
Leukocyte count, cells/mm^3	5,430 (4,210–7,310)	9,890 (6,210–12,390)	<0.001
C-reactive protein, mg/mL	6.2 (3–14)	8.1 (3.8–14.2)	0.41
D-dimer, ng/mL	644 (440–1,155)	1,382 (678–3,467)	<0.001
Creatinine, mg/dL	0.85 (0.7–1.0)	0.9 (0.7–1.3)	0.15
hs-TnI, pg/mL	NA	104 (48–479)	NA

[a]p<0.05 indicates statistical significance (bold values); [b]On ambient air; [c]Chest computed tomography analysis. BMI: body mass index; hs-TnI: high-sensitivity troponin I; IQR: interquartile range; NA: not applicable; URL: upper reference limit.

COVID-19, Xie et al. found an increased risk of multiple cardiovascular complications after the first 30 days of infection. The higher risk persisted up to 12 months after the initial diagnosis and included the occurrence of ischemic heart disease, heart failure, cerebrovascular disease, thromboembolism, and arrhythmias. These findings strongly suggest that cardiovascular complications may manifest at a much later stage and could represent the main cause of morbidity and mortality following the acute phase of COVID-19[7].

Troponin elevation is identified in approximately 30% of hospitalized patients with COVID-19 and is associated with a higher risk of both fatal and non-fatal short-term outcomes. However, abnormal TnI levels may persist months after hospital discharge and have been implicated in the long-term complications of the disease[8]. Our results further support this concept, indicating that initial myocardial injury is predictive of survival after discharge.

The exact mechanisms involved are unclear, though arrhythmias, heart failure, and atherosclerotic disease are all potential late consequences that seem to be associated with the extension of myocardial and vascular damage during the acute phase of the disease. COVID-19 endotheliopathy is a well-known factor associated with increased thrombogenicity and may persist beyond the initial stages of infection, leading to thromboembolic, coronary, and cerebrovascular events[9]. In addition, the extension of myocardial scarring following viral myocarditis is another potential mechanism that could affect long-term outcomes and has been previously described in imaging studies performed months after infection[10].

Troponin values on admission could also perform as an indirect marker of non-cardiovascular damage during the disease's inflammatory cascade. Previous studies have demonstrated an association between higher troponin levels, greater total pulmonary opacification percentages, and lower lung volumes in hospitalized patients with COVID-19[11]. In the current study, those with hs-TnI elevation also presented with significantly greater lung involvement on computed tomography. Such an association may have contributed to the adverse prognosis associated with the diagnosis of early myocardial injury, in addition to the subsequent cardiovascular complications. Most importantly, hs-TnI elevation in the acute phase represents a promising surrogate for a variety of outcomes and can be considered an early prognostic marker before hospital discharge.

This study has limitations, which have to be acknowledged. Data were retrospectively collected from a single center and may not reflect the same results as other institutions. Since patients were managed before evidence-based treatments and vaccines were available, current survivors discharged after hospitalization for COVID-19 could have different prognoses. Finally, the number of events was low, which limited a thorough statistical analysis of the results.

CONCLUSION

Although most patients have favorable outcomes after hospitalization for COVID-19, hs-TnI elevation on admission appears to remain predictive of long-term survival after discharge. Such cases should be carefully followed, and until novel preventive strategies are developed, appropriate vaccination and management of traditional modifiable cardiovascular risk factors remain essential.

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AUTHORS' CONTRIBUTIONS

GSSO: Data curation, Resources, Software, Writing – original draft. **RMF:** Data curation, Investigation, Project administration, Writing – review & editing. **JMF:** Conceptualization, Resources, Supervision. **RACL:** Conceptualization, Project administration, Supervision. **LHAS:** Formal Analysis, Methodology, Validation. **NASS:** Formal Analysis, Methodology, Supervision, Visualization.

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ORIGINAL ARTICLE

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

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SUMMARY

OBJECTIVE: This study aimed to examine the relationship between body muscle and adipose tissue composition in clear cell renal cell carcinoma patients with PBRM1 gene mutation.

METHODS: Cancer Genome Atlas Kidney clear cell renal cell carcinoma and Clinical Proteomic Tumor Analysis Consortium clear cell renal cell carcinoma collections were retrieved from the Cancer Imaging Archive. A total of 291 clear cell renal cell carcinoma patients were included in the study retrospectively. Patients' characteristics were obtained from Cancer Imaging Archive. Body composition was assessed with abdominal computed tomography using the automated artificial intelligence software (AID-U™, iAID Inc., Seoul, Korea). Body composition parameters of the patients were calculated. To investigate the net effect of body composition, the propensity score matching procedure was applied over age, gender, and T-stage parameters.

RESULTS: Of the patients, 184 were males and 107 were females. Mutations in the PBRM1 gene were detected in 77 of the patients. While there was no difference in adipose tissue areas between the PBRM1 mutation group and those without PBRM1 mutation, statistically significant differences were found in normal attenuated muscle area parameters.

CONCLUSION: This study shows that there was no difference between adipose tissue areas in patients with PBMR1 mutation, but normal attenuated muscle area was found to be higher in PBRM1 patients.

KEYWORDS: Carcinoma. Renal cell. Sarcopenia. Propensity score.

INTRODUCTION

Owing to the emergence of new genetic sequencing techniques and the increasing availability of open-source genetic and radiological datasets, a recent field of research called radiogenomics is facing rapid development[1]. Radiogenomics is primarily based on the relationship between the imaging features of diseases (imaging phenotypes) and gene expression patterns, gene mutations, and other genome-related features[2]. This field aimed to obtain preliminary predictive data for diagnostic, noninvasively prognostic, and, finally, ideal therapeutic evaluation[3,4].

The recent developments in genetics have led to the discovery of multiple mutations or genetic changes in clear cell renal cell carcinomas (ccRCCs), including mutations or alterations of genes encoding von Hippel-Lindau (VHL), polybromo-1 protein (PBRM1), BRCA1-associated protein m 1, SET domain containing 2 enzymes, and lysine-specific demethylase 5C[5,6]. Inactivation of the VHL tumor suppressor gene is the most common oncogenic event in ccRCC. Although the most widespread and famous mutation identified in ccRCCs is the VHL tumor suppressor gene (VHL), the ultimate meta-analysis has shown that there is no clear consensus on the prognostic or predictive effect of a VHL mutation in patients[7]. The second most commonly identified mutation in ccRCC involves the tumor suppressor PBRM1 gene. A recent meta-analysis reported that a mutation or decreased expression of a gene in PBRM1 was associated with poorer survival, advanced tumor, node, metastasis categories, tumor stage, and a higher Fuhrman nuclear grade in patients with RCC[8]. The latest studies have investigated the relationship between the success of immunotherapy and targeted therapy in advanced-stage RCC patients and the PBRM1 mutation[9].

Obesity is the real pandemic of today's world. According to a meta-analysis, obesity increases the incidence of RCC[10]. In contrast, some studies have shown that obesity improves prognosis, even if it increases frequency[11]. There are also studies showing that it worsens prognosis and increases surgical complications[12]. This interesting situation encountered in some malignant and nonmalign processes besides RCC is called the "obesity paradox"[13]. In most of these studies, it is said that patients should be evaluated with radiological measurements,

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although it is cumbersome, so that the paradox can be understood more deeply[14]. It would be more correct to evaluate this situation not only in terms of obesity, but also in terms of the holistic aspect of body composition. In RCC, parameters such as skeletal muscle area and distribution and amount of adipose tissue based on radiological measurements are associated with overall and cancer-specific survival, treatment-related toxicity, and survival after radical nephrectomy[15,16].

It is more accurate to investigate the complex effect of body composition at the genomic level in a heterogeneous tumor group such as RCC. Therefore, we aimed to examine the relationship between RCC and the body composition of the PBRM1 gene mutation, which we think affects both survival and response to treatment.

METHODS

Patient selection

A total of 236 RCC patients from the Cancer Genome Atlas Kidney Renal Clear Cell Carcinoma (TCGA-KIRC) dataset and 63 RCC patients from the Clinical Proteomic Tumor Analysis Consortium Clear Cell Renal Cell Carcinoma [CPTAC-CCRCC] collection were retrieved from the Cancer Imaging Archive TCIA[17-19]. Patients' characteristics were obtained from TCIA, including age, gender, pathologic grade, the American Joint Committee on Cancer (AJCC) stage, and PBRM1 genomic profile. Informed consent was not required since TCIA data contained no personally identifying information.

Inclusion criteria were as follows: (a) a diagnosis of pathologically proven ccRCC, (b) pre-operative abdominal CT examination, and (c) the images were complete and the necessary clinical information was complete. The exclusion criteria were as follows: (a) patients receiving pre-operative chemotherapy or radiotherapy treatment, (b) patients inadequate for an assessment of CT images, and (c) patients with lumbar surgical material. As a result of the criteria, 57 ccRCC patients from the CPTAC-CCRCC dataset and 234 from the TCGA-KIRC dataset, totaling 291 ccRCC patients, were included in the study.

Assessment of body composition

Body composition was evaluated by abdominal CT using automated artificial intelligence software (AID-U™, iAID Inc., Seoul, Korea), which was advanced using a fully convolutional network segmentation technique[20]. An abdominal radiologist specialist, blind to the clinical information, semi-automatically selected the axial CT sections at the level of the L3 vertebral lower end plaque with the help of sagittal reconstructed images. Later, the selected images were automatically segmented to generate the border of total abdominal muscles, subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT). For muscle quality assessment, the cross-sectional area of selected axial muscle images (i.e., psoas, paraspinal, transversus abdominis, rectus abdominis, quadratus lumborum, and internal and external obliques) were onward segmented by predetermined Hounsfield units (HU) thresholds as follows: (i) normal attenuation muscle area (NAMA; +30 to +150 HU), reflecting healthy muscle with little intramuscular fat; (ii) low attenuation muscle area (LAMA; -29 to +29 HU), reflecting unhealthy muscle with intramuscular lipid pool; and (iii) intramuscular adipose tissue (IMAT; -190 to -30 HU), reflecting the apparent fat tissue between muscle groups and muscle fibers[21,22]. Total abdominal muscle area (TAMA, -190 to +150 HU) was defined as a whole area including all skeletal muscles and fat tissues (TAMA=NAMA+LAMA+IMAT). An example of the interface of the tool can be seen in Figure 1.

Statistical analysis

Continuous variables were given as mean (±standard deviation [SD]), and categorical variables were given as a number (ratio). Normality tests were made for continuous variables Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparisons between groups were made using the following statistical tests: chi-square test for categorical variables, Student's t-test for normal-distributed continuous variables, and Mann-Whitney U test for non-normal-distributed continuous variables.

We also used propensity score matching (PSM) with a 1:1 ratio to minimize selection bias and adjust the imbalance between groups. SPSS R plug-in (SPSS R Essentials) was applied for matching. We used the SPSS "PS Matching" feature to perform propensity score-matched analysis. Matching factors include age, gender, grade, and stage. Patients with PBRM1 mutations and patients without mutation and unknown mutation status were matched 1:1 in a multivariable logistic analysis using stepwise regression based on a greedy matching algorithm with a caliper of 0.05 times the SD of the logit. After applying 1:1 PSM, 76 eligible patients were matched to each group.

RESULTS

Of the patients, 184 were males and 107 were females. In all, 134 of the patients were of low grade (grades 1–2), and 157 were of high grade (grades 3–4). According to the AJCC staging, 148 patients were noted as stage 1, 27 patients as stage 2, 74 patients as stage 3, and 42 patients as stage 4.

Mutations in the PBRM1 gene were detected in 77 of the patients. When the distribution of PBRM1 mutations was examined, no statistically significant difference was found according to gender, grade, and stage, but the frequency of PBRM1 mutations increased in advanced stage and stage disease (p=0.143, p=0.146, and p=0.304, respectively). The mean age was 60.04 (11.0) in the PBRM1 mutation group and 60.2 (12.7) in the other group, so no difference was found (p=0.875).

When the PBRM1 mutation group and the other group were examined according to body composition parameters, statistically significant differences were found in NAMA and total muscle area parameters, p=0.002 and 0.006, respectively. More detailed evaluation according to other body composition parameters is given in Table 1.

DISCUSSION

In this study, we investigated for the first time the relationship of PBRM1, one of the genetic mutations of ccRCC, with fat and muscle tissue distribution in patients matched for age, sex, nuclear grade, and disease stage. In our study, no difference was found between patients with and without PBRM1 mutations in the SAT, VAT, and TAT areas. In a few studies conducted in ccRCC patients, regardless of genetic mutations, there is evidence that SAT and VAT values can be used as prognostic factors in predicting survival and nuclear grade[23,24]. In some of these studies, it has been shown that adipose tissue has a positive contribution to survival. However, in our study, we found that there was no relationship between the PBRM1 mutations of adipose tissue components. This may be due to the lack of matching in previous studies or the failure to evaluate genetic mutations. To reveal the importance of adipose tissue, prospective studies with large participation are needed, considering the genetic conditions.

Another body component we evaluated in our study is muscle tissue. IMAT and LAMA were not associated with mutation either before or after matches. However, we found that NAMA values were higher in patients with mutations in the evaluation made before and after matching in all cases. We find it interesting that the normal attenuation muscle mass

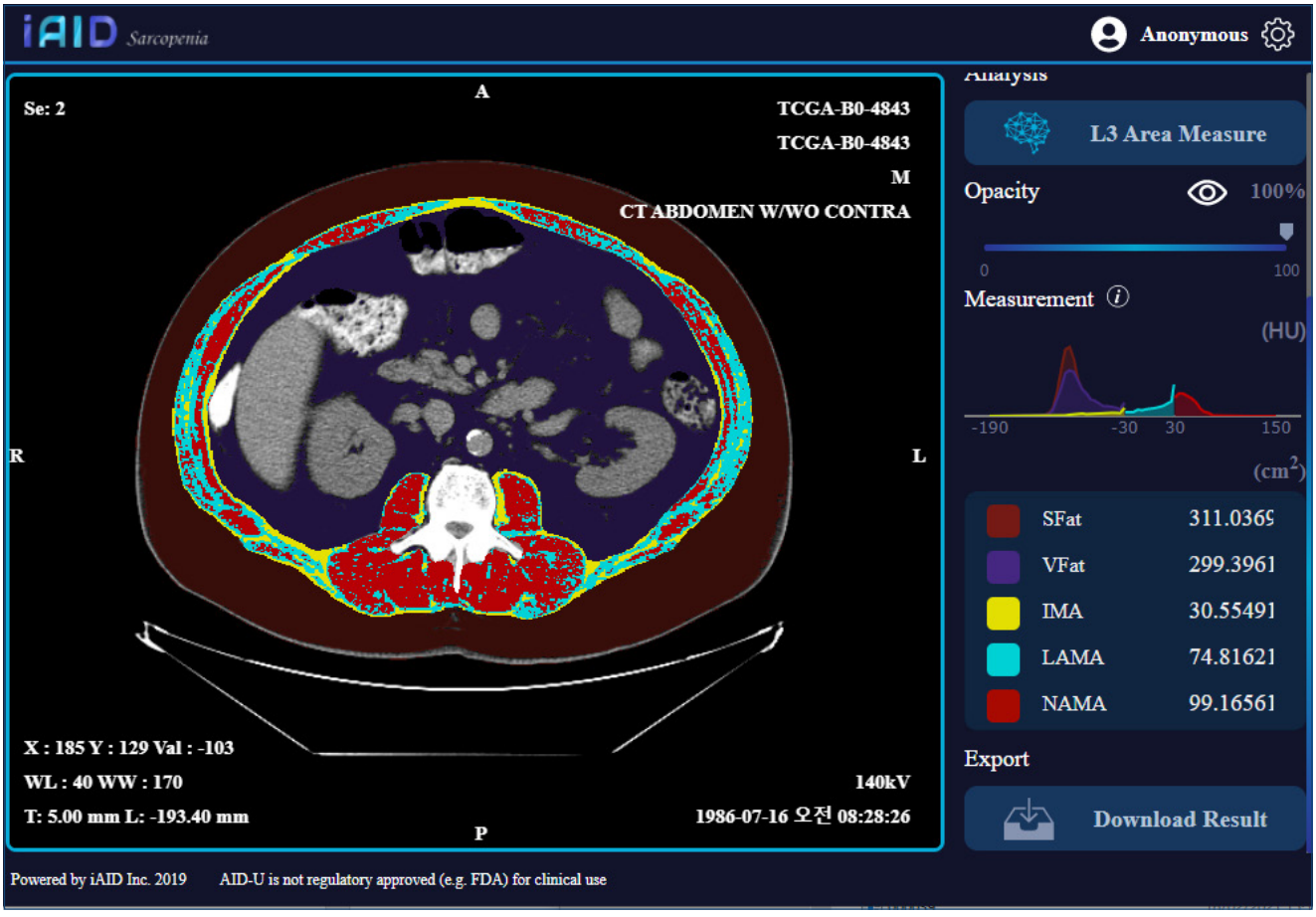

Figure 1. iAID sarcopenia interface.

is higher in patients with PBMR1 mutation. Studies evaluating the relationship between ccRCC survival and sarcopenia in the literature have shown that nonsarcopenic patients have a longer survival[25]. However, most previous survival studies have been performed without considering the genetic mutations of ccRCC patients. The evaluation of patients with genetic mutations in our study was a different aspect of this study compared to others. The relative increase in normal-density muscle area in patients with PMBR1 mutation may be an issue that needs to be investigated.

Mutations in the PBRM1 gene are the second most common mutation in ccRCC development[26]. The PBRM1 mutation acts as a direct effector as it influences the expression of proteins. In recent years, a few studies have shown that PBRM1 expression can serve as a promising biomarker in predicting the survival of various tumors. However, another study showed that reduced expression of PBRM1 is a poor predictor of overall survival, cancer-specific survival, progression-free survival, and recurrence-free survival in patients with RCC[27]. In contrast, contrary to studies showing that PBRM1 mutation is a poor prognostic factor, studies showing that this mutation can be a good predictor of response to both antiangiogenic and immunotherapy create a paradox[28]. McDermott et al.[29] found that patients with PBRM1 mutations may have increased neoangiogenesis. Miao et al.[30] found decreased expression of immune inhibitory ligands in those with intact PBRM1. We think that this paradox should be examined further, considering body composition.

There are studies examining the effects of sarcopenia and other body composition parameters in RCC with very different results[25]. This may be because the body composition is formed as a result of quite complex genetic, epigenetic, and environmental factors. For example, if we look at our study from this perspective, it is unclear whether the increased muscle area in patients with PBRM1 mutations is a cause or an effect. On the contrary, it is not clear how the biology of the tumor changes when the PBMR1 gene mutation occurs and how this change affects body metabolism. For this reason, we think that body composition may contain much more information and secrets than we can imagine. There is a need to investigate patients with PBRM1 mutation with prospectively planned studies including a normal control group, which will explain the increased muscle area even in patients with all conditions matched. A more in-depth study of the net effect of body composition on tumor behavior still remains.

There are some limitations to our study, namely, the retrospective nature of our study, lack of height and weight information of our patients, and lack of race information of all patients.

CONCLUSION

Our study shows that NAMA is greater in patients with PBMR1 mutation, even after PSM. We find that body composition plays a critical role in understanding the complex effect of PBRM1.

Practical application

Many studies have investigated the effects of body composition and genomic profile on survival and treatment response in RCC. Previous studies sought to evaluate without matching parameters such as tumor stage, grade, patient sex, and metastasis.

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

	Before matching (n=291)			After matching (n=152)		
	PBRM1 mutation (+) (n=77)	PBRM1 mutation (-) (n=214)	p-value	PBRM1 mutation (+) (n=76)	PBRM1 mutation (-) (n=76)	p-value
	Mean±Sd	Mean±Sd		Mean±Sd	Mean±Sd	
Age	60.1±11.1	60.3±12.7	0.883	59.9±11.1	59.8±13.4	0.979
SAT (cm^2)	231.2 ±125.8	226.2±116.8	0.763	232.4±126.2	225.4±115.1	0.719
VAT (cm^2)	229.4±119.3	212.3±115.8	0.269	230.4±119.8	219.4±120.7	0.654
TAT (cm^2)	460.6±214.2	438.6±192.2	0.405	462.9±214.6	444.8±200.6	0.212
IMAT (cm^2)	27.2±14.6	29.6±15.2	0.243	27.3±14.7	29.7±17.7	0.359
LAMA (cm^2)	58.2±24.2	55.8±22.3	0.445	58.1±24.4	57.2±26.2	0.831
NAMA (cm^2)	104.2±38.7	88.9±35.6	0.002	104.3±38.9	90.9±37.3	0.031
TAMA (cm^2)	189.6±40.9	174.3±40.8	0.006	189.7±41.2	177.8±42.1	0.079

SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; TAT: total adipose tissue; IMAT: intramuscular adipose tissue; LAMA: low attenuation muscle area; NAMA: normal attenuation muscle area; TAMA: total abdominal muscle area. p<0.05 found in bold values.

INFORMED CONSENT AND PATIENT DETAILS

The authors declare that this report does not contain any personal information that could lead to the identification of the patients.

AUTHORS' CONTRIBUTIONS

ED: Conceptualization, Data curation, Methodology, Software, Writing – original draft. **OD:** Conceptualization, Formal Analysis, Supervision, Writing – original draft, Writing – original draft.

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ORIGINAL ARTICLE

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Analysis of appendiceal neoplasms in 1,423 appendectomy specimens: a 10-year retrospective cohort study from a single institution

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SUMMARY

OBJECTIVE: This study aimed to reveal the incidence, clinicopathological, and oncological outcomes of appendiceal neoplasms.

METHODS: This is a retrospective cohort study from a single institution. Patients with a pathological diagnosis of malignancy who underwent appendectomy between January 2011 and 2021 were included in the study, and groups were formed according to pathological type. Clinical, pathological, and oncological results were compared in these groups.

RESULTS: The incidence of neoplasia was 2.38% (n=34) in a cohort of 1,423 appendectomy cases. Of the cases, 56% (n=19) were female. The median age in the entire cohort was 55.5 (range: 13–106) years. In the cohort, the rate of neuroendocrine tumor mucinous cystadenoma adenocarcinoma, and low-grade appendiceal mucinous neoplasm, according to the American Joint Committee on Cancer classification of appendiceal neoplasms, was 32.3% (n=11), 26.4% (n=9), 26.4% (n=9), and 14.7% (n=5), respectively. Neuroendocrine tumor patients (median age: 35 years) were younger than the other groups (p=0.021). Secondary complementary surgery was performed in 66.7% (n=6) of adenocarcinoma patients and 27.3% (n=3) of neuroendocrine tumor patients. Right hemicolectomy was performed in all neuroendocrine tumor patients requiring secondary surgery, while right hemicolectomy was performed in three adenocarcinoma patients and cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in three adenocarcinoma patients. After a median follow-up of 44.4 months (95% confidence interval: 18.6–70.1), the mean survival rate was 55% in appendiceal adenocarcinoma patients compared to 100% in neuroendocrine tumor patients.

CONCLUSION: Appendiceal neoplasms are rare but remain an important cause of mortality. Appendiceal adenocarcinomas are associated with poorer oncological outcomes compared to other neoplasms.

KEYWORDS: Appendectomy. Appendiceal neoplasms. Incidence. Pathology.

INTRODUCTION

The estimated incidence of appendiceal tumors was 0.12 cases per 1,000,000 people per year; however, recent large database studies have reported the incidence to be as high as 0.97 cases per 100,000 people. It is unclear whether this increase reflects an actual change in disease occurrence or simply more identification and reporting. Appendiceal tumors are rare but remain an important clinical problem in terms of optimal management. Surgeons should be familiar with the effects of appendiceal pathology[1-4].

Current classification of mucinous tumors, PSOGI (Peritoneal Surface Oncology Group International) 2012 Diagnostic and Staging Criteria for Epithelial Appendiceal Neoplasms, and the American Joint Committee on Cancer Staging Manual (AJCC, 8th edition) have been updated[5,6].

The five main histopathological subtypes of appendiceal neoplasms are as follows: neuroendocrine neoplasms (NENs), which are nonepithelial tumors; mucinous neoplasms; goblet cell adenocarcinomas; colonic-type (nonmucinous) adenocarcinomas; and signet ring cell adenocarcinomas, which are epithelial tumors. Due to the nature of the clinical presentation of acute appendicitis, preoperative or intraoperative diagnosis of appendiceal neoplasms is very rare. Although appendectomy for acute appendicitis is usually the adequate treatment for most of these neoplasms, clinical management is highly dependent on tumor type, histological grade, pathological stage, and the status of resection margins and can range from radical surgery to systemic chemotherapy or surveillance[4].

The risk factors for the presence of an underlying malignancy in a patient presenting with acute appendicitis are not

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well established. Complicated appendicitis by itself has been associated with an increased risk of underlying malignancy. The risk is even higher in patients presenting with a periappendiceal abscess compared with patients with uncomplicated appendicitis[7,8]. Neoplasms of the appendix are usually not suspected before surgery and are found during surgery or on pathological examination. Increasing awareness of the disease, its pathophysiology, and its presentation has led to increased interest in the fields of surgery and medical oncology regarding the treatment of diseases with peritoneal dissemination. An understanding of the histologic features, imaging appearances, and staging of appendiceal neoplasms facilitates an accurate radiologic description, which guides surgical and oncologic management. This requires evaluation of the appendix and mesoappendix in the setting of acute appendicitis, the peritoneum and organ surfaces in patients with mucinous tumors, and lymph nodes and solid organs in nonmucinous and NENs. Although there are studies in the literature on the biological behavior of appendiceal tumors, the evidence contains various inconsistencies, and limited data exist on the long-term outcomes of appendiceal neoplasms[9,10].

The present study aimed to assess the incidence and long-term outcomes of appendiceal neoplasms according to their histological types.

METHODS

After the approval (date: 10.09.2021, No: 114/36) was granted by our ethics committee, a retrospective analysis was made on the Çukurova University patient database from January 2011 to January 2021. While creating the database, electronic records, nurse observation forms, pathology records, and survival information obtained from the population directorate were created. Our institution is a third-level university hospital and serves as the reference hospital of a city with a population of 2 million. All adult patients aged ≥18 years with evidence of acute appendicitis or an appendiceal mass on preoperative imaging were included in the study. The final pathological diagnoses of the patients were retrospectively reviewed from the pathology records. The results of patients with neuroendocrine tumor (NET), mucinous cystadenoma (MC), adenocarcinoma, and low-grade appendiceal mucinous neoplasm (LAMN) were analyzed. Inflammatory conditions and negative appendectomy patients were not included. Imaging modalities included ultrasound, computed tomography, and magnetic resonance imaging. In our routine practice, an ultrasound examination was performed on each patient, and a computed tomography or MRI examination was performed for every suspicious finding, one of the advanced imaging methods. Age alone was not a criterion for the selection of imaging modalities. Demographic information included age, gender, tumor marker levels (measured in the postoperative period), the type of surgery (emergency or elective), intraoperative findings (perforation or mesoappendiceal invasion), pathological TNM stages, tumor size (obtained from pathology records), need for additional surgical intervention, and survival. The patients were classified into four groups: group 1 (low-grade mucinous neoplasia); group 2 (adenocarcinoma); group 3 (MC); and group 4 (NET). The data were compared between these groups.

We applied conventional or laparoscopic appendectomy to the patients. We routinely performed mesoappendix resections for all patients.

The follow-up of the cases included wound healing assessment at postoperative week 1 in our clinic and re-admission with pathology results. Patients with a pathology report of malignancy were followed up by the Colorectal Surgery department.

The main aim of the study was to identify different patterns of unusual histopathological findings in patients with provisional diagnosis of acute appendicitis and to assess their prevalence as well as their clinical significance.

The authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki, "Ethical Principles for Medical Research Involving Human Subjects."

Statistical assessment

The study data were analyzed using SPSS (Statistical Package for the Social Sciences, Inc.; Chicago, IL, USA) version 23.0. The study data were evaluated using descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, and maximum). The Shapiro-Wilk test was used to assess the normality of the data. The Kruskal-Wallis test was used to compare the non-normally distributed data, while Tamhane's T2 test, one of the post hoc tests, was used to analyze the intergroup differences. Categorical variables were compared using Pearson's chi-square test, Fisher-Freeman-Halton exact test, and Fisher's exact test. Survival was analyzed using Kaplan-Meier analysis and log-rank test. Since the number of patients was small, we did not look for prognostic factors. The statistical significance level was set to 0.05 for all tests.

RESULTS

The incidence of neoplasia was 2.38% (n=34) in a cohort of 1,423 appendectomy cases, 1,412 of which were performed under emergency conditions during the study period. Of the

cases, 56% (n=19) were female. The median age in the entire cohort was 55.5 (range: 13–106) years. In the cohort, the rate of NET, MC, adenocarcinoma, and LAMN, according to the AJCC (8th edition) classification of appendiceal neoplasms, were 32.3% (n=11), 26.4% (n=9), 26.4% (n=9), and 14.7% (n=5), respectively. Gender distribution was similar in the groups (p=0.223). Patients in group 4 were younger (median age: 35 years) (p=0.021). The elective surgery rate was higher in group 2 than in other groups (66.7%) (p=0.048). Two patients in group 3 and one patient in group 4 had intraoperative perforation. Demographic and clinical data are presented in Table 1.

Tumor size was similar in the groups (p=0.274). There was mesoappendiceal invasion only in group 2 (33%) (p=0.027). In group 2, 55.6% of the patients had metastases (p=0.001). Right hemicolectomy was performed in all NET patients requiring secondary surgery, while right hemicolectomy was performed in three adenocarcinoma patients and cytoreductive surgery and hyperthermic intraperitoneal chemotherapy (HIPEC) in three adenocarcinoma patients. Table 2 shows the pathology data.

After a median follow-up of 44.4 months (95% confidence interval [CI]: 18.6–70.1), the mean survival rate was 55% in appendiceal adenocarcinoma patients compared to 100% in NET patients. Survival was shorter in group 1 (22.7 vs. 43.6 vs. 55.6 vs. 49.1; p=0.011). The results are presented in Table 3.

DISCUSSION

The present study, which presented the clinical characteristics and oncological outcomes of appendiceal neoplasms in a cohort of appendectomy cases, identified NETs as the most common type of tumor. In our female-predominant population, adenocarcinoma patients had an advanced age and underwent appendectomy mostly under elective conditions. This group also had a high rate of mesoappendiceal invasion and therefore required additional surgical interventions. This group of patients tended to be metastatic and after a median follow-up of 44.4 months (95%CI 18.6–70.1), the mean survival rate was 55% in appendiceal adenocarcinoma patients compared to 100% in NET patients. We found appendiceal adenocarcinoma to have aggressive biology and exhibit poor oncological outcomes compared to other appendiceal tumor types.

The incidence of incidental appendiceal neoplasms is increasing. This may be due to the increased use of new imaging

Table 1. Demographic and clinical characteristics of patients who underwent appendectomy between 2011 and 2021 according to the classification of appendiceal neoplasms.

	Low grade	Adenocarcinoma	Cystadenoma	Neuroendocrine tumor	Total	p-value
Gender n (%)						
Male	3 (60)	2 (22.2)	3 (33.3)	7 (63.6)	15 (44.1)	0.223
Female	2 (40)	7 (77.8)	6 (66.7)	4 (36.4)	19 (55.9)	
Age, Med (95%CI)	50 (50–50)	60 (46–84)	54 (54–84)	35 (30–55)	50.5 (38–70)	**0.021***
Emergency/elective, n (%)						
Emergency	4 (80)[a,b]	3 (33.3)[b]	6 (66.7)[a,b]	10 (90.9)[a]	23 (67.6)	**0.048**
Elective	1 (20)[a,b]	6 (66.7)[b]	3 (33.3)[a,b]	1 (9.1)[a]	11 (32.4)	
Intraoperative perforation, n (%)						
Yes	0 (0)	0 (0)	2 (22.2)	1 (9.1)	3 (8.8)	0.339
No	5 (100)	9 (100)	7 (77.8)	10 (90.9)	31 (91.2)	
Diagnosis, n (%)						
Intraoperitive	1 (20)	3 (33.3)	2 (22.2)	0 (0)	6 (17.6)	0.119
Perioperitive	1 (20)	0 (0)	0 (0)	0 (0)	1 (2.9)	
Postoperitive	3 (60)	6 (66.7)	7 (77.8)	11 (100)	27 (79.4)	
CEA, **Med (95%CI)**	4.38 (4.38–4.38)	15.75 (2.04–37.2)	18.04 (1.89–34.19)	0.92 (0.59–1.77)	2.09 (1.53–26)	**0.020****
CEA, 19.9 **Med (95%CI)**	12.6 (12.6–12.6)	35.5 (2.7–70)	3.15 (0.8–5.5)	6.7 (2.4–22.5)	7.65 (3.5–32)	0.419

CEA: carcinoembryonic antigen. Post hoc pair-group analysis was performed using Bonferroni correction. *b–d, p=0.048; **a–d, p=0.015. Bold values indicate statistical significance at the p<0.05 level.

Table 2. Comparison of pathological data and operation techniques in groups.

	Low-grade	Adenocarcinoma	Cystadenoma	Neuroendocrine tumor	Total	p-value
Tumor size	2.5 (1.5–3.0)	3.1 (2.5–10.8)	6.4 (2–20)	6 (2–12)	4 (1.5–20)	0.274
Mesoappendiceal invasion, n (%)						
Yes	0 (0)[a]	3 (33.3)[b]	0 (0)[a]	0 (0)[a]	3 (8.8)	**0.027**
No	5 (100)[a]	6 (66.7)[a]	9 (100)[a]	11 (100)[a]	31 (91.2)	
R0/R1 n (%)						
R0	5 (100)	7 (77.8)	9 (100)	10 (90.9)	31 (91.2)	0.339
R1	0 (0)	2 (22.2)	0 (0)	1 (9.1)	3 (8.8)	
T stage, n (%)						
T0	5 (100)[a]	0 (0)[b]	9 (100)[b]	1 (9.1)[a]	15 (44.1)	**<0.001**
T1	0 (0)[a]	3 (33.3)[a]	0 (0)[a]	2 (18.2)[a]	5 (14.7)	
T2	0 (0)[a]	1 (11.1)[a]	0 (0)[a]	2 (18.2)[a]	3 (8.8)	
T3	0 (0)[a]	0 (0)[a]	0 (0)[a]	4 (36.4)[a]	4 (11.8)	
T4	0 (0)[a]	5 (55.6)[a]	0 (0)[a]	2 (18.2)[a]	7 (20.6)	
N stage n (%)						
N0	5 (100)	5 (55.6)	9 (100)	11 (100)	30 (88.2)	0.050
N1	0 (0)	2 (22.2)	0 (0)	0 (0)	2 (5.9)	
N2	0 (0)	2 (22.2)	0 (0)	0 (0)	2 (5.9)	
M stage, n (%)						
M0	5 (100)[a,b]	4 (44.4)[b]	9 (100)[a,b]	11 (100)[a]	29 (85.3)	**0.001**
M1	0 (0)[a,b]	5 (55.6)[b]	0 (0)[a,b]	0 (0)[a]	5 (14.7)	
Ki-67, n (%)						
1–2%	0 (0)	0 (0)	0 (0)	1 (9.1)	1 (2.9)	0.148
<1%	0 (0)	0 (0)	0 (0)	3 (27.3)	3 (8.8)	
No	5 (100)	9 (100)	9 (100)	7 (63.6)	30 (88.2)	
Advanced surgery for cancer, n (%)						
Yes	0 (0)[a,b]	6 (66.7)[b]	0 (0)[a]	3 (27.3)[a,b]	9 (26.5)	**0.006**
No	5 (100)[a,b]	3 (33.3)[b]	9 (100)[a]	8 (72.7)[a,b]	25 (73.5)	
Surgery, n (%)						
Right hemicolectomy	NA	3	NA	3	6	
Cytoreductive surgery	NA	2	NA	0	2	
Cytoreductive surgery+HIPEC	NA	1	NA	0	1	

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

Table 3. Comparison of mean monthly overall survival in groups.

	Mean	SD	95%CI		p-value
			Lower bound	Upper bound	
Low-grade mucinous neoplasm	22.7	12.4	0.0	46.9	**0.011**
Mucinous adenocarcinoma	43.6	11.3	21.3	65.8	
Mucinous cystadenoma	55.6	14.1	27.9	83.3	
Neuroendocrine tumors	49.1	7.9	33.7	64.5	

Bold value indicates statistical significance at the p<0.05 level.

modalities in health screening in relatively recent times[11]. Our appendix neoplasia rate was slightly higher than 1–2%, which is the rate in the literature. We attribute this to the fact that we are a tertiary university hospital and therefore a center where patients are referred. Appendiceal cancer is rare; however, preoperative diagnosis is difficult due to limited preoperative diagnostic tests. Furthermore, the diagnosis may be missed when some patients are treated without surgery. Previous studies have failed to identify radiological factors that may predict the presence of underlying malignancy in patients presenting with acute appendicitis[12,13]. The rate of appendiceal neoplasms in our series was 2.38%, which did not include the patients who were followed up non-operatively. The diagnosis was postoperatively established in 79% of the patients. We found results that support the literature.

The serum tumor markers CEA, CA19-9, and CA125 are frequently obtained in the diagnosis of appendiceal mucinous neoplasms and are routinely monitored to assess disease remission or progression. Although the individual predictability of disease recurrence has not been well characterized, most high-volume institutions routinely combine tumor markers with imaging at baseline, during chemotherapy, and after surgery, if present. Elevated baseline CA19-9 has also been identified as an independent predictor of worse progression-free survival and may be useful in diagnosing disease relapse after cytoreductive surgery (CRS) and HIPEC[2]. Taflampas et al. showed that disease-specific survival was significantly longer in treated patients with normal preoperative markers and suggested that tumor marker elevation may help tailor the need for perioperative systemic chemotherapy. However, surveillance imaging appears to be more sensitive than tumor markers alone for detecting peritoneal disease recurrence[14]. In our series, we found increased tumor marker levels in adenocarcinoma and cystadenoma.

Previous studies have identified several factors associated with malignancy, including female gender and age. It has been argued that malignancies should also be suspected in all patients presenting with an underlying inflammatory mass or abscess[15-17]. Our series also supports the literature; we found a higher rate of female gender and higher age, especially in the appendiceal adenocarcinoma subgroup, than in other groups. Accordingly, we believe that more common use of perioperative imaging methods in advanced-age patients will increase the success of the treatment.

Survival has improved in patients with pseudomyxoma peritoneii or peritoneal metastases with the introduction of cytoreductive surgery and HIPEC. Right hemicolectomy is indicated for invasive adenocarcinoma that allows regional lymph node resection, but this should be done at the same time as HIPEC[18]. In our series, two-thirds of the patients with adenocarcinoma required advanced surgical procedures. We also had patients who applied HIPEC within the indication.

According to the National Comprehensive Cancer Network and ENETS protocols, many factors affect the selection of treatment in appendiceal tumors, including but not limited to tumor size, tumor location, and mesoappendiceal invasion[19,20]. NENs also metastasize to the peritoneal cavity. As are other causes of carcinomatosis, CRS with or without HIPEC may improve disease control and survival in well-selected patients[21]. Adjuvant therapy should be considered in patients with surgically resected neuroendocrine carcinoma. Neoadjuvant therapy may be considered for patients with locally advanced or metastatic, resectable disease[20]. In our series, the rate of additional surgical interventions for cancer was 26%. Considering available guidelines, we determined that additional surgical intervention was required, especially in appendiceal adenocarcinoma.

The limitations of our study were the limited number of patients and its retrospective design. In addition, there may be overlooked data in the follow-up of patients. There are also problems such as ignoring poor oncological results. However, considering the scarcity of comparative studies in the literature, we believe that the present study contributes to the literature.

Appendiceal neoplasms are a rare group of malignancies with a wide variety of biological characteristics and malignant behaviors. Appendiceal adenocarcinomas are associated with poorer oncological outcomes compared to other neoplasms. Our understanding of these tumors and treatment options has enhanced dramatically in recent years, and many patients have improved survival as a result of more aggressive surgical treatments and improved systemic treatment options.

AUTHORS' CONTRIBUTIONS

AR: Conceptualization, Methodology, Project administration, Supervision, Writing – original draft. **CA:** Conceptualization, Methodology, Project administration, Writing – original draft. **UT:** Conceptualization, Methodology, Writing – original draft. **AGS:** Data curation, Resources, Writing – review & editing. **OY:** Investigation, Validation, Visualization. **KD:** Investigation, Validation, Visualization. **ICE:** Investigation, Validation, Visualization.

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ORIGINAL ARTICLE

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Analysis of possible risk predictors in patients with coronavirus disease 2019: a retrospective cohort study

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SUMMARY

OBJECTIVE: This study aimed to analyze the clinical-epidemiological profile, possible risk predictors, and outcomes of patients with coronavirus disease 2019 admitted to the ward of a tertiary care hospital in southern Brazil. Specifically, we describe the demographic characteristics, comorbidities, baseline laboratory findings, clinical course, and survival of these patients.

METHODS: This is an observational, retrospective cohort study, performed from January to March 2022, on medical records of patients hospitalized between April 2020 and December 2021 in the coronavirus disease 2019 ward of a tertiary hospital in southern Brazil.

RESULTS: Data from 502 hospitalized patients were analyzed, of which 60.2% were male, with a median age of 56 years and 31.7% were over 65 years old. The main symptoms presented were dyspnea/respiratory discomfort (69.9%) and cough (63.1%). The most common comorbidities were obesity, systemic arterial hypertension, and diabetes mellitus. A proportion of 55.8% of 493 patients had PaO_2/FiO_2<300 mmHg in the first examination performed after admission and 46.0% had a neutrophil/lymphocyte ratio>6.8. Oxygen therapy by Venturi mask or mask with reservoir was used in 34.7% of the patients, and non-invasive ventilation was used in 10.0% of the patients. The majority of the patients (98.4%) used corticosteroids, and the outcome of 82.5% of the hospitalized patients was home discharge.

CONCLUSION: After analyzing the clinical and epidemiological profile, it can be concluded that age greater than 65 years and pulmonary involvement >50% are predictors of a worse prognosis for coronavirus disease 2019, as is the need for high-flow oxygen therapy. Corticotherapy, however, proved to be beneficial in the treatment of the disease.

KEYWORDS: COVID-19. Risk factors. Prognosis. Disease management.

INTRODUCTION

A new coronavirus was identified in Wuhan, China, in December 2019, when several cases of severe pneumonia were reported. The disease caused by this virus was later named coronavirus disease 2019 (COVID-19)[1-3].

The clinical picture of COVID-19 is quite variable. Patients may be asymptomatic or may start with a flu-like syndrome that can progress to pneumonia or severe acute respiratory syndrome in a short time[1,4]. Numerous risk factors can contribute to serious diseases, such as comorbidities, advanced age, changes in physiological enzyme levels, and inflammatory markers[1,4-6].

According to the World Health Organization data, among symptomatic patients, about 80% recover without the need for hospital treatment, whereas 15% become seriously ill, requiring oxygen therapy and hospitalization and 5% progress to need for intensive care[1].

Thus, knowing the clinical and epidemiological profile of patients and their main in-hospital outcomes allows for the identification of individuals at risk of a worse prognosis. This allows the institution of a more targeted line of treatment and may contribute to improving the flow of care, avoiding system overload, and leading to a reduction in the rate of mortality.

In this regard, this study aimed to analyze the clinical-epidemiological profile, possible risk predictors, and outcomes of patients with COVID-19 hospitalized in the ward of a tertiary care hospital in southern Brazil. Specifically, we describe the demographic characteristics, comorbidities, baseline laboratory findings, clinical course, and survival of these patients.

METHODS

This is an observational, retrospective cohort study. Data were obtained from the medical records of patients hospitalized in the COVID-19 ward of a tertiary hospital in southern Brazil, after prior authorization from the institution and approval from the

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Centro Universitário para o Desenvolvimento do Alto Vale do Itajaí Research Ethics Committee (assent number 5.046.434).

The Hospital Infections Control Commission of the hospital in question was asked to provide a list of patients hospitalized due to the disease, from which the inclusion and exclusion criteria of the study were applied. Data were collected from January to March 2022.

Patients over 18 years of age, with a positive diagnosis of COVID-19, hospitalized in the ward from April 2020 to December 2021 were included. Pregnant women and patients in previous palliative care were excluded.

No sample size calculation was performed as this was a census study. All medical records of patients who met the inclusion criteria were analyzed.

The variables analyzed were age, gender, weight, height, symptoms presented at hospital admission, COVID vaccination history, use of medications from the COVID kit (composed of hydroxychloroquine, azithromycin, ivermectin, and corticoid), previous comorbidities presented by the patient, type of oxygen therapy performed during hospitalization in the ward [nasal cannula, Venturi mask or reservoir mask, non-invasive ventilation (NIV), orotracheal intubation (OTI)], the extent of pulmonary impairment on chest tomography, and laboratory tests of an inflammatory, infectious, and thrombotic character, including blood count, C-reactive protein (CRP), lactic dehydrogenase (LDH), D-dimer, and ferritin, in addition to the blood gas test. Regarding drug treatment, the use of antibiotics, corticoids, anticoagulants, and antivirals was evaluated. The length of stay in the ward and the outcome were also analyzed.

The data from this research were initially tabulated in *Google Sheets* and later transferred to the IBM *Statistical Package for the Social Sciences* (SPSS, version 22.0) for statistical analysis.

The results of the characterization of patients' profiles were expressed as mean and standard deviation (±SD) or absolute number (n) and percentage (%). To carry out the statistical inference, the quantitative variables were initially analyzed for their normality using the *Kolmogorov-Smirnov* test. In view of the non-normality, the nonparametric *Mann-Whitney* U test was used. In the association analyses, the outcome was dichotomized into Discharge to Home and Non-Discharge, which included a composite of the need for an intensive care unit (ICU) or death on the ward, with the outcome Non-Discharge characterized as the worse prognosis. For this analysis, the test used was *Pearson's* chi-square (χ^2) or *Fisher's* exact test. When the associations were significant, the analysis of adjusted residuals *(ra)* was performed, considering *ra*>1.96 to indicate the highest prevalence. Variables with p<0.05 from the *Pearson's* chi-square or *Fisher's* exact test were candidates for the model using regression Poisson logistics [prevalence ratio (PR)].

Initially, all variables were analyzed individually – univariate analysis (gross OR) – and following that, the multivariate analysis (adjusted OR) was performed. The model for multivariate analysis was the *backward* selection method, where the least significant variable is removed, one at a time, sequentially and automatically, based on statistical criteria. Only variables with p<0.05 remained in the final model.

RESULTS

Initially, 754 patients who were hospitalized during the determined period were obtained. After applying the inclusion and exclusion criteria, 210 (27.8%) patients were excluded because they were directed directly to ICU admission, thus not going through the ward beforehand; 28 (3.7%) pregnant women; 10 (1.3%) patients in previous palliative care, and 4 (0.5%) patients younger than 18 years.

Thus, a total of 502 patients were eligible for this study. Patient demographics and clinical data, laboratory data, and therapy instituted are found in Table 1. The outcomes of patients admitted to the ward are given in Table 2.

Table 3 presents the findings of the univariate and multivariate analyses, where it is possible to note the odds ratio of an unfavorable patient outcome, represented by ICU admission or death.

DISCUSSION

In this study, 502 medical records of patients hospitalized due to COVID-19 in the ward of a tertiary hospital in southern Brazil, in a period of 20 months, which included observation of the patients' demographic, clinical and laboratory data, and the therapy instituted, as well as the outcome obtained by these patients. It was observed that dyspnea/breathing discomfort, cough, and desaturation were the most common symptoms presented by the patients, while the laboratory tests showed that more than half of the patients had a PaO_2/FiO_2 <300 mmHg, indicating hypoxemia.

The median age of patients in this study was 56 years. A study that evaluated 25,919 patients from the southern region of Brazil found a median age of 60 years[7], indicating that the analyzed patients were younger than usual. However, the median age was higher for patients who progressed to the need for ICU or death in the ward, when compared with patients who were discharged (65.5 versus 53 years). Age greater than 65 years was considered a risk factor for a worse prognosis of

Table 1. Association of possible predictor variables between patients admitted to the COVID ward discharged to home and not discharged (needed ICU or died).

Variables	Total Median (IQR) or n (%) n=502	Discharged to home Median (IQR) or n (%) n=414	Not discharged (ICU or death) Median (IQR) or n (%) n=88	p-value
Age	56 (43.0–68.0)	53 (42.0–65.8)	65.5 (50.8–78.5)	**0.01**[#a]
Age >65 years	159 (31.7)	114 (27.4)[ra=4.5]	45 (52.3)	**0.01**[#b]
Male	302 (60.2)	255 (61.3)	47 (54.7)	0.25[b]
Vaccinated with one or more doses[d]	35 (15.7)	25 (14.2)	10 (21.3)	0.24[b]
Use of any medication from Kit COVID[e]	180 (36.5)	152 (37.0)	28 (34.1)	0.63[b]
Dyspnea/respiratory discomfort	351 (69.9)	286 (68.8)	65 (75.6)	0.21[b]
Cough	317 (63.1)	271 (65.1)[ra=2.0]	46 (53.5)	**0.04***[b]
Oxygen desaturation (SpO_2≤94%)	265 (52.8)	218 (52.4)	47 (54.7)	0.70[b]
Fatigue/asthenia	229 (45.6)	196 (47.1)	33 (38.4)	0.14[b]
Fever	214 (42.6)	173 (41.6)	41 (47.7)	0.30[b]
Tachypnea (fR≥24)	205 (40.8)	156 (37.5)[ra=3.3]	49 (57.0)	**0.01**[#b]
Pulmonary impairment >50%[f]	117 (25.0)	82 (21.1)[ra=4.3]	35 (44.3)	**0.01**[#b]
Obesity (BMI≥30)[g]	232 (46.7)	196 (47.3)	36 (43.4)	0.51[b]
SAH	222 (44.2)	168 (40.4)[ra=3.8]	54 (62.8)	**0.01**[#b]
DM	104 (20.7)	85 (20.4)	19 (22.1)	0.73[b]
Dyslipidemia	58 (11.6)	44 (10.6)	14 (16.6)	0.13[b]
Chronic heart disease	52 (10.4)	39 (9.4)	13 (15,1)	0.11[b]
COPD	24 (4.8)	18 (4.3)	6 (7.0)	0.29[c]
Asthma	20 (4.0)	14 (3.4)	6 (7.0)	0.12[c]
CKD	15 (3.0)	11 (2.6)	4 (4.7)	0.30[c]
Neoplasm	8 (1.6)	4 (1.0)	4 (4.7)	**0.03**[c]
Absence of comorbidities	142 (28.3)	127 (30.5)[ra=2.5]	15 (17.4)	**0.01**[#b]
PaO_2/FiO_2<300 mmHg[e]	275 (55.8)	217 (53.2)[ra=2.5]	58 (68.2)	**0.01**[#b]
NLR >6,8	231 (46.0)	186 (44.7)	45 (52.3)	0.20[b]
CRP >100 mg/L[h]	239 (47.8)	191 (46.0)	48 (56.5)	0.08[b]
LDH >250 U/L[i]	326 (89.8)	272 (89.8)	54 (90.0)	0.96[b]
D-dimer >1,000 ng/mL[j]	72 (19.3)	54 (17.3)[ra=2.2]	18 (29.5)	**0.03***[b]
Ferritin >500 µg/L[k]	163 (71.8)	132 (70.2)	31 (79.5)	0.24[b]
Oxygen therapy nasal cannula	405 (80.7)	343 (82.5)[ra=2.2]	62 (72.1)	**0.03***[b]
Venturi mask or with reservoir	174 (34.7)	98 (23.6)[ra=11.5]	76 (88.4)	**0.01**[#b]
NIV	50 (10,0)	23 (5.5)[ra=7.3]	27 (31.4)	**0.01**[#b]
OTI	17 (3.4)	2 (0.5)[ra=7.9]	15 (17.4)	**0.01**[#c]
Corticotherapy	494 (98.4)	412 (99.0)[ra=2.5]	82 (95.3)	**0.03**[#c]
Antibiotic therapy	393 (78.3)	328 (78.8)	65 (75.3)	0.50[b]
Prophylactic anticoagulation[l]	467 (98.3)	384 (98.0)	83 (100.0)	0.36[c]
Full anticoagulation[l]	8 (1.7)	8 (2.0)	0 (0.0)	0.36[c]
Use of antivirals	69 (13.7)	51 (12.3)[ra=2.1]	18 (20.9)	**0.03***[b]

Statistical method used: [a]*Mann-Whitney* U test; [b]*Pearson's* chi-square test; [c]*Fisher's* exact test. Data are expressed as Median (IQR) or n (%). IQR: interquartile range; n: sample size; SpO2: oxygen saturation; fR: respiratory frequency; BMI: body mass index; SAH: systemic arterial hypertension; DM: diabetes mellitus; COPD: chronic obstructive pulmonary disease; CKD: chronic kidney disease; NLR: neutrophil-to-lymphocyte ratio; CRP: C-reactive protein; LDH: Lactate Dehydrogenase; NIV: non-invasive ventilation; OTI: orotracheal intubation. [#]p<0.01; *p<0.05; [d]223/502; [e]493/502; [f]468/502; [g]497/502; [h]500/502; [i]363/502; [j]374/502; [k]227/502; [l]475/502. Bold indicates statiscally significant p-values.

COVID-19, increasing by 1.85 times the PR of an unfavorable progression, a result similar to that found by Marcolino et al.[6] in a study carried out in 25 Brazilian hospitals.

Males were the most affected, corresponding to 60.2% of hospitalized patients, slightly higher than that found by Ranzani et al.[7] in a study with 254,243 patients admitted to several hospitals in Brazil where the prevalence was 56%. Male patients also had a worse prognosis, and 54.7% were not discharged home and had a higher risk of mortality[8].

Few hospitalized patients had been vaccinated with some dose of the vaccine against COVID-19, and 36.5% of 493 patients had previously used the covid kit, consisting of azithromycin, ivermectin, and hydroxychloroquine. At the beginning of the pandemic, there were still no vaccines available and many drugs have been proposed as therapeutic possibilities against COVID-19, being used on a large scale in Brazil[9]. The use of the covid kit did not result in a better patient outcome.

Among the main signs and symptoms presented by patients are dyspnea/respiratory distress, cough, desaturation, fatigue/asthenia, fever, and tachypnea. Guan et al.[5] found similar symptoms, but in different proportions in their study carried out in China at the beginning of the spread of the disease.

Pulmonary involvement at the first chest tomography was >50% in 25% of 468 patients. This characteristic was also considered a risk factor for an unfavorable outcome, was present in 44.3% of patients who were not discharged home, and increased 1.49 times the risk of ICU admission or death. The most common aspects reported were ground-glass opacities, areas of consolidation, or both, which may be unilateral or bilateral and have greater extension approximately 10 days after the onset of symptoms[2,5]. A study carried out by Santos et al.[10] identified that 55% of patients with pulmonary involvement >50% underwent mechanical ventilation, while only 31% of patients with less than this had the same outcome. The result of our study reinforces this unfavorable outcome.

The main comorbidities presented by the patients were obesity, systemic arterial hypertension (SAH), and diabetes mellitus (DM), similar to what was found by Marcolino et al.[6] It is worth noting that 28.3% of the analyzed patients had no

Table 2. Outcomes of patients hospitalized in the COVID ward.

Variables	Mean±SD or n (%) (n=502)
Discharged to home	414 (82.5)
ICU	68 (13.5)
Death	20 (4.0)
Length of stay in the ward	4.9±3.3

Data are expressed as mean±standard deviation or n (%). SD: standard deviation; n: sample size; ICU: intensive care unit.

Table 3. Multivariate analysis of factors associated with non-discharge outcome (need for ICU or death) of patients admitted to the COVID ward (n=88).

Variable	OR (gross)	95%CI	p-value	OR (adjusted)	95%CI	p-value
Age >65 years	2.37	1.62–3.46	**0.01**	1.85	1.30–2.64	0.01
Cough	0.67	0.46–0.98	**0.04**	–	-	-
Tachypnea (fR≥24)	1.92	1.30–2.83	**0.01**	–	-	-
Pulmonary impairment>50%	2.39	1.61–3.53	**0.01**	1.49	1.08–2.05	0.01
SAH	2.13	1.43–3.18	**0.01**	–	-	-
Neoplasm	3.01	1.46–6.19	**0.03**	–	-	-
Absence of comorbidities	0.54	0.32–0.90	**0.02**	–	-	-
PaO_2/FiO_2<300 mmHg	1.70	1.12–2.59	**0.01**	–	-	-
D-dímero>1,000 ng/mL	1.76	1.08–2.86	**0.02**	–	-	-
Oxygen therapy nasal cannula	0.62	0.41–0.94	**0.02**	–	-	-
Venturi mask or with reservoir	14.33	7.61–26.98	**0.01**	9.69	4.87–19.26	0.01
NIV	4.13	2.91–5.85	**0.01**	1.55	1.06–2.25	0.02
OTI	6.03	4.57–7.94	**0.01**	1.86	1.27–2.72	0.01
Did not use corticotherapy	3.01	1.47 –6.20	**0.03**	1.54	1.23–1.93	0.01
Did not use antivirals	0.60	0.38–0.95	**0.03**	–	-	-

OR: odds ratio; CI: confidence interval; fR: respiratory frequency; SAH: systemic arterial hypertension; NIV: non-invasive ventilation; OTI: orotracheal intubation. Bold indicates statiscally significant p-values.

previous comorbidity reported, and this rate is much higher than that found by Ranzani et al.[7] which was 16%.

Regarding laboratory tests, 55.8% of 493 patients had PaO_2/FiO_2<300 mmHg, therefore being classified as acute respiratory distress syndrome (ARDS), which can be mild, moderate, or severe[11]. The neutrophil/lymphocyte ratio (NLR) is a parameter used to evaluate the individual's inflammatory state and predicts outcomes in a variety of conditions. In this study, 46.0% of the patients had NLR>6.8. Prozan et al.[12] identified in their study, using an NLR=6.8 as a cut-off point, that for COVID-19, a poor clinical outcome was associated with a higher NLR. In this study, this correlation was not found.

The CRP levels were >100 mg/L in 47.8% of 500 patients, as well as LDH levels >250 U/L in 89.8% of 363, ferritin >500 µg/L in 71.8% of 227, and D-dimer >1,000 ng/mL in 19.3% of 374 patients. Kim and Gandhi[13] identified that the elevation of these markers, above the presented limits, was associated with disease severity, and a CRP≥100 mg/dL was a risk factor for higher mortality[6]. No relationship between these markers and a worse outcome was found in this study.

Individuals who required oxygen therapy by Venturi mask or reservoir bag mask or required NIV or OTI also had a higher risk of not being discharged home, demonstrating that high-flow oxygen therapy to maintain a target SpO_2≥90% in adults, refractory hypoxemia requiring NIV, or failure of non-invasive therapies are important factors to an unfavorable outcome for patients admitted to the wards[3,14].

Among these factors, the greatest risk for worse outcomes was found in patients who required oxygen therapy using a Venturi mask or reservoir bag mask. We believe that two factors may have influenced our result. The first is that some patients were very elderly, with many comorbidities, were critically ill, and family members chose not to institute invasive measures in these patients. The second is that the use of the Venturi mask or reservoir mask may have extended beyond a time considered acceptable, delaying a more invasive measure. Some studies have shown that, paradoxically, the use of these measures and the delay in the use of more invasive measures can worsen the patient's respiratory condition, due to the respiratory effort, which leads to self-inflicted lung injury by the patient (PSILI), resulting in worse outcomes[15,16]. However, further studies are needed to confirm this hypothesis.

In drug therapy, 98.4% of patients used corticosteroids, and individuals who did not use corticosteroids had a higher risk of poor prognosis. The use of corticosteroids in low doses for 10 days was recommended during the pandemic for patients hospitalized with COVID-19 using supplemental oxygen[17], and our result corroborates this recommendation.

Among the limitations found, it can be considered that the study was carried out in a single hospital, where some medical records were not very detailed. There were changes in the tests requested according to severity and length of stay, which led to the need to use the relative frequency for the analysis of results. Furthermore, the method for measuring CRP levels only accounts for values up to 159.9 mg/L, with values above these presented as >160 mg/L, so it is not possible to know the accuracy of these values. Vaccination analysis was limited, given that the survey comprises a large period when there were no vaccines against COVID-19 available.

CONCLUSIONS

Age older than 65 years and a lung involvement extension greater than 50% are predictors of poor prognosis for COVID-19, as well as the need for high-flow oxygen therapy, NIV, and OTI. Corticosteroid therapy, on the contrary, proved to be beneficial in the treatment of the disease.

AUTHORS' CONTRIBUTIONS

BN: Conceptualization, Data curation, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **MVG:** Conceptualization, Formal Analysis, Project administration, Supervision, Visualization. **FRR:** Methodology, Software, Visualization. **EDM:** Data curation, Visualization. **IS:** Data curation, Visualization. **FP:** Data curation, Visualization. **PSSD:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

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ORIGINAL ARTICLE

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Identification of novel variants in retinitis pigmentosa genes by whole-exome sequencing

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SUMMARY

OBJECTIVE: Retinitis pigmentosa is an inherited degenerative disorder causing severe retinal dystrophy and visual impairment, mainly with onset in the first or second decades. The next-generation sequencing has become an efficient tool to identify disease-causing mutations in retinitis pigmentosa. The aim of this retrospective study was to investigate novel gene variants and evaluate the utility of whole-exome sequencing in patients with retinitis pigmentosa.

METHODS: The medical records of 20 patients with retinitis pigmentosa at Eskişehir City Hospital between September 2019 and February 2022 were analyzed retrospectively. Peripheral venous blood was obtained, followed by the extraction of genomic DNAs. The medical and ophthalmic histories were collected, and ophthalmological examinations were performed. Whole-exome sequencing was performed to determine the genetic etiology of the patients.

RESULTS: The proportion of genetically solved cases was 75% (15/20) in the patients with retinitis pigmentosa. Molecular genetic testing identified 13 biallelic and 4 monoallelic mutations in known retinitis pigmentosa genes, including 11 novel variants. According to ***in silico*** prediction tools, nine variants were predicted as pathogenic or possibly pathogenic. We identified six previously reported mutations to be associated with retinitis pigmentosa. The age of onset of the patients ranged from 3 to 19, with a mean age of onset of 11.6. All patients had a loss of central vision.

CONCLUSION: As the first study of the application of whole-exome sequencing among patients with retinitis pigmentosa in a Turkish cohort, our results may contribute to the characterization of the spectrum of variants related to retinitis pigmentosa in the Turkish population. Future population-based studies will enable us to reveal the detailed genetic epidemiology of retinitis pigmentosa.

KEYWORDS: Night blindness. Frameshift mutation. Mutation. Retinitis pigmentosa. Sequence analysis.

INTRODUCTION

Retinitis pigmentosa (RP) is a group of genetic disorders resulting in inherited blindness due to the degeneration of rod and cone photoreceptors[1]. RP is associated with significant genotypic and phenotypic heterogeneity, with more than 89 genes causing RP reported so far[2,3]. Despite this heterogeneity, RP patients have some common clinical features: progressive loss of photoreceptors, typically involving the rod system. The characteristic phenotype includes retinal bone-spicule pigmentation, pallor of the optic disk, and attenuation of the retinal vessel[1-3]. It is estimated to affect about 1 in 3,000 to 1 in 4,000 people worldwide[4]. The genetic condition may be autosomal dominant RP (15–25%), autosomal recessive (31–41%), or X-linked recessive trait (12–22%) Moreover, approximately 50% of RP cases are sporadic[5]. In recent years, the application of next-generation sequencing (NGS), mostly as targeted exome sequencing (TES) and whole-exome sequencing (WES), has greatly increased the genetic diagnosis rates of different forms of RP[6-9]. The diagnosis rate of TES in RP patients ranges from about 30 to 65%[10-12]. Despite the large number of disease-related genes identified, the majority of patients with RP do not appear to have any genetic defects in all known genes[13,14]. Nevertheless, WES is useful for identifying novel disease-related genes, albeit at a higher cost than TES[15,16]. As a result of the use of new-generation genetic technologies, the rapidly increasing new information leads both to illuminate the genetic etiology and to define new clinical entities with diagnosis and treatment options[14-16]. This retrospective study aims to describe the phenotype and genotype of Turkish patients with RP. This is the first comprehensive molecular diagnosis of a Turkish RP patient cohort using WES. Here, we report the genetic and ophthalmological findings in 20 Turkish patients with RP with 17 variants, including 11 novel mutations in RP genes.

METHODS

This retrospective single-center study included the subjects who were investigated at the Department of Ophthalmology.

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Between September 2019 and February 2022, the patients were evaluated by an ophthalmologist and clinically diagnosed with RP. The patient's age, gender, age of onset, family history, clinical, and ocular examination findings were noted. The diagnosis of RP was based on the detection of topographically limited retinal abnormalities consistent with corresponding sectorial visual field defects. Best-corrected visual acuity (BCVA), fundus color pictures as well as fundus autofluorescence (FAF), spectral-domain optical coherence tomography (SD-OCT), full-field electroretinography (ERG), color vision, and fundus photography were retrospectively collected and analyzed. The study was approved by the Ethics Committee of the Eskişehir Osmangazi Medical Faculty (Protocol number: 2022-111, Decision date/number: April 24, 2022/42). This study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all the patients. Prior to genetic testing, a diagnosis of RP was made based on a history of structural retinal changes and/or visual field defects consistent with the disease. Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. After the clinical diagnosis of RP, we proceeded with next-generation sequencing of the whole exome in the probands, performing the TWIST© Human Core Exome® kit with 97.11% of targeted regions covered at ≥20×. Variants were filtered against dbNSFP v2.0, dbSNP v137, and population databases including the Genome Aggregation Database (gnomAD), the Exome Aggregation Consortium (ExAC), and the 1000 Genomes Project. All variants with a MAF ≤0.01 were evaluated and classified as pathogenic (P), likely pathogenic (LP), variants of uncertain significance (VUS), likely benign (LB), and benign (B) according to the criteria and guidelines of the American College of Medical Genetics and Genomics (ACMG). The variants identified as pathogenic in ClinVar and/or Human Genome Mutation Database were considered to explain the phenotype. Deleterious effect prediction of the variants used multiple algorithms, including Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen2), and MutationTaster. The statistical analyses were done using the SPSS 15.0 software. In this analysis, clinical data were expressed in percentages.

RESULTS

In total, 20 patients from 18 families with RP were included in this study. There was a male preponderance, forming 80% of the total cases (16/20). The mean age of the patients was 38.46 years (a range of 19–57). The mean age at disease onset was 11.6 years (a range of 3–19). Whole-exome sequencing revealed one or more RP disease-causing alleles in 15/20 (75%) of the patients. In 5 of 20 cases (25%), a genetic diagnosis was not achieved. Table 1 shows demographic characteristics, age at

Table 1. Clinical and demographic characteristics in 15 patients with retinitis pigmentosa.

Patients	Sex, age (years)	Age of onset	Consanguinity in parents	Fundoscopy	Gene	Genetic diagnosis-inheritance
Case 1	Male, 54	14	Yes	ONP, ARA, PBSL	ARL2BP	RP 82 (AR)
Case 2	Male, 29	5	Yes	ARA, CA with foveal sparing, ONP	PCARE	RP 54 (AR)
Case 3	Male, 32	7	Yes	ONP, GRC, ARA	PCARE	RP 54 (AR)
Case 4	Male, 31	14	No	ARA, CA with foveal sparing, PBSL	CERKL	RP 26 (AR)
Case 5	Female, 30	4	Yes	GRC, ONP, PBSL	NR2E3	RP 37 (AR)
Case 6	Female, 45	9	Yes	PF, ARA, PBSL	EYS/ RP1	RP 25 (AR) / RP 1 (AD/AR) (digenic inheritance)
Case 7	Female, 57	16	No	PF, ARA, ONP, PBSL	CERKL	RP 26 (AR)
Case 8	Female, 54	6	Yes	CA, ONP, ARA, PBSL	CRB1	RP 12 (AR)
Case 9	Female, 19	3	Yes	CA with foveal sparing, ARA, PBSL	ABCA4	RP 19 (AR)
Case 10	Male, 19	3	Yes	CA, PF, ARA	ABCA4	RP 19 (AR)
Case 11	Male, 37	17	No	CA, ARA, PBSL	EYS	RP 25 (AR)
Case 12	Male, 40	4	Yes	ONP, GRC, ARA, PBSL	MERTK	RP 38 (AR)
Case 13	Male, 44	16	Yes	ONP, GRC, ARA, PBSL	USH2A	RP 39 (AR)
Case 14	Male, 52	19	No	CA with foveal sparing, PF, PBSL	RPGR	RP 3 (XR)
Case 15	Male, 32	4	Yes	CA, ONP, ARA, PBSL	RPE65	RP 20 (AR)

ARA: attenuated retinal arteries; GRC: gray retinal color; ONP: optic nerve pale; PBSL: pigment bone spicule-like; CA: central atrophy; PF: pale fundus.

onset of disease, clinical findings, and the diagnoses of patients with mutations detected in genetic test results. A total of 17 variants were found that could explain the RP phenotype. Among these, 11 were novel variants (4 missense, 3 nonsense, 3 frameshift mutations, and 1 intronic variant). Of these 15 probands, 12 were homozygous for causative variants (80%). Two probands had compound heterozygous mutations in recessive-RP-related genes (*EYS/RP1* and *USH2A*), and one patient had hemizygous for an X-linked gene (RPGR) (Table 2).

The *in silico* protein prediction results of the novel mutations are presented in Tables 2 and 3. Pathogenicity was interpreted in accordance with MutationTaster, PolyPhen-2, and SIFT. According to the prediction tools, one variant (*USH2A*: c.4348G>A) was predicted as of uncertain significance and one variant (*RP1*: c.2386G>A) was predicted as tolerable/benign. Nine of the 11 novel variants were predicted as pathogenic or likely pathogenic (81%) (Table 3).

A mutation in the *RPGR* gene was detected in only one patient with X-linked RP (Tables 1 and 2). In 14 patients with autosomal recessive RP, several mutations were revealed in *ARL2BP*, *PCARE*, *EYS/RP1* (biallelic variants), *CRB1*, *ABCA4*, *EYS*, *CERKL*, *MERTK*, *RPE65*, *USH2A*, and *NR2E3* (compound heterozygous) (Table 2). We also identified six previously reported mutations related to RP (*NR2E3*, *CRB1*, *ABCA4*, and *EYS*) (Table 2). The presence of attenuated retinal arteries was detected in 13 patients (86.6%), bone spicule pigmentation in 12 patients (80%), and pallor of the optic nerve or fundus in 11 patients (73.3%) of genetically diagnosed patients (Table 1).

DISCUSSION

The present study recruited 20 patients who had received a clinical diagnosis of RP and had them undergo whole-exome sequencing with the aim of identifying pathogenic variants. A genetic diagnosis was possible in 15 cases in this study. To the best of authors' knowledge, this is the first report to evaluate the diagnosis rate and causative genes among Turkish patients with RP using whole-exome sequencing. Previous results showed that the detection rate of genetic diagnosis in patients with RP by targeted exome sequencing ranged from 30 to 65%[17-19]. We have identified 17 gene variants out of 15 Turkish patients with RP; of these, 11 (64.7%) were novel. The rate found in our study was found to be compatible with recent

Table 2. The disease-associated variants identified in 15 patients.

Patient	Family	Gene	Zygosity	Allele 1	Publication	Allele 2	Publication
P1	F1	ARL2BP	Homozygous	c.403C>T, p.Arg135Ter	Novel	c.403C>T, p.Arg135Ter	Novel
P2	F2	PCARE	Homozygous	c.1541delC, p.Pro514HisfsTer27	Novel	c.1541delC, p.Pro514HisfsTer27	Novel
P3	F2	PCARE	Homozygous	c.1541delC, p.Pro514HisfsTer2	Novel	c.1541delC, p.Pro514HisfsTer2	Novel
P4	F3	CERKL	Homozygous	c.1566_1567insCCAA-GACTTATCAGTCTTTA, p. Gly523ProfsTer14	Novel	c.1566_1567insCCAA-GACTTATCAGTCTTTA, p. Gly523ProfsTer14	Novel
P5	F4	NR2E3	Compound heterozygous	c.309C>A, p.Cys103Ter	Reported	c.227G>A, p.Arg76Gln	Reported
P6	F5	EYS/ RP1	Biallelic (digenic) Heterozygous	EYS: c. 2949delC, p.Tyr983Ter	Novel	RP1:c.2386G>A, p.Gly796Ser	Novel
P7	F6	CERKL	Homozygous	c.271G>T, p.Glu91Ter	Novel	c.271G>T, p.Glu91Ter	Novel
P8	F7	CRB1	Homozygous	c.2230C>T, p.Arg744Ter	Reported	c.2230C>T,p.Arg744Ter	Reported
P9	F8	ABCA4	Homozygous	c.1804C>T, p.Arg602Trp	Reported	c.1804C>T, p.Arg602Trp	reported
P10	F8	ABCA4	Homozygous	c.1804C>T, p.Arg602Trp	Reported	c.1804C>T, p.Arg602Trp	reported
P11	F9	EYS	Homozygous	c.8793_8796delATCA, p.Gln2931HisfsTer43	Clinvar	c.8793_8796delATCA, p.Gln2931HisfsTer43	Clinvar
P12	F10	MERTK	Homozygous	c.1604+5G>A	Novel	c.1604+5G>A	Novel
P13	F11	USH2A	Compound heterozygous	c.5386T>C, p.Cys1796Arg	Novel	c.4348G>A, p.Val1450Ile	Novel
P14	F12	RPGR	Hemizygous	c.2234_2237delGAGA, p.Arg745LysfsTer69	Novel	Not determineed	(-)
P15	F13	RPE65	Homozygous	c.314C>T, p.Thr105Ile	Novel	c.314C>T, p.Thr105Ile	Novel

Table 3. Pathogenicity predictions for the 11 novel variants in RP genes reported in the present study.

Gene	Nucleotide change	Protein change	MutationTaster	PolyPhen2	SIFT
ARL2BP	c.403C>T (nonsense variant)	p.Arg135Ter	Disease causing	Damaging	Pathogenic
PCARE	c.1541delC (frameshift variant)	p.Pro514HisfsTer27	Disease causing	–	Pathogenic
CERKL	c.1566_1567insCCAAGACTTATCAGTCTTTA (frameshift variant)	p.Gly523ProfsTer14	Disease causing	–	Pathogenic
EYS	c.2949delC (nonsense variant)	p.Tyr983Ter	Likely Pathogenic	Probably damaging	Likely pathogenic
RP1	c.2386G>A (missense variant)	p.Gly796Ser	Polymorphism	Likely benign	Tolerated
CERKL	c.271G>T (nonsense variant)	p.Glu91Ter	Disease causing	Damaging	Pathogenic
MERTK	c.1604+5G>A (intronic variant)	-	Likely Pathogenic	Probably damaging	Likely pathogenic
USH2A	c.5386T>C (missense variant)	p.Cys1796Arg	Disease causing	Damaging	Pathogenic
USH2A	c.4348G>A (missense variant)	p.Val1450Ile	Uncertain Significance	Uncertain Significance	Uncertain significance
RPGR	c.2234_2237delGAGA (frameshift variant)	p.Arg745LysfsTer69	Disease causing	-	Pathogenic
RPE65	c.314C>T (missense variant)	p.Thr105Ile	Likely Pathogenic	Probably damaging	Likely pathogenic

SIFT: sorting intolerant from tolerant; PolyPhen2: polymorphism phenotyping v2.

studies reporting novel gene mutation rates ranging from 62 to 68%[20,21]. Variants in four genes (*NR2E3*, *CRB1*, *ABCA4*, and *EYS*) have been reported to be responsible for RP12 (AR), RP19 (AR), RP25 (AR), and RP37 (AR), respectively. Based on the genetic findings, inheritance turned out to be autosomal recessive in 93.3% (14 out of 15) and X-linked in 6.7% (1 out of 15) of patients. The AR RP (93.3%) was detected in the majority of the patients in our study. No proband was found with AD RP in this study.

The mutations in *ARL2BP* are a known cause of RP82 (AR)[22]. To the best of authors' knowledge, approximately 10 cases have been reported with RP82 due to a homozygous mutation in *ARL2BP* in the medical literature[23]. Herein, we report the 11th patient with RP82 in the world and the first patient from Turkey.

The *EYS* mutations can cause RP25 (AR). The *RP1* mutations have been associated with RP1 (AR/AD). The segregation analysis showed that the parents were carriers of this variant[24]. Segregation analyses pointed toward a digenic inheritance. Gao et al. reported the co-existence of *EYS* c.7723+1G>A and *LRP5* c.3361A>G heterozygous mutations in a patient with RP[25]. Herein, this is the first study in which *EYS* and *RP1* gene variants were found together in an RP patient with a digenic biallelic disease.

In this study, we present a comprehensive clinical and genetic evaluation of individuals with RP. To the best of authors' knowledge, this is the first retrospective study that includes a cohort of subjects of Turkish origin with RP. The genetic results of the present study conducted with a Turkish population showed that most of the patients were predominantly compatible with the diagnosis of AR RP (93.3%). The rate of genetically resolved cases was 75% in our study. The overall diagnostic yield of targeted gene sequencing is 55–65%[11].

Herein, we also identified 11 novel variants in RP-related genes. These results will contribute to expanding the mutational spectrum of RP genes. Approximately 81% (9/11) of the identified novel variants are pathogenic or likely pathogenic. The rate in this study is higher than that observed in similar studies from Europe and the Far East, where approximately 45 and 63% of the pathogenic alleles were novel[18,19]. These results confirm the utility of WES as a powerful method for mutation identification in the diagnosis of RP.

The limitations of our study are represented by the relatively small sample size, the retrospective nature of the study, and, as explained above, the fact that we did not use the same section as a reference for all follow-up examinations.

CONCLUSION

The WES analysis may help to provide a more accurate clinical diagnosis in the detection of genetic diseases with high heterogeneity, such as RP. Meanwhile, we are highlighting the importance of comprehensive NGS-based tests in screening genetically unresolved cases for known RP genes as well as other retinal disease genes. Our current knowledge of the mutation spectrum underlying RP in other populations is limited, as most studies of RP have been conducted with patients of European origin. Identification of the molecular diagnosis of RP patients in different populations will expand the global spectrum of RP-associated gene mutations.

AUTHOR CONTRIBUTIONS

AK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing.

İAÖ: Data curation, Investigation, Writing – review & editing.

NDU: Data curation, Investigation, Writing – review & editing.

BP: Investigation, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Factors associated with complications after percutaneous nephrolithotomy: an analysis of 1,066 cases

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SUMMARY

OBJECTIVE: The aim of this study was to identify predictive factors for complications after percutaneous nephrolithotomy.

METHODS: We prospectively analyzed patients who underwent percutaneous nephrolithotomy from June 2011 to October 2018. The association of preoperative and intraoperative factors with the presence of complications was assessed using univariate and multivariate analyses. The significance level was set at p<0.05.

RESULTS: A total of 1,066 surgeries were evaluated, and the overall complication rate was 14.9%. In all, 105 (9.8%) surgeries were performed in the prone position, and 961 (90.2%) were performed in the supine position. Univariate analysis demonstrated that surgical position, upper pole puncture, surgical time, number of tracts, and Guys Stone Score were associated with complications. In multivariate analyses, prone position (odds ratio [OR] 2.10; p=0.003), surgical time ≥90 min (OR 1.76; p=0.014), upper pole puncture (OR 2.48; p<0.001), and Guys Stone Score 3 or 4 (OR 1.90; p=0.033) were independent predictive factors for complications after percutaneous nephrolithotomy.

CONCLUSION: Performing percutaneous nephrolithotomy in the supine position, in under 90 min, and avoiding upper pole punctures may reduce complications during the treatment of large kidney stones.

KEYWORDS: Percutaneous nephrolithotomy. Kidney stones. Risk factors. Complications.

INTRODUCTION

Since its first description in 1976 by Fernström and Johansson[1], percutaneous nephrolithotomy (PCNL) has become the standard procedure for the treatment of renal stones>20 mm or complex and multiple kidney stones[2,3]. Technological advances have increased the success rates of PCNL, and major complications are less common today than in the past[4,5]. Minor postoperative complications account for the majority of cases, with a rate between 7.1 and 40.2%. Otherwise, major postoperative complications have been reported with rates of up to 17.1%[6,7]. In a large review, the most common complications were fever and bleeding. Other complications such as urinary leakage, hydrothorax, hematuria, urinary tract infection, and urinary fistula were also present but less frequent[8].

The reported risk factors for bleeding include an upper pole puncture, a solitary kidney, a staghorn stone, multiple punctures, and inexperienced surgeons[9]. Wang et al.[10] also analyzed the risk factors for bleeding and septic shock and reported the prevalence of septic shock and severe bleeding to be 2.4 and 1%, respectively.

Recent studies have evaluated the risk factors for specific complications, but there are insufficient data regarding the predictors of general complications after PCNL. In the present study, we aimed to report the risk factors for all perioperative deviations, rather than specific complications, in a very large sample.

METHODS

We performed a retrospective analysis of prospectively collected data pertaining to all patients who underwent PCNL between June 2011 and October 2018 at a single center. Informed consent was obtained from patients preoperatively, and the study protocol was approved by the Institutional Review Board.

Indications for surgery were renal stones >2 cm in size and symptomatic stones <2 cm for which first-line techniques (shockwave lithotripsy or ureterorenoscopy) failed. Considering the cases of failure after initial treatment, cases of multiple stones, inferior polar stones (>15 mm), and unfavorable anatomical conditions were eligible for percutaneous nephrolithotripsy.

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The following clinical and operative variables were collected: age, gender, body mass index (BMI), American Society of Anesthesiologists (ASA) physical status classification, hemoglobin level, stone size, laterality, number of PCNL, surgical position, surgical time, number of tracts, upper pole puncture, and Guy's Stone Score (GSS)[11]. The GSS was determined by a urologist during the preoperative consultation by analyzing computerized tomography (CT) findings, and it was rechecked immediately before surgery. All urologists were trained in evaluating the GSS.

Operating technique

All PCNL procedures were performed under general anesthesia. The patient was positioned in the prone or supine position based solely on the surgeon's preference. The surgeons were trained in performing PCNL in both the prone and supine positions. For prone positioning, we followed the classic method described by Clayman et al.[12] For supine positioning, we used the modified complete supine position described by Vicentini et al.[13] The main surgeon performed the calyceal puncture under fluoroscopic guidance. Subcostal skin punctures were preferred, but supracostal punctures through the 11th and 10th intercostal spaces were also used when necessary. A semirigid plastic dilators set (Amplatz dilators[a]) was used to sequentially dilate the tract up to 30 Fr. Nephroscopy was performed using a 26 Fr nephroscope (Karl Storz, Germany), and stone fragmentation was performed using an ultrasonic lithotripter (Swiss Lithoclast Master[a], EMS, Switzerland).

Intraoperative stone-free status was verified using fluoroscopy and flexible nephroscopy. A 16 Fr nephrostomy tube was placed at the end of the procedure in cases of bleeding, residual stones, solitary kidney, pelvic injury, or multiple tracts. Routinely, a 6 Fr ureteral catheter and an 18 Fr bladder catheter were left in place until the first postoperative day (POD1); in cases of ureteropelvic junction edema or injury, a 4.8 Fr´26 cm ureteral stent was used for 3 weeks. Ropivacaine 1% (20 mL) was injected into the tracts at the end of the surgery.

Outcome evaluation

All patients underwent an abdominal non-contrast-enhanced CT on POD1 to evaluate the surgical complications and residual stones. Finally, during the postoperative period, we analyzed the postoperative hemoglobin level 12 h after the surgery, the need for red blood cell transfusion, and complications (using the Clavien-Dindo classification adapted to PCNL)[14].

Statistical analysis

SPSS for Windows (version 21.0; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Continuous variables are expressed as mean and standard deviation. Categorical variables are described using simple and relative frequencies. Clinical and operative variables and complication status were compared using Pearson's chi-squared test. Variables with an expected frequency of less than five were analyzed using Fisher's exact test. The Cox proportional hazards model was used to determine the variables that influenced the presence of complications. The confidence interval was set to 5%.

RESULTS

We performed 1,066 PCNLs on 891 patients. The mean age of the patients was 48.6 years (range, 14–87 years). A total 105 (9.8%) surgeries were performed in the prone position, and 961 (90.2%) were performed in the supine position. GSS 3 or 4 (complex cases) were found in 47.7% of cases.

The overall complication rate was 14.9%. The Clavien grade of the complications was grade 1 in 36 (3.4%) patients, grade 2 in 60 (5.6%) patients, grade 3 in 39 (3.7%) patients, grade 4 in 20 (1.9%) patients, and grade 5 in 4 (0.4%) patients. Table 1 shows intra- and postoperative complications according to the Clavien classification (minor and major complications).

Table 1. Intra- and post-operative complications.

Type of complication*	n=1,066 (% of total)
Severe bleeding (transfusion)	48 (4.5)
Urinary tract infection	30 (2.8)
Pain	19 (1.7)
Tract leakage (persistent fistula)	15 (1.4)
Stone migration to ureter	14 (1.3)
Pleural injury	11 (1.2)
Acute kidney injury	7 (0.6)
Colon injury	6 (0.5)
Pseudoaneurysm	3 (0.28)
Liver injury	2 (0.1)
Thromboembolism	2 (0.2)
Duodenal injury	1 (0.09)
Spleen injury	1 (0.09)
Deaths (severe sepsis, septic shock, severe bleeding)	4 (0.37)
Clavien classification	**n=159 (% of complications)**
Clavien I	36 (22.6)
Clavien II	60 (37.7)
Clavien III	39 (24.5)
Clavien IV	20 (12.6)
Clavien V	4 (2.5)

*Multiple events may have occurred in a single patient.

The largest kidney stone diameter was significantly larger in patients with complications than in those without (31.7 vs. 28.6 mm, p=0.007). The complication rate progressively increased according to the GSS (p<0.001).

We also performed a univariate analysis of clinical and operative variables according to the complications (Table 2). Of the 105 patients who underwent surgery in the prone position, 25.7% had complications, while the complication rate for the patients in the supine position was 13.7% (p<0.001). Other variables such as surgical time ≥90 min (p<0.001), number of tracts (p<0.001), and upper pole puncture (p<0.001) were associated with the presence of complications.

In the multivariate analysis, the variables that remained as independent predictors of complications after PCNL were complex kidney stones (GSS 3 or 4) (OR 1.90; p=0.033), surgical time ≥90 min (OR 1.76; p=0.014), prone position (OR 2.10; p=0.003), and upper pole puncture (OR 2.48; p<0.001) (Table 3).

Table 2. Clinical and operative variables according to overall complication.

Variables	Overall complications		p-value
	Yes	No	
Age (years), mean (SD)	48.3 (12.7)	48.7 (12.5)	0.700
Largest stone diameter (mm), mean (SD)	31.7 (14.6)	28.4 (11.9)	**0.007**
BMI, mean (SD)	27.4 (5.18)	27.3 (5.14)	0.451
Gender (female), n (%)	102 (16.4)	520 (83.6)	0.108
ASA, n (%)			
1–2	145 (14.9)	830 (85.1)	
3–4	14 (15.4)	77 (84.6)	0.896
Number of PCNL, n (%)			
1	110 (14.4)	650 (85.6)	
2	31 (15.3)	171(84.7)	
3 or more	18 (17.3)	86(82.7)	0.707
Guys stone score, n (%)			
1	16 (7.4)	199 (92.6)	
2	38 (13)	255 (87)	
3	65 (17.4)	308 (82.6)	
4	40 (21.6)	145 (78.4)	**<0.001**
Surgical position, n (%)			
Supine	132 (13.7)	829 (86.3)	
Prone	27 (25.7)	78 (74.3)	**0.001**
Surgical time, n (%)			
<90 min	30 (8.5)	325 (91.5)	
≥90 min	129 (18.1)	582 (81.9)	**<0.001**
Number of tracts, n (%)			
1	100 (12.3)	714 (87.7)	
2	46 (23)	154 (77)	
3 or more	13 (25)	39 (75)	**<0.001**
Upper pole puncture			
Yes	44 (29.1)	107 (70.9)	
No	115 (12.6)	800 (87.4)	**<0.001**

Bold indicates statistically significant p-values.

DISCUSSION

PCNL remains the procedure of choice for kidney stones >2 cm and is associated with a high stone-free rate[15]. Despite its high potential for overall complications, PCNL is considered a safe procedure, mainly due to technological advances[15,16]. However, most complications are minor and do not require any additional treatment[7,8].

Our study aimed to identify the predictive factors for complications after PCNL for the treatment of kidney stones in a large number of patients from a single reference center. A retrospective single-center review reported an overall complication rate of 18.3%[16]. These results are very similar to our own findings, in which 159 (14.9%) patients had any kind of complication. In total, 9% had minor complications (Clavien 1–2) and 5.6% had major complications (Clavien ≥3).

Concerning the major complications in our series, 14 (1.3%) had septic shock or severe sepsis requiring management

Table 3. Cox regression analysis for overall complications.

Variables	OR (95%CI)	p value
Position (prone vs. supine)	2.10 (1.28–3.44)	**0.003**
Surgical time (≥90 min vs.<90 min)	1.76 (1.12–2.78)	**0.014**
Upper pole puncture	2.48 (1.63–3.75)	**<0.001**
Guys stone score (GSS)		
GSS 1	1 (reference)	0.097
GSS 2	1.55 (0.82–2.90)	0.169
GSS 3 or 4	1.90 (1,05–3.44)	**0.033**
ASA (3–4 vs. 1–2)	1.13 (0.604–2,14)	0.690
BMI (≥30 vs.<30)	1.18 (0.78–1.79)	0.424
Largest stone diameter	1.00 (0.994–1.02)	0.273
Number of tracts		
1	1 (reference)	0.303
2	1.37 (0.88–2.13)	0.152
3 or more	0.96 (0.44–2.11)	0.935

OR: odds ratio; CI: confidence interval. Bold indicates statistically significant p-values.

in the intensive care unit (ICU). Bleeding requiring transfusion occurred in 48 patients (4.5%). Of these patients, seven had severe bleeding and were also treated in the ICU. It is important to report that severe sepsis, septic shock, and bleeding were the causes of death in four patients in our series. Calculus migration to the ureter occurred in 14 (1.3%) patients and was treated using an endoscopic approach. A total of 15 patients (1.4%) had persistent urinary tract leakage, and eight of them also required double-J stent placement.

We identified several factors associated with the presence of complications, including surgical position, surgical time, number of tracts, GSS classification, and upper pole puncture. In multivariate analysis, the prone position, surgical time ≥90 min, upper pole puncture, and the presence of complex cases (GSS 3 or 4) were independent predictors of complications. It is important to note that surgical characteristics were more common predictors of complications than clinical characteristics.

Prospective and retrospective studies have revealed that patient demographics are not risk factors for complications after PCNL[15]. Thus, although age and BMI are generally considered to be risk factors in all surgeries, they were not statistically significant risk factors for complications after PCNL in this or previous studies.

Female sex has been reported to be an independent predictive factor for complications after PCNL in previous studies[17,18], which is contradictory to our own observations. The complication rates in men and women in our study were 12.8 and 16.4%, respectively (p=0.108).

The ASA classification is a widely accepted method to evaluate perioperative risk and a predictor of postoperative outcome[19]. However, this classification is not specific to urological procedures or to the risk of postoperative complications. In PCNL, the overall rate of complications was similar in patients who were identified as high-risk (ASA III or IV) or low-risk (ASA I or II)[20]. These findings are similar to our own; we found that the ASA score was not a predictive factor of complications after percutaneous surgery (p=0.690). In contrast, Labate et al.[7] showed that each increase in the ASA score increases the risk of complications as well as the chance of major complications in PCNL. It is important to note that all ASA 3 and 4 patients have a specific care protocol that includes invasive arterial blood pressure control, central intravenous access, and postoperative intensive care, developed by the anesthesiologists from our hospital. This protocol may aid in controlling complications in this group of patients.

It is well established that complex stones (GSS 3 and 4) are independent predictive factors for percutaneous complications[21], mainly due to the prolonged procedure time and the need for multiple punctures, including punctures in the upper renal pole. Falahatkar et al.[22] concluded that multiple punctures during PCNL were also predictive factors for complications. In our study, 151 patients (14.2%) underwent upper-pole puncture. The overall complication rate in the group with an upper pole puncture was 29.1%, compared to 12.6% in the group without this puncture (p<0.001). Among patients with an upper pole puncture, 73.5% had GSS 3 or 4. The treatment of complex cases (GSS 3–4) remains a challenge, and staged surgery may decrease complication rates.

Our study demonstrated that surgical time was a predictive factor for complications after PCNL. The overall complication rate for patients whose operating time was longer than 90 min was 18.1% compared to 8.5% among those with a surgical time of less than 90 min (p<0.001). Interestingly, the proportion of males with a score of 3 or 4 was also higher among those with surgical time ≥ 90 min (65.5 vs. 25.9%, p<0.001). Similarly, Labate et al.[7] reported that the risk of more severe postoperative complications increased in those with surgical times greater than 115 min (OR 2.06). It is important to mention that infections are common complications in the treatment of complex kidney stones. Thus, the stones are often colonized by bacteria, and the prolonged fragmentation associated with the irrigation fluid and hydrostatic pressure can translocate bacteria and endotoxins into the circulatory system. Treatment of complex stones is difficult, often requiring multiple punctures, puncture of the upper pole, and longer surgery times.

Regarding surgical position, the prone position has been the preferred position for PCNL in the last few decades. In 1998, Valdivia et al.[23] described the first series of patients who underwent surgery in the supine position. The association between surgical position and complication rates remains unclear. In two recent meta-analyses, surgical position was not associated with the overall complication rate; however, blood loss and fever rates were proportionally lower in the supine position[22,24]. A recent non-randomized prospective study demonstrated a higher rate of overall complications in the prone position compared to the supine position (18 vs. 8%)[25]. In our series, the complication rate in patients that underwent prone PCNL was twice as high as that noted in patients that underwent supine PCNL (OR, 2.10; 95% confidence interval 1.28–3.44, p=0.003). The proportion of complex cases (GSS 3 or 4) in the two groups was similar (p=0.401), but the surgical time ≥90 min was proportionally higher in those that underwent surgery in the prone position (82.9 vs. 64.9%) (p<0.001). Of note, only 105 patients underwent surgery in the prone position at the beginning of our series, which may represent a potential bias. Currently, this approach is reserved for specific cases and randomized studies.

Finally, this study has some limitations worth noting. It is a historical series from a single reference center with the limitations of a retrospective study. In addition, the analyses were not performed after adjustment for stone features and clinical parameters. Nevertheless, the results from this single-center study are valuable as the analysis was performed using data from the largest database in Brazil to date.

CONCLUSION

Performing PCNL in the supine position, reducing surgical time to less than 90 min, and avoiding upper pole punctures may reduce complications during the treatment of large kidney stones.

ETHICAL APPROVAL

All procedures performed in the study were in accordance with the ethical standards of the local Research Committee and with the 1964 Helsinki Declaration and its later amendments.

INFORMED CONSENT

Informed consent was obtained from patients.

AUTHORS' CONTRIBUTIONS

DFS: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **DBF:** Conceptualization, Data curation, Formal Analysis, Software, Writing – original draft, Writing – review & editing. **KKREH:** Data curation, Formal Analysis, Investigation. **RP:** Data curation, Formal Analysis, Methodology. **PKVM:** Data curation. **DJC:** Data curation. **CAB:** Data curation. **CBM:** Data curation, Project administration, Visualization. **JFAC:** Supervision. **FCV:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Effect of the prone position on recruitability in acute respiratory distress syndrome due to COVID-19 pneumonia

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SUMMARY

OBJECTIVE: This study aimed to assess the effect of prone position on oxygenation and lung recruitability in patients with acute respiratory distress syndrome due to COVID-19 receiving invasive mechanical ventilation.

METHODS: This prospective study was conducted in the intensive care unit between December 10, 2021, and February 10, 2022. We included 25 patients admitted to our intensive care unit with acute respiratory distress syndrome due to COVID-19 who had undergone prone position. We measured the respiratory system compliance, recruitment to inflation ratio, and PaO_2/FiO_2 ratio during the baseline supine, prone, and resupine positions. The recruitment to inflation ratio was used to assess the potential for lung recruitability.

RESULTS: In the prone position, PaO_2/FiO_2 increased from 82.7 to 164.4 mmHg (p<0.001) with an increase in respiratory system compliance (p=0.003). PaO_2/FiO_2 decreased to 117 mmHg (p=0.015) in the resupine with no change in respiratory system compliance (p=0.097). The recruitment to inflation ratio did not change in the prone and resupine positions (p=0.198 and p=0.621, respectively). In all patients, the median value of respiratory system compliance during supine was 26 mL/cmH_2O. In patients with respiratory system compliance<26 mL/cmH_2O (n=12), respiratory system compliance increased and recruitment to inflation decreased from supine to prone positions (p=0.008 and p=0.040, respectively), whereas they did not change in those with respiratory system compliance ≥26 mL/cmH_2O_8 (n=13) (p=0.279 and p=0.550, respectively) (ClinicalTrials registration number: NCT05150847).

CONCLUSION: In the prone position, in addition to the oxygenation benefit in all patients, we detected lung recruitment based on the change in the recruitment to inflation ratio with an increase in respiratory system compliance only in acute respiratory distress syndrome due to COVID-19 patients who have <26 mL/cmH_2O baseline supine respiratory compliance.

KEYWORDS: COVID-19. Acute respiratory distress syndrome. Mechanical Ventilation. Prone position.

INTRODUCTION

Prone positioning improves oxygenation by distributing ventilation more homogeneously, improving ventilation-perfusion matching, decreasing venous admixture, reducing lung compression, and limiting ventilator-induced lung injury in patients with acute respiratory distress syndrome (ARDS)[1-7]. Early data on COVID-ARDS showed severe hypoxemia with near-normal respiratory compliance[8]. However, the physiological effects of the prone position on static compliance and oxygenation were not differentiated between the patients with and without COVID-ARDS[9,10].

The prone position may affect respiratory mechanics by varying lung recruitability and compliance[5,10-13]. Static compliance of the respiratory system increases during the prone position when accompanied by high positive end-expiratory pressure (PEEP) levels but not with low PEEP in non-COVID-ARDS[5]. In a study with COVID-19 patients, the prone position did not improve static compliance[10]. In a study by Cour et al., including COVID-19 patients with ARDS, high recruiters had better compliance of the respiratory system in addition to oxygenation in the prone position, while low recruiters had better oxygenation only[12]. The measurement of recruitability was proposed to predict alveolar recruitment induced by PEEP[14]. A novel bedside technique, known as the recruitment to inflation (R/I) ratio, can estimate the high or poor potential for lung recruitment in patients receiving invasive mechanical ventilation (IMV)[14]. In a study by Pan et al., including COVID-19 patients, the R/I ratio increased with prone ventilation[15]. In another study with COVID-19 patients, the R/I ratio decreased in high recruiters during prone ventilation with increased Cs and oxygenation[12]. The decrease in R/I ratio with increased Cs and oxygenation was explained by accurate lung recruitment with prone ventilation[12]. We conducted this study to assess the effect of the prone position on oxygenation, Cs, and the R/I ratio in patients with COVID-ARDS receiving IMV.

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METHODS

Patients with COVID-19 older than 18 years of age who were intubated, sedated, and receiving IMV due to moderate-to-severe ARDS between December 10, 2021, and February 10, 2022, were screened if they met the ARDS criteria according to the published consensus conference on the definition of ARDS[16]. The patients who had partial oxygen/inspired oxygen fraction (PaO_2/FiO_2) less than 150 mmHg and had undergone prone positioning were included. Prone positioning was accepted to be indicated if ARDS patients receiving IMV had PaO_2/FiO_2 of less than 150 mmHg. Exclusion criteria were the presence of obstructive lung disease history, chest wall abnormalities, interstitial lung disease, pneumothorax, pregnancy, hemodynamic instability refractory to a vasoactive drug (mean arterial blood pressure <65 mmHg lasting more than 1 h, not responsive to noradrenaline>0.5 μg/kg/min), and a history of pneumonectomy or lobectomy. This prospective study was conducted in a tertiary hospital's intensive care unit (ICU). The Tepecik Training and Research Hospital Local Ethics Committee approved the study protocol (No: 2021/11-02), and written informed consent was obtained from the patients and/or the nearest kin of the patients (ClinicalTrials registration number: NCT05150847).

All patients received volume-controlled mechanical ventilation with a tidal volume (VT) of 6–8 mL/kg of predicted body weight, keeping the inspiratory plateau pressure (Pplat) below 30 cmH_2O, respiratory frequency of 12–20 breaths/min, inspiratory time to expiratory time ratio (I/E) 1:2, and FiO_2 level that kept arterial PaO_2 between 60 and 80 mmHg. If the pH was less than 7.25 with low VT and adequate breathing frequency, the Pplat limit was allowed to reach up to 35 cmH_2O. In all patients, clinically set PEEP was the minimum PEEP associated with PaO_2 ranging from 60 to 80 mmHg, aiming a FiO_2 of ≤0.60 while avoiding adverse effects such as hypotension, severe acidosis, and Pplat>30 cmH_2O. All patients were deeply sedated. Patients received neuromuscular blocking agents when needed.

Patients were included in the study within 48 h of intubation. Prone positioning was performed over 16 h in patients whose PaO_2/FiO_2 was less than 150 mmHg. The oxygenation and respiratory mechanics were monitored in the supine, prone, and resupine positions. Respiratory mechanics and arterial blood gas (ABG) measurements were repeated at 6–8 h in the supine position, 12–16 h in the prone position, and 6–8 h in the resupine position. The R/I ratio, ABG measurements, Pplat, static compliance [Cs; VT/(Pplat-PEEP)], and driving pressure (Pplat-PEEP) were recorded in each season. Pplat was obtained using an inspiratory pause maneuver. Airway opening pressure (AOP) was determined during a low-flow insufflation (4 L/min) period of the pressure-volume curve, as described previously[14]. The R/I ratio measurement was performed based on a study by Chen et al.[14]. According to this technique, PEEP was changed from the baseline level to 15 cmH_2O. Then, the change in end-expiratory lung (ΔEELV) volume was measured by a single-breath PEEP reduction from 15 to 5 cmH_2O[14] with a respiratory rate of 10/min to eliminate possible auto-PEEP. If the AOP detected by a low-flow pressure-volume curve was higher than>5 cmH_2O PEEP, this measured AOP was used for measurement. During the single-breath PEEP reduction maneuver, ΔEELV was calculated by subtracting the expired tidal volume from the first expired volume detected when PEEP decreased abruptly from 15 to 5 cmH_2O[14]. The recruited lung volume (Vrec) was calculated as ΔEELV—minimal predicted ΔEELV. The minimally predicted ΔEELV was calculated as Cs at 5 cmH_2O PEEP (or AOP)×ΔPEEP (i.e., 15 cmH_2O - 5 cmH_2O (or AOP)). The recruited lung compliance (CRec) was calculated as Vrec/ΔPEEP. The R/I ratio was calculated as Crec/Cs at low PEEP (5 cmH_2O or AOP). During the single breath maneuver for measuring and calculating the R/I ratio, Vrec, Cs, the same tidal volume, and respiratory rate settings were used. In post hoc analysis, we classified patients into two groups according to the median Cs at baseline supine position. The primary endpoint was the improvement in the PaO_2/FiO_2 ratio, and the secondary endpoints were the Cs and the R/I ratio.

The results are presented as the number (%), the mean±SD or median [interquartile range]. The data in the figures were drawn based on the median, interquartile range, and minimum-maximum range. A chi-square test was used for categorical variables. The t-test was used for continuous variables when data were normally distributed, and when the data were not normally distributed, the Mann-Whitney U test was used for comparing two groups. Paired measurements taken from the same individuals were compared using the paired samples t-test or Wilcoxon rank test, where appropriate. p-values ≤0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

We screened 35 patients with laboratory-confirmed COVID-19 and moderate-to-severe ARDS for whom prone positioning was indicated during the study period. Three patients were hemodynamically unstable, two had septic shock requiring vasopressors, one had lung cancer, two had chronic obstructive lung disease, and two had pneumothorax. After excluding these 10 patients, 25 patients with moderate and severe

ARDS who had undergone prone positioning were included in the study. The median time between ICU admission and inclusion was 6 [2–7] days. Baseline patient characteristics are summarized in Table 1.

In the prone position, PaO_2/FiO_2 increased from a median of 73 [65–102] mmHg at baseline in the supine position to 156 [118–204] mmHg (p<0.001). In the resupine position, PaO_2/FiO_2 decreased to 117 [95–151] mmHg (p=0.015) (Table 2). In the prone position, the plateau pressure decreased from 24 [23–27] to 23 [21–24] cmH_2O (p<0.001). In the resupine position, the plateau pressure increased to 24 [22–26] cmH_2O (p=0.001). In the prone position, the driving pressure decreased from 13 [11–15] to 11 [9–13] cmH_2O (p<0.001). In the resupine position, the driving pressure increased to 13 [11–17] (p=0.002). In the prone position, the Cs increased from 26 [19–32] to 28 [22–38] mL/cmH_2O (p=0.003). In the resupine position, the Cs did not increase (p=0.097) (Table 2). There was no change in the Vrec (79 [49–154] for supine vs. 99 [67–122] for prone, p=0.393), Crec (8.2 [4.3–15.1] mL/cmH_2O for supine, 10.4 [6.7–12.9] mL/cmH_2O for prone, p=0.339), and R/I ratio in the prone position (0.39 [0.12–0.64] for supine vs. 0.36 [0.10–0.45] for prone, p=0.198). There was no change in the R/I ratio in the resupine position (0.36 [0.10–0.45] for prone, 0.24 [0.10–0.50] for resupine, p=0.621) (Table 2).

In post hoc analysis, patients were classified into two groups according to the median value of baseline supine Cs as Cs ≥26 mL/cmH_2O (n=13, median 31 [26–36] mL/cmH_2O) and <26 mL/cmH_2O (n=12, median 19 [16–20] mL/cmH_2O). We compared these two groups according to the median baseline Cs value, as there is no accepted threshold to consider Cs as high or low. There were no differences in age, sex, APACHE II score, body mass index, heart rate, or mean arterial pressure at inclusion between the two groups (Table 1). The median time between ICU admission and inclusion was 2 [1.5–6] days in the group with Cs ≥26 mL/cmH_2O and 7.5 [3–12] days in those with Cs<26 mL/cmH_2O (p=0.006). The days on noninvasive ventilation support before intubation were 2 [1–5] days in the group with Cs ≥26 mL/cmH_2O and 7 [2–11] days in those with Cs<26 mL/cmH_2O (p=0.009).

There was a higher R/I ratio in the baseline supine position in patients with Cs <26 mL/cmH_2O (p=0.050). The PEEP, $PaO_2/F\dot{I}O_2$, Vrec, and Crec were not differentiated in Cs <26 mL/cmH_2O versus Cs ≥26 mL/cmH_2O at the baseline supine position (p=0.293, 0.814, 0.828, and 0.731, respectively). The PaO_2/FiO_2 was higher in Cs ≥26 mL/cmH_2O group than in Cs <26 mL/cmH_2O during the prone position (p=0.003). The PaO_2/FiO_2 increased from the supine to the prone position in both groups (p=0.001 for Cs ≥26 mL/cmH_2O and p=0.012 for Cs <26 mL/cmH_2O; Figure 1A, Table 2). The Cs increased from the supine to the prone position in both groups, but statistical significance was detected only in the Cs <26 mL/cmH_2O (p=0.008 vs. p=0.279). Vrec and Crec were not differentiated in Cs <26

Table 1. Baseline characteristics of all patients and comparison between the group with Cs ≥26 and <26 mL/ cmH_2O.

	All patients (n=25)	Cs ≥26 mL/cmH_2O group (n=13)	Cs <26 mL/cmH_2O group (n=12)	p-value*
Age, years	62.4±13.3	67.4±9.7	57.0±14.9	0.052
Female gender, n (%)	14 (56)	5 (38)	9 (75)	0.063
Body mass index, kg/m^2	32.0±5.7	32.2±5.7	31.9±6.3	0.977
APACHE II score	19.9±4.0	18.7±5.8	18.5±7.4	0.915
Heart rate, /min	86±20	83±19	93±21	0.196
Mean arterial pressure, mmHg	80 [70–88.5]	78 [69–86]	80 [71–91]	0.612
Preexisting disease				
Diabetes mellitus	10 (40)	6 (46)	4 (33)	0.688
Hypertension	11 (44)	5 (38)	6 (50)	0.561
Chronic renal failure	2 (8)	1	1	1.000
None	3 (12)	2	1	1.000
Between ICU admission to inclusion, days	6 [2–7]	2 [1.5–6]	7.5 [3–12]	0.006
Noninvasive support before intubation, days	5 [1–7]	2 [1–5]	7 [2–11]	0.009

Data are presented as the number (%), mean±standard derivation or median [interquartile range]. ICU: intensive care unit; APACHE: acute physiology and chronic health evaluation. *p-values refer to the comparison between the Cs ≥26 and Cs<26 groups.

Table 2. PaO_2/FiO_2, Cs, and R/I between Cs ≥26 and Cs <26 groups during supine, prone, and resupine positions.

	All patients	Cs ≥26	Cs<26	p-value*
PaO_2/FiO_2, mmHg				
Supine	73 [65–102]	75 [63–98]	72 [64–108]	0.814
Prone	156 [118–204]	200 [153–250]	124 [90–163]	0.003
Resupine	117 [95–151]	115 [99–153]	118 [81–128]	0.733
p-value**	<0.001	0.001	0.012	
p-value***	0.015	0.005	0.044	
Cs, mL/cmH_2O				
Supine	26 [19–32]	31 [26–36]	19 [16–20]	<0.001
Prone	28 [22–38]	35 [30–40]	22.5 [21–25]	<0.001
Resupine	29 [21–34]	33 [29–42]	21.3 [18–23.3]	<0.001
p-value**	0.003	0.279	0.008	
p-value***	0.097	0.613	0.090	
R/I ratio				
Supine	0.39 [0.12–0.64]	0.19 [0.12–0.49]	0.56 [0.10–0.75]	0.050
Prone	0.36 [0.10–0.45]	0.32 [0.10–0.44]	0.37 [0.11–0.47]	0.943
Resupine	0.24 [0.10–0.50]	0.37 [0.13–0.55]	0.29 [0.10–0.37]	0.164
p-value**	0.198	0.550	0.040	
p-value***	0.621	0.792	0.178	

Data are presented as the median [interquartile range]. PaO_2/FiO_2 mmHg: arterial oxygen partial pressure/fraction of inspired oxygen; Cs: static compliance; R/I ratio: recruitment to inflation ratio. *p-values detected by using Mann-Whitney U test refer to the comparison between the Cs ≥26 and Cs<26 groups. **p-values detected by using Wilcoxon rank test refer to the comparison of the parameter from supine to prone. ***p-values detected by using Wilcoxon rank test refer to the change between parameters from prone to resupine.

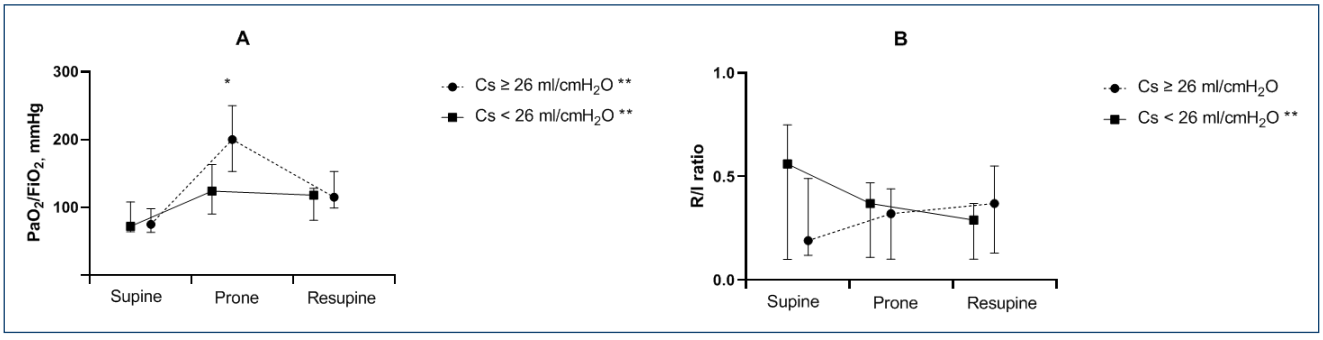

Figure 1. PaO_2/FiO_2 and R/I ratio between groups with Cs ≥26 mL/cmH_2O and Cs <26 mL/cmH_2O during supine, prone, and resupine positions. (A) *The PaO_2/FiO_2 was higher in Cs ≥26 mL/cmH_2O group than in Cs <26 mL/cmH_2O during the prone position (p=0.003). **The PaO_2/FiO_2 increased from the supine to prone position in both groups (p=0.001 for Cs ≥26 mL/cmH_2O and p=0.012 for Cs <26 mL/cmH_2O). (B) **The R/I decreased from the supine to prone position only in the Cs <26 mL/cmH_2O group (p=0.04), whereas it did not change in those with Cs ≥26 mL/cmH_2O (p=0.55). PaO_2/FiO_2 mmHg: arterial oxygen partial pressure/fraction of inspired oxygen; Cs: static compliance; R/I ratio: recruitment to inflation ratio.

mL/cmH_2O versus Cs ≥26 mL/cmH_2O at the baseline supine position (p=0.295, 0.819, 0.823, and 0.737, respectively). The R/I decreased from the supine to the prone position only in the Cs <26 mL/cmH_2O group (p=0.040), whereas it did not change in those with Cs ≥26 mL/cmH_2O (p=0.550, Figure 1B, Table 2).

DISCUSSION

This study found that the PaO_2/FiO_2 ratio increased both in the patients with higher and lower baseline supine compliance (Cs ≥26 and Cs<26 mL/cmH_2O). The recruitment to inflation ratio decreased in the prone position only in those with static compliance <26 mL/cmH_2O.

The group with Cs<26 mL/cmH_2O had a longer stay in the ICU on inclusion than those with Cs ≥26 mL/cmH_2O. All patients received noninvasive ventilation before intubation. The longer stays in the ICU with more prolonged use of noninvasive ventilation before intubation might explain the lower compliance due to impaired lung parenchyma. In COVID-19 patients, the oxygenation response to prone positioning and recruitment decreases over time, possibly due to the predominance of consolidation in the late stages compared with the early stages[17].

Measurement of the R/I ratio estimates the potential for lung recruitment at the bedside in mechanically ventilated ARDS patients[14-18]. The prone position may help recruit the injured lung even in patients with low potential for lung recruitment[5]. In this study, the low-compliance (median Cs <26 mL/cmH_2O) group exhibited a decreased R/I ratio with better oxygenation in the prone position than in the supine. Lung recruitment is consistent with a reduced R/I ratio and increased Cs during the prone position[12].

The decrease in the R/I ratio in Cs<26 mL/cmH_2O, but not in Cs ≥26 mL/cmH_2O, might reflect the differences in Cs and lung volume changes between the high- and low-compliance groups during the prone position. Although not statistically significant, there was a trend toward a reduction in the volume and compliance of the recruited lung in Cs<26 mL/cmH_2O during the prone position. According to these results, in Cs ≥26 mL/cmH_2O, the effect of prone positioning on the improvement of oxygenation could not be explained by lung recruitment, as there was no change in the R/I ratio and Cs. The oxygenation response may be primarily due to a more homogenous perfusion distribution in patients with Cs ≥26 mL/cmH_2O[13].

In their physiologic study, Pelosi et al. found no correlation between the change in Cs and the increase in PaO_2 during prone positioning in non-COVID-ARDS[19]. They also found significant improvements in Cs in the resupine position compared to the baseline supine. They concluded that improved oxygenation during prone positioning might be explained by regional lung volume changes, perfusion, and inflation/ventilation[19]. In a study with COVID-ARDS, improvement in oxygenation in the prone position was not associated with a change in Cs[10]. In this study, in addition to the significant improvement in Cs from the supine to the prone position, we found no change in Cs when returning to the supine from the prone position. The oxygenation slightly declined but was still higher than the baseline value, and the R/I ratio was maintained in the resupine position.

Our study had some limitations. It is a single-center study with a small sample size, and therefore confirmation of the results is required. The study was unblinded, and bias cannot be excluded. The severity of the disease and the influence of additional clinical conditions may be different in patients. The length of noninvasive support is a confounder that may influence the respiratory mechanics measured within 48 h postintubation.

In conclusion, in addition to the oxygenation benefit in all patients with prone position, we found that the R/I ratio was significantly reduced in the prone position with an increase in Cs, indicating recruitment benefit, only in patients with baseline compliance <26 cmH_2O in patients with COVID-ARDS requiring invasive mechanical ventilation.

ETHICAL STATUS

The Tepecik Training and Research Hospital Local Ethics Committee approved the study protocol (No: 2021/11-02), and written informed consent was obtained from the patients and/or nearest kin of the patients (ClinicalTrials registration number: NCT05150847). All authors declare that the study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of the country of origin.

AUTHORS' CONTRIBUTIONS

ÖE: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. **KR:** Conceptualization, Formal Analysis, Methodology, Resources, Supervision, Visualization, Writing – review & editing. **HY:** Conceptualization, Data curation, Writing – review & editing. **RE:** Conceptualization, Data curation, Writing – review & editing. **IKG:** Conceptualization, Supervision, Writing – review & editing.

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ORIGINAL ARTICLE

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Effects of hepatitis C virus genotypes and viral load on glucose and lipid metabolism after sustained virological response with direct-acting antivirals

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SUMMARY

OBJECTIVE: The objective of this study, carried out at the university hospital of the Federal University of Rio Grande, was to assess whether the treatment of chronic hepatitis C with direct-acting antivirals and the sustained virological response will affect the metabolic influences of the hepatitis C virus and whether these effects will vary according to genotypes and virus load.

METHODS: This is an intervention pre-post study, carried out from March 2018 to December 2019, evaluating 273 hepatitis C virus patients treated with direct-acting antivirals. Inclusion criteria included being monoinfected with hepatitis C virus and achieving sustained virological response . Exclusion criteria included the presence of decompensated cirrhosis or co-infected with hepatitis B virus or human immunodeficiency virus. Genotypes, genotype 1 subtypes, and hepatitis C virus viral load were analyzed. Glucose metabolism was evaluated by the Homeostasis Model Assessment-insulin resistance indices: Homeostasis Model Assessment-β, TyG, and HbA1c, measured at the beginning of treatment and in sustained virological response. Statistical analysis with a T test by paired comparison of the means of the variables in the pretreatment and in the sustained virological response.

RESULTS: Homeostasis Model Assessment-insulin resistance analysis: there were no significant differences between pretreatment and sustained virological response. Homeostasis Model Assessment-β analysis: significant increase in genotype 1 patients ($p<0.028$). TyG index analysis: significant increase in genotype 1b ($p<0.017$), genotype 3 ($p<0.024$), and genotype non-1 with low viral load ($p<0.039$). HbA1c analysis: significant decrease in genotype 3 ($p<0.001$) and genotype non-1 patients with low viral load ($p<0.005$).

CONCLUSION: We detected significant metabolic influences after sustained virological response: impairment in lipid profile and improvements in the glucose metabolism. We found significant differences in genotype dependence, genotype 1 subtypes, and viral load.

KEYWORDS: Genotype. Hepatitis C. Lipid metabolism. Insulin resistance. Viral load.

INTRODUCTION

Hepatitis C virus (HCV) can induce insulin resistance (IR) regardless of the severity of liver disease, demonstrating that this virus can induce this metabolic effect even in the preliminary stages of the disease[1]. The influence of HCV on lipid metabolic pathways has also been demonstrated, with evidence of significant changes after sustained virological response (SVR), suggesting a direct viral effect[2]. The viral cycle depends on cholesterol metabolism in host cells, which causes hypolipidemia during chronic infection[3]. The possible influence of HCV genotypes on lipid and glucose metabolism remains not well defined[4]. Overall, there is a great variability in the geographical distribution of HCV genotypes. In Brazil, the most prevalent genotype is 1 (G1) (64%), followed by genotype 3 (33%), and genotypes 2 and 4 (3%)[5]. There is evidence that the eradication of G1 is more beneficial in relation to IR than the eradication of genotypes 2 and 3[3,4].

In addition to the genotype, some studies associate elevated levels of viral load (VL) with the presence of IR in patients with chronic hepatitis C[3,6]. However, other studies found no association between VL value, IR, and type 2 diabetes mellitus (T2DM)[7,1].

The most commonly used method to evaluate the influence of SVR on glucose metabolism employs the Homeostasis Model Assessment (HOMA) indexes. The HOMA index estimates IR (HOMA-IR) and cell-β function (HOMA-β)[8]. Another proposed method for measuring IR is the TyG index, a product of fasting triglyceride levels and blood glucose[9]. Glycated hemoglobin (HbA1c)[10], widely used in daily practice, provides an additional analysis of metabolic effects.

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Considering the effectiveness of direct-acting antivirals (DAAs) and the association of HCV with systemic disorders, it can be expected that the eradication of the virus will improve metabolic parameters and reduce the rates of IR and T2DM in patients with chronic infection[11]. However, the effect of SVR on glucose control is still not clear[12-14]. The present study aimed to compare glucose and lipid metabolism in patients with chronic hepatitis C treated with DAAs in pretreatment and SVR, with special attention to genotypes and VL.

METHODS

This was an intervention pre-post study that initially evaluated 481 patients eligible for treatment with DAAs, treated at the Dr. Miguel Riet Corrêa Jr. University Hospital of the Federal University of Rio Grande (FURG) from March 2018 to December 2019. The study was approved by the ethics and research committee in the health area of FURG (CEPAS), under the process number: 23116.00516/2018-56. The CAAE registration number generated on the Brazil platform is 82698018.0.00005324. All patients read and signed free and informed consent forms before participating in the study. The treatment followed the inclusion and duration criteria of the Clinical Protocol and Therapeutic Guidelines for Hepatitis C and Co-infections 2018/2019[15].

Inclusion criteria were as follows: monoinfected by HCV, achieving SVR, and living in the Rio Grande or São José do Norte municipalities. Exclusion criteria were as follows: the presence of decompensated cirrhosis, patients co-infected with hepatitis B virus or human immunodeficiency virus (HIV), severe psychiatric or cognitive disorders, chronic renal failure, type 1 DM, steroid or anabolic use, and alcohol consumption. According to these criteria, Figure 1 shows the number of patients who were included in this study. The diagnosis of T2DM, pre-diabetes, and normoglycemic followed the criteria adopted in the Guidelines of the Brazilian Society of Diabetes, 2019–2020[10].

The genotypes and subtypes of G1 and VL were evaluated by the real-time polymerase chain reaction technique. Undetectable VL, after the 12th week of treatment, was considered as SVR[15].

For the evaluation of glucose metabolism, the following indices were used: HOMA-IR[16], HOMA-β[16], TyG[9], and HbA1c. The parameters evaluated were measured at the beginning of treatment and in SVR. For comparison analysis, the patients were subdivided into groups according to genotypes and subtypes of genotype 1 and VL to demarcate their respective influences on glycidic and lipid metabolism.

Preliminary data analysis consisted of checking the frequency in search of extreme values, categorization, and creation of derived variables, and then using the T test to compare means. Next, the analysis of the means and their respective standard deviations of the variables in the pretreatment and the SVR were carried out. The confidence interval of the means was also calculated, followed by the T test for paired comparison of the means, adopting a $p<0.05$ as significant. All these analyses were performed using the statistical package.

This project was approved by the ethics and research committee in the health area of FURG (CEPAS) under the process number: 23116.00516/2018-56.

RESULTS

A total of 273 patients participated in this study. The mean age was 57 years old, 70.7% were white, and 52.7% were male. Regarding liver injury, 78 (28.6%) were cirrhotic, and 92 (33.7%) were classified as F0 or F1. About the glucose profile, 125 (45.8%) patients were prediabetic and 50 (18.3%) were diabetic. As for laboratory tests, we highlight a significant increase in the values of triglycerides, total cholesterol (TC), and low-density lipoproteins (LDL), but not in high-density lipoproteins (HDL) in SVR (Table 1).

The HOMA-IR index did not have significant differences between pretreatment and SVR (Table 2). Using the HOMA-β, we found a significant increase in those with G1 (89.29–103.97; p=0.028), a trend to significance in genotype 3 (87.51–99.61; p=0.058), and in subgroup G1 with low VL (85.15–96.34; p=0.05), there was a significant increase (Table 1).

Table 2 shows a significant increase in the TyG index in G1b patients (4.51–4.57; p=0.017), genotype 3 (4.48–4.54; p=0.024), and non-1 genotype (N1G) with low VL (4.51–4.57; p=0.039). Regarding HbA1c values in pretreatment and SVR, there was a significant decrease in patients with genotype 3 (5.85–5.54; p=0.001) and N1G with low VL (5.90–5.59; p=0.005).

DISCUSSION

It is estimated that approximately two-thirds of patients with chronic hepatitis C may experience extrahepatic manifestations, which are especially important in metabolic alterations[17]. Some clinical studies have suggested improvements, after antiviral treatment, in glucose metabolism[11,18]. In addition, the effect of HCV modulating the metabolic pathways of intrahepatic cholesterol biosynthesis to promote viral replication can generate significant changes in lipid

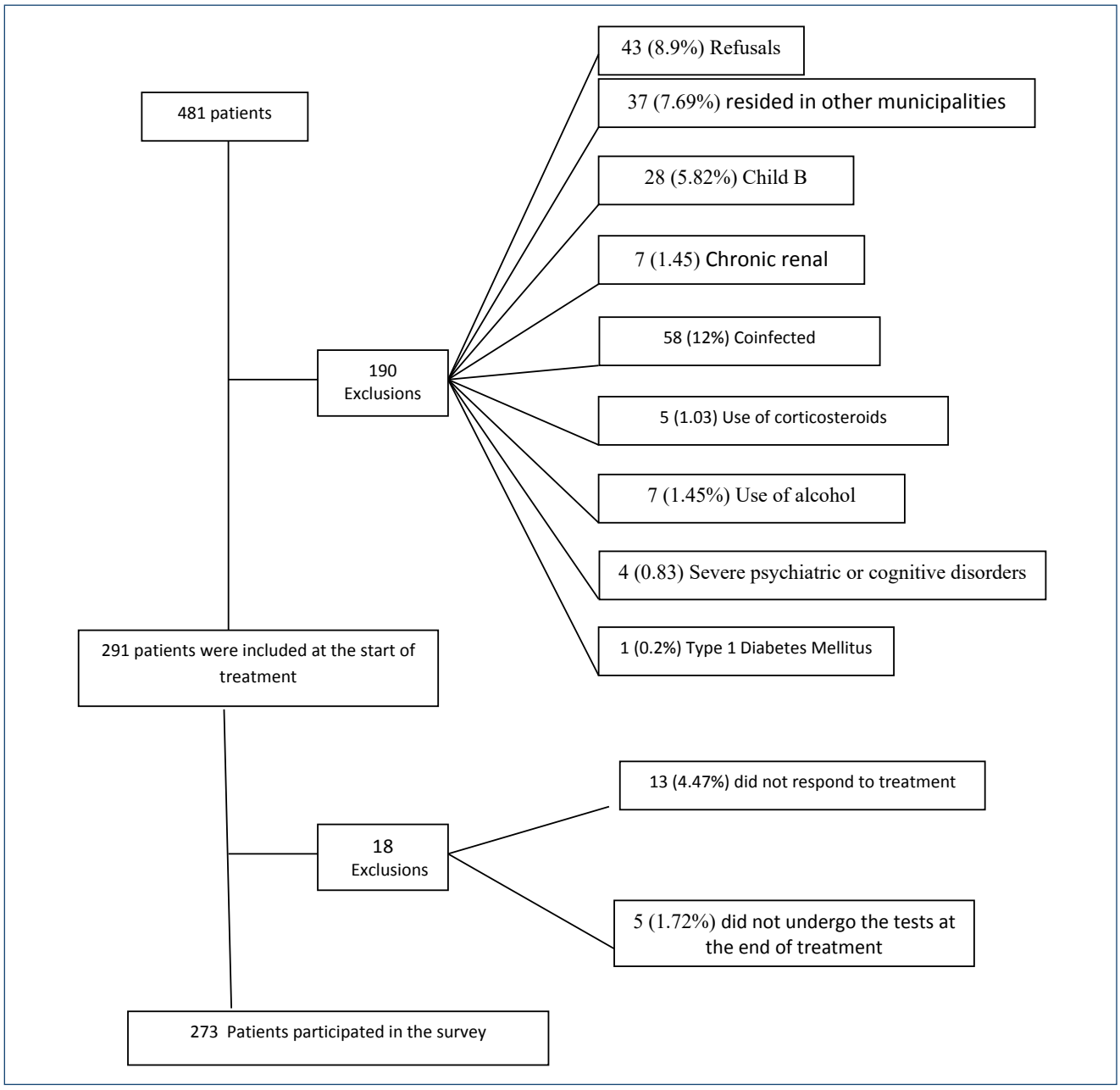

Figure 1. Flowchart of excluded patients.

metabolism in SVR[2]. However, positive and negative metabolic changes were described with treatment with DAAs[11,19], and a better understanding of this issue is necessary in different populations.

We draw attention to the fact that 64.1% of the patients surveyed have lipid metabolism disorders, while in the general Brazilian population, it is estimated that 7.5–18.5% are prediabetic and 7.7% diabetic[20], which is in accordance with the hypothesis of the association of HCV with IR and T2DM and in agreement with other authors[5,21].

In this study, after SVR, there was a significant increase in TC, LDL, and triglycerides, but not in HDL. Studies conducted with patients with genotype 1 mostly observed a significant increase in the rates of TC, LDL, and triglycerides after SVR[22,23]. Similarly, Jain et al.[24], in a prospective study with 50 individuals, all HCV genotype 3, demonstrated that TC and LDL increased significantly with SVR, but there were no changes in HDL and triglycerides. As antiviral treatment would affect lipid metabolism, it is not well established[2]. We highlight that the worsening observed in lipid metabolism

Table 1. Anthropometric and laboratory data and comparison parameters.

	n (%)	Pretreatment	SVR	p-value
Age (mean±SD)		57.03 (±11.11)		
≤54 years	98 (35.9)			
55–64 years	113 (41.4)			
≥65 years	62 (22.7)			
Color				
White	193 (70.7)			
Nonwhite	80 (29.3)			
Genre				
Male	144 (52.7)			
Female	129 (47.3)			
Weight (mean±SD)			73.54 (±14.69)	0.127
WC Abdominal (mean±SD)		73.21 (±14.72)	94.04 (±11.99)	0.206
Adequate	73 (29.3)	94.38 (±11.87)		
Inadequate	200 (70.7)			
BMI (mean±SD)		25.59 (±4.76)	27.73 (±4.84)	0.093
≤24.9 (81)	81 (29.7)			
25–29.9 (116)	117 (42.9)			
≥30 (74)	75 (27.5)			
Glycemic profile (mean±SD)		101.84 (±27.29)	107.79 (±31.70)	0.976
With normal blood glucose	98 (35.9)			
With prediabetes	125 (45.8)			
With diabetes	50 (18.3)			
Glycemic/genotypes (mean±SD)				
Genotype 1 (146)		105.02 (±21.01)	104.27 (±22.23)	0.541
enotype 2 (38)		115.47 (±42.31)	116.00 (±37.23)	0.889
Genotype 3 (87)		109.28 (±28.10)	110.25 (±40.57)	0.775
Profile triglycerides (mean±SD)				
Genotype 1 (146)		101.87 (±66.30)	105.30 (±66.47)	0.416
Genotype 2 (38)		104.95 (±49.45)	109.51 (±54.57)	0.563
Genotype 3 (87)		79.85 (±31.56)	100.94 (±76.15)	0.031
Laboratories (mean±SD)				
Total cholesterol		166.37 (±36.91)	185.52 (±38.41)	<0.001
LDL		97.30 (±33.86)	111.80 (±36.66)	<0.001
HDL		49.59 (±14.23)	50.26 (±13.66)	0.652
Triglycerides		95.44 (±55.67)	104.47 (±67.50)	0.006

BMI: body mass index; WC: waist circumference; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SD: standard deviation; SVR: sustained viral response.

Table 2. Analysis of the homeostasis model assessment-insulin resistance index, homeostasis model assessment-β cell index, TyG index, and HbA1c in relation to viral characteristics.

	HOMA-IR pretreatment Mean (SD)	HOMA-IR pretreatment 95%CI	HOMA-IR SVR Mean (SD)	HOMA-IR SVR 95%CI	p-value		HOMA-β pretreatment Mean (SD)	HOMA-β pretreatment 95%CI	HOMA-β SVR Mean (SD)	HOMA-β SVR 95%CI	p-value
Genotypes (G)						**Genotype (G)**					
Genotype 1 (146)	2.42 (±1.76)	2.13–2.72	2.65 (±1.87)	2.37–2.99	0.079	Genotype 1 (146)	89.29 (±62.85)	79.42–99.37	103.97 (±79.97)	91.27–117.30	0.028
G1 a (67)	2.36 (±1.60)	2.02–2.79	2.53 (±1.83)	2.12–3.00	0.457	G1 a (67)	85.81 (±53.67)	73.25–99.82	98.27 (±91.03)	79.46–121.54	0.223
G1 b (78)	2.63 (±2.44)	2.14–3.19	3.06 (±3.76)	2.34–3.94	0.098	G1 b (78)	91.12 (±62.94)	77.56–105.43	104.16 (±61.73)	90.66–117.47	0.086
Genotype 2 (38)	2.75 (±2.03)	2.17–3.40	2.53 (±1.80)	2.00–3.10	0.323	Genotype 2 (38)	79.34 (±54.38)	63.93–99.16	72.78 (±43.84)	59.75–87.58	0.516
Genotype 3 (87)	2.56 (±2.33)	2.09–3.14	2.96 (±3.50)	2.34–3.80	0.117	Genotype 3 (87)	87.51 (±56.82)	76.00–101.42	99.61 (±57.16)	87.76–11.01	0.058
Viral load (VL)	2.51 (±1.80)	2.22–2.84	2.68 (±2.05)	2.36–3.06	0.300	**Viral load** (VL)	87.85 (±65.71)	76.86–99.40	98.62 (±79.30)	86.28–113.14	0.147
≥600,000 – High (132)	2.51 (±1.81)	2.22–2.85	2.69 (±2.06)	2.34–3.07	0.294	≥600,000 – High (132)	87.40 (±65.76)	77.61–98.48	99.23 (±79.29)	86.33–113.98	0.108
≤599,999 – Low (131)	2.50 (±1.87)	2.09–2.93	2.76 (±1.80)	2.33-3.16	0.239	≤599,999 – Low (131)	92.17±70.27	77.17–108.12	110.80±94.10	90.41–134.05	0.089
Genotype 1 (G1)	2.31 (±1.66)	1.96–2.77	2.52 (±1.98)	2,10–3.07	0.148	**Genotype 1 (G1)**	85.15±52.49	72.99–99.07	96.34±60.84	82.02–110.66	0.050
High VL (76)	2.54 (±1.74)	2.10–3.04	2.60 (±2.38)	2.38–3.29	0.813	High VL (76)	82.02±59.63	66.46–98.06	81.67±49.57	68.98–94.74	0.966
Low VL (66)	2.67 (±2.57)	2.11–3.32	3.00 (±3.55)	2.35–3.85	0.181	Low VL (66)	87.40±53.29	75.30–100.29	99.14±57.62	86.12–114.00	0.080
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
High VL (55)						High VL (55)					
Low VL (70)						Low VL (70)					
	TyG pretreatment Mean (SD)	**TyG pretreatment 95%CI**	**TyG RVS Mean (SD)**	**TyG RVS 95%CI**	**p-value**		**HbA1c pretreatment Mean (SD)**	**HbA1c pretreatment 95%CI**	**HbA1c RVS Mean (SD)**	**HbA1c RVS 95%CI**	**p-value**
Genotypes (G)						**Genotypes**					
Genotype 1 (144)	4.56 (±0.26)	4.52–4.60	4.58 (±0.27)	4.53–4.62	0.510	Genotype 1 (146)	5.63 (±0.92)	5.49–5.80	5.54 (±0.78)	5.42–5.69	0.144
G1 a (66)	4.58 (±0.24)	4.51–4.63	4.56 (±0.31)	4.49–4.64	0.651	G1 a (68)	5.78 (±1.02)	5.54–6.04	5.69 (±0.98)	5.46–5.94	0.391
G1 b (74)	4.51 (±0.25)	4.46–4.57	4.57 (±0.30)	4.51–4.64	0.017	G1 b (78)	5.59 (±0.77)	5.43–5.79	5.47 (±0.66)	5.33–5.61	0.155
Genotype 2 (37)	4.63 (±0.31)	4,53–4.73	4.64 (±0.29)	4.54–4.74	0.771	Genotype 2 (38)	5.83 (±0.94)	5.56–6.15	5.91 (±1.05)	5.59–6.27	0.400
Genotype 3 (83)	4.48 (±0.23)	4,43–4.52	4.54 (±0.33)	4.48–4.62	0.024	Genotype 3 (87)	5.85 (±1.06)	5.65–6.11	5.54 (±1.08)	5.34–5.79	0.001
Viral load (VL)	4.55 (±0.27)	4,51–4.60	4.56 (±0.31)	4.51–4.62	0.639	**Viral load (VL)**	5.71 (±0.91)	5.57–5.88	5.61 (±1.02)	5.44–5.80	0.117
≥600,000 – High (128)	4.55 (±0.27)	4,50–4.60	4.56 (±0.31)	4.50–4.62	0.634	≥600,000 – High (132)	5.72 (±0.92)	5.56–5.88	5.61 (±1.03)	5.45–5.80	0.119
≤599,999 – Low (127)	5.56 (±0.26)	4.50–4.62	4.55 (±0.27)	4.49–4.61	0.813	≤599,999 – Low (131)	5.68 (±0.97)	5.47–5.92	5.53 (±0.88)	5.35–5.75	0.108
Genotype 1 (G1)	4.56 (±0.26)	4.50–4.63	4.59 (±0.26)	4.52–4.65	0.389	**Genotype (G1)**	5.58 (±0.83)	5.40–5.79	5.52 (±0.63)	5.37–5.67	0.462
G1+High VL (76)	4.54 (±0.28)	4.46–4.62	4.57 (±0.37)	4.47–4.67	0.323	G1+High VL (76)	5.78 (±0.84)	5.58–6.01	5.73 (±1.20)	5.44–6.05	0.609
G1+Low VL (66)	4.51 (±0.26)	4.45–4.57	4.57 (±0.29)	4.51–4.64	0.039	G1+Low VL (66)	5.90 (±1.14)	5.65–6.19	5.59 (±0.99)	5.38–5.85	0.005
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
GN1+High VL (55)						GN1+High VL (55)					
GN1+Low VL (68)						GN1+Low VL (70)					

Continue...

Table 2. Continuation.

	HOMA-IR pretreatment Mean (SD)	HOMA-IR pretreatment 95%CI	HOMA-IR SVR Mean (SD)	HOMA-IR SVR 95%CI	p-value		HOMA-β pretreatment Mean (SD)	HOMA-β pretreatment 95%CI	HOMA-β SVR Mean (SD)	HOMA-β SVR 95%CI	p-value
Genotypes (G)						**Genotype (G)**					
Genotype 1 (146)	2.42 (±1.76)	2.13–2.72	2.65 (±1.87)	2.37–2.99	0.079	Genotype 1 (146)	89.29 (±62.85)	79.42–99.37	103.97 (±79.97)	91.27–117.30	0.028
G1 a (67)	2.36 (±1.60)	2.02–2.79	2.53 (±1.83)	2.12–3.00	0.457	G1 a (67)	85.81 (±53.67)	73.25–99.82	98.27 (±91.03)	79.46–121.54	0.223
G1 b (78)	2.63 (±2.44)	2.14–3.19	3.06 (±3.76)	2.34–3.94	0.098	G1 b (78)	91.12 (±62.94)	77.56–105.43	104.16 (±61.73)	90.66–117.47	0.086
Genotype 2 (38)	2.75 (±2.03)	2.17–3.40	2.53 (±1.80)	2.00–3.10	0.323	Genotype 2 (38)	79.34 (±54.38)	63.93–99.16	72.78 (±43.84)	59.75–87.58	0.516
Genotype 3 (87)	2.56 (±2.33)	2.09–3.14	2.96 (±3.50)	2.34–3.80	0.117	Genotype 3 (87)	87.51 (±56.82)	76.00–101.42	99.61 (±57.16)	87.76–11.01	0.058
Viral load (VL)	2.51 (±1.80)	2.22–2.84	2.68 (±2.05)	2.36–3.06	0.300	**Viral load** (VL)	87.85 (±65.71)	76.86–99.40	98.62 (±79.30)	86.28–113.14	0.147
≥600,000 – High (132)	2.51 (±1.81)	2.22–2.85	2.69 (±2.06)	2.34–3.07	0.294	≥600,000 – High (132)	87.40 (±65.76)	77.61–98.48	99.23 (±79.29)	86.33–113.98	0.108
≤599,999 – Low (131)	2.50 (±1.87)	2.09–2.93	2.76 (±1.80)	2.33-3.16	0.239	≤599,999 – Low (131)	92.17±70.27	77.17–108.12	110.80±94.10	90.41–134.05	0.089
Genotype 1 (G1)	2.31 (±1.66)	1.96–2.77	2.52 (±1.98)	2,10–3.07	0.148	**Genotype 1 (G1)**	85.15±52.49	72.99–99.07	96.34±60.84	82.02–110.66	0.050
High VL (76)	2.54 (±1.74)	2.10–3.04	2.60 (±2.38)	2.38–3.29	0.813	High VL (76)	82.02±59.63	66.46–98.06	81.67±49.57	68.98–94.74	0.966
Low VL (66)	2.67 (±2.57)	2.11–3.32	3.00 (±3.55)	2.35–3.85	0.181	Low VL (66)	87.40±53.29	75.30–100.29	99.14±57.62	86.12–114.00	0.080
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
High VL (55)						High VL (55)					
Low VL (70)						Low VL (70)					

	TyG pretreatment Mean (SD)	TyG pretreatment 95%CI	TyG RVS Mean (SD)	TyG RVS 95%CI	p-value		HbA1c pretreatment Mean (SD)	HbA1c pretreatment 95%CI	HbA1c RVS Mean (SD)	HbA1c RVS 95%CI	p-value
Genotypes (G)						**Genotypes**					
Genotype 1 (144)	4.56 (±0.26)	4.52–4.60	4.58 (±0.27)	4.53–4.62	0.510	Genotype 1 (146)	5.63 (±0.92)	5.49–5.80	5.54 (±0.78)	5.42–5.69	0.144
G1 a (66)	4.58 (±0.24)	4.51–4.63	4.56 (±0.31)	4.49–4.64	0.651	G1 a (68)	5.78 (±1.02)	5.54–6.04	5.69 (±0.98)	5.46–5.94	0.391
G1 b (74)	4.51 (±0.25)	4.46–4.57	4.57 (±0.30)	4.51–4.64	0.017	G1 b (78)	5.59 (±0.77)	5.43–5.79	5.47 (±0.66)	5.33–5.61	0.155
Genotype 2 (37)	4.63 (±0.31)	4,53–4.73	4.64 (±0.29)	4.54–4.74	0.771	Genotype 2 (38)	5.83 (±0.94)	5.56–6.15	5.91 (±1.05)	5.59–6.27	0.400
Genotype 3 (83)	4.48 (±0.23)	4,43–4.52	4.54 (±0.33)	4.48–4.62	0.024	Genotype 3 (87)	5.85 (±1.06)	5.65–6.11	5.54 (±1.08)	5.34–5.79	0.001
Viral load (VL)	4.55 (±0.27)	4,51–4.60	4.56 (±0.31)	4.51–4.62	0.639	**Viral load (VL)**	5.71 (±0.91)	5.57–5.88	5.61 (±1.02)	5.44–5.80	0.117
≥600,000 – High (128)	4.55 (±0.27)	4,50–4.60	4.56 (±0.31)	4.50–4.62	0.634	≥600,000 – High (132)	5.72 (±0.92)	5.56–5.88	5.61 (±1.03)	5.45–5.80	0.119
≤599,999 – Low (127)	5.56 (±0.26)	4.50–4.62	4.55 (±0.27)	4.49–4.61	0.813	≤599,999 – Low (131)	5.68 (±0.97)	5.47–5.92	5.53 (±0.88)	5.35–5.75	0.108
Genotype 1 (G1)	4.56 (±0.26)	4.50–4.63	4.59 (±0.26)	4.52–4.65	0.389	**Genotype (G1)**	5.58 (±0.83)	5.40–5.79	5.52 (±0.63)	5.37–5.67	0.462
G1+High VL (76)	4.54 (±0.28)	4.46–4.62	4.57 (±0.37)	4.47–4.67	0.323	G1+High VL (76)	5.78 (±0.84)	5.58–6.01	5.73 (±1.20)	5.44–6.05	0.609
G1+Low VL (66)	4.51 (±0.26)	4.45–4.57	4.57 (±0.29)	4.51–4.64	0.039	G1+Low VL (66)	5.90 (±1.14)	5.65–6.19	5.59 (±0.99)	5.38–5.85	0.005
Non-1 Genotype (GN1)						**Non-1 Genotype (GN1)**					
GN1+High VL (55)						GN1+High VL (55)					
GN1+Low VL (68)						GN1+Low VL (70)					

HOMA-IR: homeostasis model assessment-insulin resistance; HOMA-β: homeostasis model assessment-β cell; TyG: product of triglycerides and glucose; HbA1c: glycated hemoglobin; SD: standard deviation; SVR: sustained viral response; CI: confidence interval.

makes an evolutionary control of this profile in patients who obtained SVR necessary.

Regarding the influence of genotypes on IR, when SVR was calculated, the HOMA-IR index did not detect significant differences between genotypes, but it was observed that the HOMA-β index improved significantly in patients with G1. This finding is in agreement with the research by Huang et al.[25], in which 72.3% of the patients were G1, and also verified a significant improvement in β-cell function, suggesting that this genotype may have an important action on the β cells of the pancreas. Regarding the TyG index, we saw worsening in patients with G1b and genotype 3, suggesting a more hypolipidemic action of these agents. About HbA1c, there was significant improvement only in patients with genotype 3, suggesting a greater beneficial metabolic effect of SVR in these individuals. This result is in accordance with the research by Jain et al.[24]. However, we agree with the statement that more studies are needed for a better understanding of this phenomenon[3].

In relation to the different genotypic influences on IR, there are specific associations with genotypes, but the causal relationship remains unclear[4]. Thus, we see that these results are complex and sometimes contradictory, suggesting that the analysis of the influences of genotypes may be obscured by other factors acting on glucose metabolism. However, from a practical point of view, the improvement of HbA1c obtained with SVR in patients with genotype 3 suggests the importance of this treatment on glucose metabolism.

When analyzing the isolated influence of VL, no significant role was detected in relation to glucose metabolism. Other researchers[3,6] indicated that higher VL levels are associated with the presence of IR in patients with chronic hepatitis C. In contrast, in another study[7] with nondiabetic patients, IR was not associated with VL. The present study agrees with these findings, because even in a population of normoglycemic, prediabetic, and diabetic patients, no association between SVR and change in glucose metabolism was found. However, when we analyzed the association of G1 with low VL, we observed a significant increase in HOMA-β, improvement in the TyG index, and HbA1c of these patients. These associations with low VL could suggest a more deleterious and permanent effect on individuals with high VL, who did not improve in any index studied and, in any association, investigated.

Regarding limitations, the high percentage of overweight and obese patients may have made it difficult to find a more generalized beneficial effect of SVR in this sample. There may have been diet-related variations for the analysis of fasting triglyceride levels, which could affect the reliability of the TyG index, making it difficult to interpret the effects of decreased lipolysis suppression with HCV cure. Another limitation is the relatively short follow-up time of these patients.

The observations of this study suggest variable metabolic influences after SVR, indicating differences in the effects of HCV genotypes, genotype 1 subtypes, and VL in specific situations but not in the entire sample, with possible damage to the lipid profile and benefits in the glucose profile of these individuals.

AUTHORS' CONTRIBUTIONS

JMHS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ACS:** Conceptualization, Data curation, Investigation, Methodology, Project Project administration, Visualization, Writing – original draft, Writing – review & editing. **AAP:** Data curation, Investigation, Methodology. **ALMP:** Data curation, Investigation, Methodology. **LG:** Data curation, Investigation, Methodology. **CVG:** Conceptualization, formal analysis, methodology, writing – original draft. **JMHS, ACS, and CVG:** Participated in the writing – original draft, writing – review & editing.

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ORIGINAL ARTICLE

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Knowledge level of healthcare professionals regarding hepatitis B immunizatıon of newborns: example of Turkey

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate healthcare professionals' knowledge level for hepatitis B immunization of term and preterm newborns.
METHODS: The study was conducted with 213 midwives, nurses, and physicians between October 2021 and January 2022 in a province of Turkey.
RESULTS: Participants had the least knowledge about the management of newborns with low birth weight born to hepatitis B-infected mothers (16%).
CONCLUSION: The study revealed some knowledge gaps among healthcare professionals regarding hepatitis B immunization of newborns.
KEYWORDS: Hepatitis B vaccines. Health knowledge, attitudes, practice. Infant, newborn. Immunization. Health personnel.

INTRODUCTION

It is estimated that 2 billion people have been exposed to the hepatitis B virus (HBV) and over 400 million chronic hepatitis B (HepB) infections worldwide. In a study conducted in Turkey, HBsAg positivity over 18 years old was 4%, and anti-HBc positivity was 31%[1]. The most important route of transmission of HepB infection is from mother to baby.

The World Health Organization (WHO) has reported that it aims to reduce the prevalence of HBsAg below 0.1% by 2030. It is accepted that vaccination is the cornerstone of achieving this goal[2]. The vaccine protects newborns born to HBsAg(+) mothers who were not screened before birth and who were not identified as HBsAg(+) due to test errors and delay[3]. Three doses of vaccine against HepB develop an adequate antibody response and protect for 20–30 years[2]. In Turkey, the HepB vaccine entered the childhood vaccination calendar in 1998 and is administered in three doses. The rate of vaccination has increased gradually in our country, and the rate of vaccination, which was 64% in 1999, increased to 98% as of 2016. The incidence of HepB disease under the age of 5 years has decreased to less than 1/100,000. The HepB vaccination rate in Turkey is above the WHO standards[4]. It has been announced that the vaccination rate, which is 42% globally, is 4% in the African Region[5]. A study conducted in Vietnam showed that only 45.2% of newborns were vaccinated at birth within 24 h[6]. A study in Washington State reported that 75.5% of newborns received the HepB vaccine while still in the hospital[7]. It has been reported that the HepB vaccine dose is 45% in India[8].

A study from Turkey showed that HepB immunoglobulin (HBIG) and HepB vaccines were administered to all babies of mothers with positive HBsAg tests[9]. Healthcare professionals need a basic understanding of the subject to administer an effective vaccine and immunoglobulin. Evidence shows that vaccine and immunoglobulin administration is influenced by knowledge, attitude, and practice. However, there are few studies in this area. Some studies investigate the knowledge of healthcare professionals or students about the transmission and prevention of the disease and their HepB vaccination status[10-13]. A study conducted in Sudan showed that midwives and nurses have moderate knowledge and safe practices about the HepB vaccine[14]. In Papua New Guinea and China, it has been reported that insufficient knowledge of the healthcare professionals is an obstacle to the birth dose vaccine[15].

Moreover, in an African study with physicians and midwives in the eastern region of Ghana, participants had some knowledge gaps about HBIG[16]. A study in Saudi Arabia strangely revealed that some healthcare professionals did not know that the HepB vaccine should be given to newborn babies[17]. Papers on the immunization of newborns in Turkey are scarce[9]. For this reason, this study was planned to assess the gap in knowledge and the necessity for in-service training of healthcare professionals that are likely to do and give advice on newborn immunization.

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METHODS

Study design

The study was conducted as a cross-sectional, descriptive-correlational study. The research was conducted at public hospitals in a province of Turkey. The participants of the study consisted of 213 midwives, nurses, and doctors.

Data collection

Data were collected between October 2021 and January 2022. An anonymous self-administered questionnaire was used. A study form was provided to those who accepted to participate.

Data analyses

Statistical evaluation was performed via IBM SPSS 20.0 package program. The data were analyzed using chi-square test, $p<0.05$.

Ethical considerations

Ethics committee approval (approval no.: 2021/107) and official permission from the hospital were obtained before starting the study. We informed and interviewed healthcare professionals on the first page of the questionnaire and included information about the aim and content of this study. This study has been conducted in accordance with the principles set forth in the Declaration of Helsinki.

RESULTS

The study was completed with 94% of the participants: 32.4% were midwives, 42.7% were nurses, and 24.5% were doctors. Most participants were between the ages of 28–37 years; 59.2% were undergraduates, 65.7% were married, and 55.9% had an experience of 0–10 years. More than half of the healthcare professionals received in-service training for the HepB immunization after graduation, 53.5% reported that they needed in-service training for the HepB immunization, 69.5% administered the HepB vaccine, and 60.1% administered HBIG.

Almost all (93.9%) of the healthcare professionals, even those who had not received any education about hepatitis other than school education (p=0.014), knew that the HepB vaccine was safe and effective in preventing the disease. Especially those aged 38 and over (p=0.042) and those who did not apply the vaccine (p=0.011) knew that the vaccine was more effective than cesarean section. Physicians (p=0.022), postgraduate education (p=0.026), and those who administered immunoglobulin (p=0.000) knew that the vaccine dose should not be more than 1 mL. Healthcare professionals (p=0.004) who received HepB education from school have more information about the necessity of administering vaccines and immunoglobulins to babies of HepB-infected mothers. Those aged 28–37 years (p=0.004), physicians (p=0.044), those with postgraduate education (p=0.004), those who were married (p=0.022), and those who applied immunoglobulin (p=0.011) had more knowledge.

Nurses (p=0.022), those who need other HepB-related training (p=0.020) and those who administered immunoglobulin (p=0.016), stated that consent should be obtained before vaccination and immunoglobulin administration (Table 1).

Overall, 89.7% of the participants knew that newborns ≥2,000 g and their HBsAg(-) mothers should be vaccinated within 24 h (within 72 h at the latest). Those who administer vaccines have more knowledge about these issues (p=0.036). In all, 87.8% of the participants knew that newborns ≥2,000 g and their HBsAg(+) mothers should be vaccinated within 12 h. Those who administer immunoglobulin have more knowledge about these issues (p=0.001). Those who administered the vaccine (p=0.005) were more aware that a newborn ≥2000 g born to an unknown HBsAg status mother should only be vaccinated within 12 h (Table 2).

Overall, 63.4% of the participants know that the vaccine dose should not be reduced if the birth weight of newborns is 2,000 g or less. Those who received HepB training other than school education (p=0.000), those who administered vaccines (p=0.001), and those who administered immunoglobulin (p=0.000) had more knowledge on the subject. Those between the ages of 18 and 27 years (p=0.002), those who have experience for 0–10 years (p=0.000), and those who did not apply the vaccine (p=0.008) did not know that newborns ≤2,000 g born to HBsAg(+) mothers should be vaccinated and applied HBIG within first 12 h. Those between the ages of 18 and 27 years (p=0.027), physicians (p=0.007), postgraduate students (p=0.005), those who worked between 0 and 10 years (p=0.007), and those who administered immunoglobulin (p=0.000) knew that a newborn ≤2,000 g born to unknown HBsAg status should only be vaccinated within 12 h (Table 3).

DISCUSSION

In this study, less than half of the participants were knowledgeable of the characteristics that the effect of the HepB vaccine in preventing the disease was no inferior to that of cesarean section (38.0%), that HepB vaccine could be administered to low-birth-weight newborns (44.1%), and that the vaccine and HBIG should be administered to newborns ≤2,000 g born to HBsAg(+) mothers within 12 h (16.0%).

Almost all (93.9%) healthcare professionals knew that the HepB vaccine was safe and effective in preventing the disease.

Table 1. General information of healthcare professionals about the HepB vaccine.

	HepB vaccine is safe and effective in preventing disease		The effect of the HepB vaccine in preventing the disease is no less than cesarean section		Vaccine dosage for the newborn		Both vaccine and HBIG should be administered to a newborn born to an HBsAg(+) mother		HepB vaccine and HBIG should not be administered to the same injection site		Informed consent of the mother should be obtained before administering the vaccine and HBIG	
	Knowing n (%)	p-value	Knowing n (%)	p-value	Knowing n (%)	p-value	Knowing n (%)	p-value	Knowing n (%)	p-value	Knowing n (%)	p-value
Profession												
Midwives	65 (94.2)	χ^2=0.787 p>0.05	22 (31.9)	χ^2=0.317 p>0.05	36 (52.2)	**χ^2=0.022 *p<0.05**	63 (91.3)	χ^2=0.411 p>0.05	52 (75.4)	**χ^2=0.044 *p<0.05**	45 (65.2)	**χ^2=0.022 *p<0.05**
Nurse	88 (96.7)		35 (38.5)		61 (67.0)		82 (90.1)		62 (68.1)		73 (80.2)	
Physician	47 (88.7)		24 (45.3)		40 (75.5)		51 (96.2)		46 (86.8)		32 (60.4)	
Age (years)												
18–27	46 (93.9)	χ^2=0.787 p>0.05	13 (26.5)	**χ^2=0.042 *p<0.05**	29 (59.2)	χ^2=0.736 p>0.05	45 (91.8)	χ^2=0.793 p>0.05	28 (57.1)	**χ^2=0.004 *p<0.05**	36 (73.5)	χ^2=0.467 p>0.05
28–37	94 (94.9)		36 (36.4)		65 (65.7)		90 (90.9)		80 (80.8)		72 (72.7)	
38 and older	60 (92.3)		32 (49.2)		43 (66.2)		61 (93.8)		52 (80.6)		42 (64.6)	
Education status												
Graduate	138 (95.2)	χ^2=0.256 p>0.05	54 (37.2)	χ^2=0.601 p>0.05	86 (59.3)	**χ^2=0.026 *p<0.05**	130 (89.7)	χ^2=0.063 p>0.05	101 (69.7)	**χ^2=0.004 *p<0.05**	108 (74.5)	χ^2=0.590 p>0.05
Postgraduate	62 (91.2)		27 (39.7)		51 (75.0)		66 (97.1)		59 (86.8)		42 (61.8)	
Marital status												
Married	133 (95.0)	χ^2=0.352 p>0.05	55 (39.3)	χ^2=0.601 p>0.05	90 (64.3)	χ^2=0.989 p>0.05	132 (94.3)	χ^2=0.091 p>0.05	112 (80.0)	**χ^2=0.022 *p<0.05**	100 (71.4)	χ^2=0.590 p>0.05
Single	67 (91.8)		26 (35.6)		47 (64.4)		64 (87.7)		48 (65.8)		50 (68.5)	
Experience												
0–10 years	110 (93.2)	χ^2=0.646 p>0.05	41 (34.7)	χ^2=0.271 p>0.05	77 (65.3)	χ^2=0.751 p>0.05	106 (89.8)	χ^2=0.189 p>0.05	84 (71.2)	χ^2=0.139 p>0.05	89 (75.4)	χ^2=0.575 p>0.05
>10 years	90 (94.7)		40 (42.1)		60 (63.2)		90 (94.7)		76 (80.0)		61 (64.2)	
Received training on HepB vaccine other than school education												
Yes	99 (90.0)	**χ^2=0.014 *p<0.05**	46 (41.8)	χ^2=0.239 p>0.05	71 (64.5)	χ^2=0.943 p>0.05	107 (97.3)	**χ^2=0.004 *p<0.05**	85 (77.3)	χ^2=0.452 p>0.05	80 (72.7)	χ^2=0.446 p>0.05
No	101 (98.1)		35 (34.0)		66 (64.1)		89 (86.4)		75 (72.8)		70 (68.0)	
Administration of vaccine												
Yes	137 (92.6)	χ^2=0.221 p>0.05	48 (32.4)	**χ^2=0.011 *p<0.05**	97 (65.5)	χ^2=0.575 p>0.05	135 (91.2)	χ^2=0.514 p>0.05	112 (75.7)	χ^2=0.776 p>0.05	110 (74.3)	χ^2=0.060 p>0.05
No	63 (96.9)		33 (50.8)		40 (61.5)		61 (93.8)		48 (73.8)		40 (61.5)	
Administration of HBIG												
Yes	118 (92.2)	χ^2=0.201 p>0.05	44 (34.4)	χ^2=0.178 p>0.05	97 (75.8)	**χ^2=0.000 *p<0.05**	119 (93.0)	χ^2=0.530 p>0.05	104 (81.2)	**χ^2=0.011 *p<0.05**	98 (76.6)	**χ^2=0.016 *p<0.05**
No	82 (96.5)		37 (43.5)		40 (47.1)		77 (90.6)		56 (65.9)		52 (61.2)	

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

Table 2. Knowledge level of healthcare professionals about HepB vaccine and immunoglobulin administration to above ≥2,000 g weight newborns.

	Newborns ≥2,000 g and born to HBsAg(-) mother should be vaccinated within 72 h		Vaccine and HBIG should be administered within 12 h to newborns ≥2,000 g born to HBsAg(+) mother		A newborn ≥2,000 g born to a mother with HBsAg status unknown should be vaccinated within 12 h	
	Knowing n (%)	p-value	Knowing n (%)	p-value	Knowing n (%)	p-value
Profession						
Midwives	61 (88.4)	χ^2=0.912 p>0.05	61 (88.4)	χ^2=0.376 p>0.05	51 (73.9)	χ^2=0.203 p>0.05
Nurse	82 (90.1)		77 (84.6)		55 (60.4)	
Physician	48 (90.6)		49 (92.5)		35 (66.0)	
Age (years)						
18–27	46 (93.9)	χ^2=0.104 p>0.05	43 (87.8)	χ^2=0.872 p>0.05	31 (63.3)	χ^2=0.468 p>0.05
28–37	91 (91.9)		88 (88.9)		65 (65.7)	
38 and older	54 (83.1)		56 (86.2)		45 (69.2)	
Education status						
Graduate	129 (89.0)	χ^2=0.621 p>0.05	126 (86.9)	χ^2=0.559 p>0.05	97 (66.9)	χ^2=0.753 p>0.05
Postgraduate	62 (91.2)		61 (89.7)		44 (64.7)	
Marital status						
Married	125 (89.3)	χ^2=0.798 p>0.05	126 (90.0)	χ^2=0.173 p>0.05	92 (65.7)	χ^2=0.837 p>0.05
Single	66 (90.4)		61 (83.6)		49 (67.1)	
Professional eχperience						
0–10 years	108 (91.5)	χ^2=0.322 p>0.05	104 (88.1)	χ^2=0.865 p>0.05	76 (64.4)	χ^2=0.538 p>0.05
>10 years	83 (87.4)		83 (87.4)		65 (68.4)	
Received training on HepB vaccine other than school education						
Yes	98 (89.1)	χ^2=0.774 p>0.05	100 (90.9)	χ^2=0.151 p>0.05	77 (70.0)	χ^2=0.225 p>0.05
No	93 (90.3)		87 (84.5)		64 (62.1)	
Administration of vaccine						
Yes	137 (92.6)	**χ^2=0.036 *p<0.05**	134 (90.5)	χ^2=0.065 p>0.05	107 (72.3)	**χ^2=0.005 *p<0.05**
No	54 (83.1)		53 (81.5)		34 (52.3)	
Administration of HBIG						
Yes	120 (93.8)	**χ^2=0.016 *p<0.05**	121 (94.5)	**χ^2=0.001 *p<0.05**	91 (71.1)	χ^2=0.064 p>0.05
No	71 (83.5)		66 (77.6)		50 (58.8)	

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

Contrary to the study findings in Vietnam, some medical students (61%) thought the HepB vaccine was safe[10]. Knowing that the HepB vaccine is safe and effective in our study may be because the study was conducted with professionals in practice and experience. It has been reported that the risk of HepB transmission from a HepB-infected mother to her baby does not differ according to the delivery method, whether vaginal or caesarian[18]. In addition, there is insufficient evidence that cesarean section does not prevent transmission and provides additional protection against HepB transmission compared to HepB immunoglobulin and vaccination[18,19]. In this study, those aged 38 and over (p=0.042) and those who did not apply the vaccine (p=0.011) knew that the vaccine was effective. It may be because the participants received adequate training on the subject during school education and did not need training, and some information was better comprehended as the age progressed. Having received training other than school education in our study made the participants more knowledgeable about administering both vaccine and HBIG to an HBsAg(+) newborn (p=0.004) and not reducing the vaccine dose for newborns born under ≤2,000 g (p=0.000). This made us think that the education received after graduation is effective in comprehending information. In a study conducted in a city in the state of Amazonas, it was shown that, similar to the findings

Table 3. Knowledge level of healthcare professionals about HepB vaccine and immunoglobulin administration to under ≤2,000 g weight newborns.

	Vaccine dose should not be reduced		HBV vaccine should not be administered within 12 h to newborns ≤2,000 g born to HBsAg(-) mother		Vaccine and HBIG both should be administered within 12 h to newborns ≤2,000 g born to HBsAg(+) mother		A newborn who is ≤2,000 g born to a mother with an HBsAg status unknown should not be vaccinated within 12 h	
	Knowing n (%)	p	Knowing n (%)	p-value	Knowing n (%)	p-value	Knowing n (%)	p-value
Profession								
Midwives	43 (62.3)	χ^2=0.638 p>0.05	43 (62.3)	χ^2=0.638 p>0.05	13 (18.8)	χ^2=0.638 p>0.05	44 (63.8)	**χ^2=0.007 *p<0.05**
Nurse	56 (61.5)		65 (71.4)		16 (17.6)		63 (69.2)	
Physician	36 (67.9)		42 (79.2)		5 (9.4)		47 (88.7)	
Age (years)								
18–27	29 (59.2)	χ^2=0.590 p>0.05	40 (81.6)	**χ^2=0.040 *p<0.05**	3 (6.1)	**χ^2=0.002 *p<0.05**	39 (79.6)	**χ^2=0.027 *p<0.05**
28–37	63 (63.6)		71 (71.7)		12 (12.1)		76 (76.8)	
38 and older	43 (66.2)		39 (60.0)		19 (29.2)		39 (60.0)	
Education status								
Graduate	91 (62.8)	χ^2=0.783 p>0.05	97 (66.9)	χ^2=0.100 p>0.05	26 (17.9)	χ^2=0.252 p>0.05	95 (65.5)	**χ^2=0.005 *p<0.05**
Postgraduate	44 (64.7)		53 (77.9)		8 (11.8)		59 (86.8)	
Marital status								
Married	92 (65.7)	χ^2=0.327 p>0.05	98 (70.0)	χ^2=0.852 p>0.05	26 (18.6)	χ^2=0.150 p>0.05	99 (70.7)	χ^2=0.474 p>0.05
Single	43 (58.9)		52 (71.2)		8 (11.0)		55 (75.3)	
Eχperience								
0–10 years	75 (63.6)	χ^2=0.952 p>0.05	92 (78.0)	**χ^2=0.007 *p<0.05**	10 (8.5)	**χ^2=0.000 *p<0.05**	94 (79.7)	**χ^2=0.007 *p<0.05**
>10 years	60 (63.2)		58 (61.1)		24 (25.3)		60 (63.2)	
Received training on HepB vaccine other than school education								
Yes	83 (75.5)	**χ^2=0.000 *p<0.05**	78 (70.9)	χ^2=0.872 p>0.05	18 (16.4)	χ^2=0.869 p>0.05	80 (72.7)	χ^2=0.886 p>0.05
No	52 (50.5)		72 (69.9)		16 (15.5)		74 (71.8)	
Administration of vaccine								
Yes	105 (70.9)	**χ^2=0.001 *p<0.05**	102 (68.9)	χ^2=0.468 p>0.05	30 (20.3)	**χ^2=0.008 *p<0.05**	108 (73.0)	χ^2=0.741 p>0.05
No	30 (46.2)		48 (73.8)		4 (6.2)		46 (70.8)	
Administration of HBIG								
Yes	96 (75.0)	**χ^2=0.000 *p<0.05**	98 (76.6)	**χ^2=0.025 *p<0.05**	21 (16.4)	χ^2=0.828 p>0.05	105 (82.0)	**χ^2=0.000 *p<0.05**
No	39 (45.9)		52 (61.2)		13 (15.3)		49 (57.6)	

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

of our study, the knowledge, attitudes, and practices of physicians and nurses about the vertical transmission of HepB increased with postgraduate education[20]. In this study, doctors had more knowledge of the vaccine dose to be administered to newborns (p=0.022), that the HepB vaccine and HBIG should not be administered to the same injection site at the same time (p=0.044), and that newborns ≤2,000 g born to mothers with HBsAg status unknown should not be only vaccinated within 12 h (p=0.007). The reason physicians are more knowledgeable compared to the other professions may be that more than half (62.3%) of the physicians have received training on the HepB vaccine other than school education, very few (28.3%) need training on the subject, and more than half (62%) of the postpartum care of babies is performed by physicians[21]. Unlike the study results in Khartoum, Sudan, two-thirds of nurses and midwives had a safe practice of HepB vaccine[14]. It may be due to the different education systems. Nurses were more knowledgeable about the need to obtain informed consent from the mother before administering only the HepB vaccine and HBIG (p=0.022). WHO recommends obtaining verbal consent from the mother before administering the HepB vaccine[18]. In our findings, it is a positive feature that nurses consider it necessary to obtain informed consent from the mother before administering vaccine and immunoglobulin. Administering the immunoglobulin and vaccine has made healthcare professionals more knowledgeable about some issues. Healthcare professionals with direct hands-on care experience are expected to have good practices[22]. In this study, the knowledge of the proper education by those who administered the vaccine and immune globulin may be due to the understanding of the correct information by practice.

In this study, the participants who did not apply the vaccine did not know that the vaccine and HBIG should be administered within 12 h to newborns ≤2,000 g born to HBsAg(+) mothers. Similar to the findings of the Ghana study, 12.7% of the participants knew that there was a vaccine that could prevent the transmission of HepB infection from mother to newborn when administered with HBIG to newborns born to mothers infected with HepB[16]. The lack of information in our study may be because healthcare professionals mostly care for newborns with average weight. In a study conducted in Turkey, babies' birth weight (92.3%) was more than 2,500 g[23]. Healthcare professionals who lack knowledge about newborn immunization may miss vaccination opportunities. Therefore, improving the education and training of health workers is critical. Evaluations of hospitals in Papua New Guinea and peripheral health facilities in China have reported that insufficient knowledge among medical staff is an obstacle to timely and correct dose administration of vaccines. In the study evaluating the impact of medical staff training in the Philippines, it was seen that the coverage of the birth dose increased from 19 to 74% within 2 months of the training, and attention was drawn to the effect of the training. Similarly, in the Chinese province of Qinghai, it has been reported that the rate of term delivery increased from 40 to 70% with the training of healthcare workers[15].

CONCLUSION

This study points out a lack of knowledge among midwives, nurses, and physicians about the immunization of some newborns and that some newborns may be exposed to the risk of transmission of HepB from their mother. It may be recommended to provide education to healthcare professionals about vaccines and immunoglobulins.

AUTHORS' CONTRIBUTIONS

PŞ: Conceptualization, Writing – original draft, Writing – review & editing. **KD:** Investigation, Writing – review & editing.

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ORIGINAL ARTICLE

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The effect of COVID-19 fear on prenatal distress and childbirth preference in primipara

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SUMMARY

OBJECTIVE: The purpose of this study was to examine the effect of COVID-19 fear on prenatal distress and childbirth preference in primipara.
METHODS: This descriptive and cross-sectional study was conducted with 206 primipara women in Istanbul between June and December 2021. The data were collected with an information form, "The Fear of COVID-19 Scale" and "The Prenatal Distress Questionnaire."
RESULTS: The median of the Fear of COVID-19 Scale was 14.00 (7–31) and the median of the Prenatal Distress Questionnaire was 10.00 (0–21). A statistically significant positive and weak correlation was found between "The Fear of COVID-19 Scale" and "The Prenatal Distress Questionnaire" (r=0.21; p=0.00). Overall, 75.2% of pregnant women preferred normal (vaginal) delivery. There was no statistically significant relationship between "The Fear of COVID-19 Scale" and childbirth preference (p>0.05).
CONCLUSION: It was determined that fear of coronavirus increases prenatal distress. Women should be supported to cope with fear of COVID-19 and prenatal distress, both during the preconceptional and antenatal periods.
KEYWORDS: Natural childbirth. Cesarean section. COVID-19. Fetal distress.

INTRODUCTION

The novel coronavirus (SARS-CoV-2), which emerged in Wuhan, China, in December of 2019, was identified as a causative agent of a series of atypical respiratory diseases. The SARS-CoV-2 disease, called COVID-19, was declared a pandemic by the World Health Organization on March 11, 2020[1]. Pregnant women may be at higher risk of becoming infected with SARS-CoV-2 and developing more complex clinical events due to physiological and immune changes[2].

Pregnant women are among the groups that are most affected psychologically due to the stress and fear they experience during the COVID-19 pandemic[3]. In the literature, it is stated that pregnant women have a high COVID-19 phobia[4], most of the pregnant women concern about infecting their babies during delivery[5], and during the COVID-19 period, it was reported that depression symptoms were high during pregnancy[6]. The possibility of not being with their families during childbirth and the fear of dying because of COVID-19 increase the level of anxiety in pregnant women[7].

The COVID-19 pandemic is creating a new source of stress with unique implications for parents and those preparing for childbirth. There is evidence that this stress leads to additional stress during pregnancy[8]. Factors such as isolation measures, limitations on pregnancy controls, not being with family members during pregnancy controls, feeling the need to protect their babies as well as protecting themselves, the probability of the infection to be transmitted to the fetus, and the economic and social effects of the pandemic increase the fear of COVID-19 and the level of prenatal stress in pregnant women[9,10]. It is known that increased stress during pregnancy increases the risk of cesarean delivery. Pregnant women do not prefer vaginal birth because of the pain factor, the long duration of delivery, and the inability to be with spouses or other family members during childbirth. It is stated that health professionals prefer cesarean delivery in pregnant women who have COVID-19, although it has not been proven yet, to reduce the possibility of transmitting COVID-19 to the baby[10,11]. The purpose of this study was to examine the effect of COVID-19 fear on prenatal distress and childbirth preference in primipara.

METHODS

Study design

The study was conducted as descriptive cross-sectional type.

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Population and sample of the study

The universe of the study consisted of primipara women who applied to the maternity polyclinics of a training and research hospital in Istanbul, Turkey, between June 2021 and December 2021. The G*Power 3.1.2.9.7 package program has been used to determine the sufficient sample size. For the sample of the research, the results of the study conducted by Aksoy Derya et al.[9] were taken as basis. The effect size was calculated by using the mean and standard deviation values of the total score variable related to the prenatal distress questionnaire of the participants in this study. The sample size was determined as 198 women, considering the degree of confidence (95%), margin of error (5%), effect size (0.5), and ability test (80%), and the data were collected from 206 pregnant women. The data were obtained by conducting face-to-face interviews. It takes 15–20 min to complete each questionnaire.

Inclusion criteria of the study

The inclusion criteria of the study were ³18 years old, willingness to participate, being primipara, knowing Turkish language, and not having communication barriers.

Exclusion criteria of the study

The exclusion criteria were pregnant women who refused to participate in the study and were unable to complete the data collection instrument.

Data collection tools

The study data were collected with a 13-question information form containing demographic and obstetric data, conducted by the researcher in line with the literature[9-11], "The Fear of COVID-19 Scale" and "The Prenatal Distress Questionnaire."

The Fear of COVID-19 Scale (FCV-19S): It was developed by Ahorsu et al.[12] and adapted to the Turkish language by Bakioğlu et al.[13] It is a 5-point Likert-type scale with seven items. The Cronbach's alpha was 0.82, and in this study it was found to be 0.81.

The Prenatal Distress Questionnaire (PDQ): It was developed by Yali and Lobel[14], and the scale was revised by Lobel[15], increasing the number of items from 12 to 17. The Turkish validity and reliability of the scale were established by Yüksel et al.[16] The scale has no cutoff score. The Cronbach's alpha was reported as 0.85[16], and in this study it was found to be 0.79.

Data analysis

The SPSS (Windows 22.0) software was used for data analysis. Descriptive statistical methods (mean, standard deviation, mode, median, frequency, minimum, and maximum) were used for statistical analysis of data, and Mann-Whitney U, chi-square, and Spearman's correlation tests were calculated for determining the relationship between the descriptive tests and scales. No missing data were found in the study. While analyzing the demographic and obstetric data of the participants, median and percentage values were taken as basis. The median value was also determined while analyzing the scale scores.

Ethical considerations

Ethics committee approval was obtained from the Social and Humanities Research and Publication Ethics Committee of a university (decision no.: 2021/29; date: 17.05.2021). Data were collected after ethics committee approval and institutional permission. Verbal and written consent was obtained from the participants who met the criteria for being included in the sample and agreed to participate in the research.

RESULTS

The sociodemographic and obstetric data of women are presented in Table 1. The mean age of women was 26.78±5.28 years. It was found that 57.3% of the pregnant women were in the third trimester, 43.7% had health problems related to pregnancy, and 85.4% had planned pregnancy (Table 1).

The mean scale scores of the pregnant women were as follows: the FCV-19S scores were moderate (median: 14.00; min: 7; and max: 31); and the PDQ scores were also moderate (median: 10.00; min: 0; and max: 21).

The correlation between FCV-19S and PDQ is shown in Table 2. A statistically significant positive and weak correlation was found between FCV-19S and PDQ (r=0.21; p=0.00) (Table 2).

It was found that 75.2% of the pregnant women preferred normal (vaginal) delivery. The reason why most of them (71.0%) preferred normal delivery was that normal delivery was healthier than caesarean delivery. The most important reason for preferring caesarean delivery was the fear of labor pain (88.2%).

The comparison of the characteristics of the pregnant women and their scale scores is presented in Table 3. It was determined that the pregnancy trimester affected the PDQ scores. When FCV-19S and pregnancy trimester were compared, it was found that the fear of coronavirus was higher in the first trimester than in the second and third trimesters (χ^2=25.374; p=0.00). A statistically significant relationship was found between planned pregnancy status (Zmwu=-2.192; p=0.02), health problems in the baby (Zmwu=-3.366; p=0.00), and PDQ. When the relationship between preferred type of delivery and FCV-19S and PDQ scores were examined, it was determined that there was

Table 1. Sociodemographic and obstetric characteristics of pregnant women (n=206).

Variables		X±(SD)	Min–Max
Age (years)		**26.78±5.28**	**17.00–41.00**
		n	**%**
Marital status	Married	206	100.0
	Single	0	0.0
Education level	Literate	5	2.4
	Primary School	37	17.9
	Secondary School	36	17.4
	High School	56	27.1
	Graduate and Master	72	34.9
Employment status	Employed	17	8.3
	Unemployed	179	86.9
	Unemployed because of pregnancy	10	4.9
Health insurance	Yes	146	70.9
	No	60	29.1
Income status	Lower than expenditure	89	43.2
	Equal to expenditure	109	52.9
	Higher than expenditure	8	3.9
Trimester	1. Trimester	41	19.9
	2. Trimester	47	22.8
	3. Trimester	118	57.3
Health problems during pregnancy	Yes No	90 116	43.7 56.3
Health problems type during pregnancy	Nausea-vomiting	26	12.6
	Urinary tract infection	18	8.7
	Gestational diabetes	5	2.4
	Hypertension	5	2.4
	Pain	5	2.4
	Premature labor risk	11	5.3
	Placenta previa totalis	6	2.9
	COVID-19 infection	6	2.9
	Premature membrane rupture	4	1.9
	Hypothyroidism	4	1.9
Planned pregnancy	Yes	176	85.4
	No	30	14.6
Wanted having baby	Both parents want baby	198	96.1
	Mother wants, father doesn't	5	2.4
	Father wants, mother doesn't	3	1.5
Health problem in baby	Yes	18	8.7
	No	188	91.3
Health problem Type in baby	Polihidramnios	6	2.9
	Intrauterine growth restriction	4	1.9
	Vaginal bleeding	4	1.9

Mean±SD: mean±standard deviation; Min: minimum; Max: maximum.

no statistically significant relationship (p>0.05). It was determined that those who preferred cesarean section had higher concerns about health care and health status, as well as about baby care and postpartum period (Table 3).

DISCUSSION

In this study, it was found that pregnant women had a moderate level of fear of COVID-19 infection. There are studies in the literature showing that pregnant women have a higher than moderate fear of COVID-19[3,17]. In line with this study's findings and the literature, it can be said that the COVID-19 pandemic caused a moderate or higher level of fear of coronavirus in primiparous women.

In this study, it was determined that pregnant women experienced a moderate level of prenatal distress. There are results in the literature showing that primiparous women experienced moderate-to-high levels of prenatal stress during the pandemic[18-22]. In line with these findings, it can be said that the COVID-19 epidemic may affect pregnant women negatively and cause stress and anxiety. The stress during pregnancy can adversely affect the blood pressure and heart rate of the fetus, cause premature birth, and lead to low birth weight[3]. For this reason, it is important to determine the risk factors that cause prenatal distress for mother-baby health and a healthy pregnancy and postpartum period.

In this study, it was found that as the fear of COVID-19 increased, prenatal stress increased, and the most important predictors of the fear of COVID-19 were the PDQ's "physical and social changes due to pregnancy," "concerns about baby care and postpartum period," and "financial concerns," and subdimensions were determined. There are studies with similar findings

Table 2. Correlation between scales.

	Fear of COVID-19 Scale	
	r*	p-value
Prenatal Distress Questionnaire	0.21*	0.00
Physical and social changes due to pregnancy	0.14*	0.03
Concerns about health care and health status	-0.08	0.23
Concerns about baby care and postpartum period	0.40*	0.00
Financial concerns	0.25*	0.00

*p≤0.05, Spearman's correlation. Bold values indicate statistical significance at the p<0.05 level.

Table 3. The comparison of pregnant women's characteristics and scale scores.

Scales		FCV-19S		PDQ		Physical and social changes due to pregnancy		Concerns about health care and health status		Concerns about baby care and postpartum period		Financial concerns	
Variables		Median (Min–Max)	Test p-value	Median (Min–Max)	Test p-value	Median (Min–Max)	Test p-value	Median (Min–Max)	Test p-value	Median (Min–Max)	Test p-value	Median (Min–Max)	Test p-value
Trimester	1. Trimester	25.00 (7–28)	25.374** 0.00	10.00 (0–15)	7.263** 0.02	7.00 (0–12)	5.571** 0.05	1.00 (0–2)	4.888** 0.08	1.00 (0–2)	13.044** 0.00	1.00 (0–3)	8.785** 0.01
	2. Trimester	16.00 (11–27)		10.00 (0–19)		6.00 (0–11)		1.00 (0–2)		1.00 (0–4)		1.00 (0–3)	
	3. Trimester	13.00 (7–31)		8.00 (2–21)		5.50 (0–13)		0.50 (0–5)		0.00 (0–3)		0.00 (0–3)	
Planned pregnancy	Yes	15.00 (7–31)	-1.291* 0.19	10.00 (0–21)	-2.192* 0.02	6.00 (0–13)	-2.096* 0.03	0.00 (0–5)	-2.253* 0.02	0.00 (0–4)	-2.677* 0.00	1.00 (0–3)	-2.007* 0.04
	No	14.00 (11–21)		13.00 (5–15)		10.00 (3–11)		1.00 (0–2)		1.00 (0–2)		0.00 (0–3)	
Health problems in baby	Yes	19.00 (13–21)	-1.340* 0.18	19.00 (0–21)	-3.366* 0.00	10.00 (0–13)	-3.293* 0.00	2.00 (0–5)	-3.295* 0.00	3.00 (0–4)	-4.589* 0.00	0.00 (0–3)	-1.977* 0.04
	No	14.00 (7–31)		9.00 (0–15)		6.00 (0–12)		1.00 (0–3)		0.00 (0–2)		1.00 (0–3)	
Preferred type of delivery	Normal (vaginal) delivery	15.00 (7–28)	-0.543* 0.58	10.00 (0–21)	-0.097* 0.92	6.00 (0–13)	-0.912* 0.36	0.00 (0–5)	-2.623* 0.00	0.00 (0–3)	-2.619* 0.00	1.00 (0–3)	-1.620* 0.10
	Caesarean delivery	14.00 (11–31)		8.00 (2–19)		6.00 (0–11)		1.00 (0–2)		1.00 (0–4)		1.00 (0–3)	

*Mann-Whitney U test; **Kruskall-Wallis test, p<0.05.

in the literature[3,19]. It is important that pregnant women are not exposed to COVID-19 to optimize their health. For this, it is recommended to take all available measures (vaccination, hygiene practices, wearing a mask, and maintaining social distance)[23]. Fear of being infected with COVID-19 during pregnancy, uncertainties, delaying health checks due to lack of information, being away from work due to the pandemic, and loss of economic income may increase prenatal stress.

In this study, it was found that the trimester of pregnancy affected prenatal distress. In the literature, different results have been found in this regard[24,25]. It is thought that the fact that the women in our study had their first pregnancies, and the individual characteristics of the women may be the reason for the difference with the results of other studies.

In the study, it was determined that women who had an unplanned pregnancy and had health problems in their baby had a higher level of prenatal distress. In a study by Yılmaz and Şahin[25], it was determined that the prenatal distress level of pregnant women who had high-risk pregnancies and had health problems during pregnancy was high and that planned pregnancy was not associated with prenatal distress. It is stated that planned pregnancy has an effect on pregnancy stress[3]. In the case of a planned and healthy pregnancy, it is thought that pregnant women can adapt better psychosocially to both the pregnancy process and motherhood.

In this study, the majority of pregnant women reported that they preferred normal delivery. Pregnant women who had higher scores for "Concerns about health care and health status" and "Concerns about baby care and postpartum period" subdimensions of the PDQ stated that they would prefer cesarean delivery. It has been reported by the American College of Obstetricians and Gynecologists (ACOG) that cesarean delivery should be based on obstetric (fetal or maternal) indications, not just on COVID-19 status[23]. It is important to learn about the birth preferences of pregnant women and their concerns about mother-baby health during pregnancy, birth, and the postpartum period that will affect birth preferences.

Limitations of the study

This study may not be generalized to all pregnant women. Since the findings of the study can only be generalized to the research sample, it is recommended to conduct similar studies with larger and different sample groups. The research data were obtained from a questionnaire consisting of closed-ended questions. It is thought that qualitative studies or studies with open-ended questions can more deeply examine the subject.

CONCLUSION

This study revealed that primiparous women experienced moderate fear of coronavirus and prenatal distress. It was determined that fear of coronavirus increases prenatal distress. There was no statistically significant relationship between fear of coronavirus and childbirth preference. Our findings suggest that pregnant women who have prenatal distress and COVID-19 fear can be easily determined by simple questionnaires during prenatal visits, and this can help in having a better pregnancy period.

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AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Relation of impulse oscillometry and spirometry with quantitative thorax computed tomography after COVID-19 pneumonia

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SUMMARY

OBJECTIVE: This study aimed to investigate if there is any correlation between the quantitative computed tomography and the impulse oscillometry or spirometry results of post-COVID-19 patients.

METHODS: The study comprised 47 post-COVID-19 patients who had spirometry, impulse oscillometry, and high-resolution computed tomography examinations at the same time. The study group consisted of 33 patients with quantitative computed tomography involvement, while the control group included 14 patients who did not have CT findings. The quantitative computed tomography technology was used to calculate percentages of density range volumes. The relationship between percentages of density range volumes for different quantitative computed tomography density ranges and impulse oscillometry-spirometry findings was statistically analyzed.

RESULTS: In quantitative computed tomography, the percentage of relatively high-density lung parenchyma, including fibrotic areas, was 1.76±0.43 and 5.65±3.73 in the control and study groups, respectively. The percentages of primarily ground-glass parenchyma areas were found to be 7.60±2.86 and 29.25±16.50 in the control and study groups, respectively. In the correlation analysis, the forced vital capacity% predicted in the study group was correlated with $DRV\%_{[(-750)-(-500)]}$ (volume of the lung parenchyma that has density between (-750)-(-500) Hounsfield units), but no correlation with $DRV\%_{[(-500)-0]}$ was detected. Also, reactance area and resonant frequency were correlated with $DRV\%_{[(-750)-(-500)]}$, while X_5 was correlated with both $DRV\%_{[(-500)-0]}$ and $DRV\%_{[(-750)-(-500)]}$ density. Modified Medical Research Council score was correlated with predicted percentages of forced vital capacity and X_5.

CONCLUSION: After COVID-19, forced vital capacity, reactance area, resonant frequency, and X_5 correlated with the percentages of density range volumes of ground-glass opacity areas in the quantitative computed tomography. X_5 was the only parameter correlated with density ranges consistent with both ground-glass opacity and fibrosis. Furthermore, the percentages of forced vital capacity and X_5 were shown to be associated with the perception of dyspnea.

KEYWORDS: COVID-19. Oscillometry. Tomography. Spirometry.

INTRODUCTION

Computed tomography (CT) abnormalities may last for months following COVID-19 pneumonia. Patients with pneumonia who develop sequelae require clinical, radiological, and functional follow-up[1]. It has been shown that CT data can be utilized to evaluate patients for disease severity and follow-up[2,3]. The most common radiographic findings are ground-glass opacities (GGO), consolidation, and fibrosis. Thin-section spiral volumetric CT is a common imaging modality used in the diagnosis and follow-up of COVID-19 pneumonia patients[4]. Quantitative CT (qCT) was reported to be used to evaluate the extent of COVID-19 pneumonia and in follow-up of the patients[5]. Spirometry and lung diffusion tests are recommended in routine clinical follow-ups of patients, especially in severe disease[6]. Furthermore, sound wave-based tests [forced oscillation technique and impulse oscillometry (IOS)] are employed to evaluate obstructive and restrictive disorders, particularly in obstructive diseases[7]. In obstructive diseases, IOS was shown to be more sensitive than spirometry in identifying minor airway obstruction[8]. Another study in patients who recovered from COVID-19 showed that IOS might detect aberrant findings even when spirometry was normal[9].

This study aimed to investigate the functional equation of qCT results in patients with COVID-19 pneumonia, as well as their relationship with IOS and spirometry values. Our hypothesis was that relatively high-density lung fields in qCT due to COVID-19 involvement would correlate with IOS and spirometry parameters. As far as we know, no research has been undertaken to explore the correlation of qCT and IOS measurements.

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METHODS

Study population

Institutional review board approval was obtained for the study from University of Health Sciences, Ankara Ataturk Sanatorium Training and Research Hospital. A retrospective analysis was performed on 84 consecutive post-COVID-19 patients who applied to our center between November 1, 2020, and January 31, 2021, whose follow-ups were performed using CT, IOS, and spirometry because of the prolongation of their symptomatic periods (to an average of 6 weeks) after the conclusion of therapy. Patients who had more than one week between their qCT and IOS-spirometry dates, as well as those who had poor IOS and spirometry measurement quality, were excluded from the study. Poor measurement quality was defined according to the American Thoracic Society – European Respiratory Society (ATS/ERS) guidelines recommendations for spirometry and ERS task force recommendations for IOS[10,11]. A total of 47 patients' data were retrieved. The study group included 33 patients who had qCT results consistent with COVID-19 pneumonia, while the control group included 14 patients who did not have COVID-19-related CT findings (Figure 1). The predicted percentages of FEV1, FVC, and FEV1/FVC from spirometry measurements were recorded for the control and study groups. The IOS parameters reactance area (AX), resonant frequency (Fres), R_{20}, R_5, R_{5-20}, and X_5 were recorded, and the predicted percentages of these values were determined using Shulz et al.'s reference formulae for Caucasians[12]. Percentages of volumes of certain predefined density ranges (DRV%), within a maximum density of 0 Hounsfield units (HU) and a minimum density of -850 HU, were obtained from qCT using a computer program. The modified Medical Research Council (mMRC) score was used to assess the patients' dyspnea perception scores.

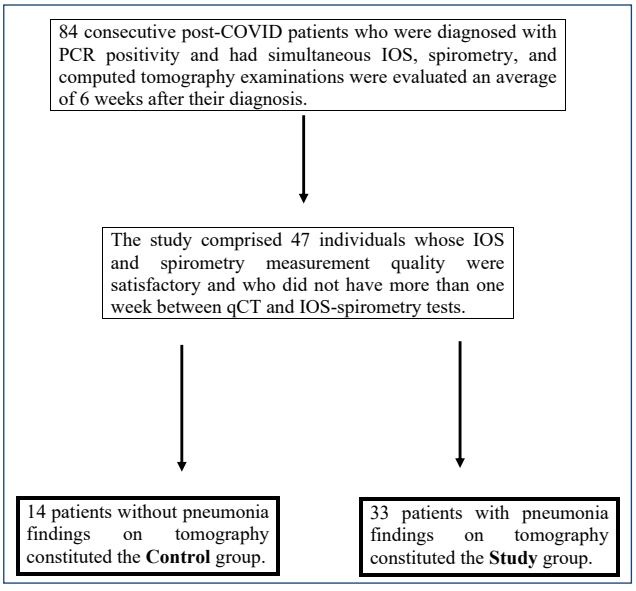

Figure 1. Patient selection.

Impulse oscillometry and spirometry measurements

All patients first underwent IOS, followed by spirometry (Carefusion Vyntus Jaeger IOS, Germany). Initially, the patient was informed about the measurement technique that would be used in order to improve compliance with the test. Oscillometric tests were performed with the patients sitting comfortably and straight, with the head and neck in a neutral or slightly extended posture, and with no forward head flexion. A nasal clip was used to close the nose, and individuals were asked to grip the mouthpiece of the device tightly with their lips and externally support their cheeks with their palms while breathing normally. It was checked visually by the chest physician, who performed the test to see if there was leakage from the edges of the mouthpiece and nose clip and whether the tongue was in the correct position. The tests were repeated at least three times, and the best results were recorded when the coherence at 5 Hz was greater than 0.8 or the coherence at 20 Hz was greater than 0.9[13]. Swallowing, laryngeal closure, leaks around the mouthpiece, inappropriate location of the nasal clip, irregular breathing, and acute hyperventilation during the test are reasons for invalidating the data. Most of these events may be detected by the flow signal, which should be displayed on the screen during measurement. During and after the test, the practitioner controlled each of these conditions visually. The measurements were taken in accordance with the ERS recommendations[11]. Spirometry was used to measure forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and FEV1/FVC in accordance with the ATS-ERS recommendations[10].

Computed tomography

CT examinations were performed using a multi-detector spiral CT scanner (Philips Ingenuity 128 slice) in a single breath hold during deep inspiration. All CT scans obtained after intravenous contrast administration were excluded because contrast material could interfere with density measurements of lung parenchyma. CT acquisitions were performed utilizing a 120 kV tube voltage and current modulation technique, and images were acquired with a 1.5 mm reconstruction thickness and a "B filter."

The obtained thin-section volumetric CT images were quantitatively analyzed using the Philips IntelliSpace Portal

software, and all steps of this analysis were supervised by a 20 year experienced radiologist. This program can determine the overall volume of parenchyma areas and the volume below a particular threshold density value after automatically recognizing both lungs and their lobes on CT images. The percentages of parenchymal volumes in the whole lung volume that were below the predetermined threshold density values were measured by using density mask technique, a qCT technique that is widely used to quantify emphysema. In this technique, all voxels (the volume element of a CT slice that corresponds to a pixel of CT image) that have a density less than a predefined threshold are identified and masked by a color (Figure 2). Since the volume of a single voxel of a CT slice is known (it is pixel area multiplied by slice thickness), it is possible to calculate the total volume of all "masked densities."

After measuring parenchymal volumes that have densities below seven predefined threshold density levels (0, -500, -600, -700, -750, -800, and -850 HU), we obtained volumes of lung parenchyma regions that have densities between certain thresholds by simply subtracting the volume of the lower threshold value from the volume of the higher one. In this way, we obtained an absolute volume value of a parenchymal density range, and when we divided it into total lung volume, we got the percentage of a certain density range volume (DRV%), such as $DRV\%_{[(-750)-(-500)]}$, which means the percentage of lung parenchyma areas that have a density between -750 and -500 HU.

Although different density ranges are utilized in the literature for lung fibrosis and ground glass opacities, in our study, $DRV\%_{[(-500)-0]}$ was accepted to represent parenchymal areas including fibrosis, atelectasis, and consolidation, and $DRV\%_{[(-750)-(-500)]}$ was accepted to represent GGO[14,15].

Correlation between predicted percentages of IOS parameters (AX, Fres, R_{20}, R_5, R_{5-20}, and X_5), spirometry measurements (FEV1, FVC and FEV1/FVC), and qCT results ($DRV\%_{[(-850)-0]}$) were statistically analyzed.

Statistical analysis

In our study, statistical analyses were performed by IBM SPSS version 26.0. To determine if the variables were normally distributed, visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov, Skewness, and Kurtosis tests) were used. Normally distributed independent data were analyzed using the Independent-Samples t-test. Non-normally distributed independent data were analyzed using the Mann-Whitney U test. The correlation between variables that did not show normal distribution was evaluated using Spearman's test. $p<0.05$ was considered statistically significant.

RESULTS

A total of 47 patients (32 males and 15 females) with a mean age of 54 years were included in the study. The study and control groups were similar regarding their body mass indices (BMIs),

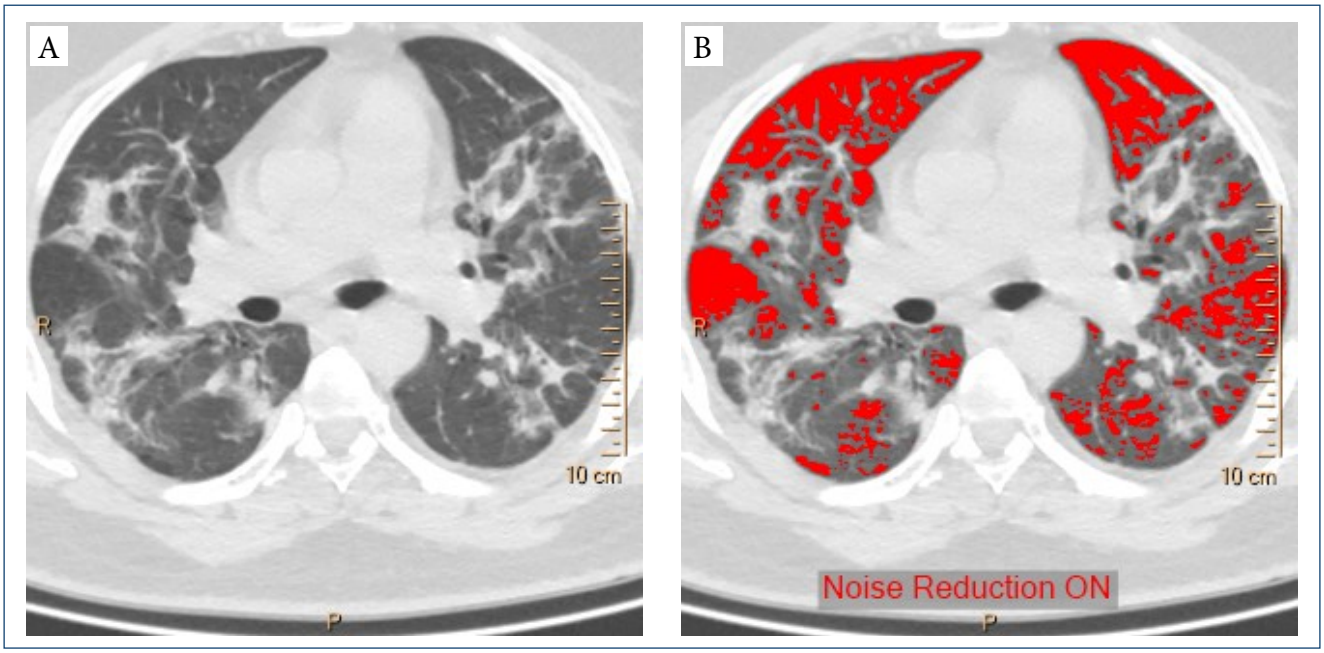

Figure 2. (A) Computed tomography image and (B) density mask with -750 Hounsfield units.

comorbidity rates, and smoking durations. In the control group, the hospitalization rate was 28%, and the average number of hospitalization days was 2.36 days. In the study group, the rate of hospitalized patients was 78%, and the average length of stay was 9.79 days. In addition, the proportion of patients using long-term oxygen therapy (LTOT) was higher in the study group. Detailed demographic data and outcome measures are shown in Table 1 and 2.

Table 1. Demographic data, clinical data, and quantitative computed tomography results of patients.

	All patients n=47 Mean±SD	Control group n=14 Mean±SD	Study group n=33 Mean±SD	p-value
Gender n m/f (%)	32 (68.1)/15 (31.9)	6 (42.9)/8 (57.1)	26 (78.8)/7 (21.2)	**0.017**
Age	54.23±8.51	49.21±5.92	56.36±8.62	**0.007**
Comorbidities y/n	25 (53.2)/22 (46.8)	6 (42.9)/8 (57.1)	19 (57.6)/14 (42.4)	0.360
Smoking history (p/y)	6.71±6.68	5.93±6.39	7.1±6.90	0.664
BMI	28.14±4.29	26.72±4.39	28.75±4.17	0.139
mMRC	1.80±0.72	1,43±,514	1,97±,752	**0.024**
LTOT y/n	15 (31.9)/32 (68.1)	1 (7.1)/13 (92.9)	14 (42.4)/19 (57.6)	**0.003**
qCT (DRV%)				
[(-500)-0] HU	4.49±3.60	1.76±0.43	5.65±3.73	**<0.001**
[(-750)-(-500)] HU	22.80±17.08	7.60±2.86	29.25±16.50	**<0.001**

BMI: body mass index; DRV%: percentages of density range volumes; HU: Hounsfield units; LTOT: long-term oxygen therapy; m/f: male/female; mMRC: modified Medical Research Council score; n: number; p/y: pack year; qCT: quantitative computed tomography; SD: standard deviation; y/n: yes/no. Statistically significant p-values were given as bold.

Table 2. Spirometry and impulse oscillometry results according to the groups.

	All patients n=47 Mean±SD	Control group n=14 Mean±SD	Study group n=33 Mean±SD	p-value
FVC, %pred	92.19±19.86	106.93±13.205	85.94±19.002	**<0.001**
FEV_1, %pred	91.43±18.54	100.57±14.070	87.55±19.013	**0.026**
FEV_1/FVC, %	81.30±10.47	77.2750±8.40269	83.0130±10.90094	0.086
Fres, Hz	16.13±4.31	14.25±3.19	16.93±4.51	0.050
Fres, %pred	120.99±33.16	105.95±31.90	127.37±32.04	**0.042**
AX, kPa/L	0.58±0.41	0.48±0.46	0.62±0.38	0.278
AX, %pred	180.45±110.30	129.67±93.22	201.99±111.14	**0.038**
R_5, kPa/L/s	0.35±0.09	0.351±0.10	0.358±0.09	0.560
R_5, %	112.62±29.99	100.44±20.280	116.54±31.817	0.165
R_{20}, kPa/L/s	0.27±0.07	0.275±0.08	0.276±0.07	0.825
R_{20}, %pred	101.70±27.21	92.85±19.595	105.18±29.210	0.169
R_{5-20}, kPa/L/s	0.079±0.043	0.076±0.047	0.088±0.035	0.104
R_{5-20}, %	126.22±47.45	112.39±48.29	132.08±46.58	0.306
X_5, kPa /L/s	-0.122±0.05	-0.096±0.02	-0.133±0.06	**0.035**
X_5, %pred	121.74±58.41	99.73±20.39	131.08±66.58	**0.044**

AX: reactance area; FEV_1: forced expiratory volume in the first second; Fres: resonant frequency; FVC: forced vital capacity; IOS: impulse oscillometry; pred: predicted; R: respiratory resistance; R_{5-20}: R_5-R_{20}; SD: standard deviation; X: respiratory reactance. Statistically significant p-values were given as bold.

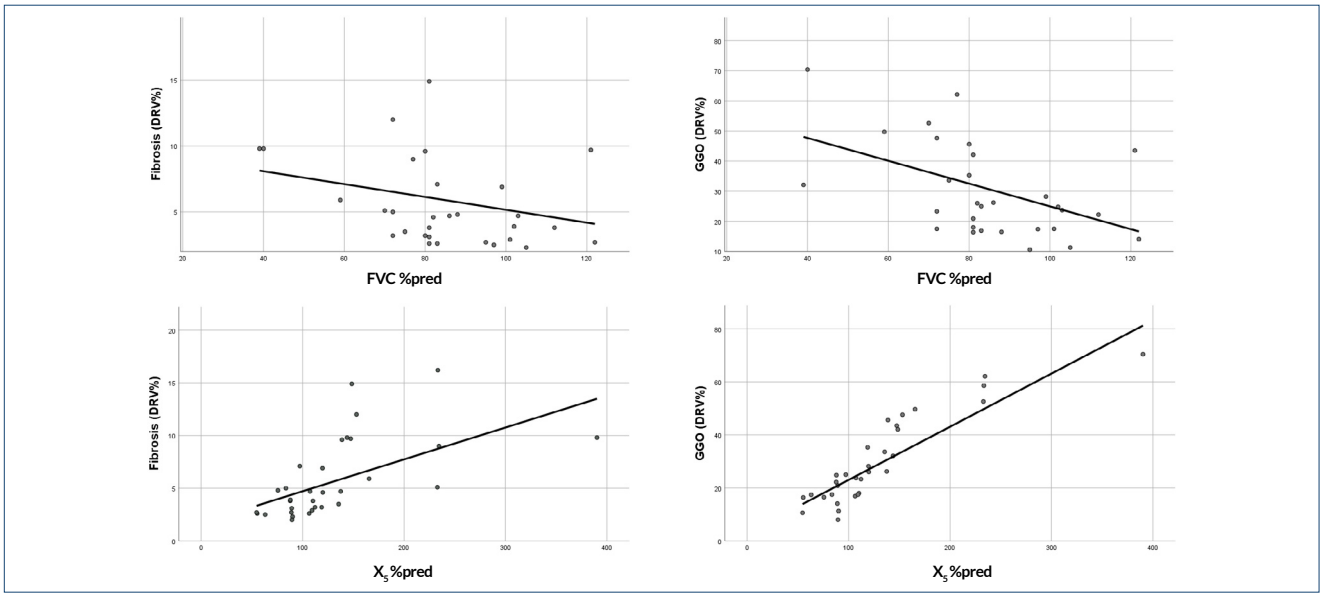

Figure 3. The relationship among FVC%pred and X_5%pred values with fibrosis ($DRV\%_{[(-500)-0]}$) and ground-glass opacities ($DRV\%_{[(-750)-(-500)]}$).

In the correlation analysis, none of the spirometry and IOS parameters in the control group were correlated with any of the qCT-derived DRV% values that were within the range of CT densities between 0 and -750 HU. Predicted FVC percentages were correlated with $DRV\%_{[(-750)-(-500)]}$ in the study group, but not with $DRV\%_{[(-500)-0]}$ (Table 3; Figure 3). In the correlation analysis between IOS parameters and DRV% values, AX and Fres were correlated with $DRV\%_{[(-750)-(-500)]}$, while X_5 was correlated with both $DRV\%_{[(-500)-0]}$ and $DRV\%_{[(-750)-(-500)]}$. The perception of dyspnea measured by mMRC was correlated with both FVC%pred and X_5%pred.

DISCUSSION

In this study, the correlation of qCT-derived DRV% with spirometry and IOS measurement results was investigated in patients with COVID-19 pneumonia 6 weeks after the conclusion of therapy.

Previous studies have shown that patients with COVID-19 can develop a restrictive ventilatory defect associated with the severity of the disease[6]. In another study, CT findings were observed even 3 months after the disease, and a decrease in diffusion capacity was found even when lung volumes were within normal ranges[16]. In both obstructive and restrictive diseases, IOS parameters AX, Fres, and R_5 generally increase, while X_5 decreases[17,18]. However, studies about COVID-19 are very limited. In our study, FVC%pred was only correlated with $DRV\%_{[(-750)-(-500)]}$. $DRV\%_{[(-500)-0]}$ did not correlate with spirometry parameters. This may be because the percentage of $DRV\%_{[(-500)-0]}$ is relatively low. Fres and AX values (%pred),

Table 3. Correlations between spirometry-impulse oscillometry parameters with quantitative computed tomography values and dyspnea perception.

		DRV% [(-500)-0] HU	DRV% [(-750)-(-500)] HU	mMRC
FVC, %pred	r	-0.251	-0.453	-0.403
	p	0.174	**0.011**	**0.030**
FEV_1, %pred	r	-0.080	-0.171	-0.211
	p	0.668	0.356	0.272
Fres, Hz	r	0.002	0.057	0.190
	p	0.990	0.751	0.306
Fres, %pred	r	0.310	0.452	0.323
	p	0.079	**0.008**	0.077
X_5, kPa/L/s	r	-0.493	-0.716	-0.257
	p	**0.004**	**<0.001**	0.163
X_5, %pred	r	0.607	0.773	0.376
	p	**0.001**	**<0.001**	**0.037**
AX, kPa/L	r	0.108	0.217	-0.045
	p	0.549	0.225	0.808
AX, %pred	r	0.304	0.430	0.334
	p	0.086	**0.012**	0.067
R_{5-20}, kPa/L/s	r	0.155	0.232	0.143
	p	0.390	0.193	0.442
R_{5-20}, %pred	r	0.026	-0.017	-0.001
	p	0.887	0.926	0.997

AX: reactance area; DRV%: percentages of density range volumes; FEV_1: forced expiratory volume in the first second; Fres: resonant frequency; FVC: forced vital capacity; HU: Hounsfield units; IOS: impulse oscillometry; mMRC: modified Medical Research Council score; pred: predicted; qCT: quantitative computed tomography; R: respiratory resistance; R_{5-20}: R_5-R_{20}; X: respiratory reactance. Statistically significant p-values were given as bold.

which are IOS parameters, are also correlated with $DRV\%_{[(-750)-(-500)]}$. Of these parameters, only X_5 is correlated with both $DRV\%_{[(-750)-(-500)]}$ and $DRV\%_{[(-500)-0]}$. X_5 is associated with elastic recoil as the out-of-phase component of lung impedance. Lung diseases that reduce the elastance of the lung (fibrosis and hyperinflation) lead to more negative X_5[13]. It is also a useful parameter for the assessment of the peripheral regions of the lungs. The reactance at 5 Hz is likely to detect small amounts of fibrosis-induced elastic recoil changes. The rate of $DRV\%_{[(-500)-0]}$, which is supposed to represent mainly fibrotic areas, was relatively low in our patients, and we believed that X_5 might be more sensitive to functional disorders that cannot be detected by FVC.

Studies on the use of IOS in restrictive diseases are relatively few. Soave et al. reported that reactance can be used in the functional follow-up of interstitial lung disease (ILD)[19]. In both obstructive and restrictive diseases, AX, Fres, and R_5 increase, and X_5 decreases. It has been claimed that a normal R_{20} level can be used for discrimination in ILD[20]. The mean R20%pred in our patients was also normal. Intrapulmonary airway and alveolar destruction, basal cell proliferation in the airways, and fibrinous exudates have all been seen in autopsy series of COVID-19 patients[21,22]. This shows that some individuals may also have airway obstruction. However, in addition to the normal R_{20} and R_5 percentages in our study group, there was no statistically significant difference when compared to the control group. Although the R_{5-20} mean was higher in the study group than in the control group, no statistically significant difference was identified between the two groups. Iwamoto et al. reported that the inspiratory X_5, being more negative than the expiratory X_5, may be a guide to distinguish restrictive diseases from obstructive pathologies, and a single breath reactance measurement would not discriminate[23]. Our study was designed retrospectively, and patients did not have delta X_5 results.

The mMRC dyspnea score has been proposed as a simple and valid method for classifying COPD-related disability[24]. In patients with idiopathic pulmonary fibrosis whose restriction is prominent, the mMRC score has been shown to correlate with major functional parameters of both maximal and submaximal exercise tests, which are known to be associated with disease severity and survival, as well as ventilatory impairment and exercise limitation[25]. Correlation of mMRC score with spirometry and IOS findings shows the importance of functional follow-up of patients and suggests that IOS can be used in the follow-up of patients.

IOS is a test that requires minimal patient cooperation. Oscillometry is fundamentally a different measurement from traditional lung function measurements, spirometry, and lung volumes. IOS detects small airway obstructions more sensitively than spirometry and has a strong correlation with the degree of obstruction. Furthermore, it can reveal the location of the obstruction. However, spirometry was found to be more sensitive in cases of large airway obstruction[8]. Lu et al. showed that IOS may be more sensitive than spirometry in the diagnosis of small airway disease in people with normal lung functions. However, in patients with abnormal lung function, spirometry may be more sensitive than IOS to detect patients with clinical symptoms and CT lesions[26]. It can detect lung involvement in patients with ILDs who have mild or even normal spirometry changes[27]. Our findings showed that IOS parameters, especially X_5 value, were associated with some qCT-derived DRV%s. It may be useful to use the IOS test together with spirometry in the functional evaluation of post-COVID patients.

As a result of this study, we hope that general pulmonologists will remember that the findings on quantitative thoracic CT of patients with COVID-19 pneumonia correlated with spirometry and IOS parameters, with the strongest correlation being with X_5 from the IOS parameters.

Our study had some limitations. As it was a retrospective study, some results could not be reached, and the number of our patients was small. However, as far as we know, this is the first study to investigate the functional equivalence of qCT findings with IOS measurements.

CONCLUSION

In this study, 6 weeks after COVID-19 pneumonia, the spirometry parameter FVC, as well as the IOS parameters AX and Fres (%predicted), was correlated with the qCT-derived $DRV\%_{[(-750)-(-500)]}$. The percentage of X_5 relative to what was predicted was the sole parameter associated with both $DRV\%_{[(-750)-(-500)]}$ and $DRV\%_{[(-500)-0]}$. Furthermore, the predicted percentages of FVC and X_5 were correlated to the perception of dyspnea. IOS can be used in combination with spirometry to assess pulmonary function in individuals with COVID-19 pneumonia.

AUTHORS' CONTRIBUTIONS

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

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

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ORIGINAL ARTICLE

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24th hour vasoactive inotrope score is associated with poor outcome in adult cardiac surgery

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SUMMARY

OBJECTIVE: The aim of this study was to investigate the efficacy of vasoactive inotrope score at the 24th postoperative hour for mortality and morbidity in elective adult cardiac surgery.

METHODS: Consecutive patients who underwent elective adult coronary artery bypass and valve surgery in a single tertiary center for cardiac surgery between December 2021 and March 2022 were prospectively included. The vasoactive inotrope score was calculated with the dosage of inotropes that were continuing at the 24th postoperative hour. Poor outcome was defined as any event of perioperative mortality or morbidity.

RESULTS: The study included 287 patients, of whom 69 (24.0%) were on inotropes at the 24th postoperative hour. The vasoactive inotrope score was higher (21.6±22.5 vs. 0.94±2.7, p=0.001) in patients with poor outcome. One unit increase in the vasoactive inotrope score had an odds ratio of 1.24 (95% confidence interval: 1.14–1.35) for poor outcome. The receiver operating characteristic curve of vasoactive inotrope score for poor outcome had an area under the curve of 0.857.

CONCLUSION: Vasoactive inotrope score at the 24th hour can be a very valuable parameter for risk calculation in the early postoperative period.

KEYWORDS: Cardiac surgical procedures. Inotropic agents. Outcome assessment. In-hospital mortality.

INTRODUCTION

Cardiac surgical procedures are performed with increasing volumes and better outcomes[1]. Nevertheless, patients undergoing cardiac surgery are at risk of mortality and morbidity in the perioperative period. Prolonged intubation, extended intensive care unit (ICU) stay, acute renal injury, and cerebrovascular events are common major risks encountered following cardiac surgery[2,3].

During weaning from cardiopulmonary bypass (CPB) at the end of cardiac surgery and in the early postoperative period, inotropes are utilized to stabilize hemodynamics and improve cardiac function. Depending on the patient's preoperative comorbidities, the extent of Ischemia-reperfusion damage, and intraoperative variables, severe myocardial dysfunction can arise, leading to low cardiac output syndrome and end-organ malperfusion[4]. Inotropic and vasopressor agents are the first-line treatments for low cardiac output syndrome[5]. The dosing and number of these agents are managed according to the hemodynamic and metabolic requirements of the patient with higher doses denoting a worse condition[6].

Inotropic and vasopressor agents are associated with distinct complications, including vasoconstriction, arrhythmia, pulmonary, and hepatic complications. Patients who require high doses of inotropes are more prone to postoperative complications. The vasoactive inotropic score (VIS) is a score calculated from the doses of administered inotropic agents and reflects the level of total inotrope requirement of the patient, which allows for objective quantification of the level of inotropes required by a patient[7]. Although originally developed to include dopamine, dobutamine, and epinephrine, it was subsequently expanded to include more agents. VIS has been shown to be a marker of disease severity and a prognostic factor for mortality and morbidity. It was initially used in the pediatric age group for prognostic purposes but has also been used in adult cardiac surgery patients[8].

Several risk scoring systems have been developed for outcome prediction following cardiac surgery. The current European System for Cardiac Operative Risk Evaluation (EuroSCORE II) reflects the risk of a planned cardiac operation using patient factors and operation type. Although it provides very useful information, the operative and early postoperative periods are also important in the final state of the patient. No current risk score incorporates direct or indirect data that reflect intraoperative parameters[9]. The level of inotropes necessary in the early postoperative period may reflect both the patient's preoperative state and the intraoperative parameters. Therefore, we aimed to investigate the efficacy of VIS for predicting mortality and morbidity after elective adult cardiac surgery.

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METHODS

The study was designed as a single-center prospective study. Approvals were obtained from the hospital academic board and the local ethics committee (approval number HNHEAH-KAEK 2021/KK/291). The study was conducted in full compliance with the ethical principles of the Declaration of Helsinki. Consecutive patients who underwent elective adult cardiac surgery at our tertiary cardiac center between December 2021 and March 2022 were included in the study. Patients who required urgent surgery and who required extracorporeal membrane oxygenation (ECMO) during the weaning period or early postoperative period were excluded. Operations were performed by different surgical teams of the hospital following routine surgical protocols, and patients were treated in the ICU by a single anesthesiology team.

Demographic parameters, preoperative echocardiography results, EuroSCORE II calculations, and operative data including CPB and cross clamp (CC) times were recorded. VIS was calculated at the first 24th hour of the postoperative ICU stay. The time to extubation, renal injury, need for mechanical support with intra-aortic balloon pump (IABP), stroke, reoperation, and death were recorded. Renal injury was determined according to the RIFLE classification[10]. EuroSCORE II was calculated for each patient using the online calculator[11]. A cerebrovascular event was defined as a new-onset neurological deficit in the postoperative period, as evidenced by radiological imaging. Acute renal failure was defined as the need for renal replacement therapy in the intensive care unit. Extended ICU stay was defined as longer than 2 days of ICU stay. Reoperation for bleeding included all patients reoperated for excessive chest tube output in the postoperative period. A poor outcome was defined as any perioperative mortality or morbidity.

Calculation of vasoactive inotrope score

As a routine protocol of perioperative management in our institute, inotropes were started, targeting a mean arterial pressure of >65 mmHg. In patients with high pulmonary capillary wedge pressure and pulmonary artery pressure (PAP), milrinone was started at 0.2–0.4 μg/kg/min. An IABP was placed if a low cardiac output state was present despite maximum doses of inotropes with a systolic arterial pressure<100 mmHg, mean PAP>25 mmHg, central venous pressure>15 mmHg, and cardiac index <2.1 L/min/m^2.

Inotrope and vasopressor doses were recorded to calculate VIS with the following formula: dopamin (mcg/kg/min)+dobutamine (mcg/kg/min)+100×epinephrine (mcg/kg/min)+100×norepinephrine (mcg/kg/min)+10×milrinone (mcg/kg/min)+10,000×vasopressin (munits/kg/min). VIS calculation was performed with the dosage of inotropes continuing at the 24th postoperative hour[12].

Statistical analysis

IBM SPSS 22 software was used for statistical analysis. Continuous parameters are given as mean±standard deviation, while categorical parameters are given as numbers and percentages. The normal distribution of continuous parameters was assessed using the Shapiro-Wilk test. For group comparison, continuous variables with normal distribution were compared using the Student's t-test, continuous variables without normal distribution were compared using the Mann-Whitney U test, and categorical variables were compared using the chi-squared test. Factors significant in univariate analysis were carried onto multivariate analysis for the assessment of risk factors. Receiver operating characteristic (ROC) curves were constructed to compare the efficacy of VIS and EuroSCORE II in predicting poor outcome.

RESULTS

The records of 287 consecutive patients who met the inclusion criteria during the study period were evaluated. The mean age of the patients was 60.0±10.7, 199 (69.3%) were males, and 88 (30.7%) were females. The mean EuroSCORE II was 1.89±1.34. The baseline patient characteristics are presented in Table 1. In the 24th postoperative hour, vasoactive agents were necessary for 69 (24.0%) patients. The mean VIS on the first operative day was 3.82±11.26. The mortality rate among the study patients was 4.2%. The composite endpoint of poor outcome was observed in 40 (13.9%) patients. The observed morbidities are summarized in Table 1.

Patient factors were compared between patients with and without poor outcome (Table 2). Chronic obstructive pulmonary disease was more frequent, the mean preoperative ejection fraction was lower, and CPB and CC times were longer in patients with mortality (p=0.005, p=0.011, p=0.001, and p=0.013, respectively). Combined coronary artery bypass grafting (CABG) and valve procedures were more common among patients with poor outcome (p=0.005). VIS (p<0.001) and EuroSCORE II (p<0.001) were higher in patients with poor outcome. The factors that were significant between the groups were all represented by the EuroSCORE II. After controlling for EuroSCORE II and CPB time, VIS was found to be independently associated with poor outcome with an odds ratio (OR) of 1.24 (95% confidence interval [CI]: 1.14–1.35). The same analysis was repeated for isolated CABG, where VIS

Table 1. Summary of patient characteristics.

Variables	n (%)
Age	60.0±10.7
Gender	
Male	199 (69.3%)
Female	88 (30.7%)
EuroSCORE II	1.89±1.34
Diabetes mellitus	147 (51.2%)
Chronic obstructive pulmonary disease	34 (11.8%)
Left ventricular ejection fraction (%)	52.6±8.6
Left ventricular ejection fraction ≤50%	113 (39.4%)
Pulmonary artery pressure (mmHg)	26.2±12.4
Cardiopulmonary bypass time (min)	125.8±46.0
Cross clamp time (min)	80.3±33.8
Operation type	
CABG	203 (70.7%)
Valve	65 (22.6%)
CABG+valve	19 (6.6%)
VIS	3.82±11.3
Intra-aortic balloon pump use	15 (5.2%)
Poor outcome	40 (13.9%)
Mortality	12 (4.2%)
Prolonged intubation	10 (3.5%)
Prolonged ICU stay	22 (7.7%)
Acute renal failure	4 (1.4%)
Cerebrovascular event	8 (2.8%)
Reoperation for bleeding	9 (3.1%)

CABG: coronary artery bypass graft; EuroSCORE II: European System for Cardiac Operative Risk Evaluation II; ICU: intensive care unit; VIS: vasoactive inotrope score.

was independently associated with poor outcome with an OR of 1.21 (95%CI: 1.10–1.33). Individual morbidities of prolonged ICU stay, prolonged intubation, cerebrovascular events, and reoperation for bleeding were also significantly associated ($p<0.001$) with higher VIS means.

The efficacy of VIS was assessed and compared against EuroSCORE II using ROC analysis. Area under the curve (AUC) was greater for VIS (0.857) compared to EuroSCORE II (0.788). A value of 4.5 for VIS had a sensitivity of 77.5% and a specificity of 92.7% for poor outcome (Figure 1). The AUC of VIS for poor outcome in CABG-only patients was 0.814 and in valve-only patients was 0.870.

DISCUSSION

After weaning off CPB and the initial stabilization period in the ICU, the variety and dose of inotropes and vasopressors required represent both the extent of low cardiac output syndrome and myocardial dysfunction. Although acting to increase cardiac contractility and systemic perfusion, the use of inotropes and vasopressors has been associated with increased mortality and organ dysfunction. With more severe myocardial dysfunction and low cardiac output, higher doses of inotropic exposure will be necessary for the patient, with a high associated VIS[6,13].

The VIS quantifies the total dose of inotropes and effectively reflects the patient's risk of mortality and morbidity during their hospital stay. The VIS is a numerical score that

Table 2. Patients factors in patients with and without poor outcome.

	No poor outcome (n=247)	Poor outcome (n=40)	p-value
Age	59.7±10.6	62.0±10.7	0.203
Gender			0.167
Male	175 (70.9%)	24 (60.0%)	
Female	72 (29.1%)	16 (40.0%)	
EuroSCORE II	1.70±1.16	3.06±1.70	<0.001
Diabetes mellitus	128 (51.8%)	19 (47.5%)	0.612
Chronic obstructive pulmonary disease	22 (8.9%)	12 (30.0%)	0.001
Left ventricular ejection fraction (%)	53.2±8.0	49.0±10.8	0.022
Left ventricular ejection fraction ≤50%	91 (36.8%)	22 (55.0%)	0.029
Pulmonary arterial pressure (mmHg)	25.4±11.5	30.8±16.0	0.048
Cardiopulmonary bypass time (min)	120.1±40.2	161.0±62.0	<0.001
Cross clamp time (min)	77.7±32.0	95.8±40.1	0.002
VIS	0.94±2.7	21.6±22.5	<0.001
Operation type			0.005
CABG	181 (73.3%)	22 (55.0%)	
Valve	54 (21.9%)	11 (27.5%)	
CABG+valve	12 (4.9%)	7 (17.5%)	

CABG: coronary artery bypass graft; EuroSCORE II: European System for Cardiac Operative Risk Evaluation II; VIS: vasoactive inotrope score.

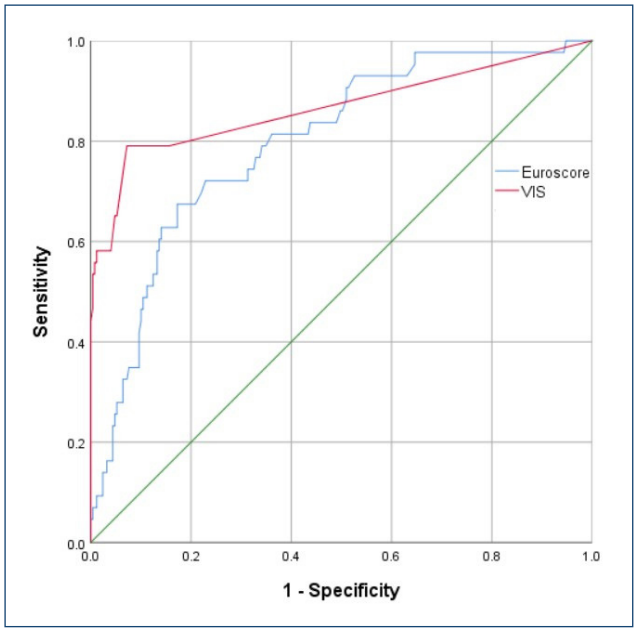

Figure 1. Receiver operating characteristic curves of European System for Cardiac Operative Risk Evaluation II and vasoactive inotropic score for poor outcome.

was first used in the pediatric patient group and was later studied in adult cardiac surgery[7,14,15]. Our results show that VIS is an effective indicator of poor outcome in adult cardiac surgery patients undergoing elective CABG and valve surgery. Studies on VIS have chosen different time points to determine the score and its relationship with outcomes. In a prospective multicenter study on pediatric patients below the age of 1 by Gaies et al., the maximum VIS value during the first postoperative 24 h was used[7], whereas in another study, the VIS at the end of surgery was used[14]. Koponen et al. calculated the maximal VIS (VISmax) during the first 24 h after surgery using the highest doses of vasoactive and inotropic drugs administered[15]. In another study, the highest VIS value was obtained from the data recorded in the first and next 24th hours after intensive care admission[16].

The optimal timing for the VIS value that best predicts patient outcomes is debatable. In this study, we calculated the VIS at the 24th postoperative hour. The very early postoperative period (i.e., the first 6 h) during the initial stabilization of the patient may be misleading due to mechanisms such as concurrent fluid and electrolyte imbalance, varying levels of systemic vascular resistance, and hypothermia, which influence the choice and dosage of anesthetics. Any persistent cardiac dysfunction that requires inotropic and vasopressor support at the 24th hour would be associated with a higher risk of poor outcome in the postoperative course. Future studies may compare the VIS at different time points in a single cohort to determine the best interval associated with outcomes.

The level of VIS above which there is increased risk differs with the study population. Gales et al. have found a VIS above 20 to be associated with poor outcomes[7]. In a study on patients operated on for infective endocarditis, a VIS>10 was accepted as a high value[17]. In another cardiac surgery study, a cutoff value of 5.5 for VIS had 0.83 sensitivity and 0.54 specificity[14]. High VIS values have been associated with morbidity in pediatric cardiac surgery patients, and the higher cutoff value for VIS in the pediatric population has been explained by the decreased beta-adrenergic receptors with lower ages[6]. Higher cutoff values at 10–15 have been reported in a different study[17]. In our study, a cutoff value of 4.5 had a sensitivity of 77.5% and a specificity of 92.7% for adult CABG and valve surgery patients.

Maximum VIS in the first 24 h has been demonstrated to be an independent predictor of renal failure[18]. In our cohort, a high VIS was associated with an increased occurrence of the composite endpoint of any comorbidity. Although the number of each specific comorbidity was low, a higher VIS could be demonstrated for the occurrence of each comorbidity. A high VIS was associated with a prolonged ICU stay, renal failure, cerebrovascular events, and reoperation for bleeding. Future studies can be designed to determine cutoff values for VIS above which the risk of these morbidities is increased.

The EuroSCORE II is a prevalent scoring system that incorporates preoperative patient data, preoperative cardiac parameters, and the type of planned operation to predict perioperative risk[19]. In our study, the VIS performed better than the EuroSCORE II for demonstrating the risk of poor outcome. The EuroSCORE II is a highly validated risk score that utilizes preoperative factors to suggest a risk profile for patients undergoing cardiac procedures[20]. On the contrary, patient factors and the type of planned operation play significant roles in the risks faced by the patient in the perioperative period. Furthermore, perioperative complications are affected by factors that become evident during the operation. These include the duration of CPB, CC, and myocardial contractility at the end of the operation. These factors are not included in preoperative risk calculations. The dosage of inotropes necessary in the postoperative period may reflect the operative factors that influence outcomes. This state is better quantified by the VIS, which may explain its better performance for poor outcomes.

Our study has certain limitations. This study was performed at a single center with a limited number of patients. Urgent cases and those that required an ECMO were excluded to form a homogenous patient group. With a larger patient group, the

predictive ability of the VIS for individual morbidities can be better evaluated. The use of inotropes may vary across institutions, which may limit the external validity of our results.

CONCLUSION

This study showed that a higher VIS is associated with an increased risk of poor outcome following elective cardiac surgery in adult patients. Our results emphasize that the VIS at the 24th hour can be a very valuable parameter for risk calculation in the early postoperative period. Further risk analysis studies can determine the ideal time for score calculation, the potential benefit of its use alongside traditional risk scores, and the ideal cutoff values for individual postoperative complications.

AUTHORS' CONTRIBUTIONS

EMTM: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **MB:** Formal Analysis, Investigation, Methodology, Visualization, Writing – review & editing. **MA:** Conceptualization, Data curation, Methodology, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of the association between silent ischemic lesions and stent design in carotid stenting applications

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SUMMARY

OBJECTIVE: Minor ischemic events and silent ischemic lesions are more common in carotid stenting than in endarterectomy. These silent ischemic lesions are also associated with stroke risk and cognitive impairment, so it is important to understand the factors that increase the risk and develop strategies to reduce the risk. We aimed to evaluate the association between carotid stent design and silent ischemic lesion development.

METHODS: The files of the patients who underwent carotid stenting between January 2020 and April 2022 were scanned. Patients with diffusion MR images taken within the postoperative 24 h were included in the study, while those undergoing acute stent placement were excluded. The patients were divided into two groups: those with open-cell stents and those with closed-cell stents.

RESULTS: A total of 65 patients, including 39 patients undergoing open-cell stenting and 26 patients undergoing closed-cell stenting, were included in the study. There was no significant difference in demographic data and vascular risk factors between the groups. New ischemic lesions were detected in 29 (74.4%) patients in the open-cell stent group and 10 (38.4%) patients in the closed-cell stent group and were significantly higher in the open-cell group. There was no significant difference between the two groups in terms of major and minor ischemic events and stent restenosis at the 3-month follow-up.

CONCLUSION: The rate of new ischemic lesion development was found to be significantly higher in carotid stent procedures performed with an open-cell Protégé stent than in those performed with a closed-cell Wallstent stent.

KEYWORDS: Embolism. Carotid stenosis. Diffusion MRI. Stent. Stroke.

INTRODUCTION

One of the common causes of stroke is carotid atherosclerosis. Current guidelines recommend carotid stenting (CAS) as an alternative treatment to endarterectomy (CEA), especially in high-risk patients for endarterectomy[1-3]. Multicenter randomized studies reported that periprocedural disabling stroke and death rates historically declined from 4.4 to 0.8% as the materials used began to change, the techniques employed improved, and the experience in this field increased[4,5]. Despite such decreasing rates, the frequency of minor strokes in the treatment of CAS is still slightly higher compared to CEA[4]. In recent years, studies have reported that the transcarotid artery revascularization (TCAR) method has a lower risk of periprocedural stroke and death compared to transfemoral carotid stenting (TFCAS), but no clear recommendation has been found in the guidelines to date[1,6]. In the ESVS 2023 guideline, which is in the process of publication, it is recommended that the transradial access or TCAR method should be considered in patients who are planned for carotid stenting and that the transfemoral access may increase the risk of complications, as class IIa level B[2]. However, in many centers, including our center, TCAR still cannot be performed and TFCAS is widely applied. Therefore, improvements are needed to reduce complications after CAS.

Cerebral infarction as a perioperative complication related to CAS is an issue, and previous studies reported that risk factors for cerebral infarction included emboli protection devices (EPD), the operator's skill, patient age, plaque properties, stent design, and statin use[2,7-11]. EPD, balloon angioplasty, and stent design are material-related factors that may affect procedural complications. Although current guidelines recommend the use of EPD, numerous studies have found no significant difference between the clinical outcomes and newly detected ischemic lesions on diffusion-weighted magnetic resonance imaging (DWI) images of patients for whom an EPD has been used or not[12-14]. Even there are reports in the literature that new ischemic lesion development as detected in DWI is more

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common in patients for whom a distal protection type EPD has been used[13].

Carotid stents include open-cell stents with fewer interconnections and larger empty cells and closed-cell stents with more frequent interconnections. There are also controversial results on the effect of stent design on clinical outcomes, and the guidelines have not made a clear decision on this subject yet[12]. While some studies have reported a higher risk of stroke in patients using open-cell stents, there are also studies reporting the opposite result[7,15-17]. There are a relatively small number of studies evaluating the association between stent design and post-stenting microembolization. Therefore, we wanted to evaluate whether the stent design has any effect on the microembolic lesions as detected in DWI and on the 3rd month clinical outcome in patients on whom we performed CAS in our own clinic.

METHODS

The data of the patients who underwent CAS in our Interventional Neurology clinic at Bolu Abant Izzet Baysal Training and Research Hospital between January 2020 and April 2022 were evaluated retrospectively after obtaining the approval of the ethics committee. (Ethics Committee of Bolu Abant Izzet Baysal University (2022-236) 27/09/2022). Patients older than 18 years of age who had a DWI check within 24 h (16–32 h) after the procedure were included in the study. Those undergoing acute CAS were excluded from the study. DWI scans were performed on the same 1.5-T device (Signa Explorer, GE Healthcare, Chicago, IL, USA) in all patients who underwent CAS before and at least 24 h (16–32) after the procedure. Patients who could not undergo follow-up imaging due to reasons such as failure to make an appointment, device malfunction, or maintenance were excluded from the study. The files of 104 patients to whom we applied CAS during the study date range were scanned. A total of 65 patients who met the inclusion and exclusion criteria were included in the study (Figure 1). The patients were divided into two groups: those with open-cell stents and those with closed-cell stents. The patients' age, gender, vascular risk factors, antiaggregant treatments, rate of stenosis in the ICA, and contralateral ICA, which side was operated on, and arch types were noted. Residual stenosis rates, complications during the procedure, and cardiac and cerebrovascular events in the postoperative 3-month follow-up, patients with more than 50% residual stenosis on the 3rd month Doppler USG were noted. It was noted whether predilatation or postdilatation was performed in the procedure and whether EPD was used or not.

Procedure

All patients were operated on under local anesthesia with acetylsalicylic acid and clopidogrel treatment. After an 8F 11-cm sheath was placed, the bilateral extracranial and intracranial vessels were evaluated using a diagnostic catheter in at least two planes. A 6F guide catheter was placed in the CCA. There was no operator preference bias in the stent selection since the stent design available in the hospital on the day of the procedure applied to the stenosis segment was used. The reason for the change in the type of stent used was the purchase from the company that gave the lowest bid in the tender held by the hospital. An open-cell Protégé (Medtronic Corp.; Minneapolis, MN, USA) stent was available in our hospital between January 2020 and March 2021 and a closed-cell Wallstent (Boston Scientific, Marlborough) between March 2021 and April 2022. After stenting, images of ipsilateral intracranial vessels and the ICA were obtained from at least two planes. Residual stenosis rates were noted.

Statistical analysis

Data were evaluated by the SPSS 21.0 (IBM Corp., Armonk, NY, USA) software.

Categorical variables were expressed as numbers and percentages, and countable variables as mean±SD. Between the two independent groups, countable variables showing normal distribution were evaluated by the independent sample T test, and variables not showing normal distribution were evaluated by the Mann–Whitney U test. Chi-square test was used when comparing categorical variables. $p<0.05$ was considered significant.

RESULTS

The data of a total of 65 patients, including 39 patients undergoing open-cell stenting and 26 patients undergoing closed-cell stenting, who met the inclusion criteria, were evaluated. Age, gender, and vascular risk factors in both groups are given in Table 1. There was no difference in terms of demographic data and vascular risk factors.

Pre-procedural stenosis rates, contralateral stenosis rates, residual stenosis rates, which side ICA was treated, arch types, balloon angioplasty rates, balloon sizes, distal filter usage rates, and symptomatic/asymptomatic patient rates are given in Table 2. Preoperative stenosis rates were found to be significantly higher in the closed-cell stent group (80.69±12.37% vs. 73.0±11.0% respectively; $p=0.011$). The rate of predilatation and double dilatation was found to be higher in the closed-cell stent group (for all, $p<0.01$). There was no difference in the other data between the two groups.

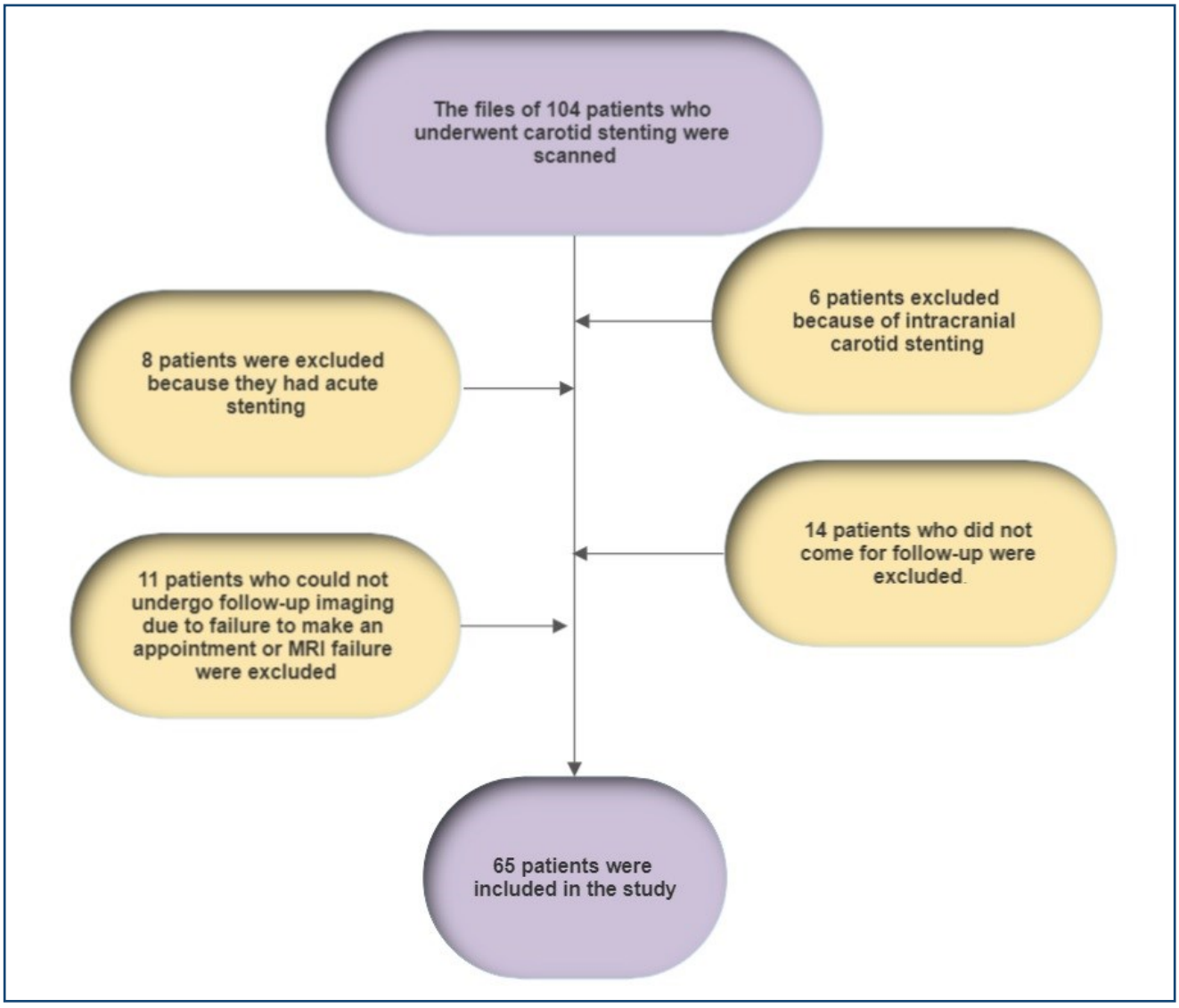

Figure 1. Flowchart.

Table 1. Comparison of patients' demographic data and vascular risk factors.

	Open-cell stent n=39	Closed-cell stent n=26	p
Age	68.02±11.45	67.42±9.08	0.82
Gender M/F	25/14	20/6	0.27
HT n (%)	28 (71.8)	19 (73.1)	0.91
DM	18 (46.2)	13 (50)	0.76
HL	26 (66.7)	20 (76.9)	0.37
CAD	17 (43.6)	11 (42.3)	0.91
Previous stroke	14 (35.9)	11 (42.3)	0.60
Smoker	20 (51.3)	12 (46.2)	0.68

HT: hypertension; DM: diabetes mellitus; HL: hyperlipidemia; CAD: coronary artery disease.

New ischemic lesions were detected in 29 (74.4%) patients in the open-cell stent group and 10 (38.4%) patients in the closed-cell stent group, and they were significantly higher in the open-cell group (p=0.004). No significant difference was identified between the two groups in terms of stent thrombosis, restenosis, and major cardiac and cerebrovascular events within the first 3 months postoperatively (Table 3).

DISCUSSION

In this study, we found that the rate of new ischemic lesion development in diffusion MRI was significantly lower in patients undergoing closed-cell stenting.

Table 2. Comparison of patients' radiological data.

	Open-cell stent (39)	Closed-cell stent (26)	p
Stenosis rate	73.0±11.04	80.69±12.37	**0.011**[a]
Contralateral stenosis rate	20.28±25.43	30.76±34.57	0.16
Leftf (%)/Right (%)	25 (64.1)/14(35.9)	16 (61.5)/10 (38.5)	0.83
Symptomatic/Asymptomatic (%)	29 (74.4)/10 (25.6)	21 (80.8)/5 (19.2)	0.54
Residual stenosis	14.69±10.67	11.11±11.6	0.19
Balloon angioplasty (%)	27 (69.2)	21 (80.7)	0.30
DF (%)	22 (56.4)	14 (53.8)	0.83
Predilatation (%)	6 (15.3)	17 (65.3)	**0.00**[b]
Postdilatation (%)	25 (64.1)	18 (69.2)	0.66
Double angioplasty vs others (%)	4 (10.2)	13 (50)	**0.001**[b]
Prediltatation balloon diameters (n)	2 mm (2) 3 mm (4)	2 mm (1) 2.25 mm (1) 2.5 mm (2) 2.75 mm (3) 3 mm (5) 3.5 mm (5)	
Posdiltatation balloon diameters	3 mm (1) 3.5 mm (2) 4 mm (2) 4.5 mm (3) 5 mm (18)	4 mm (2) 4.5 mm (11) 5 mm (11)	

DF: distal filter; [a]Independent sample T test; [b]Chi square test; p<0.05.

Table 3. Comparison of clinical outcome data of patients during 3-month follow-up.

	Open-cell stent (39)	Closed-cell stent (26)	p
Stent thrombosis	1 (2.5)	0	0.41
Restenosis>50%	0	0	–
Minor ischemic stroke	2 (5.1)	1 (3.8)	1.00
Major ischemic stroke	2 (5.1)	0	0.51
Intracerebral hemorrage	0	0	–
Death	1 (2.5)	0	0.41
MACCE	2 (5.1)	0	0.51
Silent ischemic lesion	29 (74.3)	10 (38.4)	**0.004**[a]

[a]Chi square test; p<0.05. MACCE: major adverse cardiac and cerebrovascular events.

Carotid stents consist of cascading rings connected in a helical fashion by bridges. The free cell area between the bridges varies according to the bridge density between the rings. Stents with a free cell area of less than 5 mm^2 are called closed-cell stents, while those with a free cell area of more than 5 mm^2 are called open-cell stents. Closed-cell stents provide a higher level of support to the vessel wall, and the radial force applied by the stent reduces the likelihood of thrombogenic material passing into the circulation. Open-cell stents, on the other hand, have fewer bridges, allowing them to be more flexible and to be applied to tortuous vessels[15]. In our study, a Wallstent (Boston Scientific, Marlborough) stent with a free cell spacing of 1.08 mm^2 from the closed-cell stent group and a Protégé (Medtronic Corp., Minneapolis, MN, USA) stent with a free cell spacing of 10.71 mm^2 from the open-cell stent group were employed.

Timaran et al.'s randomized controlled study conducted on 40 high-risk patients for endarterectomy reported that new ischemic lesions were detected in 53% of the patients in the open-cell group and 47% in the closed-cell group and that no difference was found between microembolic signals detected by transcranial Doppler and the rates of new ischemic lesion development of the two groups[15]. Bijuklic et al.'s observational study identified new ischemic lesions in 26% of the patients in both the open-cell and closed-cell stent groups[18]. Leal et al.'s study evaluating 45 patients undergoing closed-cell stenting and 19 patients undergoing open-cell stenting detected new ischemic lesions in 18% and 37% of patient, respectively[19]. Park et al.'s study evaluating 91 CAS cases reported a significantly higher number of new ischemic lesions for the open-cell stent group[17]. In a meta-analysis of 930 cases in total, which evaluated the data of 8 studies assessing postoperative MR images, the probability of developing both ipsilateral and contralateral new ischemic lesions was found to be significantly higher in the open-cell stent group. It has been reported that the probability of developing a new ischemic lesion is 25% higher when CAS is performed with an open-cell stent (RR, 1.25 95%)[7]. In our study, a new ischemic lesion was identified in 74.4% of the

open-cell stent group, which is significantly higher, than the 38.5% of the closed-cell stent group. In our study, a higher rate of new ischemic lesions was found in the open-cell stent group compared to previous studies. In the study conducted by Park et al., it was reported that 51.1% of new ischemic lesions were detected in which the Precise stent was used[17]. This may be related to the wider free cell spacing of the Protégé stent (10.71 vs. 5.89 mm^2).

Hart et al.'s observational study conducted on 701 CAS patients found a significantly low rate of new neurological event development in patients undergoing closed-cell stenting (3.4% vs. 1.3%)[20]. A study by Bosiers et al. investigating 3,179 stent cases reported a new neurological event and death within 30 days in 3.4% of the patients undergoing open-cell stenting and 1.2% of the patients undergoing closed-cell stenting[11]. A meta-analysis including 46728 CAS cases identified no significant association with stent design in terms of major events at 30-day and 1-year follow-ups[7]. In another meta-analysis evaluating only 1,557 CAS cases performed on symptomatic patients, the risk of stroke within 30 days was found to be 10.3% in those undergoing open-cell stenting and 6% in those undergoing closed-cell stenting[16]. In our study, a major cerebrovascular event developed within 90 days in two (5.1%) patients in the open-cell group but in none (%0) in the closed-cell group.

Today, hybrid and dual-layer mesh-covered stents (DLS) are also available. Although hybrid stents are thought to theoretically combine the advantages of both stents, a meta-analysis that included 4,182 cases of stroke and death within 30 days found no difference between open-cell and closed-cell stents, nor did it find any significant difference between the two groups in the comparison of hybrid stents and closed stents involving 5,987 cases[7]. It was reported by Montorsi et al. that less microembolic signal was detected in cases using DLS than those using closed-cell stents[21]. In the ESVS 2023 guideline, consideration of DLS in cases of elective carotid stenting has been added as a new recommendation at Class 2b level C[2]. Hybrid or DLS stents were not used in our study. In DLSs, the very small cell sizes of the inner mesh cover the plaque better and reduce the risk of prolapse. DLS may be preferred, especially in cases where plaque structure is more risky, but its higher cost is a factor limiting its use.

De Viries et al.'s meta-analysis identified no significant difference between the rates of restenosis and stent fracture between open-cell and closed-cell patients; however, they reported a rate of restenosis of 5% for open-cell patients and 3.2% for closed-cell stent patients[7]. In our study, stent fractures and significant restenosis were not observed in the 3-month follow-up of the patients. Acute stent thrombosis developed in one patient in the open-cell stent group.

Balloon angioplasty is another factor that may be associated with complications in carotid stenting. It is thought that the plaque may break up with the effect of a "cheese grater" and cause embolism, especially in the postdilatation stage. In the meta-analysis study conducted by Ziapour et al., it was determined that avoidance of postdilatation reduces the risk of hemodynamic instability and that both postdilatation and predilatation do not have an independent effect on the development of new neurological events or mortality. However, it has been reported that the risk of developing neurological events is higher in patients who have undergone two dilatations, regardless of the type of dilatation[22]. In our study, although the proportion of patients who underwent two dilatations was higher in the group receiving closed-cell stents, the number of silent ischemic lesions was lower. In the ESVS guideline, it is recommended to prefer a balloon size of <5 mm if predilatation is to be made and to avoid postdilatation if residual stenosis is <30%. In our study, balloons with sizes between 2 and 3.5 mm were used for predilatation and between 3 and 5 mm for postdilatation, in accordance with the recommendations of the guidelines[2]. New ischemic lesions developing after carotid stenting are clinically important, even if they do not give any signs. In a long-term follow-up study conducted by Gensicke et al. on 62 patients who developed new ischemic lesions after CAS and 62 patients who did not, the 5-year risk of TIA or stroke was found to be significantly higher in those with new ischemic lesions, as shown on DWI (22.8 vs. 8.8%)[23]. It has also been reported that silent ischemic lesions increase the risk of cognitive decline and dementia[24]. During carotid revascularization procedures, iatrogenic and atherosclerotic microemboli and cerebral blood flow variability could cause cognitive deficits. The RAVLT (Rey Auditory Verbal Learning Test) test the success of total volumes of microemboli developing after CAS was found to be negatively correlated in short- and long-term follow-ups. Localization of silent ischemic lesions has also been noted to be important[25]. Therefore, it is critical to develop techniques that will reduce the possibility of silent ischemic lesion development in the CAS procedure. In our study, no cognitive evaluation was made, and both patients who had a stroke within a month had a silent ischemic lesion in the postoperative DWI scan.

The limitations of our study include its retrospective nature, a relatively small number of cases, and short follow-up periods. Although ours is a retrospective study, there was no bias in stent preference because the stent design available in the hospital at that time was used. Further randomized controlled studies with

high case numbers and long follow-up periods, also involving patients with hybrid and DLS stent designs and making cognitive evaluations, are needed.

CONCLUSION

Although it is thought that silent ischemic lesions don't show any clinical signs, such lesions are known to be associated with long-term stroke risk and cognitive impairment[23,24]. Therefore, it is essential to develop strategies aimed at reducing the development of silent ischemic lesions. In this study, we evaluated the effect of stent design, a factor that might influence procedural complications, on new ischemic lesions in DWI, and the rate of new ischemic lesion development was found to be significantly higher in CAS with an open-cell Protégé stent than in those performed with a closed-cell Wallstent stent.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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Factors influencing neonatal outcomes in twin pregnancies undergoing cesarean section: a cross-sectional study

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SUMMARY

OBJECTIVE: This study aimed to evaluate maternal and fetal characteristics and factors affecting fetal outcomes in twin pregnancies delivered by cesarean section.

METHODS: This was a cross-sectional study in a tertiary care referral hospital. The primary outcome was to ascertain the effects of independent factors on the 1st and 5th minute APGAR scores, neonatal intensive care unit admissions, the need for mechanical ventilation, and neonatal mortality.

RESULTS: A total of 453 pregnant women and 906 newborns were included in the analysis. The final logistic regression model revealed that early gestational weeks and neonates <3rd weight percentile at the time of delivery were the most significant predictors of all poor outcome parameters in at least one of the twins ($p<0.05$). General anesthesia for cesarean section was associated with 1st minute APGAR<7 and the need for mechanical ventilation, and emergency surgery was correlated with the need for mechanical ventilation ($p<0.05$) in at least one of the twins.

CONCLUSION: General anesthesia, emergency surgery, early gestational weeks, and birth weight <3rd weight percentile were strongly associated with poor neonatal outcomes in at least one of the twins delivered by cesarean section.

KEYWORDS: Anesthesia, obstetrical. Pregnancy twin.

INTRODUCTION

While the rate of multiple pregnancies varies significantly among societies and individuals, it has shown a significant rise worldwide, especially in middle- and high-income countries. The growing use of assisted reproductive procedures due to increased maternal age and decreased fertility is another factor contributing to multiple pregnancies[1]. As a result, multiple pregnancies constitute approximately 2–4% of all births[2].

Multiple pregnancies are associated with greater maternal and fetal risks compared to singleton pregnancies[3]. The maternal mortality associated with a twin pregnancy is 2.5 times higher than that for a singleton pregnancy[4], and adverse neonatal outcomes such as perinatal mortality, fetal growth restriction, and low birth weight are two to three times higher among twins[5]. Moreover, neonatal near-miss, which refers to cases that almost resulted in death, has been found to be associated with multiple pregnancies[6,7].

However, cesarean delivery is associated with a higher risk of maternal morbidity and poor neonatal outcomes[8,9]. From this perspective, we aimed to evaluate maternal and fetal characteristics and factors affecting fetal outcomes in twin pregnancies delivered by cesarean section.

METHODS

After the approval of the local Ethics Committee (2011-KAEK-25 2019/05-26), our study was conducted following the principles of the Declaration of Helsinki. The study was registered at www.clinicaltrials.gov under the number NCT05104255. This single-center, cross-sectional chart review comprised twin pregnancies and newborns delivered by cesarean section. The main a priori objective was to evaluate four outcome parameters among neonates: APGAR scores, neonatal intensive care unit (NICU) admissions, the need for non-invasive or invasive mechanical ventilation (MV), and neonatal death. We then analyzed mothers' and newborns' demographic data and characteristics from the electronic medical records. Multiple pregnancies involving triplets or more and twins delivered through the vaginal route were excluded. All neonates were examined by a neonatologist in the operating room after delivery.

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General anesthesia was applied to patients with emergency Category 1 (which means "immediate threat to the life of the mother or baby")[10], when there was not enough time for regional anesthesia. Spinal anesthesia was administered through the L3-4 or L4-5 interspinous space with a 25G Quincke spinal needle by injecting 10–12 mg of hyperbaric bupivacaine, following the free flow of the cerebrospinal fluid. Ephedrine (5–10 mg) was administered intravenously if the blood pressure fell 20% or more below the baseline. Propofol (2–2.5 mg/kg) and rocuronium (0.6–1 mg/kg) were used for induction. Fentanyl (1 μg/kg) was administered immediately after clamping the cord, and then the anesthesia was continued with sevoflurane (1–2%) in an oxygen and air mixture.

Maternal age, predelivery body mass index (BMI), parity, gestational age at delivery, emergency of the cesarean section, anesthesia method, use of intraoperative antihypotensive agents (ephedrine), preoperative and intraoperative use of blood products, and neonatal weight percentile distribution for a given gestational age at delivery were considered as independent variables affecting neonatal outcomes. The primary outcome of the study was to ascertain the effects of independent factors on poor outcomes in newborns. A newborn was considered to have a poor outcome if any of the following variables were present: 1st and 5th minute APGAR scores<7, NICU admission, need for non-invasive or invasive MV, or neonatal mortality within the first 28 days after birth.

The data of the neonates included in the correlation analysis and multivariate logistic regression models were dichotomized as 0 (none of the twins) or 1 (at least one of the twins). The neonatal weight percentile distribution for a given gestational age at delivery was categorized as <3rd weight percentile or ≥3rd weight percentile. The APGAR scores at the 1st and 5th minutes were dichotomized as APGAR scores <7 or ≥7.

The statistical data were analyzed using the SPSS Statistics for Windows, version 19.0, 2010 (IBM Corp., Armonk, NY). The normality of the distribution was analyzed with the Shapiro-Wilk test. The patients' demographic and clinical characteristics are presented as median (min–max) and frequency (proportion). Pearson's chi-squared test was used to compare the categorical variables, and the Mann-Whitney U test was performed to compare the continuous variables. A nonparametric Spearman test was conducted to determine the associations between maternal and neonatal factors. Accordingly, a logistic regression model was built, and a multivariate analysis was performed for each significant factor influencing neonatal outcomes as determined by the correlation analysis. After the multicollinearity analysis (tolerance>0.4), the Hosmer-Lemeshow test was run to check the model's fitness. The effect sizes are presented as odds ratios (OR) and 95% confidence intervals (CIs). All tests were performed with two-tailed, and $p<0.05$ was considered significant.

RESULTS

Between January 2017 and January 2020, 478 (86.7%) of 551 multiple pregnancies were delivered by cesarean section. Triplets and more (4 patients), and patients with a congenital anomaly in at least one of the twins (21 patients) that were delivered by cesarean section were excluded from the study. Accordingly, a total of 453 pregnant women and 906 newborns were included in the further analysis.

The general characteristics of the patients are presented in Table 1. The median gestational week at the time of delivery was 35 (min. 24 to max. 39) weeks.

Table 1 shows the intraoperative care characteristics and complications during pregnancy and delivery. Of 453 (68.9%), 312 patients underwent emergency cesarean section, and of 453 (81.5%), 369 women received spinal anesthesia. Six patients who received combined spinal and epidural anesthesia were included in the spinal anesthesia group because no additional drugs were administered through epidural catheters during the surgery. No maternal mortality was observed in the following postoperative 1-month period.

Table 2 shows the comparison of physical characteristics, APGAR scores at delivery, and data regarding poor outcomes for the first- and second-born twins. The second-born twins had a significantly lower birth weight than the first-born twins ($p=0.008$). Also, the number of neonates with <3rd weight percentile was significantly higher among the second-born twins than the first-born twins ($p=0.001$). However, we found no difference between the first- and second-born neonates in terms of poor outcomes. According to the correlation analysis, which was performed for fetal and maternal parameters that were considered to affect neonatal outcomes, early gestational weeks, emergency surgery, general anesthesia administration for cesarean section, and neonates <3rd weight percentile were correlated with the predetermined poor neonatal outcomes ($p<0.01$). Among these parameters, the early gestational weeks strongly correlated with NICU admissions ($r=0.566$) and the need for MV ($r=0.534$). Besides, early gestational weeks had a moderate correlation with the 1st and 5th minute APGAR scores ($r=0.430$ and 0.322, respectively) and neonatal mortality ($r=0.365$).

The significant parameters from the correlation test were included in the final regression model (Table 3). The logistic regression was repeated to ascertain the effects of spinal anesthesia, gestational weeks, emergency surgery, and neonates <3rd

Table 1. Patient characteristics and perioperative care characteristics.

Age, years; median (min–max)	28 (15–48)
Weight, kg; median (min–max)/ Height, cm; median (min–max)	80 (56–121)/160 (150–175)
BMI, kg m^{-2}; median (min–max)	31.2 (21.9–50.4)
Gravidity, n; median (min–max)/ Parity, n; median (min–max)	2 (1–10)/1 (0–9)
Gestational weeks, weeks; median (min–max)	35 (24–39)
Extreme preterm; 24 (+0) –27 (+7); n (%)	18 (4.0)
Very early preterm; 28 (+0)–31 (+7); n (%)	52 (11.5)
Early preterm; 32 (+0) –33 (+7); n (%)	51 (11.3)
Late preterm; 34 (+0)–36 (+7); n (%)	233 (51.4)
Early term and term; >37 (+0); n (%)	99 (21.9)
Platelet count, mcl; median (min–max)	202 (37–539)
Hemoglobin, g dL^{-1}; median (min–max)	11 (6.3–15)
Anemia; <11 g dL^{-1}; n (%)	197 (43.5)
Comorbidities; n (%); Thyroid disease	12 (2.7)
Hypertension	9 (2.0)
Diabetes mellitus	6 (1.3)
Other*	9 (1.9)
Perioperative care characteristics and complications during pregnancy and delivery.	
Surgical admission; n (%); Emergent/ Elective	312 (68.9)/141 (31.1)
Anesthesia method; n (%); Spinal/ General	369 (81.5)/84 (18.5)
Intraoperative ephedrine use; n (%)	147 (32.5)
Postoperative follow-up; n (%); Ward/ ICU	430 (94.9)/23 (5.1)
Complications during pregnancy and delivery; n (%)	
Preeclampsia	22 (4.9)
Gestational diabetes mellitus	18 (4.0)
Premature rupture of membranes	7 (1.6)
Other**	22 (4.9)
Duration of surgery, min; median (min–max)	50 (30–90)
Intraoperative bleeding, mL; median (min–max)	300 (100–1200)
Blood products; n (%)	53 (11.7)
Hospital stay time, days; median (min–max)	3 (1–13)

BMI: body mass index. Other*: Familial Mediterranean fever, facial paralysis, epilepsy, chronic respiratory disease. ICU: intensive care unit. Other**: uterine atony, uterine rupture, rectus sheath hematoma, placental abruption, vaginal bleeding, cholestasi.

weight percentile on each individual dependent factor: APGAR 1st minute <7, APGAR 5th minute <7, NICU admission, the need for MV, and neonatal death. The model for APGAR 1st minute <7 correctly classified 90.7% of the cases with a specificity of 97.7% (R^2=0.470); the model for APGAR 5th minute <7 correctly classified 95.1% of the cases with a specificity of 98.4% (R^2=0.452); the model for NICU admission correctly classified 81.7% of the cases with a specificity of 89.8% (R^2=0.542); the model for the need for MV correctly classified 87.6% of the cases with a specificity of 95.6% (R^2=0.558); and the model for neonatal death correctly classified 94.7% of the cases with a specificity of 98.1% (R^2=0.499). Early gestational weeks and neonates <3rd weight percentile at the time of delivery were found to be the most significant predictors of all poor outcome parameters in at least one of the twins ($p<0.05$). General anesthesia was associated with APGAR 1st minute <7 and the need for MV ($p<0.05$), and emergency surgery showed an association with the need for MV ($p<0.05$).

Table 2. Characteristics of the twins.

	1st twin	2nd twin	p
Weight; median (min–max)	2320 (450–3680)	2200 (470–3650)	0.008*
Height; median (min–max)	46 (24–52)	46 (24–54)	0.057
Gender; n (%)			
Women/man	230 (50.8)/223 (49.2)	233 (51.4)/220 (48.6)	0.894
Weight percentiles; n (%)			
<3rd	45 (9.9)	85 (18.8)	0.001*
3rd–10th	64 (14.1)	56 (12.4)	
>10th	344 (75.9)	312 (68.9)	
APGAR scores <7; n (%)			
1st minute	36 (7.9)	43 (9.5)	0.480
5th minute	15 (3.3)	15 (3.3)	1
APGAR scores <7 in general anesthesia (n=84); n (%)			
1st minute	15 (17.9)	17 (20.2)	0.694
5th minute	8 (9.5)	7 (8.5)	0.073
NICU admission; n (%)	176 (38.9)	193 (42.6)	0.279
Non-invasive and invasive MV; n (%)	74 (16.3)	79 (17.4)	0.723
Intubated in the operating room; n (%)	50 (8.8)	48 (10.6)	0.432
Died within the first 28 days; n (%)	20 (4.4)	24 (5.3)	0.536

*p<0.05; MV: mechanical ventilation; NICU: neonatal intensive care unit.

Table 3. Logistic regression analysis of the significant independent factors.

	Spinal anesthesia	Gestational weeks	Emergent surgery	<3rd percentile
APGAR 1st min <7				
OR, [95%CI]	0.356, [0.163, 0.776]	0.622, [0.553, 0.699]	1.621, [0.554, 4.739]	3.778, [1.744, 8.185]
Wald	6.744	62.952	0.779	11.355
p	0.009*	0.000*	0.378	0.001*
APGAR 5th min <7				
OR, [95%CI]	0.530, [0.183, 1.537]	0.636, [0.551, 0.734]	2.647, [0.315, 22.257]	5.678, [1.957, 16.473]
Wald	1.365	38.268	0.803	10.212
p	0.243	0.000*	0.370	0.001*
NICU				
OR, [95%CI]	0.925 [0.488, 1.754]	0.455, [0.384, 0.539]	1.456, [0.837, 2.533]	7.144, [3.898, 13.093]
Wald	0.057	82.913	1.769	40.461
p	0.811	0.000*	0.184	0.000*
MV				
OR, [95%CI]	0.420, [0.202, 0.875]	0.563, [0.493, 0.642]	4.178, [1.446, 12.075]	5.699, [2.785, 11.661]
Wald	5.364	73.524	6.975	22.691
p	0.021*	0.000*	0.008*	0.000*
Death				
OR, [95%CI]	0.772, [0.271, 2.196]	0.591, [0.510, 0.684]	1.391, [0.281, 6.896]	5.263 [1.934, 14.321]
Wald	0.236	49.641	0.163	10.573
p	0.627	0.000*	0.686	0.001*

*p<0.05. CI: confidence interval; min: minute; MV: mechanical ventilation; NICU: neonatal intensive care unit; OR: odds ratio.

DISCUSSION

The main finding of this study was that general anesthesia administration for cesarean section, early gestational weeks, emergency surgery, and neonates <3rd weight percentile were the strongest predictors for any of the poor neonatal outcomes in at least one of the twins delivered by cesarean section.

Planned cesarean section was found to be associated with increased poor neonatal outcomes between the 32nd and 37th gestational weeks[8,11-13]; on the contrary, cesarean section was suggested to be safer after 37 weeks of gestation[14]. Despite this evidence, the cesarean section rate for twin pregnancies is still very high, which may be due to preconceptions that a cesarean section may prevent inevitable complications and medico-legal issues[12].

Yielding data suggests using regional anesthesia for cesarean sections for better neonatal outcomes[15,16]. Theoretically, prolonging the inter-delivery interval during general anesthesia could have worsened the APGAR scores of second-born twins; however, in contrast to our expectations, no significant difference was found between the first and second-born twins in terms of APGAR scores. On the contrary, when all twins were taken into account, general anesthesia was positively correlated with low 1st and 5th minute APGAR scores, a higher need for MV and NICU admissions, and a higher neonatal mortality rate. Regional anesthesia was the first choice for obstetrical anesthesia. However, general anesthesia was mainly applied to patients with emergency Category 1. Thus, the reason for poor outcomes related to general anesthesia is more likely associative than causative.

Neonatal near-misses enable identifying the group of newborns who have a high risk of death due to morbidity but who survive the first 27 days of life under these conditions. Generally, the criteria of birth weight <1,750 g, 5th minute APGAR score <7, and gestational age <33 (+7) weeks were recommended for defining neonatal near-miss[17]. Previous studies found a relationship between advanced maternal age and neonatal near-miss in nulliparous and multiparous women[6]. In addition, it was stated that neonatal near-miss risk in twins was associated with parity, an early gestational week, and intrauterine growth restriction[18]. Although maternal age affects the prevalence of twins, it does not appear to affect twin pregnancy outcomes; furthermore, the preterm birth risk was higher among younger mothers[19]. In the present study, we observed that maternal age, parity, and predelivery BMI did not affect neonatal outcomes. Also, general anesthesia administration, early gestational weeks, emergency surgery, and neonates <3rd weight percentile at the time of delivery were the strongest predictors of any of the poor neonatal outcomes.

Spontaneous or medically indicated preterm birth complicates twin pregnancies[1]. Fetal lung maturation is mostly completed in the 32 weeks of gestation; births before 32 weeks have high rates of perinatal morbidity and mortality; and preterm infants from multiple births are at increased risk compared with singletons born at the same gestational age[4,5,17]. In the present study, early gestational weeks were strongly correlated with the need for MV and NICU admissions. Early gestational weeks also showed a moderate correlation between 1st and 5th minute APGAR scores and neonatal death. Therefore, efforts should be intensified to prevent avoidable twin pregnancy complications like preterm labor to achieve better neonatal outcomes.

Previous studies suggested that second-born twins had worse outcomes than first-born twins regardless of the route of delivery[2]. Besides, Luo et al. attributed the increased mortality risk of the second-born twin to their relatively smaller birth weight than the first-born twin[20]. We also found that the second-born twins had significantly lower weight percentiles and median birth weights compared to the first-born twins. While neonates <3rd weight percentile was one of the strongest factors influencing poor neonatal outcomes, we could not find a significant difference in poor outcomes between first- and second-born twins in terms, contrary to a previous study.

The main limitations of this study were the lack of data regarding assisted reproductive techniques and chorionicity. Therefore, neonates were evaluated according to their birth weight and neonatal weight percentile distribution for a given gestational age at delivery.

One strength of our research is that the number of patients included in the analysis is sufficient to show the correlation between the predetermined factors.

CONCLUSION

This study evaluated the factors associated with poor neonatal outcomes (such as low APGAR scores, NICU admissions, the need for MV, and neonatal death) among twins delivered by cesarean section. Our findings revealed that general anesthesia administration for cesarean sections, emergency surgeries, low weight percentiles, and early gestational weeks was correlated with the aforementioned variables of poor neonatal outcomes in at least one of the twins. Still, the order of birth did not affect neonatal outcomes.

AVAILABILITY OF DATA AND MATERIAL

All the data generated or analyzed during this study are included in this article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval for this study (Ethical Committee protocol No. 2011-KAEK-25) was provided by the Ethical Committee of Bursa Yuksek Ihtisas Training and Research Hospital in Bursa, Turkey. The study was registered at www.clinicaltrials.gov under the number NCT05104255.

AUTHORS' CONTRIBUTIONS

NK: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **HG:** Conceptualization, Writing – original draft, Formal Analysis, Methodology. **UK:** Conceptualization, Data curation, Investigation, Methodology. **DK:** Conceptualization, Methodology, Writing – review & editing. **FNT:** Data curation, Formal Analysis, Investigation. **MG:** Writing – review & editing.

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ORIGINAL ARTICLE

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Relationship between villous atrophy and Wnt pathway gene expressions in pediatric celiac patients

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SUMMARY

OBJECTIVE: Celiac disease is an autoimmune disease characterized by an abnormal immune response occurring in the small intestine linked to consumption of food containing gluten in individuals with a genetic predisposition. Dysregulation of Wnt signal transduction plays a role in the pathogenesis of many diseases including autoimmune diseases like celiac disease. In this study, the correlation of Wnt pathway gene expressions with each other and the correlation with clinical data were researched in pediatric celiac disease cases grouped according to the Marsh classification.

METHODS: Gene expression levels of *FZD8*, *DVL2*, *LRP5*, *RHOA*, *CCND2*, *CXADR*, and *NFATC1*, which are involved in the Wnt pathway, were determined using quantitative real-time polymerase chain reaction in 40 celiac disease and 30 healthy individuals.

RESULTS: All cases with the short height symptom were observed to be in Marsh 3b\3c groups (p=0.03). The gene expressions of *DVL2*, *CCND2*, and *NFATC1* were high in the Marsh 3b group, and these genes showed positive correlation with each other (p=0.002). *LRP5* and *CXADR* gene expressions were lower in the Marsh 3b group compared to other Marsh groups, and these genes showed a positive correlation with each other (p=0.003). *CCND2* gene expression was associated with Marsh 3b group, diarrhea, and vomiting symptoms. *DVL2* gene expression was correlated with Marsh 2 group and constipation symptom (p<0.05).

CONCLUSION: Wnt signaling in the early stages of the disease of Marsh 1–2 involves high expression of *LRP5* and *CXADR* genes, while expression of these two genes reduces, and *DVL2*, *CCND2*, and *NFATC1* gene expressions clearly increase with a transduction variation observed from Marsh 3a stage when villous atrophy begins to form. It appears that the Wnt pathway may contribute to disease progression through expression changes.

KEYWORDS: Celiac disease. Wnt signaling pathway. Gene expression. Autoimmune diseases.

INTRODUCTION

Celiac disease (CD) is an autoimmune disease occurring with infection and chronic atrophy of the small intestine linked to intake of foods containing gluten and some prolamins by individuals with genetic predisposition. Generally, symptoms in children are growth and development retardation, chronic diarrhea, loss of appetite, abdominal bloating, malabsorption, and gastrointestinal irregularities. Disease symptoms may begin at 6 months of age. CD diagnosis is made by observing human leukocyte antigen *(HLA)-DQ2 HLA-DQ8* haplotypes of HLAs, antibodies specific to CD, and the presence of enteric atrophy[1,2]. Playing an important role in many autoimmune diseases including CD, the Wnt signaling pathway participates and regulates many biological processes like cell proliferation, differentiation, regulation of transcription of a variety of target genes, and cell adhesion in both embryonic and adult periods[3,4]. Wnt signal transduction begins with frizzled (FZD) transmembrane receptors and low-density lipoprotein receptor-related protein (LRP) coreceptors triggering canonic and noncanonic signal transductions[5,6]. With FZD mediation, noncanonic Wnt signals activate disheveled segment polarity protein (DVL)-dependent Ras homolog family member A (RHOA)-ROCK, G-protein-dependent calcineurin-nuclear factor of activated T cells (NFAT), and RTK-dependent P13K-AKT[7]. The *Cyclin D2* (*CCND2)* gene on the Wnt signaling pathway interacts with cyclin-dependent kinases in the cell cycle, playing an important role especially in G1/S transition[8]. CXADR Ig-like cell adhesion molecule (CXADR) is effective in cell adhesion via β-catenin inactivation[9]. Abnormalities in Wnt signal transduction play roles in pathogenesis of many diseases. Therefore, in this case-control study, it was aimed to determine the correlations of *FZD8*, *DVL2*, *LRP5*, *RHOA*, *CCND2*, *CXADR*, and *NFATC1* gene expressions with disease symptoms and Marsh classification, as well as with *HLA-DQ2/8* haplotypes and other clinical data.

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METHODS

Study subjects and ethics

The study included a total of 70 children attending the Haseki Education Research Hospital Pediatric Gastroenterology Clinic. Of these children, 40 received diagnosis of CD and 30 had normal gastrointestinal endoscopy results. The diagnosis of CD was made using the criteria of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[1] according to the results of the histopathological examination of the endoscopic tissues of the cases and the Marsh classification. The age, gender, clinical findings, hematological, and biochemical parameters at diagnosis along with symptoms and signs were recorded. Patients with other chronic gastrointestinal system diseases such as inflammatory bowel disease and autoimmune diseases other than chronic gastrointestinal system disease were excluded from the study. Ethics permission was received from Aydın Adnan Menderes University Non-Interventional Clinical Research Ethics Committee. Volunteers were informed about the study and were included after providing consent forms before the study. This study was completed in accordance with the standards determined by the ethics committee and the Declaration of Helsinki.

DNA isolation

Peripheral blood samples were used to isolate genomic DNA according to the manufacturer's instructions (Qiagen, Hilden, Germany). DNA concentration and purity were determined by the absorbance value at 260 nm (A260) and the ratio of A260/A280, respectively, using a spectrophotometer (NanoDrop, Thermo Scientific, USA).

Detecting *HLA-DQ* genotypes

The primary susceptibility genotype for CD is *HLA-DQ2* consisting of *HLA-DQA1*05* and *DQB1*02*. The remainder of the cases were associated with *HLA-DQ8* consisting of *HLA-DQA1*03* and *DQB1*03:02*. Case DNA samples were genotyped according to the sequence-specific primers-polymerase chain reaction (SSP-PCR) method for *HLA-DQA1* and *DQB1* according to the manufacturer's instructions (Olerup SSP® DQ low-resolution AB, Sweden). The commercial diagnostic kit includes 22 primer mixes and one negative control for the *DQA1* and *DQB1* alleles. The typing was interpreted with the lot-specific interpretation and specificity tables from kit.

RNA isolation and cDNA synthesis

Sections taken from FFPE blocks belonging to volunteers had RNA isolation completed using a RNeasy FFPE Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Complementary DNA (cDNA) synthesis was completed using an RT2 First Strand Kit (SA Bioscience, Frederick, MD, USA) in line with the manufacturer's instructions.

Q-PCR primer assay

Expression levels for seven genes acting on the Wnt signaling pathway of *FZD8*, *DVL2*, *LRP5*, *RHOA*, *CCND2*, *CXADR*, *NFATC1*, and hypoxanthine phosphoribosyl transferase 1 (*HPRT1*) as a housekeeping gene were determined using a Rotor-Gene 3000 (Corbett Research, Qiagen, Germany) device in accordance with the manufacturer's directions.

Data analysis

Normalization of expression data and data analysis were completed using an online data analysis robot offered by the manufacturer (https://geneglobe.qiagen.com/us/analyze). ΔΔCt method was used for the quantification of gene expression.

Statistical analysis

Demographic characteristics and clinical data were analyzed using IBM SPSS Statistics Version 25 (IBM Company, New York, USA) using the χ^2 test or Fisher-exact χ^2 and correlation tests. ΔCt values for each gene were calculated based on a Student's t-test. $p<0.05$ was considered statistically significant.

RESULTS

There were no statistical differences in terms of age distribution between the CD group (10.71±5.63) and control group (11.03±5.49) ($p>0.05$). In the CD group, the proportion of boys was 37.5% and that of girls was 62.5%, while in the control group the proportion of boys was 40% and that of girls was 60% (Table 1).

In CD cases, 75% had *HLA-DQ2* and 15% had *HLA-DQ8*, with 10% having both *HLA-DQ2* and *HLA-DQ8*. Among cases, 90% had abdominal pain, 65% had an inability to gain weight, 55% had anemia, 30% had short height, 25% had constipation, 20% had diarrhea, and 10% had vomiting symptoms (Table 1). All cases with the short height symptom were in the Marsh 3b and 3c groups ($p=0.031$). Although not statistically significant, it was observed that the inability to gain weight and anemia symptoms intensified in the Marsh 3b and 3c groups (Table 2).

LRP5 and *CXADR* gene expressions were higher in Marsh 1-2 and 3a groups compared to controls, and displayed a clear reduction in the Marsh 3b group. Expression of these genes

showed a significant positive correlation in terms of Marsh classification (p=0.002). Expressions of *DVL2*, *CCND2*, and *NFATC1* genes were close to or below controls in the Marsh 1-2 and 3a groups, with a pronounced elevation in the Marsh 3b group. These gene expressions showed significant positive correlation in terms of Marsh classification (*DVL2-CCND2* p=0.004), (*DVL2-NFATC1* p<0.001), and (*CCND2-NFATC1* p<0.001). *CCND2* and *DVL2* genes displayed statistically significant expression in terms of Marsh classification (p=0.04 and p=0.04, respectively). *CXADR* gene expression showed statistically significant negative correlation with the expression of *DVL2*, *CCND2*, and *NFATC1* genes (p=0.026, p=0.006, and p=0.038, respectively). *LRP5* gene expression displayed a statistically significant negative correlation with *DVL2* and *CCND2* gene expressions (p=0.011 and p=0.008, respectively); however, there was no statistically significant correlation with *NFATC1* gene expression (p=0.068) (Figure 1).

Table 1. Demographic features.

Characteristics	Group	Number of patients	Percentage
Gender	Female	25	62.5
	Male	15	37.5%
Age	2–12	19	47.5
	13–20	21	52.5
HLA	DQ2/-	30	75
	DQ8/-	6	15
	DQ2/DQ8	4	10
Marsh classes	Marsh 1–2	8	20
	Marsh 3a	8	20
	Marsh 3b	10	25
	Marsh 3c	14	35
Symptom	Abdominal pain	34	85
	Inability to gain weight	26	65
	Anemia	22	55
	Short height	12	30
	Constipation	10	25
	Diarrhea	8	20
	Vomiting	4	10

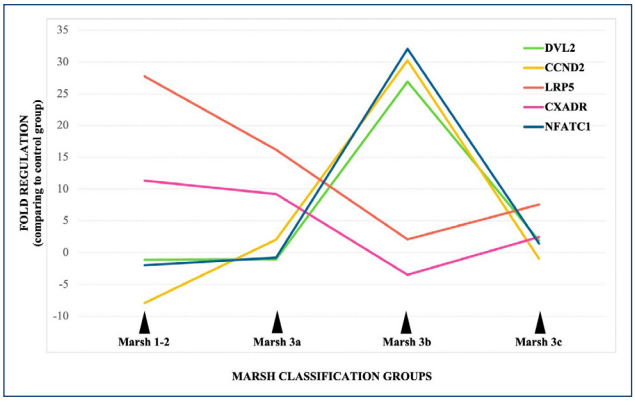

Figure 1. Fold regulations of gene expressions comparing to the control group. The changes in gene expressions in Marsh groups compared to the control group and their correlations with each other are shown. It is observed that some genes act together in the formation of mucosal damage. *DVL2*, *CCND2*, and *NFATC1* gene expressions act together in the formation of mucosal damage and are expressed at the highest level in the Marsh 3b group. *LRP5* and *CXADR* genes also act together and are expressed high level in Marsh 1 and 2 groups, which are the initial stages of mucosal damage, and decrease to their lowest levels in Marsh 3b stage.

Table 2. Comparison of Marsh classification by symptoms and HLA-DQ haplotype.

Total n=40	Marsh 1–2 (n=8)	Marsh 3a (n=8)	Marsh 3b (n=10)	Marsh 3c (n=14)	p-value
Abdominal pain (n=34)	6	7	9	12	0.668
İnability to gain weight (n=26)	4	4	8	10	0.176
Anemia (n=22)	2	4	8	8	0.106
Short height (n=12)	0	0	5	7	0.031*
Constipation (n=10)	2	1	3	4	0.711
Diarrhea (n=8)	1	2	3	2	1.000
Vomiting (n=4)	0	1	2	1	0.638
HLA-DQ2 (n=30)	6	6	7	11	0.998
HLA-DQ8 (n=6)	1	1	2	2	0.998
HLA-DQ2/DQ8 (n=4)	1	1	1	1	0.998

*Significant p<0.05.

In terms of disease symptoms, *CCND2* gene expression was associated with vomiting and diarrhea symptoms at statistically significant levels (p=0.001 and p=0.028, respectively). *DVL2* gene expression was found to be associated with the constipation symptom at a statistically significant level (p=0.003).

DISCUSSION

In our study, there were higher rates for the female sex, similar to the literature[10-12]. Similar to the literature, the *HLA-DQ2* rate was identified to be dominantly higher[10,12,13]. Symptoms like chronic diarrhea, abdominal pain, and growth retardation in addition to vomiting, constipation, and anemia were observed in our cases, similar to the literature[2,14]. The finding of short height was identified in all cases in the Marsh 3b and 3c groups, and similarly, cases with lack of weight gain and anemia were observed more intensely in these two groups compared to the other groups. We think these symptoms may be caused by disruption of small intestine tissue function in these two Marsh groups, where the highest levels of villous atrophy and crypt hyperplasia are observed.

When our study is examined in terms of the detected gene expressions, expression levels among cases in the Marsh 3c class were identified at levels close to those of the control group. We think this may be due to Wnt signaling returning to normal levels after completing the task of total atrophy of villous and intense hyperplasia in crypts. In our study, three genes had increased expression (*DVL2*, *CCND2*, and *NFATC1*), and two genes had reduced expression (*LRP5* and *CXADR*) from Marsh 3a class on, when villous atrophy and crypt hyperplasia began to occur. These data create the idea that the Wnt signaling pathway responds to increased lymphocyte infiltration at the onset of disease by displaying an expression pattern where *LRP5* and *CXADR* genes are effective. With the continuation of pathological status, cells adapt to the situation with *DVL2*, *CCND2*, and *NFATC1* gene expressions on the Wnt signal gaining efficacy and the Wnt signal transduction pattern causing villous atrophy and crypt hyperplasia being adopted. When we examine the literature, we think small intestinal cells may show expression on the LRP5/β-catenin/CCND1 axis for renewal against stress caused by lymphocyte infiltration induced by the immune response[15], while *CXADR* gene expression activates CDC42, supporting cell adhesion[9]. Increased cell renewal and adhesion may have begun to disrupt the villous architecture by slowing the migration of cells toward the villous tip. In the Marsh 3a class where villus atrophy formation starts, it is seen that the expression of these two genes started to decrease, and the expression of *DVL2*, *CCND2*, and *NFATC1* genes started to increase. There is a strong correlation between these genes because they are similarly negatively correlated in Marsh 3b class. This suggests that they may be involved in the regulation of each other and that there may be a Wnt signal transduction pattern specific to villus atrophy. *NFATC1* gene expression has an important role in the non-canonical Wnt signal pathway of Wnt/Ca^{+2} signal transduction and especially comes to the agenda during embryogenesis[16] and additionally undertakes important duties in the immune response induced by T cells and the activation of B cells[17]. We think the high *NFATC1* gene expression we identified in Marsh 3b cases is due to T cells and causes induction of the immune response at high levels. *CCND2* gene expression is known to suppress the G1/S stage of the cell cycle, stopping proliferation and allowing the opportunity for differentiation[18]. *CCND2* gene expression at high levels in our Marsh 3b cases brings to mind the formation of villous atrophy as a result of suppression of proliferation. On the contrary, *DVL2* expression increases cell proliferation in crypts and is known to regulate tight junctions directly[19]. Increased tight junctions and dysregulated proliferation may be effective in the formation of crypt hyperplasia.

Limitations

Studies with higher case numbers including gene expression will support our findings and contribute to understanding molecular mechanisms of disease and creation of treatment targets.

CONCLUSION

We think that all these gene expressions act in accordance with a certain order to create pathogenesis in small intestinal tissue. Our findings suggest that villus atrophy and crypt hyperplasia occur as a result of increased activity of the non-canonical Wnt pathway, which plays a role in cytoskeleton and cell adhesion. Suppression of the noncanonical Wnt pathway can be considered a treatment strategy to prevent small intestine tissue damage.

AUTHORS' CONTRIBUTIONS

MC: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **GD:** Resources, Writing – review & editing. **SOB:** Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Repeated adolescent pregnancy in Brazil from 2015 to 2019

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SUMMARY

OBJECTIVE: The aim of this study was to assess the rate of repeated pregnancy in adolescence and its association with early marriage and education level.

METHODS: This is a cross-sectional study conducted by searching the Live Births Data System. The study included all adolescents in the age group 10–19 years with live births from 2015 to 2019 (n=2,405,248), divided into three groups: G1: primiparas; G2: with 1 previous pregnancy; and G3: with two or more previous pregnancies.

RESULTS: Total repeated pregnancies remained stable, along the years. In the age group 10–14 years, the decrease in the period was from 5.0 to 4.7%, whereas in the age group 15–19 years, it was from 27.8 to 27.3%. Being married or in a stable union increases by 96% the chance of repeated pregnancy in the age group 10–14 years (p<0.001; OR=1.96; 95% confidence interval [CI] 1.85–2.09). In the age group 15–19 years, the chance of repeated pregnancy among the married or in stable union increased 40% (p<0.001; OR=1.40; 95%CI 1.39–1.41)). Girls aged 10–14 years with an education level of<8 years had a 64% higher chance of repeated pregnancy (p<0.001; OR=1.64; 95%CI 1.53–1.75), and among those aged 15–19 years, there was a 137% higher chance of repeated pregnancy (p<0.001; OR=2.37; 95%CI 2.35–2.38).

CONCLUSION: Repeated pregnancy in adolescence in Brazil remains very high over the years. There is an association between low education level and early marriage with repeated pregnancies in adolescence.

KEYWORDS: Pregnancy. Adolescent. Recurrence. Maternal age.

INTRODUCTION

Repeated pregnancy in adolescence is defined as a new pregnancy in the age group 10–19 years, being considered fast when a second delivery or a new pregnancy occurs within 2 years from the last pregnancy[1]. This is a matter of concern because it increases the risk for materno-fetal health[2].

In the United States of America (USA), approximately 12–49% of adolescent repeated pregnancies occur within 1 year of the previous pregnancy[3], reaching 63% within 18 months. Among girls with repeated pregnancy, two-third reported that it was a unplanned pregnancy[4-6].

The younger the adolescent mother is, the greater the socioeconomic vulnerability and the materno-fetal complications[2,6,7]. Experiencing another delivery before the age of 20 years may lead to unfavorable perinatal outcomes in a higher proportion than in the first childbirth. Repeated pregnancy in adolescence is more common in contexts of poverty, low education level, sexual initiation before the age of 15 years, early union, no use of effective contraceptive methods, and previous abortion or dead fetus birth[8-10].

As a single event, pregnancy in adolescence causes an important impact in the life of the adolescent and her family. Comparing with the first pregnancy, repetition leads to a greater risk of preterm birth, low-weight birth, greater perinatal and neonatal mortality, and child developmental disorders[5-7]. This reflects the lack of capacity of health systems to supply the basic needs of health education and social well-being of adolescents following the first pregnancy. Because of the effects throughout life, it is essential to identify the causes of repeated pregnancy in adolescence in order to develop appropriate prevention strategies to reduce its occurrence[9].

The aim of this study was to assess repeated pregnancy among Brazilian adolescents in the period from 2015 to 2019 and its association with marital status and educational level.

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METHODS

This is a cross-sectional study conducted with data obtained from the Live Births Data System (SINASC), through the server of the Informatics Department of the Unified Health System (DATASUS) with the purpose of gathering epidemiological data on informed births in the national territory[11]. The following variables were used to perform this study: birth according to the mother's place of residence, birth according to region of the country, year of birth, maternal age, and number of pregnancies.

The variable that assesses the number of previous pregnancies is named QTDGESTANT in the DATASUS database. The download of data from SINASC was performed from the DATASUS page for the years from 2015 to 2019 (Brazil, DATASUS). For each year and Federative Unity, there is a file in dBase File Compacted (*.dbc) format, which was converted into dBase File (*.dbf) via batch script (.bat) using the application TabWin developed by DATASUS (Brazil, DATASUS). Since the database comprises millions of registers, it was necessary to use the Database Management System (DBMS) to analyze and manipulate such a large amount of data. The option was for DBMS open source PostgreSQL, version 11.8. Data were imported into PostgreSQL from DBF files by means of scripts developed in Python language, version 3.8.

The study encompassed all women in the age group 10–19 years who had live births (LB) in Brazil in the period 2015–2019, with data available in the SINASC database (n=2,405,248). Data obtained included the total number of LB in age groups 10–14 and 15–19 years, to calculate LB rate of repeated pregnancies among adolescent mothers. The adolescents were placed in three groups: group 1: primiparas; group 2: with one previous pregnancy (second pregnancy); and group 3: with two or more previous pregnancies. There was also the assessment of sociodemographic data referring to marital status and education level of adolescent mothers, with the purpose of relating them to the prevalence of repeated pregnancy. Data were analyzed by the Epi-Info 3.5.4 software.

The research project that resulted in this article was sent to Plataforma Brasil, received the number CAAE 04209418.1.0000.5259, and was approved by the Research Ethics Committee of Pedro Ernesto University Hospital of Rio de Janeiro State University (UERJ).

RESULTS

The total of repeated pregnancies remained stable along the years. In the age group 10–14 years, the decrease in the period was from 5.0 to 4.7%, whereas in the age group 15–19 years, there was a decrease from 27.8 to 27.3% (Table 1). Repeated pregnancy two or more times among all adolescents presented a slight decrease (from 5.9% in 2015 to 5.5% in 2019) with higher frequency in the age group 15–19 years (Table 2).

Among adolescents aged from 10 to 14 years, in group 1 (primiparas), 19.1% were married or in a consensual union, and 63.6% had an education level lower than 8 years. In group 2 (second pregnancy), the rates were 31.1 and 73.3%, and in group 3 (two or more previous pregnancies), the rates were 35 and 70.1%, respectively. Among adolescents aged from 15 to 19 years, in group 1, 31.6% were married or in a consensual union, and 22.7% had an education level<8 years. In group 2, these rates were 38.4 and 38.0%, and in group 3, 42 and 51.7%, respectively (Table 3). It was observed that as the number of pregnancies increases, the same occurs with the rates of early marriage and low education level.

Being married or in a stable union increases the chance of repeated pregnancy by 96% in the age group 10–14 years (p<0.001; OR=1.96; 95% confidence interval [CI] 1.85–2.09) and by 40% (p<0.001; OR=1.40; 95%CI 1.39–1.41) in the age group 15–19 years.

Girls aged 10–14 years with an education level <8 years had a 64% higher chance of repeated pregnancy (p<0.001; OR=1.64; 95%CI 1.53–1.75), whereas in the age group 15–19 years, there was a 137% higher chance of repeated pregnancy (p<0.001; OR=2.37; 95%CI 2.35–2.38) (Table 3).

DISCUSSION

This is the first study to present data on repeated pregnancy in adolescence in the entire country, using official current data obtained from the full SINASC database of the Ministry of Health[11]. The available studies conducted until the present time are punctual and focused on the reality of the researchers' own states.

Despite the decrease of 37.2% in the frequency of adolescent pregnancy in the past years[12], repeated pregnancy does not occur in the same way. It can be verified that in Brazil, between 2015 and 2019, there was no significant decrease in repeated pregnancy.

It is observed that the occurrence of repeated pregnancy remains a great challenge in Brazil, as well as in several other countries.

In Uruguay, despite a 50% decrease in the rate of adolescent pregnancy (from 72 to 36‰)[13], repeated pregnancy in adolescents aged<15 years is maintained at 20%, and as the age raises, there is an increase in the number of planned pregnancies[14]. In Chile, there was a decrease from 57.5‰ in 2010 to 26.5‰ in 2017[13]; however, repeated pregnancy is an issue

Table 1. Distribution of repeated pregnancy among adolescents in the period 2015 to 2019 in Brazil.

Year	Repeated pregnancy age 10–14	%	Total births age 10–14	Repeated pregnancy age 15–19	%	Total births age 15–19
2015	1,146	5.0	23,016	129,969	27.8	467,583
2016	1,144	5.4	21,272	121,922	28.0	436,010
2017	1,064	5.3	19,988	118,698	27.9	426,104
2018	920	4.7	19,472	112,882	27.5	410,616
2019	849	4.7	17,942	103,495	27.3	379,723
Year	Age 10 to 14 years					
	Primiparas		1 previous pregnancy		2 or more previous pregnancies	
	Freq. (%)		Freq. (%)		Freq. (%)	
2015	21,870 (95.0)		1,058 (4.6)		88 (0.4)	
2016	20,128 (94.6)		1,058 (5.0)		86 (0.4)	
2017	18,924 (94.7)		980 (4.9)		84 (0.4)	
2018	18,552 (95.3)		847 (4.3)		73 (0.4)	
2019	17,093 (95.3)		786 (4.4)		63 (0.4)	
	Age 15 to 19 years					
2015	337,614 (72.2)		104,466 (22.3)		25,503 (5.5)	
2016	314,088 (72.0)		98,141 (22.5)		23,781(5.5)	
2017	307,406 (72.1)		95,627 (22.4)		23,071(5.4)	
2018	297,734 (72.5)		91,315 (22.2)		21,567 (5.3)	
2019	276,228 (72.7)		84,034 (22.1)		19,461(5.1)	

Source: The authors.

Table 2. Distribution of marital status in adolescence per age group and association of marital status with repeated pregnancy in adolescence in Brazil (2015–2019).

Frequency of marital status			
Marital status	Age 10 to 14 years		
	Primiparas	1 previous pregnancy	2 or more previous pregnancies
	Freq. (%)	Freq. (%)	Freq. (%)
Single/widow/separated	76,916 (79.6)	3.172 (70.1)	250 (63.5)
Married/consensual union	18,416 (19.1)	1,470 (31.1)	138 (35.0)
Ignored	1,235 (1.3)	87 (1.8)	6 (1.5)
	Age 15 to 19 years		
Single/widow/separated	1,033,401 (67.4)	285,680 (60.4)	64,176 (56.6)
Married/consensual union	484,865 (31.6)	182,068 (38.4)	47,640 (42.0)
Ignored	14,804 (1.0)	5,835 (1.2)	1,614 (1.4)
Association of marital status with repeated pregnancy			
	Repeated pregnancy – age 10 to 14 years		
Marital status		**Yes**	**No**
Single/widow/separated		1,608	18,416
Married/consensual union		3,422	76,916
Total		5,030	95,332
p<0.001; OR=1.96; 95%CI (1.85–2.09)			
	Repeated pregnancy – age 15 to 19 years		
Marital status		**Yes**	**No**
Single/widow/separated		229,708	484,865
Married/consensual union		349,856	1,033,401
Total		579,564	1,518,266
p<0.001; OR=1.40; 95%CI (1.39–1.41)			

Source: The authors

Table 3. Distribution of education level in adolescence per age group and association of education level with repeated pregnancy in adolescence in Brazil (2015–2019).

Frequency of education level			
Education level	Age 10 to 14 years		
	Primiparas	1 previous pregnancy	2 or more previous pregnancies
	Freq. (%)	Freq. (%)	Freq. (%)
<8 years	61,394 (63.6)	3,465 (73.3)	276 (70.1)
8 years or more	33,841 (35.0)	1,155 (24.4)	105 (26.6)
ignored	1,332 (1.4)	109 (2.3)	13 (3.3)
	Age 15 to 19 years		
<8 years	347,632 (22.7)	180,162 (38.0)	58,628 (51.7)
8 years or more	1,166,350 (76.1)	285,855 (60.4)	52,641 (46.4)
ignored	19,088 (1.2)	7,566 (1.6)	2,161 (1.9)
Association of education level with repeated pregnancy			
		Repeated pregnancy – age 10 to 14 years	
Education level		Yes	No
<8 years		3,741	61,394
8 years or more		1,260	33,841
Total		5,001	95,235
p<0.001; OR=1.64; 95%CI (1.53–1.75)			
		Repeated pregnancy – age 15 to 19 years	
< 8 years		238,790	347,632
8 years or more		338,496	1,166,350
Total		577,286	1,513,982
p<0.001; OR= 2.37; 95%CI (2.35–2.38)			

Source: The authors.

that has not yet been resolved and is a challenge for public policies for adolescent health[15].

In the USA, repeated pregnancy in adolescence decreased from 53.8 to 16.9% between 2004 and 2015[16]. In 2017, 16.3% of North-American girls in the age group 15–19 years became pregnant again during adolescence[17].

In Australia and Canada, the prevalence of repeated pregnancy in adolescence is of 33 and 15.2%, respectively[18].

In Uganda, repeated childbirth in adolescence has not decreased in the past 30 years, and the average number of live births among women aged<20 years remains at 2.2[7].

Available specific data for some states of Brazil reveal that in Piauí, the prevalence of repeated pregnancy within the period of 2 years after the end of a pregnancy was 25.9% in the capital and 35.4% in the hinterland[19]. A study conducted in Ceará presented 61% of adolescent pregnancy 5 years after the first pregnancy, and 40% of them had become pregnant more than once within this period[20].

Early marriage is an important cause of a new pregnancy, because it provides family structure, which in many cases leads to nonregular use of contraception methods, hence the occurrence of a new pregnancy. Brazil is ranked fourth worldwide in absolute numbers, with more child marriages. In the country, 26% of the female population marries before the age of 18 years[21]. The Federal Government published Law No. 13.811/2019, which prohibits the marriage of adolescents younger than 16 years, aiming to minimize this problem, but it is not possible to avoid consensual unions[22].

Often, marriage and maternity are the way in which those adolescents are inserted in adult life and have a more important role in their families, thus creating a vicious circle of poverty and repeated pregnancy.

According to the World Health Organization, the problem of pregnancy in adolescence becomes greater as the adolescent's age decreases. The United Nations Population Fund showed that of 7.3 million pregnant adolescents worldwide,

2 million are aged less than 14 years. This situation may induce the aforementioned vicious circle of poverty and low education level, with a decrease of three times in the opportunity of achieving a university degree, and an income that is on average 24% lower than that of women who are the same age without children[23].

Confirming the findings of this research, Maravilla et al., in a meta-analysis, showed that continuing at school and having more years of education are protective factors for the prevention of repeated pregnancy in adolescence[9].

This situation was even more aggravated with the COVID-19 pandemic. It has been highlighted that schools were closed in 194 countries due to pandemic restrictions, thus increasing social inequality. In sub-Saharan Africa, approximately 1 million girls did not return to school due to pregnancy during the period of COVID-19 restriction measures[24].

Systematic reviews suggest that the most efficient strategies to prevent repeated pregnancy in adolescence are the promotion of access to highly effective methods, especially long-acting reversible contraceptives (LARC), which include hormonal intrauterine devices (IUD), copper IUD, and etonogestrel implants. This should be followed up by means of motivational interviewing conducted by skilled nurses, who provide individualized education on birth control options based on the adolescents' preferences, besides guiding them toward effective contraception[9,25]. The offer of LARC in the immediate postpartum results in a higher rate of permanence of its use at 3, 6, and 12 months, and the supply of LARC before hospital discharge increases the chances of effective contraception in the postpartum, significantly reducing repeated pregnancy in adolescence[3].

As limitations of this study, we point out the fact that it was conducted using SINASC data, which made it impossible to assess the interval between pregnancies, if it was a planned or unplanned pregnancy, if the new pregnancy was from the same partner, and previous abortion history. However, the finding of a high rate of repeated pregnancy in adolescence in Brazil highlights the dimension of the problem and the need for effective public policies for its reduction.

CHECKLIST

STROBE.

AUTHORS' CONTRIBUTIONS

DLMM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **NCPR:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology. **FRDM:** Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **IMSL:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **MBC:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **ZVB:** Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **JASR:** Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Association between lower urinary tract symptoms and polycystic ovary syndrome

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SUMMARY

OBJECTIVE: The aim of this study was to analyze the association between lower urinary tract symptoms and polycystic ovary syndrome.

METHODS: A total of 180 women were enrolled in this prospective study. Demographic data, body mass index, waist circumference, modified Ferriman–Gallwey scores, biochemical parameters, ultrasonographic findings, and maximum urinary flow rate (Q max) were analyzed. In addition, the Beck Depression Inventory, Beck Anxiety Inventory, and Bristol Female Lower Urinary Tract Symptom Scored Form questionnaires were evaluated for each subject.

RESULTS: The mean age of patients was calculated as 23.78±3.04 years, which was similar for both groups (p=0.340). Body mass index, waist circumference, Beck Depression Inventory, Beck Anxiety Inventory, Bristol Female Lower Urinary Tract Symptom Scored Form, and modified Ferriman–Gallwey scores were significantly higher in group 2 (p<<0.001). Hyperandrogenism, lipid profile, and glucose metabolism disorders were more frequent in group 2 (p<<0.05). Bladder capacity (Q max), bladder wall thickness, and post-void residual volume values were similar in both groups (p>>0.05).

CONCLUSION: In our study, a close relationship was observed between polycystic ovary syndrome and lower urinary tract symptoms. In this context, we think that a detailed urinary system evaluation of women with polycystic ovary syndrome is extremely important.

KEYWORDS: Lower urinary tract symptoms. Women. Polycystic ovary syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrinological disorder that occurs in women of reproductive age[1]. Approximately 6–20% of women in this period are affected. Two-thirds of the criteria for oligomenorrhea or amenorrhea, clinical or biochemical signs of hyperandrogenism, and polycystic ovarian morphology, known as the Rotterdam consensus, are considered diagnostic[2]. Today, PCOS syndrome is one of the most complex health problems that require intense attention by healthcare professionals due to its multifactorial etiopathogenesis and progression and its consequences in different medical disciplines[3].

Clinical analyses have shown that PCOS is closely associated with various organic pathologies such as impaired glucose tolerance, hyperinsulinemia, insulin resistance, dyslipidemia, hyperandrogenism, and obesity. Given the high prevalence and multisystemic impact of PCOS, the importance of multidisciplinary treatment modalities has recently been increasingly recognized[4].

Lower urinary tract symptoms (LUTS) are characterized by three main symptoms[1]: storage symptoms, such as urgency, frequency, nocturia, and urge incontinence[2]; voiding symptoms, such as poor and/or intermittent stream; and[3] post-voiding symptoms, such as the feeling of incomplete emptying[5]. LUTS exceeds the critical threshold, quality of life is impaired, level of physical activity is decreased, and psychological condition is negatively affected[6]. Large-scale epidemiological studies have reported that, on average, 84% of women suffer from at least one of the lower urinary tract symptoms in their lifetime[7].

PCOS leads to several psychogenic, physical, and metabolic problems and thus affects the dynamics of many different systems. Urinary system dynamics are affected quite seriously by psychogenic and organic factors. To have a healthy voiding physiology, it is critical that physical and mental health be within normal limits. This study aimed to thoroughly analyze the association between PCOS and LUTS through a multi-dimensional evaluation of organic and psychological factors.

METHODS

A total of 90 patients of reproductive age who were diagnosed with PCOS for the first time were enrolled at Tokat Gaziosmanpaşa University, Medical School, and the same number of healthy

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volunteers were included in the study. Prior to this prospective study, approval was obtained from the local ethics committee of our hospital (date: 13.08.2020/Tokat Gaziosmanpasa University approval number: 20-KAEK-216).

The diagnosis of PCOS was made in the presence of clinical or biochemical hyperandrogenism, anovulation or oligomenorrhea, the polycystic appearance of the ovaries on ultrasound, and at least 2 of these 3 major criteria. Healthy volunteers were identified as group 1 and women with PCOS as group 2. A detailed history was obtained from all patients, and a physical examination was performed. Body mass index (BMI) was calculated for each patient after measuring weight and height. Patients with a BMI of 30 kg/m^2 or more were classified as obese. In addition, waist circumference was calculated. Accordingly, a waist circumference of 88 cm and above were classified as high risk. Hirsutism is assessed with the modified Ferriman–Gallwey score (mFG)[1]. The extent of lower urinary tract symptoms in each case included in the study was analyzed using the Bristol Female Lower Urinary Tract Symptom (BFLUTS) questionnaire. After several studies with large series, the use of BFLUTS with 34 questions was replaced by BFLUTS-SF (scoring form) with 19 questions, which is an easy-to-use form that provides clearly measurable results for clinicians with better reproducibility. This scoring system includes 5 categories. BFLUTS-SF includes 4 questions defining filling symptoms (BFLUTS-FS), 3 questions defining voiding symptoms (BFLUTS-VS), 5 questions about signs of incontinence (BFLUTS-IS), 2 questions analyzing sexual function (BFLUTS-sex), and finally, 5 questions assessing the quality of life of those affected (BFLUTS-QoL)[8,9]. Because sexual dysfunction was not examined in this study, BFLUTS sex was not considered. In this regard, the BFLUTS-SF was reliably used in our study by excluding 2 questions analyzing sexual functioning. The distribution of responses among cases in the PCOS and control groups was statistically compared. In addition, the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory (BDI) were applied to the patients in both groups to better assess the psychological state of the patients[10].

Hormone analysis determined the levels of dehydroepiandrosterone sulfate (DHEA-S), LH, FSH, prolactin, and estradiol. As for the lipid profile, triglyceride, LDL, and HDL levels were measured. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) score was used to monitor glucose metabolism. Bladder ultrasonography was also performed in all patients to determine bladder capacity, residual urine volume after urination (PVR), and bladder wall thickness (BWT). The patients' maximum urinary flow rate (Q max) was also measured by a uroflowmetry test.

Diagnosis of PCOS based on the 2003 Rotterdam ESHRE/ASRM consensus criteria. Detailed medical history research was done on healthy volunteers. Only nulliparous women aged 18–49 years with no history of pelvic surgery were included in this study. In addition, patients diagnosed with a neurological or endocrine disease or metabolic disorder, patients taking medications for chronic diseases, patients with cancers of the genitourinary system, and patients with urinary tract infections were excluded from the study.

Statistical analysis of data was performed with the SPSS program (version: 22.0, SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented with median (minimum–maximum) and mean±standard deviation (SD) as a function of the normality distribution of the data for numeric variables. Descriptive statistics for categorical variables were presented as numbers and percentages (%). Testing of the normality distribution of the data for the selection of statistical tests was performed using the Kolmogorov–Smirnov test. The Mann–Whitney U test was used to compare numerical measurements between two independent research groups. Power analysis was performed to select the sample size, and it was decided to take a total of 90+90 patients for 5% error and 90% power.

RESULTS

The mean age of the patients was reported as 23.78±3.04 years, which was similar for both groups (p=0.340). A total of 33 (18.3%) patients were obese, and 41 (22.8%) had a high waist circumference. The mean BMI of patients in group 2 was calculated at 27.9±7.26 kg/m^2, and their waist circumference was 87.67±9.73 cm. These values were significantly higher compared to group 1 (p<<0.001). A total of 6 (3.3%) subjects had severe hirsutism. The mean mFG score in group 2 was 16.12±5.79, which was significantly higher than in group 1 (p<0.001). The BDI and BAI scores of patients in group 2 were 16.5±9.48 and 16.48±9.59, respectively. The psychological condition of the patients in group 2 was negatively affected based on these scoring systems (p<0.001).

A total of 42 (23.3%) subjects had impaired glucose metabolism. While only 8 (4.4%) cases in group 1 had impaired glucose metabolism, the mean HOMA-IR score was calculated to be 1.58±0.78. This score was significantly lower compared to group 2 (p<0.001). Also, abnormalities in lipid profile were more frequent in group 2 (for triglyceride, p< 0.001; for LDL, p=0.036; for HDL, p=0.021). The levels of DHEA-S and LH were significantly higher in patients in group 2 compared to those in group 1 (p<<0.001 and p=0.039, respectively). There was no statistically significant difference between groups in FSH, prolactin, and estradiol levels (p>0.05).

The mean BFLUTS-SF total scores of groups 1 and 2 were 15.59±11.06 and 27.78±14.18, respectively, except for the analysis of sexual function. The scores were significantly higher in group 2 (p<<0.001). Similarly, all categories of this scoring system for the analysis of filling, voiding, incontinence, and quality of life were significantly higher in patients in group 2 (p<0.001) (Figure 1). In addition, no significant difference was found between groups in bladder capacity, BWT, PVR, and Q max (p>0.05) (Table 1).

Correlation analysis was used for the cases in group 2. A high level of significant positive correlation was found between BMI, waist circumference, and BFLUTS-SF parameters; a moderate level of positive correlation was found between the mFG scores and BFLUTS-SF parameters. Similarly, a high level of significant positive correlation was found between the BDI and BAI scores and the BFLUTS-SF parameters. On the contrary, moderate and high levels of positive correlation were significantly found between HOMA-IR, DHEA-S, and the BFLUTS-SF parameters; a moderate level of positive correlation was significantly found between triglyceride levels and the BFLUTS-SF parameters. While weak and moderate levels of negative correlation were significantly found between the HDL levels and the BFLUTS-SF parameters, weak and moderate levels of positive correlation were found in terms of the LDL levels (Table 2).

DISCUSSION

Clinical findings vary according to age groups in PCOS cases. Patients often present with ovulation problems such as menstrual irregularities or infertility. Other clinical implications include endothelial damage, obesity, insulin resistance, and hyperandrogenism, which are closely associated with inflammatory processes in the pathogenetic pathways of PCOS[1]. For healthy maintenance of voiding physiology, anatomic factors must be in perfect harmony with neuroendocrine pathways. When this dynamic is disrupted in any way, LUTS occur. Inflammatory responses and metabolic effects of these processes lead to some consequences for the dynamics of the lower urinary system in PCOS[11-13]. This situation has a significant negative impact on the quality of life of patients with PCOS. Nevertheless, to our knowledge, there are few studies on this topic in the literature. Our study addresses the different aspects of the changes that occur in the bladder dynamics of patients with PCOS.

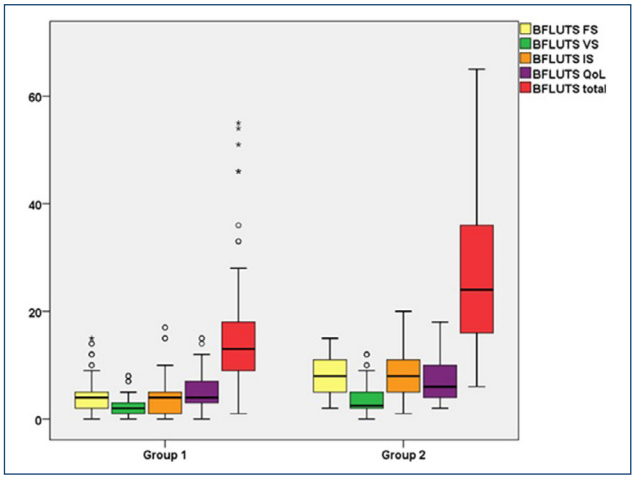

Figure 1. Distribution of voiding symptom scores between research groups.

Studies have shown that hyperandrogenism is present in more than 80% of PCOS cases. Recent studies emphasize both the microscopic and clinical implications of changes in androgen hormone levels on the urinary system. In an experimental study, Çayan et al.[14] examined rats undergoing oophorectomy. They reported that androgen and estrogen deprivation decreased bladder capacity and compliance, and function improved after hormone therapy. In a similar study, Tek et al.[15] assessed the effects of testosterone therapy on bladder functions in orchidectomized rats, reporting an elevation in bladder capacity and smooth muscle/collagen content following testosterone therapy. Antonio et al.[16] reported that pelvic floor muscle strength was evaluated in a clinical study of 79 patients diagnosed with 36 PCOS. They reported that although pelvic floor muscle strength was higher in patients with PCOS, these values did not present a statistically significant correlation. Yet, the urinary incontinence ratio was recorded at a statistically significant high level in PCOS cases. In another study, Sahinkanat et al.[11] reported a correlation between bladder symptoms, such as pelvic pain, nocturia, or urinary urgency, and testosterone levels in women with PCOS. However, in the same study, no statistical correlation was found between ultrasonographic findings such as bladder capacity, PVR, and testosterone level. Similarly, our study also showed an increase in the frequency of LUTS with elevated testosterone levels. On the contrary, no significant difference was found in Qmax, PVR, bladder capacity, and BWT.

The prevalence of obesity in PCOS is approximately 30–70%, which is quite high compared to the normal population[17]. Obesity leads to the formation of oxidative stress in the urethral mucosa, a reduction in the amount of collagen, and a loss of urethral elasticity[18]. Any increase in intra-abdominal pressure, in addition to these histopathologic changes, leads to an increase in lower urinary system symptoms in obese patients[12]. In a large series by Lai et al.,[19] researchers examined lower urinary system symptoms in subjects and found a direct relationship between obesity and overactive bladder, frequency, urinary incontinence, and stress incontinence. Our study showed a higher prevalence of obesity in patients with PCOS compared with the control

Table 1. Comparison of demographic data, modified Ferriman–Gallwey, Beck Depression Inventory, Beck Anxiety Inventory, of Bristol Female Lower Urinary Tract Symptom Scored Form values, bladder measurements, uroflowmetry test scores, and biochemical parameters between groups.

	Group 1 (n=90) Median (min–max) (Mean±SD)	Group 2 (n=90) Median (min–max) (Mean±SD)	p-value
Age (year)	23 (19–33) (23.5±2.78)	23.5 (19–34) (24±3.28)	0.340
BMI (kg/m^2)	22 (16–35) (23.4±3.68)	25 (16–45) (27.9±7.26)	**<0.001***
Waist circumference (cm)	77.5 (57–99) (76.06±10.87)	86.5 (60–106) (87.67±9.73)	**<0.001***
mFG score	7 (1–25) (7.96±5.72)	19 (7–28) (16.12±5.79)	**<0.001***
BDI	9 (1–30) (8.97±5.19)	15 (2–39) (16.5±9.48)	**<0.001***
BAI	7 (1–30) (7.94±4.79)	17 (1–39) (16.48±9.59)	**<0.001***
BFLUTS-FS	4 (0–15) (4.39±3.18)	8 (2–15) (8.76±3.41)	**<0.001***
BFLUTS-VS	2 (0–8) (2.11±2.03)	2.5 (0-12) (3.63±2.78)	**<0.001***
BFLUTS-IS	4 (0–17) (4.12±3.86)	8 (1–20) (7.98±4.14)	**<0.001***
BFLUTS-QoL	4 (0–15) (4.97±3.48)	6 (2–18) (7.41±4.12)	**<0.001***
BFLUTS-SF total	13 (1–55) (15.59±11.06)	24 (6–65) (27.78±14.18)	**<0.001***
Bladder capacity (cc)	400 (300–670) (410.6±71.7)	400 (300–750) (402.9±69.7)	0.313
PVR (cc)	10 (0–65) (14.67±16.9)	10 (0–60) (16.47±16.2)	0.198
Q max (mL/s)	31 (15–40) (29.8±5.99)	30 (14–40) (28.9±6.2)	0.157
BWT (mm)	2 (0.3–7.1) (2.59±1.31)	2.3 (0.4–7.1) (2.71±1.4)	0.556
FSH (mIU/mL)	3.8 (2–7) (3.87±0.97)	4 (2–8) (4.46±1.5)	0.064
LH (mIU/mL)	6.1 (1.9–9) (6.22±1.25)	7 (2.1–10) (7±2.14)	**0.039***
LH/FSH	1.45 (0.51–2.57) (1.46±0.44)	1.75 (0.57–3.3) (1.7±0.69)	**0.044***
HOMA-IR	1.5 (0.5–3.9) (1.58±0.78)	2.5 (0.5–4.4) (2.31±1.13)	**<0.001***
Prolactin (mIU/L)	17.5 (4–35) (17.5±5.29)	20 (4–45) (20.8±6.99)	0.089
DHEA-S (mg/Dl)	161 (65–500) (176.9±109.6)	302.5 (80–640) (289.3±159.6)	**<0.001***
Estradiol (pg/Ml)	56.5 (30–130) (66.2±28.6)	60 (27.8–149) (71.4±37.8)	0.132
Triglyceride (mg/dL)	123 (60–285) (131.3±51.5)	157.7 (70–360) (178.8±79.5)	**<0.001***
HDL (mg/dL)	41 (21–78) (42.1±10.7)	35 (21–72) (36±9.37)	**0.021***
LDL (mg/dL)	132 (80–192) (131.1±26.9)	145 (70–200) (146.8±28.5)	**0.036***

Data are presented as mean±minimum–maximum numbers and as mean±standard deviation. Mann–Whitney U test was used. BMİ: body mass index; mFG: modified Ferriman–Gallwey; BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory; BFLUTS-FS: Bristol Female Lower Urinary Tract Symptom-Filling Symptoms; BFLUTS-VS: Bristol Female Lower Urinary Tract Symptom-Voiding Symptoms; BFLUTS-IS: Bristol Female Lower Urinary Tract Symptom- Incontinence Symptoms; BFLUTS-QoL: Bristol Female Lower Urinary Tract Symptom- Quality of Life; BFLUTS-SF: Bristol Female Lower Urinary Tract Symptom- Scored Form; PVR: Post-void residual volume; Q max: Maximum urinary flow rate; BWT: Bladder wall thickness; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; DHEA-S: Dehydroepiandrosterone Sulfate; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein. *Bold values indicate statistically significant (p<0.05).

group. On the contrary, lower urinary symptoms were found to be directly related to increased waist circumference and BMI in patients with PCOS.

Broad meta-analyses have found that metabolic syndrome is 3.35 times more common in PCOS[19]. Metabolic disorders have a significant impact on bladder dynamics. Lee et al.[20]

Table 2. Correlation analysis results between age, body mass index, waist circumference, Ferriman–Gallwey Score, Beck Depression Inventory, Beck Anxiety Inventory, voiding symptom scores, bladder measurements, and biochemical parameters for group 2 (n=90).

		BFLUTS FS	BFLUTS VS	BFLUTS IS	BFLUTS QoL	BFLUTS SF Total	Bladder Capacity	PVR	Qmax	BWT
Age	r	-0.273	-0.176	-0.266	-0.152	-0.244	0.157	-0.217	-0.096	-0.208
	p	0.116	0.208	0.121	0.199	0.137	0.114	0.093	0.274	0.102
BMI	r	0.742*	0.708*	0.723*	0.710*	0.733*	-0.294*	0.457*	0.123	0.220*
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	0.248	0.037
WC	r	0.739*	0.703*	0.725*	0.707*	0.731*	-0.299*	0.437*	0.116	0.214*
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.278	0.043
FG Score	r	0.575*	0.550*	0.582*	0.508*	0.589*	-0.258*	0.424*	0.137	0.261*
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	<0.001	0.199	0.013
BDI	r	0.740*	0.711*	0.728*	0.700*	0.730*	-0.293*	0.450*	0.103	0.226*
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	0.332	0.033
BAI	r	0.727*	0.703*	0.714*	0.702*	0.715*	-0.288*	0.430*	0.094	0.215*
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	0.380	0.042
FSH	r	0.040	0.062	0.009	0.004	0.016	0.285*	-0.177	0.205	-0.026
	p	0.707	0.559	0.936	0.972	0.884	0.007	0.096	0.053	0.806
LH	r	-0.070	-0.065	-0.043	-0.033	-0.073	0.186	0.092	-0.113	-0.179
	p	0.509	0.543	0.687	0.756	0.492	0.079	0.389	0.290	0.092
LH/ FSH	r	-0.019	-0.029	0.029	0.051	0.004	-0.081	0.242*	-0.270*	-0.125
	p	0.861	0.784	0.786	0.631	0.970	0.448	0.021	0.010	0.240
HOMA-IR	r	0.719*	0.712*	0.738*	0.675*	0.705*	-0.297*	0.416*	0.062	0.183
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.561	0.084
Prolactin	r	-0.224	-0.168	-0.196	-0.130	-0.195	-0.007	-0.289	0.250	-0.058
	p	0.054	0.114	0.064	0.221	0.066	0.950	0.053	0.060	0.585
DHEA-S	r	0.736*	0.691*	0.680*	0.738*	0.702*	-0.301*	0.459*	0.122	0.234*
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	0.251	.026
Estradiol	r	0.178	0.244	0.269	0.152	0.175	-0.125	0.251*	0.112	0.122
	p	0.107	0.096	0.063	0.216	0.106	0.062	0.031	0.294	0.095
Triglyceride	r	0.667*	0.601*	0.648*	0.655*	0.641*	-0.210	0.257*	0.102	0.130
	p	<0.001	<0.001	<0.001	<0.001	<0.001	.057	0.022	.337	.223
HDL	r	-0.607*	-0.493*	-0.601*	-0.470*	-0.584*	0.210*	-0.198	0.111	-0.054
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.021	0.079	0.296	0.612
LDL	r	0.587*	0.494*	0.536*	0.569*	0.545*	-0.156	0.235*	-0.110	0.013
	p	<0.001	<0.001	<0.001	<0.001	<0.001	0.055	0.041	0.303	0.902

*Statistically significant (Spearman's correlation coefficient: p<0.05). WC: Waist circumference; mFG: modified Ferriman–Gallwey; BMI: body mass index; BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; BFLUTS-FS: Bristol Female Lower Urinary Tract Symptom-Filling Symptoms; BFLUTS-VS: Bristol Female Lower Urinary Tract Symptom-Voiding Symptoms; BFLUTS-IS: Bristol Female Lower Urinary Tract Symptom- Incontinence Symptoms; BFLUTS-QoL: Bristol Female Lower Urinary Tract Symptom- Quality of Life; BFLUTS-SF: Bristol Female Lower Urinary Tract Symptom- Scored Form; PVR: Post-void residual volume; Q max: Maximum urinary flow rate; BWT: Bladder wall thickness; FSH: Follicle Stimulating Hormone; LH: Luteinizing Hormone; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; DHEA-S: Dehydroepiandrosterone Sulfate; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein.

reported bladder dysfunction in their study on experimental animals after the creation of metabolic syndrome. The same study indicated injury in bladder smooth muscle mitochondria, the elevation of interstitial tissue leukocytes, and intense neutrophil infiltration around the endothelium. In a similar study, Tong et al.[21] highlighted the presence of findings indicating detrusor overactivity in 62.5% of rats exposed to metabolic syndrome. In our study, insulin resistance and dyslipidemia were detected commonly in women with PCOS. Besides, it was concluded that such metabolic disorders are associated with the severity of LUTS.

Psychogenic problems are also quite common in women with PCOS. Previous comprehensive studies indicate a depression rate of 28–64% in patients with PCOS. On the contrary, anxiety disorders are significantly more common in PCOS (34–57%) than in the general population[22]. In a series of 100 women followed for at least 6 months, Heidari et al.[23] concluded that there was a direct association between depressive disorders and irritability symptoms, obstructive symptoms, and urodynamic test results. However, in the same study, no association was found between obsessive-compulsive disorder and LUTS. Our study concludes that anxiety and depression scales are severely impaired in women with PCOS. The study also suggests that depression and anxiety scale scores correlate with LTUS severity.

The main limitations of our study were the performance of the analyses in a single center, the limited number of cases in a similar geography, the impossibility to assess sexual function, and the inability to perform an invasive urodynamic examination. On the contrary, although PCOS is a very common pathology, the urinary system dynamics of this patient group are mostly ignored by health professionals. In this prospective study, we believe that associating PCOS with urinary system dynamics will make important contributions to the medical literature.

CONCLUSION

According to the results of our study, a strong association was found between PCOS and LUTS. Moreover, an association was found between the severity of LUTS and the psychological problems observed in patients with PCOS, hyperandrogenism, obesity, impaired glucose, and lipid profile.

AUTHORS' CONTRIBUTIONS

EK: Conceptualization, Data curation, Formal Analysis, Investigation, Resources, Writing – original draft, Writing – review & editing. **SG:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, **FE:** Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Epstein-Barr virus in gastric cancer and association with 30 bp del-latent membrane protein 1 polymorphism

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SUMMARY

OBJECTIVE: This study aimed to determine the frequencies of Epstein-Barr virus, types 1 and 2 infection, and 30 bp del-latent membrane protein 1 viral polymorphism in gastric adenocarcinomas, as well as to investigate the association between Epstein-Barr virus infection and tumor location, type, and the patient's sex.

METHODS: Samples were collected from 38 patients treated at a university hospital in Rio de Janeiro, Brazil. Epstein-Barr virus detection and genotyping were performed by polymerase chain reaction, followed by polyacrylamide gel electrophoresis and staining by the silver nitrate method.

RESULTS: Overall, 68.4% of patients had Epstein-Barr virus-positive tumors. Of these, 65.4% presented infection by Epstein-Barr virus type 1, 23.1% by Epstein-Barr virus type 2, and 11.5% had coinfection with types 1 and 2. The 30 bp del-latent membrane protein 1 polymorphism was found in 42.3% of Epstein-Barr virus-positive tumors, 23.1% had the wild-type virus, and 23.1% had the wild-type and the polymorphism concomitantly. In 11.5% of Epstein-Barr virus-positive tumors, it was impossible to determine whether there was polymorphism or not. Tumor location in the antrum (22 of 38) and diffuse type (27 of 38) were predominant. There was no significant difference in Epstein-Barr virus infection or the 30 bp del-latent membrane protein 1 polymorphism between men and women.

CONCLUSION: Epstein-Barr virus infection was found in 68.4% of tumors investigated in this study. To the best of our knowledge, this is the first article showing the coinfection of Epstein-Barr virus types 1 and 2 in gastric carcinoma in Brazil.

KEYWORDS: Stomach neoplasms. Herpesvirus 4, human. Coinfection.

INTRODUCTION

Gastric cancer (GC) is the fifth most common and fourth most lethal malignant tumor worldwide[1]. For 2020, there were an estimated 1,089,103 new cases and 768,793 deaths for GC globally[1]. In Brazil, there were an estimated 21,480 new cases in 2022[2]. In 2020, 13,850 died because of the disease in the country[2]. Several risk factors are associated with GC, including chronic *Helicobacter pylori* infection, family history, diet, alcohol consumption, smoking, and infection by Epstein-Barr virus (EBV)[3]. Nearly 10% of gastric carcinomas are associated with EBV, and the virus infects more than 90% of the global population[4]. EBV is classified into two major types: 1 and 2 (or types A and B) based on differences in viral nuclear antigen (EBNA) genes, especially *EBNA2*, *EBNA3A*, *-3B*, and *-3C*[5]. Type 1 is the most prevalent worldwide. EBV-1 can convert human B-lymphocytes into lymphoblastoid cell lines more efficiently than EBV-2[5]. Several of the EBV-encoded latent proteins are involved in cellular transformation[6]. The latent membrane protein 1 (LMP1), encoded by the *BNLF1* gene, is an essential EBV protein. It can induce phenotypic changes in B-cells and epithelial cells[7,8]. The 30 base pairs (bp) deletion in the third exon of

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BNLF1 (called the 30 bp del-LMP1) causes a loss of ten amino acids. Some studies associate the 30 bp del-LMP1 polymorphism with the oncogenesis of various tumors, including nasopharyngeal carcinoma and GC[8].

This study aimed to determine the frequency of EBV types (1 and 2) and the prevalence of the viral 30 bp del-LMP1 polymorphism in gastric adenocarcinomas in 38 patients treated at a university hospital in Rio de Janeiro, Brazil. Moreover, the study investigated the association between EBV type and the 30 bp del-LMP1 polymorphism with the tumor location, patients' sex, and histological tumor type.

METHODS

This was a hospital-based study. Samples were collected from 38 patients (26 men and 12 women) diagnosed with primary gastric adenocarcinoma at the Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro, Brazil. The mean age of patients was 63.2±10.8 years. For men, the mean age was 65.9±9.96 years, and the mean age for women was 57.4±9.66 years. After diagnosis, patients underwent surgical resection, performing total or partial gastrectomy. The histological type of tumors was classified according to the Laurén classification. Sample collection occurred between April 2013 and August 2019. The Institutional Research Ethics Committee approved the study (#23511719.0.0000.5257).

DNA extraction: Genomic DNA was extracted from fresh tumor tissue using the phenol:chloroform method, according to previously described by Maccormick et al.[9].

Detection of Epstein-Barr virus DNA: Two regions of the EBV genome were selected for polymorphism analysis by polymerase chain reaction (PCR): the U2 region encoding *EBNA-2* (to recognize type 1 or 2) and a sequence at the exon 3 of the *BNLF1* gene (to detect 30 bp del-LMP1 variant). To analyze *EBNA-2*, the method described by Kunimoto et al.[10] was adapted for multiplex PCR, using two pairs of primers. For type l: forward, 5′-ACAACCACTCATGATGCCAC-3′ and reverse, 5′-ACCGTGGTTCTGGACTATCT-3′. For type 2: forward, 5′-GGTAGCCTTAGGACATACTC-3′ and reverse, 5′-TGGAGGGAGTCCTGTACTAT-3′[10]. PCR conditions were: initial denaturation of 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The final extension was performed at 72°C for 5 min. The products generated were fragments of 240 bp (type 1) or 233 bp (type 2)[10] visualized as DNA bands by electrophoresis in 10% polyacrylamide gel, followed by silver nitrate staining according to previously described by Silva et al.[11]. For the *BNLF1* fragment, the primers used were: forward, 5′-TGGAGGGAGAGTCAGTCAGGC-3′ and reverse, 5′-ATTGACGGAAGAGGTTGAAAAC-3′[12]. Amplification conditions were: initial denaturation of 94°C for 3 min, followed by 40 cycles of 94°C for 1 min, 60°C for 30 s, and 72°C for 1 min. The final extension was performed at 72°C for 5 min. The products generated were fragments of 254 bp (wild) or 224 bp (deleted), visualized by gel electrophoresis and silver nitrate staining[11].

RESULTS

Demographic and clinical data of patients were collected from medical records and reports.

Table 1 shows the association between patients' characteristics and the presence or absence of tumoral EBV.

Table 2 includes only patients with EBV-positive tumors (n=26), associating the characteristics of patients with the EBV type and 30 bp del-LMP1 polymorphism.

Of the 38 patients included in the study, 23 had died by January 31, 2022. The overall mean survival of patients with EBV-positive tumors was 800.96 days. Furthermore, patients with EBV-negative tumors had an overall mean survival of 1,160.83 days, meaning a difference of 359.87 days.

Table 1. Characteristics of patients with gastric carcinoma and tumoral EBV infection.

Characteristics	Total	EBV-positive (%)	EBV-negative (%)
Number of patients	38	26 (68.4)	12 (31.6)
Sex			
Male	26	17 (65.4)	9 (34.6)
Female	12	9 (75)	3 (25)
Mean age (years)	63.2	62.4	64.9
Tumor location			
Cardia	5	3 (60)	2 (40)
Cardia/body/fundus	1	0 (0)	1 (100)
Antrum	22	16 (72.7)	6 (27.3)
Antrum/body	1	1 (100)	0 (0)
Body	7	4 (57.1)	3 (42.9)
Fundus	1	1 (100)	0 (0)
Cardia/body/fundus/antrum	1	1 (100)	0 (0)
Tumor type[§]			
Intestinal	10	6 (60)	4 (40)
Diffuse	27	19 (70.4)	8 (29.6)
Intestinal+diffuse	1	1 (100)	0 (0)

EBV: Epstein-Barr virus. [§]Tumor type according to the Laurén classification.

Table 2. Characteristics of patients with gastric carcinoma and genotype of EBV-positive tumors.

Characteristics	EBV type (%)			30 bp deletion (%)			
	EBV 1	EBV 2	EBV 1/2	W	D	W/D	ND
Sex							
Male (n=17)	10 (58.8)	5 (29.4)	2 (11.8)	5 (29.4)	8 (47)	2 (11.8)	2 (11.8)
Female (n=9)	7 (77.8)	1 (11.1)	1 (11.1)	1 (11.1)	3 (33.3)	4 (44.4)	1 (11.1)
Age (years)							
≥65 (n=13)	8 (61.5)	4 (30.8)	1 (7.7)	5 (38.5)	5 (38.5)	1 (7.7)	2 (15.4)
<65 (n=13)	9 (69.2)	2 (15.4)	2 (15.4)	2 (15.4)	6 (46.1)	4 (30.8)	1 (7.7)
Tumor type[§]							
Intestinal (n=6)	4 (66.7)	1 (16.7)	1 (16.7)	1 (16.7)	4 (66.7)	1 (16.7)	0 (0)
Diffuse (n=19)	12 (63.2)	5 (26.3)	2 (10.5)	6 (31.6)	7 (36.8)	3 (15.8)	3 (15.8)
Int+Diff (n=1)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Tumor location							
Cardia (n=3)	3 (100)	0 (0)	0 (0)	1 (33.3)	1 (33.3)	0 (0)	1 (33.3)
Noncardia (n=23)	14 (60.9)	6 (26.1)	3 (13)	6 (26.1)	10 (43.5)	5 (21.7)	2 (8.7)

EBV: Epstein-Barr virus (types 1, 2, or 1/2); 30 bp deletion: the 30 bp del-LMP1 polymorphism of the gene that encodes EBV protein LMP1 (latent membrane protein 1); W: wild type; D: 30 bp deletion; W/D: wild type+30 bp deletion; ND: not detectable. §Tumor type according to the Laurén classification. Int+Diff: Intestinal+Diffuse.

Among deceased patients, the mean survival was 264.06 days for those with EBV-positive tumors and 424.43 days for patients with EBV-negative tumors, a difference of 160.37 days. Figure 1 shows the survival curve of patients according to the Kaplan-Meier method.

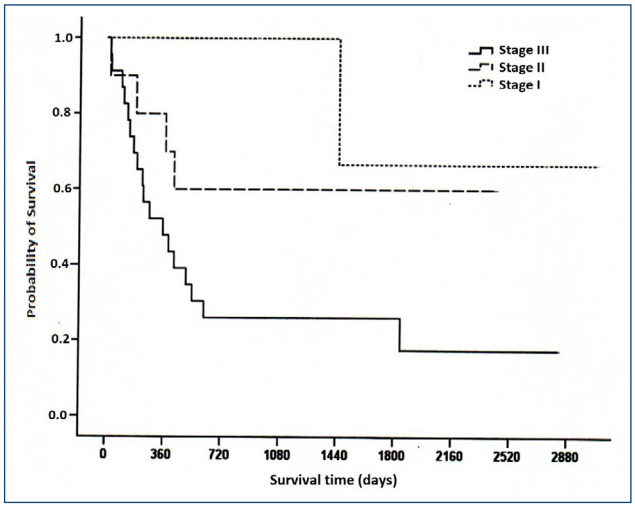

Figure 1. Kaplan-Meier curve correlating tumor staging with survival of gastric cancer patients.

DISCUSSION

Several studies have investigated the frequency of EBV-associated GCs. An investigation from "The Cancer Genome Atlas" network, with 295 tumors from North America, Europe, and Asia, found EBV in 9% of samples[4]. A study from the "Asian Cancer Research Group" with 300 GCs from Asian patients detected the virus in 6.5% of them[4]. In Brazil, research conducted in São Paulo showed 10.5% of EBV-positive among 286 tumors[13]. In contrast, a study from Amazonas, northern Brazil, using the PCR technique found EBV in 80% of the biopsies from 10 patients with GC[14]. Noteworthy is that, in the research from Amazonas, tumor biopsies were pulverized, ensuring the availability of a substantial amount of material for DNA extraction[14]. In our investigation, EBV was detected in 68% of the evaluated GC tumors. The disagreement between this study and some others may be partly explained by the use of different methodologies. Some studies used 5-µm-thick sections of paraffinized tumor tissue to detect viral DNA. On the contrary, we used tumor fragments of at least 1 cm in diameter, substantially increasing the amount of material available to assess the presence of EBV DNA. This difference in tissue collection was possibly an important factor in detecting EBV in 68% of samples. Our research showed that among EBV-positive tumors, 65.4% were of type 1, and 23.1% were of type 2. In 11.5% of the cases, there was coinfection of types 1 and 2. As far as we know, the phenomenon of EBV 1 and 2 coinfections has not yet been reported in GCs among Brazilian patients. The investigated

population had peculiar characteristics, presumably resulting from the Brazilian people's miscegenation. The LMP1 protein has a relevant contribution to cellular proliferation and survival that occur in EBV-associated malignancies[15]. EBV-positive GC tends to have a distinct clinicopathological phenotype compared to EBV-negative tumors. Some studies have indicated that EBV-positive GC was more prevalent in younger patients compared to EBV-negative tumors. Moreover, the disease is more commonly associated with males[16,17], as well as with Caucasians and Hispanics[18]. According to scientific literature, EBV-positive tumors preferentially occur in proximal portions of the stomach, more frequently in the cardia and gastric body, and are associated with diffuse histology[18]. In our study, the mean survival of patients with EBV-positive tumors was considerably shorter than the survival of those with EBV-negative tumors. Regarding overall mean survival, the difference was of 359.87 days. However, this result contradicts what is shown in the scientific literature, which shows a better prognosis for patients with EBV-positive tumors. Nonetheless, the poorer prognosis presented for EBV-positive patients found in our investigation may perhaps be explained by the fact that, in this sample, there were 27 tumors of the diffuse type against only 10 of the intestinal type and one with both. In the literature, it has been reported that diffuse tumors have a worse prognosis than intestinal ones[19,20].

CONCLUSION

The frequency of EBV-positive tumors in this study was 68.4%. The 30 bp del-LMP1 polymorphism was found in 42.3% of EBV-positive tumors. There was no significant difference in the frequency of EBV infection or the 30 bp del-LMP1 polymorphism between men and women. Tumor location in the gastric antrum and diffuse histological type were predominant. As far as we know, this is the first study to show the coinfection of EBV types 1 and 2 in gastric carcinoma in Brazil.

AUTHORS' CONTRIBUTIONS

ERCS: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MSMS:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **NHSC:** Investigation, Methodology, Validation, Writing – review & editing. **MFDG:** Conceptualization, Writing – original draft, Writing – review & editing. **ÁLVLB:** Methodology, Validation, Visualization. **WMVS:** Formal Analysis, Writing – original draft. **MGCC:** Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft. **GPBN:** Conceptualization, Data curation, Formal Analysis, Writing – original draft.

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ORIGINAL ARTICLE

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Effect of maternal cortisol levels on fetal heart rate patterns in primiparous pregnant women in the third trimester

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SUMMARY

OBJECTIVE: This study aimed to determine whether maternal cortisol levels affect fetal heart rate patterns in primiparous pregnant women in the third trimester.

METHODS: This cross-sectional descriptive study included 400 primiparous pregnant women with uncomplicated pregnancies between November and December 2022. The study included primiparous pregnant women over 18 years old in the third trimester who had not exercised for at least 2 h before the fetal heart rate monitoring and had a healthy pregnancy without consuming any food or drink. Fetuses with decelerating heartbeats and pregnant women who showed uterine contraction and cervical dilation during the fetal heart rate monitoring were excluded from the study. Research data were collected with the data collection form. The fetal heart rate data were collected using a cardiotocograph. At least two accelerations during the 20-min nonstress test period were the basis for diagnosing a reactive nonstress test. About 5 mL of maternal saliva for cortisol measurements was collected before fetal heart rate monitoring. Research data were analyzed with IBM SPSS Statistics for Macintosh, Version 28.0. A p-value of <0.05 was considered significant.

RESULTS: There were no significant differences in the comparison of the groups in terms of education and income status, family type, fetal gender, pregnancy planning status, BMI and age averages, or gestational week averages (p>0.05). The number of at least two accelerations required for the diagnosis of reactive NST was also higher in Group 1 (maternal salivary cortisol level ≤24.20). A moderately positive relationship between fetal heart rate and maternal salivary cortisol was observed (r=0.448, p=0.000). In total, 11.9% of the total change in fetal heart rate level is explained by maternal cortisol (R^2=0.119). Maternal cortisol increases fetal heart rate level (ß=0.349).

CONCLUSION: These findings suggest that stress in primiparous pregnant women with high cortisol levels may influence fetal heart rate patterns. It was revealed that the increase in cortisol level, considered a stress hormone, may be a harbinger of fetal tachycardia.

KEYWORDS: Heart rate, fetal. Hydrocortisone. Pregnancy. Saliva.

INTRODUCTION

The mental health of women during the perinatal period is affected by many factors[1]. Especially in primiparous pregnancies, pregnancy, and birth unknowns due to a lack of information can cause stress and anxiety[2]. Since pregnancy brings significant alterations in the levels and function of key endocrine systems, the role of endocrine changes across the perinatal period has been widely investigated as an influence on maternal mood and behavior as well as fetal and child development[3]. During pregnancy, dramatic changes in the functioning of the maternal hypothalamic-pituitary-adrenal (HPA) axis are observed because the placenta expresses the genes for human corticotropin-releasing hormone (hCRH) and the precursor for adrenocorticotropic hormone (ACTH) and beta-endorphin (proopiomelanocortin). Placental corticotropin-releasing hormone (pCRH) production increases dramatically over gestation, and pCRH plays a central role in the regulation of fetal maturation and the timing of parturition[4].

Stress experienced in the prenatal period can cause negative maternal and neonatal outcomes[5]. Stress and anxiety disorders experienced during pregnancy not only cause adverse effects on the course of the pregnancy but also affect the neurodevelopment of the baby[6,7]. Furthermore, stress is associated with negative outcomes such as prematurity and low birth weight in newborns[8]. Cortisol is released in response to stress and is a critical physiological marker for activation of the stress response. Cortisol is a glucocorticoid steroid hormone synthesized from cholesterol in the adrenal cortex, and its release is regulated via the HPA system[9]. Typically, in response to the cognitive appraisal of significant stressors, CRH is produced in the paraventricular nucleus of the hypothalamus and released into the pituitary gland. CRH then stimulates the release of ACTH in

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the anterior pituitary, which subsequently results in the adrenal cortex releasing several glucocorticoids, including cortisol, in humans[4,9]. Consequently, pCRH and cortisol in maternal plasma increase exponentially across pregnancy, and maternal levels can be 60 to 700 times higher than before pregnancy[10]. Cortisol can be measured through different substrates (blood, saliva, hair, and urine), and measurements of cortisol concentrations may vary based on the substrate being used[11].

Fetal heart rate (FHR) monitoring is the primary clinical technique for assessing fetal well-being and is one of the most valuable techniques for investigating fetal neurodevelopment. The nonstress test (NST) measures the FHR in response to fetal movement over time. NST test results are reported as reactive and non-reactive NST. A regular test (reactive NST) is usually associated with a neurologically sound and sufficiently oxygenated fetus. An abnormal test (non-reactive NST) is associated with negative fetal or neonatal outcomes[12,13].

Although there are results about stress levels and outcomes for pregnant women in the literature, there are no studies evaluating the effect of cortisol levels on fetal heart patterns in primiparous pregnant women in the third trimester. The present study aimed to determine whether maternal cortisol levels affect FHR patterns in primiparous pregnant women in the third trimester.

METHODS

This cross-sectional descriptive study included 400 primiparous pregnant women (Group I (n:203), maternal saliva cortisol level≤24.20 nmol; Group 2 (n:197), maternal saliva cortisol level>24.21 nmol) with uncomplicated pregnancies and a single fetus recruited from a private hospital in Istanbul, Turkey, between November 2022 and December 2022. The STROBE checklist was used in the study design's planning, implementation, and reporting[14].

The minimum sample size required in the study was decided by power analysis (G*Power Version 3.1.9.2). In the calculation, the effect size was taken as 0.35 (as the primiparous mother rate)[15]. Type 1 error rate (α)=0.05, and the power of the study (1-β) was 0.95 (Type II error=0.05). Accordingly, the minimum number of samples to be reached was calculated as a total of 356. It aimed to reach 392 samples considering the 10% loss risk, and the research was completed with 400 participants. After maternal saliva analyses, based on the mean daytime saliva concentrations (24.20 nmol/L) of pregnant women between the 31st and 37th gestational weeks[7], pregnant women with maternal saliva averages ≤24.20 nmol/L were evaluated as group 1 (n:203), >24.21 nmol/l, and pregnant women with 2 (n:197).

The study included primiparous pregnant women over 18 years old in the third trimester who had not exercised for at least 2 h before the FHR monitoring and had a healthy pregnancy without consuming any food or drink. In all patients, the well-being and growth of the fetus were normal, and the amniotic fluids were also normal. Fetuses with decelerating heartbeats and pregnant women who showed uterine contractions and cervical dilation while the NST procedure was in progress were excluded from the study (n=12).

Research data were collected with the data collection form. The data collection form included 10 questions in total on the socio-demographic characteristics (BMI, age, education status, family type, and income status) of women and their obstetric history (gestational week, fetal sex, pregnancy planning status, NST result, and the number of accelerations).

After collecting the data collection, the pregnant woman was asked to give a saliva sample into a 5-mL Eppendorf tube. The sampling time took 3–5 s. Saliva for cortisol measurements was collected between 08:00 and 10:00. The salivary cortisol samples were taken into Eppendorf tubes and centrifuged at 3,000 RPM for 10 min, and the supernatant portions were stored in a –80°C cabinet for 7 days. The samples were delivered to the biochemistry laboratory of a private university for weekly analysis. Cortisol values in supernatant samples were determined by ELISA-based commercially available kits [Human Salivary Cortisol ELISA kit (DRG International, Inc., USA, Cat Num:SLV-2930); Human Adrenocorticotropic Hormone (ACTH) ELISA kit (Elabscience Inc., USA, Cat Num:E-EL-H0137)] and were measured on a microplate reader (Thermo Scientific Multiskan FC, 2011-06, USA).

The FHR data were collected using a cardiotocograph (Philips Avalon FM20, Koninklijke Philips Electronics N.V., The Netherlands). Pregnant women admitted to the clinic before the research were checked routinely by the obstetrician in the clinic. The researcher included pregnant women who met the inclusion criteria in the study and voluntarily participated. Philips Avalon FM 20 brand NST Device US probe (where the fetal heartbeat is taken) and Toco probe (uterine fundus) were placed. All pregnant women were rested in the left lateral semi-fowler position in a quiet room for 30 min before the study to avoid being affected by external factors that cause stress. The NST process was continued in the quiet room where the pregnant women were resting and in the left lateral semi-fowler position in all groups. At least two accelerations (elevation of basal rhythm over 15 beats for 15 s) during a 20-min NST period were the basis for diagnosing reactive NST. The patients who did not meet the criteria for normal cardiotocography considered by the researchers continued to receive routine care.

Statistical analysis

Research data were analyzed with IBM SPSS Statistics for Macintosh, Version 28.0. Mean, median, standard deviation, and interquartile range were used to evaluate statistical data. Kurtosis and skewness values were examined to determine whether the research variables showed a normal distribution. In the relevant literature, it is accepted as a normal distribution that the results regarding the kurtosis skewness values of the variables are between +1.5 and -1.5, +2.0 and -2.0[16,17]. Accordingly, it was determined that the data were distributed in accordance with the normal distribution. Chi-square analysis and independent groups T-test were used for parametric data. Pearson's correlation test was carried out to determine the relationship between the variables. A p-value of <0.05 was considered significant.

Ethical aspects

The Ethics Committee approval was obtained from XX University (Ethics Committee no: E-10840098-772.02-7253; date: 24/11/2022). The study was registered at ClinicalTrials.gov (identifier: NCT05503433). The study was conducted in accordance with the Declaration of Helsinki and followed the ethical standards of the country of origin. Written permission was obtained from the hospital where the research would be conducted. Written consent was retrieved from all participants before enrolment in the study.

RESULTS

The pregnant women who participated in the study were mainly primary school graduates (61.4%), lived in a nuclear family (79.5%), had an income equivalent to their expenses (61.4%), were pregnant women with a baby girl (63.6%), and planned their pregnancy (70.5%). Furthermore, their BMI averages were 25.50±4.36 (min: 23.34; max: 29.72). Their average age was 25.67±4.92 (min: 18; max: 35), and their gestational week average was 36.91±1.93 (min: 32; max: 41). There were no significant differences in the comparison of the groups in terms of education status, family type, income status, fetal gender, pregnancy planning status, BMI averages, age averages, and gestational week averages (p>0.05) (Tables 1 and 2).

In the analysis of the NST results of the groups, it was found that the reactive NST results were mostly seen in Group 1 (maternal salivary cortisol level ≤24.20), and there was a significant difference between the groups (p=0.000) (Group 1=97%; Group 2=51%). The number of at least two accelerations required for the diagnosis of reactive NST was also higher in Group 1. There was a significant difference between the intergroup acceleration averages and between the groups (p=0.000) (Table 2) (Group 1=97%; Group 2=52%). A moderately positive relationship between FHR and maternal salivary cortisol was observed (r=0.448, p=0.000) (Table 3).

Regression analysis to determine the cause-and-effect relationship between maternal cortisol and FHR was found to be significant (F=55.045; p=0.000<0.05). In total, 11.9% of the total change in FHR level is explained by maternal cortisol (R^2=0.119). Maternal cortisol increases FHR level (ß=0.349) (Table 4; Figure 1).

The regression analysis performed to determine the cause-and-effect relationship between maternal cortisol, BMI, age, gestational

Table 1. Socio-demographic and obstetric characteristics of pregnant women (n=400).

Characteristics	n	%
Education status		
Primary	245	61.4
High school	91	22.7
University and above	64	15.9
Family type		
Nuclear	318	79.5
Extended	82	20.5
Income status		
Income more than expenses	–	–
Income equal to expenses	245	61.4
Income less than expenses	155	38.6
Fetal sex		
Girl	255	63.6
Boy	145	36.4
Pregnancy planning status		
Planned pregnancy	282	70.5
Unplanned pregnancy	112	29.5
NST result		
Reactive	298	74.5
Nonreactive	102	24.5
Number of accelerations		
0–1	102	25.5
2 and above	298	74.5
	Min–max	$\bar{X}$±SD
BMI	23.34–29.72	25.50±4.36
Age (year)	18–35	25.67±4.92
GW	32–41	36.91±1.93
FHR	120.00–175.00	140.06±13.39
Cortisol (ng/mL)	16.98–29.70	23.70±3.30

Chi-square analysis; independent groups T-test; BMI: body mass index, GW: gestational week, IQR: interquartile range.

week, fetal sex, and FHR was found to be significant (F=13.513; p=0.000<0.05). In total, 13.6% of the total change in FHR level is explained by maternal cortisol, BMI, age, gestational week, and fetal sex (girl) (R^2=0.136). Maternal cortisol increases FHR level (ß=0.313). BMI does not affect FHR level (p=0.522>0.05). Age reduces the level of FHR (ß=-0,160). The gestational week does not affect FHR level (p=0.788>0.05). Fetal sex (girl) does not affect FHR level (p=0.585>0.05) (Table 5).

DISCUSSION

The fetal heart rate pattern is a parameter that is influenced by diurnal rhythm, gestational age, maternal pulse, and movements of the baby and also indicates fetal well-being[18]. In a study, it was determined that the basal fetal heart rate between the 37th and 40th gestational weeks was 143.10±.17[19]. Similarly, in another study, it was determined that the fetal heart rate in the third trimester was 134.5 (min 128.7; max 140.0) at week 36, 134.2 at week 37 (min 130.0; max 141.2), and 134.2 at week 38 (min 129.3; max 140.3)[20]. The mean gestational week of the pregnant women participating in the study was

Table 3. Correlation of fetal heart rate with body mass index, age, gestational week, and cortisol.

		BMI	Age	GW	Cortisol
FHR	r	-0.034	-0.216	0.261	0.349
	p	0.499	0.000	0.001	0.000

Pearson correlation test.

Table 2. Comparison of socio-demographic and obstetric characteristics according to cortisol levels (n=400).

Characteristics	Group I (n:203) Maternal saliva cortisol level ≤24.20		Group 2 (n:197) Maternal saliva cortisol level >24.21		p
	n (%)		n (%)		
Primary	128 (63)		117 (59)		0.128
High school	44 (21)		47 (23)		
University and above	31 (16)		33 (18)		
Family type					
Nuclear	158 (78)		160 (81)		0.347
Extended	45 (22)		37 (19)		
Income status					
Income equal to expenses	117 (58)		128 (64)		0.143
Income less than expenses	86 (42)		69 (36)		
Fetal sex					
Girl	130 (64)		125 (63)		0.456
Boy	73 (36)		72 (37)		
Pregnancy planning status					
Planned pregnancy	134 (66)		148 (75)		0.066
Unplanned pregnancy	69 (34)		49 (25)		
NST result					
Reactive	197 (97)		101 (52)		**0.000**
Nonreactive	6 (3)		96 (48)		
Number of accelerations					
0–1	6 (3)		96 (48)		**0.000**
2 and above	197 (97)		101 (52)		
	Min–max	$\bar{X}$±SD	Min–max	$\bar{X}$±SD	p
BMI	23.34–29.72	27.92±1.92	25.51– 29.41	27.92±1.33	0.232
Age (year)	21–30	24.36±3.13	19–27	22.36±2.61	0.372
GW	35.00–40.00	36.72±1.34	32.00–40.00	37.00±1.26	0.321
FHR	120.00–158.00	137.97±9.18	120.00–175.00	142.22±16.40	**0.027**

Chi-square analysis; independent groups T-test. Bold values indicate a statistically significant difference.

36.91±1.93, and the mean fetal heart rate was 140.06±13.39. Since the pregnant women participating in the study were in the third trimester, fetal neurological maturity was sufficient; thus, the fetal heart rate parameters were within normal limits.

Stress and anxiety during pregnancy are linked with differences in FHR and fetal movement and may have implications for future emotional development[21]. However, maternal anxiety seemed to affect the duration and variability of the FHR, with prolonged accelerations often fusing into sustained tachycardia[22]. Another study reported that the fetuses of mothers with depression had an elevated baseline FHR and a 3.5-fold delay in returning to baseline FHR after vibroacoustic stimulation (VAS)[23]. Thus, the maternal environment significantly influences the fetal autonomic nervous system and the central nervous system (CNS)[24]. It was found that the fetuses of women who had a cortisol increase following an arithmetic task had higher resting (HR) and less short-term HR variability (HRV) 20 min after the stressor task ended. There was a trend finding that participants who had a cortisol increase reported higher levels of life stress[25]. In other research, higher resting maternal cortisol during the third trimester was associated with greater fetal movement amplitude and amount (time spent) during a 50-min observation period[26]. In a study aimed to determine whether there were differences in FHR reactivity associated with the mother's psychiatric status as assessed by a psychological challenge, the Stroop color–word matching task, they reported that fetuses of women with high anxiety levels had more significant FHR increases than those with low anxiety levels[7,27]. Fetuses of pregnant women who report tremendous life stress have reduced parasympathetic or increased sympathetic activation, as measured by reduced FHR variability. Fetuses of highly stressed mothers, who also have a faster baseline heart rate, show reduced FHR variability and delayed maturation of the coupling between FHR and fetal movement, which is hypothesized to reflect a less mature CNS[7,27]. Elevated stress during pregnancy, specifically stress specific to being pregnant, is associated with greater fetal reactivity, as assessed at three time points during gestation. Fetuses of highly anxious women showed an increase in heart rate when their mother was exposed to psychological stress, while fetuses of low-anxiety women did not exhibit a change in heart rate[27]. Previous studies used the Stroop task to determine maternal stress levels; in the present study, stress was evaluated using the salivary cortisol level. In this study, it

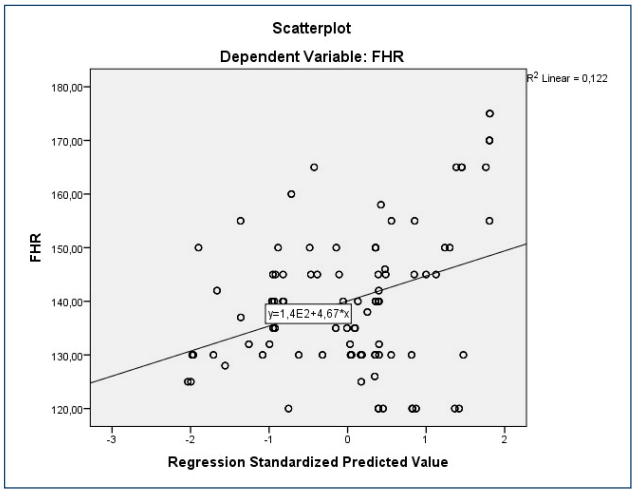

Figure 1. Effect of maternal cortisol on fetal heart rate.

Table 4. Effect of maternal cortisol on fetal heart rate.

Independent variable	Unstandardized coefficients		Standardized coefficients	t	p	95% confidence ınterval	
	B	SE	ß			Lower bound	Upper bound
Constant	106.573	4.558		23.383	0.000	97.612	115.533
Maternal Cortisol	1.413	0.190	0.349	7.419	**0.000**	1.038	1.787

Linear regression analysis; dependent variable=FHR; R=0.349; R^2=0.119; F=55.045; p=0.000; Durbin-Watson=0.442.

Table 5. Effect of independent variables on fetal heart rate.

Independent variable	Unstandardized coefficients		Standardized coefficients	t	p	95% confidence ınterval	
	B	SE	ß			Lower bound	Upper bound
Constant	114.497	13.734		8.337	0.000	87.496	141.498
Maternal cortisol	1.269	0.206	0.313	6.163	**0.000**	0.865	1.674
BMI	0.093	0.145	0.030	0.641	0.522	-0.192	0.378
Age	-0.434	0.132	-0.160	-3.286	**0.001**	-0.694	-0.174
Gestational week	0.094	0.350	0.014	0.269	0.788	-0.593	0.782
Fetal sex (girl)	0.697	1.275	0.026	0.547	0.585	-1.810	3.204

Linear regression analysis; dependent variable=FHR; R=0.383; R^2=0.136; F=13.513; p=0.000; Durbin-Watson=0.450.

was determined that FHR increased as maternal saliva cortisol levels increased. Following the literature, it can be thought that the cortisol rising with the increase in maternal stress level affects fetal reactivity, causes the acceleration of the fetal heartbeat, and therefore may be a harbinger of fetal tachycardia. In the study conducted by Kısa Karakaya et al.[28] 60–120 min after birth, it was found that maternal cortisol and fetal cord cortisol levels were affected by the mode of delivery, but there was no correlation between maternal cortisol and fetal cord cortisol levels (r=-0.192, p=0.336). According to these results, it can be said that maternal cortisol may cause fetal tachycardia in the intrauterine period, but it has no effect in the long term.

Strengths and limitations

Since there is no study evaluating FHR with cortisol levels in primiparous pregnant women who were previously in the third trimester, this study's findings will constitute the first result that will be reflected in clinical applications in this direction. The limitations are that, due to the nature of the research, the data obtained can only be generalized to primiparous pregnant women without uterine contractions, cervical dilation, or fetuses without decelerating heartbeats.

CONCLUSION

These findings suggest that stress in primiparous pregnant women with high cortisol levels may influence FHR patterns. It was found that the increase in cortisol level, which is considered a stress hormone, may be a harbinger of fetal tachycardia. To maintain fetal well-being, it is essential to minimize women's stress levels in the perinatal period.

AUTHORS' CONTRIBUTIONS

AT: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **CK:** Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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National Institute of Health Stroke Scale was associated with the immediate and long-term prognosis of patients with acute ischemic stroke treated with intravenous thrombolysis

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SUMMARY

OBJECTIVE: The objective of this study was to examine whether the National Institute of Health Stroke Scale was associated with the short- and long-term prognosis of patients with acute ischemic stroke treated with intravenous thrombolysis.

METHODS: A total of 247 patients with acute ischemic stroke admitted to the hospital from April 2019 to October 2020 were retrospectively selected as study subjects, and the immediate and long-term prognosis after thrombolysis was assessed using the modified Rankin Scale and divided into good prognosis group (119 cases) and poor prognosis group (128 cases) based on the effect of thrombolysis. Both groups were treated with alteplase, the National Institute of Health Stroke Scale of the two groups was compared, and the factors affecting the prognosis of acute ischemic stroke were analyzed.

RESULTS: After intravenous thrombolysis, 24 h, and 7 days of treatment, the National Institute of Health Stroke Scale in the poor prognosis group was higher than those of patients in the good prognosis group, and the differences were statistically significant ($p<0.05$). The results of the multivariate analysis suggested that National Institute of Health Stroke Scale before treatment was an independent factor associated with the 3-month (OR: 1.068, 95%CI 1.015–1.123, $p=0.011$) and long-term poor prognosis (OR: 1.064, 95%CI 1.012–1.119, $p=0.015$) in patients with acute ischemic stroke receiving intravenous thrombolysis after adjustment of age, gender, body mass index, smoking, alcohol consumer, onset-to-door time, door-to-needle time, and imaging score.

CONCLUSION: The National Institute of Health Stroke Scale could be a promising indicator for the prognosis, and active intervention is needed to improve the quality of life in patients with acute ischemic stroke.

KEYWORDS: Acute ischemic stroke. Prognosis. Thrombolytic therapies.

INTRODUCTION

Acute ischemic stroke (AIS) is a common cerebrovascular disease with a rapid onset and a high disability and mortality rate[1]. It is a localized disorder of blood supply to the brain tissue region due to various causes, which subsequently results in neurological deficits due to Ischemia and hypoxia of brain tissue[2]. Most of these patients have severe vascular atherosclerosis and are often combined with other systemic diseases, which results in a relatively poor prognosis. Early opening of occluded vessels and restoration of intracerebral blood circulation are the keys to saving the lives of patients with AIS and improving their prognosis. Numerous studies have demonstrated that the ischemic hemispheric zone has viable cells, and early intravenous thrombolysis can re-establish circulation in the ischemic zone and reduce neuronal cell damage[3]. In this study, we used intravenous thrombolytic therapy with alteplase to treat the patients with AIS and analyzed the factors associated with the immediate and long-term prognosis of AIS.

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METHODS

Study design and population

This study was carried out in the Neurology Department of our hospital, in the period from April 2019 to October 2020. Patients were eligible for inclusion in the study if they were ≥18 years, had received the clinical diagnosis of AIS, and presenting within 24 h of symptom onset. IV rTPA was administered based on the Chinese Stroke Rehabilitation Guidelines for the early management of the AIS 2019 scientific statement. The IV thrombolysis administered was alteplase (Actilyse) by Boehringer-Ingelheim, Germany.

Inclusion criteria were as follows: (1) clinical symptoms met the diagnostic criteria of "Chinese Stroke Rehabilitation Guidelines (2011)"; (2) a confirmed diagnosis by physical examination, imaging, and neurological examination; (3) duration of illness <4.5 h; (4) indication for thrombolysis, i.e., blood pressure not exceeding 180/100 mmHg; and (5) patient (or family) agreed to thrombolytic therapy and signed an informed consent form.

Exclusion criteria were as follows: (1) combination with cerebrovascular malformation, cerebral aneurysm, and other diseases; (2) combination with active bleeding disease; (3) combination with renal failure, liver cancer, and other serious organic diseases; (4) combination with severe hypertension; (5) major surgery within the last 2 weeks; (6) recent use of anticoagulant drugs; and (7) allergic to the drugs used in the study.

The following clinical data were collected from patients, including age, sex, baseline characteristics, time from admission to thrombolysis (DNT), time from onset to thrombolysis (ONT), past history (smoking, hypertension, hyperlipidemia, diabetes, atrial fibrillation, stroke, history of antiplatelet drug use, and history of anticoagulant drug use), and modified Rankin Scale (mRS) score assessed 3 months after thrombolysis.

Intravenous thrombolysis

Patients were given 10% alteplase (Boehringer Ingelheim Pharma GmbH & Co. KG, specification: 50 mg/stem) mixed with saline and administered intravenously at 0.9 mg/kg, with a total drug volume of <90 mg. The remaining 90% alteplase was mixed with saline and administered intravenously by drip, with the dosing time controlled within 1 h. After treatment, patients with a stable condition and stable signs after treatment were considered to have a good prognosis (mRS≤2), and patients with insignificant changes or continuous deterioration or death after treatment were considered to have a poor prognosis (mRS≥2).

Observation indexes and evaluation criteria

The National Institute of Health Stroke Scale (NIHSS) was compared between the two groups[4-7]. The neurological deficits were scored based on the NIHSS scale before, after, 24 h, and 1 week after the treatment, respectively, with the highest score of 20, and the higher score indicates the more serious neurological deficits of the patients. The clinical outcome of patients was assessed by the mRS at 3 months and 1 year after the treatment. Patients were followed up for 1 year and inquired about their health condition. The mRS is a measuring method for disability. Scores according to the mRS range from 0 (no symptoms at all) to 6 (death). Patients' clinical outcome was categorized as favorable (score 0–2) or unfavorable (score 3–6)[8]. Systolic blood pressure: normal adult systolic blood pressure should be ≤140 mmHg; the higher the value, the more serious the disease. Diabetes history taunt: human blood glucose value >3.9–6.1 mmol/L can be determined as diabetes, and the higher the value, the more serious the degree of disease.

Statistical analysis

SPSS version 18.0 statistical software was used for data analysis. The measurement data were expressed as mean±standard deviation, and the count data were expressed as counts (percentages). The chi-square test was used for comparison between groups. Potential variables associated with the poor prognosis were screened in the univariate analysis, and confounding factors were adjusted in the multivariate analysis to obtain a risk model combined with independent risk factors. The difference was considered statistically significant when $p<0.05$.

RESULTS

Comparison of basic information between groups before and after treatment

In total, 247 patients were included in this study with a mean age of 61.9±13.4 years and 161 males (65.2%). There were 128 and 129 patients with a poor prognosis at 3-month and long-term follow-ups, respectively. Compared with patients with a good prognosis, those with a poor prognosis were more likely to be older and have a higher pre-onset mRS and imaging score ($p<0.05$ for both). There was no significant difference in other baseline characteristics between the two groups (Tables 1 and 2).

Comparison of National Institute of Health Stroke Scale scores between the 3-month good prognosis and bad prognosis before and after treatment

Table 1. Baseline characteristics based on the 3-month prognosis.

	Good prognosis (n=119)	Poor prognosis (n=128)	p-value
Age, years	59.7±12.9	63.9±13.5	0.013
Male	80 (67.2%)	81 (63.3%)	0.515
Height, cm	168.4±7.7	167.8±7.35	0.500
Weight, kg	69.0±10.7	69.6±12.2	0.684
BMI, kg/m^2	24.3±3.1	24.7±4.0	0.356
Pre-onset mRS	2.18±1.44	2.65±1.49	0.014
Systolic BP, mmHg	146.3±22.6	148.4±20.9	0.452
Diastolic BP, mmHg	85.2±12.8	84.2±13.2	0.573
Hypertension	44 (37.0%)	50 (39.1%)	0.736
Diabetes mellitus	17 (14.3%)	22 (17.2%)	0.532
Smoking	45 (37.8%)	42 (32.8%)	0.411
Alcohol consumer	28 (23.5%)	25 (19.5%)	0.444
ONT, min	148.3±70.3	152.5±56.3	0.607
DNT, min	51.5±32.4	50.2±28.9	0.745
rtPA, mg	54.33±11.62	54.94±13.60	0.706
NIHSS			
Before treatment	5.55±4.67	7.51±6.37	0.006
After treatment	3.90±3.89	6.31±5.98	<0.001
24 h after treatment	3.03±3.93	5.75±6.06	<0.001
7 days after treatment	1.79±2.93	4.42±5.81	<0.001
Imaging score	1.66±0.77	1.84±0.73	0.054

Values are expressed as n (%) or mean±standard deviation.

Table 2. Baseline characteristics based on the long-term prognosis.

	Good prognosis (n=118)	Poor prognosis (n=129)	p-value
Age, years	59.7±13.0	63.9±13.5	0.013
Male	79 (66.9%)	82 (63.6%)	0.577
Height, cm	168.4±7.7	167.8±7.3	0.577
Weight, kg	69.0±10.8	69.6±12.2	0.695
BMI, kg/m^2	24.3±3.1	24.7±4.0	0.399
Pre-onset mRS	2.18±1.44	2.65±1.48	0.012
Systolic BP, mmHg	146.2±22.7	148.5±20.8	0.412
Diastolic BP, mmHg	85.1±12.8	84.3±13.1	0.616
Hypertension	44 (37.3%)	50 (38.8%)	0.896
Diabetes mellitus	17 (14.4%)	22 (17.1%)	0.569
Smoking	44 (37.3%)	43 (33.3%)	0.516
Alcohol consumer	27 (22.9%)	26 (20.2%)	0.602
ONT, min	148.7±70.5	152.1±56.2	0.672
DNT, min	51.5±32.5	50.3±28.8	0.755
rtPA, mg	54.27±11.65	54.98±13.56	0.659
NIHSS			
Before treatment	5.58±4.68	7.47±6.35	0.008
After treatment	3.91±3.90	6.29±5.96	<0.001
24 h after treatment	3.06±3.94	5.70±6.06	<0.001
7 days after treatment	1.81±2.93	4.39±5.80	<0.001
Imaging score	1.64±0.76	1.85±0.73	0.032

Values are expressed as n (%) or mean±standard deviation.

Before the treatment, the NIHSS scores of the two groups were compared, and the difference was statistically significant (p<0.05). After the treatment, 24 h, and 7 days of treatment, the NIHSS scores of both groups were lower than those before treatment, and the NIHSS scores of the group with good prognosis were lower than those of the group with poor prognosis, and the differences were statistically significant (p<0.05) (Table 1).

Comparison of National Institute of Health Stroke Scale scores between the long-term good prognosis and bad prognosis before and after treatment

Before the treatment, the NIHSS scores of the two groups were compared, and the difference was statistically significant (p<0.05). After the treatment, 24 h, and 7 days of treatment, the NIHSS scores of both groups were lower than those before treatment, and the NIHSS scores of the group with a good prognosis were lower than those of the group with a poor prognosis, and the differences were statistically significant (p<0.05) (Table 2).

Multivariate logistic regression analysis for the 3-month pprognosis of ppatients with acute ischemic stroke

After adjustment of other confounding variables, the multivariate analysis suggested that only NIHSS before treatment (OR: 1.068, 95%CI 1.015–1.123, p=0.011) was associated with the 3-month poor prognosis in patients with AIS (Table 3).

Multivariate logistic regression analysis for the long-term prognosis of patients with acute ischemic stroke

Similar to the above, the multivariate analysis suggested that NIHSS before treatment (OR: 1.064, 95%CI 1.012–1.119, p=0.015) was an independent factor associated with the long-term poor prognosis in patients with AIS receiving intravenous thrombolysis after adjustment of age, gender, body mass index (BMI), smoking, alcohol consumer, onset-to-door time (ODT), DNT, and imaging score (Table 3).

Table 3. Multivariate logistic regression analysis.

Multivariate logistic regression analysis for 3-month prognosis			
Variables	**OR**	**95%CI**	**p-value**
Age ≥60 years	1.362	0.784–2.366	0.273
Male	1.037	0.544–1.974	0.203
BMI	1.044	0.970–1.123	0.251
Smoking	0.780	0.379–1.606	0.500
Alcohol consumer	0.978	0.454–2.110	0.955
ODT	1.002	0.997–1.006	0.450
DNT	0.996	0.987–1.006	0.443
NIHSS before treatment	1.068	1.015–1.123	0.011
Imaging score	1.263	0.882–1.808	0.203
Multivariate logistic regression analysis for long-term prognosis			
Variables	OR	95%CI	p-value
Age ≥60 years	1.399	0.805–2.428	0.233
Male	1.041	0.546–1.982	0.903
BMI	1.040	0.967–1.119	0.288
Smoking	0.800	0.389–1.647	0.545
Alcohol consumer	1.051	0.488–2.266	0.899
ODT	1.001	0.997–1.006	0.543
DNT	0.997	0.987–1.006	0.507
NIHSS before treatment	1.064	1.012–1.119	0.015
Imaging score	1.312	0.916–1.881	0.139

BMI: body mass index; ODT: onset-to-door time; DNT: door-to-needle time; NIHSS: National Institute of Health Stroke Scale.

DISCUSSION

As the aging society becomes more and more severe, the proportion of patients with AIS is increasing year by year, mainly because the elderly generally need to be bedridden for a long period of time, exercise is reduced, clinical symptoms are not obvious, various bodily functions are reduced, and the metabolic level is low, thus making atherosclerosis. AIS accounts for about 70% of all strokes. Early intravenous thrombolytic therapy effectively dissolves the thrombus, promotes the recovery of nerve and blood flow, prevents the occurrence of tissue edema, and provides protection for the patient's physical health. The clinical principles of treatment are thrombolysis, protection of the nervous system, lowering blood pressure, and increasing mobility. Early intravenous thrombolytic therapy effectively dissolves the thrombus, inhibits lipid peroxidation, and protects nerve cell function in order to promote the recovery of nerve and blood flow and prevent the occurrence of tissue edema, thus changing the patient's neurological deficit[3,9].

Intravenous thrombolysis is an important method for the early treatment of AIS. The European Travel Plan recommends first-line drug alteplase within 3 h of the acute onset of ischemic stroke, and the majority of scholars believe that intravenous thrombolysis for AIS can extend the time window to 4.5 h[10,11]. Alteplase is a thrombolytic agent, unlike the traditional thrombolytic drug urokinase, which specifically binds to the fibrin on the surface of the thrombus, activates fibrinogen, and converts it into fibrin, thus exerting an antithrombotic effect. Clinical studies have demonstrated that patients with AIS can be treated in hospitals 3–6 h after the onset of stroke. The shorter the time is, the more significant the treatment effect will be. The shorter the time is, the more significant the treatment effect will be, so early interventional intravenous thrombolysis treatment is the main method. In this study, after treatment, the NIHSS scores after treatment, 24 h, and 7 days in the poor prognosis group were higher than those in the good prognosis group, and the difference was statistically significant ($p<0.05$). It is suggested that NIHSS after treatment has a certain indicative function for both future and distant prognosis, and if patients have unsatisfactory NIHSS scores after thrombolytic therapy, it should be brought to the attention of physicians.

There are many causes of AIS, such as hypertension, coronary heart disease, atrial fibrillation, chronic bronchitis, diabetes mellitus, hyperlipidemia, and bad habits such as smoking and drinking, all of which can lead to insufficient blood supply to the brain, causing necrosis of brain tissue, which leads to sclerosis or thrombosis of the blood[12,13], and then blockage of blood vessels and acute cerebral hypoperfusion, which can seriously threaten life safety. Therefore, the earlier the clinical intervention of intravenous thrombolysis for AIS, the better the recovery of patients' health, thus reducing the death and disability rate and improving the treatment effect of patients[14,15].

There are some shortcomings in this study: first, this study is retrospective; again, non-intravenous thrombolysis patients were not included in this study, and some in-hospital stroke patients may have been lost to thrombolysis because of too late detection or process delays, which may have underestimated in-hospital stroke delays. Therefore, there is a need to further expand the sample size and conduct a more in-depth and objective study of in-hospital stroke.

In summary, the use of intravenous thrombolytic therapy in patients with AIS is clinically effective, and the shorter the duration of treatment, the more beneficial it is in reducing neurological deficits in patients with AIS. The shorter the treatment time, the better the reduction in neurological impairment.

The factors associated with AIS are related to underlying conditions such as hypertension, which require clinical intervention, and NIHSS scores after thrombolysis, which can help indicate prognosis and improve the quality of life.

AVAILABILITY OF DATA AND MATERIALS

Data not directly reported in this publication can be obtained from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Xingtai People's Hospital. Written informed consent was obtained from all participants.

AUTHORS' CONTRIBUTIONS

QX: Conceptualization, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing. **YS:** Conceptualization, Resources, Validation, Writing – original draft, Writing – review & editing. **JW:** Data curation, Project administration, Software, Writing – original draft, Writing – review & editing. **YFL:** Formal Analysis, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. **YTL:** Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **YLL:** Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **XS:** Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **ZL:** Formal Analysis, Investigation, Project administration, Visualization, Writing – original draft, Writing – review & editing. **BZ:** Formal Analysis, Methodology, Writing – original draft, Writing – review & editing.

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ORIGINAL ARTICLE

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Evaluation of functional parameters of the foot and ankle in elderly with sarcopenia

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SUMMARY

OBJECTIVE: With population aging, the prevalence of sarcopenia has increased. It is a pathology often neglected, with the potential to cause great damage if not diagnosed and treated. The objective of this study was to identify sarcopenic elderly people through the SARC-F score and palm grip test and to evaluate foot and ankle functionality parameters: gait speed, plantar sensitivity, and baropodometry.

METHODS: This is a descriptive and cross-sectional study. The sample consisted of 20 sarcopenic elderly diagnosed through the SARC-F score and the handgrip strength test, from which demographic data were obtained, and the three functional tests related to the foot and ankle were performed.

RESULTS: No individual was aware of the term sarcopenia. Regarding gait speed, 20 (100%) presented values compatible with sarcopenia (average of 0.52 m/s). Regarding plantar sensitivity, five (25%) of the patients showed changes in the exam with the detection of insensitivity. Regarding baropodometry, higher pressure values were observed in the right foot (average of 52.9±7.01%) compared to the left (average of 47.10±7.01%) and in the hindfoot (average of 55.85±16.21%) compared to the forefoot (mean 44.15±15.35%). When correlating the analyzed variables with the SARC-F scores, the only association that showed statistical significance ($p<0.05$) was the dynamometry on the right.

CONCLUSION: The SARC-F score and the handgrip strength test are easy to apply in the screening of sarcopenia, and the functional parameters of the foot and ankle were shown to be altered in the studied group.

KEYWORDS: Aging. Muscles. Muscle, skeletal. Sarcopenia.

INTRODUCTION

With population aging, the prevalence of sarcopenia has increased[1]. The European Working Group on Sarcopenia in the Elderly Population (EWGSOP2) recommends, for investigation, the application of the sarcopenia form (SARC-F)[2] questionnaire, for the detection of characteristic signs of sarcopenia. Once detected, the diagnosis can be confirmed by testing the handgrip strength using a calibrated dynamometer, which is a simple and inexpensive method[3].

It is known that sarcopenia is associated with changes in muscle architecture. As muscle size reduces with advancing age, muscle fibers become shorter and less feathered, which directly interferes with muscle function[4]. Aging is associated with the degeneration of the nervous system, which may affect plantar sensitivity, an important source of information for balance control, as it encodes changes in pressure under the foot, especially during gait[5].

The relationship between sarcopenia and functional parameters of the foot and ankle, such as gait speed, plantar sensitivity, and plantar pressure, has been little explored in the literature. It is questioned whether sarcopenia, through changes in muscle architecture and metabolic changes, affects the functionality parameters described above, a situation that, in a fragile group, can be very debilitating. The objective of this study was to evaluate the tracking of sarcopenia through the SARC-F score and the handgrip test and to evaluate foot and ankle functionality parameters in elderly individuals with sarcopenia.

This project was submitted to the ethics committee and approved under protocol 5.149.988. All participants signed an informed consent form.

This is a descriptive and cross-sectional study consisting of two stages. In the first, in a tertiary hospital, in 2 months (April and May of 2021), all 180 patients treated at an orthopedic outpatient clinic were included. After applying the exclusion criteria (i.e., age less than 60 years, use of walking devices, orthopedic, dermatologic, or neurologic diseases of the lower limbs, psychiatric disorders that interfered with participation, and diabetes mellitus), 39 were elected to participate in the study, to which the SARC-F[2] questionnaire was applied. Of these, 20 obtained a score suggestive of sarcopenia (SARC-F≥4), being included in the second stage of the study.

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The second stage was carried out in the human performance laboratory of a public university, where the 20 individuals were submitted to the handgrip strength test and functionality tests related to the foot and ankle.

Handgrip strength was assessed using a Jamar®[6] dynamometer. A value of less than 27 kg in males and a value of less than 16 kg in females in handgrip are objective indicators of sarcopenia[1]. The subjects remained seated in an office-type chair. The arm was kept suspended in the air with the hand positioned on the dynamometer, which was supported by the evaluator[6]. The same was repeated 3× on the right side and 3× on the left side, with an interval of 1 min between repetitions. The highest value obtained on each side was considered for registration. Figure 1 shows the position of the volunteer to assess the handgrip strength with a dynamometry instrument.

A socio-demographic questionnaire was applied to each participant containing the following variables: age, history of falls and previous fractures, and knowledge about sarcopenia.

The manual measurement of the 4 m gait speed was performed, being the gold standard test for such an evaluation in older adults[7]. According to the EWGSOP2[1], a velocity lower than or equal to 0.8 m/s is an indicator of sarcopenia. A well-defined path of 4 m was established, with the beginning and end marked on the ground. The time count started with the first movement of the participant's foot crossing the starting line and ended as soon as the foot crossed the final line[7].

The assessment of plantar cutaneous sensitivity was performed using a Semmes-Weinstein monofilament (referring to 10 g). Sensitivity evaluation was performed in three plantar areas of each foot: hallux, plantar region of the first metatarsal head, and plantar region of the fifth metatarsal head, and repeated three times each[8].

Figure 1. Positioning of the volunteer to assess the handgrip strength with a dynamometry instrument.

The assessment of plantar pressure in the right and left foot was performed using an electronic baropodometry platform (Sensor Medica®, Guidonia Montecelio, Italy) connected to a computer (Dell All®, Texas, USA). For the baropodometrics, the participants were positioned barefoot in the orthostatic posture with bipedal support on the baropodometric platform. The platform was positioned 1 m from the wall, and the participants were instructed to keep their eyes fixed, taking as reference a fixed point on the wall at eye level, with arms relaxed along the body[9].

The quantitative variables were classified using measures of central tendency and for categorical variables, absolute and relative frequency. Data analysis was performed using Minitab version 19.1 and the Statistical Package for the Social Sciences, Inc. (SPSS) Chicago, USA, version 26.0. The significance level used was $p<0.05$. Spearman's ordinal correlation test was used to assess the correlations based on the distribution of variables.

RESULTS

Twenty individuals with a mean age of 75.6±5.9 years (ranging from 65 to 90 years) were evaluated, 95% of whom were female. A total of 100% mentioned a history of falls and 40% mentioned a history of fractures. A total of 100% of the sample reported unfamiliarity with the term sarcopenia.

Table 1 shows the descriptive analysis of the functional parameters.

Regarding gait speed, the maximum and minimum values obtained were, respectively, 0.76 and 0.2 m/s, with an average, in the sample, of 0.52 m/s and a standard deviation of 0.13. Regarding plantar sensitivity, five individuals (25%) presented alterations in the exam.

Regarding baropodometry, when evaluating the distribution of plantar pressure in the left and right feet, higher pressure values were observed in the right foot (mean of 52.9±7.01%) compared to the left (mean of 47.10±7.01%). When comparing the load distribution in the anteroposterior direction, greater pressures were observed in the hindfeet (mean of 55.85±16.21%) compared to the forefeet (mean of 44.15±15.35%).

When correlating the analyzed variables with the SARC-F scores through Spearman's correlation, the only association that showed statistical significance ($p<0.05$) was the right dynamometry, whose interpretation was that lower levels of grip strength in the right hand were associated with a higher level of SARC-F score. Table 2 exemplifies Spearman's correlation between SARC-F and the analyzed variables.

DISCUSSION

Malmstrom and Morley[10] suggested that a SARC-F score greater than or equal to 4 is a predictor of sarcopenia. In this study, of the 39 elderly people who met the inclusion criteria, 20 had a SARC-F score suggestive of sarcopenia, and of these 20, 100% had confirmation through the handgrip strength test. In an investigation carried out in Spain with 235 elderly women, Aibar-Almaz et al. found an association between sarcopenia and falls[11]. Lim et al. investigated the association between sarcopenia and falls in 147 elderly patients aged over 65 years from different hospitals in South Korea[12] with hip fractures. As a result, the authors found a significant correlation between sarcopenia and falls. Such findings are in line with the results obtained in the study in question, in which 100% of the elderly participants reported a history of falls and 40% reported a history of suffered fractures.

A study that evaluated handgrip strength in elderly people over 65 years of age in Turkey[13] in 2016, evaluating 406 individuals, found a mean strength of 25.7±8.7 kgf. In the study in question, the handgrip strength values were lower, as this was a more fragile group with a SARC-F score suggestive of sarcopenia. An average force (kgf) of 13.25±1.41 was found on the right, and when correlating the force values obtained in the dynamometry with the SARC-F score, lower levels of force were observed in higher scores of SARC-F. A systematic review analyzed the effects of resistance training on muscle strength in very elderly adults and found that participation in resistance training over 8–18 weeks with a frequency o 1–3 days per week can restore the strength that has been potentially lost over several years of inactivity[14].

It is believed that the deterioration in gait speed related to sarcopenia during aging is due to qualitative and quantitative changes in muscle structure and function[15]. A study carried out in Colombia[16] evaluated gait speed as a predictor of sarcopenia including 19,705 individuals. A higher prevalence of sarcopenia was found at older ages, a result consistent with the study in question, in which higher SARC-F scores were found in older age groups. A Brazilian study in 2016 evaluated the gait speed in hospitalized elderly people. In a total of 110 elderly people, the average speed value was 1.26±0.44 m/s, and, of these, 15

Table 1. Descriptive statistics of functional variables and handgrip strength test.

Variables	Mean	SD	1° Q	Median	3° Q
Right handgrip strength (kgf)	13.250	1.410	12.00	14.000	14.000
Left handgrip strength (kgf)	11.550	2.144	10.000	12.000	13.500
Gait speed (m/s)	0.5290	0.1398	0.4400	0.5500	0.6500
Plantar sensitivity	0.767	0.53	0.287	0.69	1.045
Right full weight bearing (%)	52.9	7.01	46.5	56.50	1.022
Left full weight bearing (%)	47.10	7.01	43.50	47.50	53.50
Right hindfoot weight bearing (%)	28.35	9.63	25.00	30.00	34.00
Left hindfoot weight bearing (%)	27.50	6.58	24.00	25.50	32.25
Right forefoot weight bearing (%)	24.55	9.09	19.50	25.00	29.00
Left forefoot weight bearing (%)	19.60	6.26	16.00	20.50	24.00

SD: standard deviation.

Table 2. SARC-F and variables correlation.

Variable 1	Variable 2	Correlation	95%CI for ρ	p-value
SARC-F	Right handgrip strength (kgf)	-0.516	(-0.792 to 0.065)	**0.020**
SARC-F	Previous fractures	0.267	(-0.207 to 0.639)	0.256
SARC-F	Gait speed	-0.152	(-0.559 to 0.314)	0.522
SARC-F	Plantar sensitivity	0.095	(-0.364 to 0.517)	0.690
SARC-F	Left handgrip strength (kgf)	-0.067	(-0.495 to 0.388)	0.780
SARC-F	Age	-0.025	(-0.463 to 0.422)	0.916

Statistically significant value is indicated in bold.

patients had a gait speed lower than or equal to 0.8 m/s, suggestive of sarcopenia[17]. Comparing the different ages, a tendency toward a reduction in gait speed values was observed in the more advanced age groups, which is also compatible with the findings of the study in question.

A Brazilian study evaluated the differences in plantar sensitivity between 19 young adults and 19 elderly people[18]. The elderly showed greater loss of plantar sensitivity compared to adults. In this study, when evaluating 20 elderly people known to have sarcopenia and without pathologies that affect plantar sensitivity, five (25%) had impaired plantar sensitivity; however, no significant correlation was established between insensitivity and the SARC-F score. A study carried out in China evaluated the correlation between diabetic neuropathy and sarcopenia in type 2 diabetics[19]. A total of 1,104 patients were included, of which 204 had sarcopenia. There was a higher prevalence of neuropathy in sarcopenic than in non-sarcopenic patients.

Sousa et al.[20] studied the distribution of plantar pressure in two groups of women of different age groups: 50–65 years and 66–88 years. In both groups, greater plantar pressure was detected in the right foot, compared to the left, and greater in the hindfoot, compared to the forefoot. Such results are in line with the study in question, with the same finding. A study by Alvaro et al.[18] evaluated the differences in plantar pressure between 19 young adults and 19 elderly people. In agreement with the study in question, both groups had higher pressures in the hindfeet regions. However, comparing both groups of different age groups, the elderly had values of plantar pressure in the forefoot higher than young adults. The hypotheses that would justify this, according to the authors, could be the natural modification that occurs in the feet of the elderly, with a reduction in the medial plantar arch, or even the posture with a greater anterior inclination of the trunk that the elderly adopt during the orthostatic position. This hypothesis is in line with the study in question. When analyzing the positioning of the center of gravity in the anteroposterior direction of this group of sarcopenic elderly, it was found that in 90% of the sample, it was detected in a pre-fixed position.

A Japanese study evaluated the use of customized insoles as a factor for improving physical activity levels in individuals with sarcopenia. The sample was divided into two groups; half used the customized insoles for 6 months, compared to the other half who did not use it. It was seen that the use of the insole improved the pain reported during walking and improved the levels of physical activity when comparing both groups, despite not having improved the muscle quantity, that is, objectively, it did not interfere with the sarcopenia status[21].

Among the limitations of the study, the following stand out: sample defined by convenience; definition of sarcopenia based on the application of a score associated with a physical test, without a complementary exam that quantitatively evaluated muscle tissue; absence of a control group to compare the data obtained; and scarcity, in the literature, of studies that evaluated the same variables explored in this work. Among the strong points, considering the high prevalence of sarcopenia, this is, as far as the author is aware, the first study that describes the parameters of functionality in this specific group in a state of Brazil.

CONCLUSION

The prevalence of sarcopenia increases with population aging but is often neglected in clinical practice and, as seen in this work, unknown to the elderly population. The SARC-F score and the handgrip strength test were considered easy to apply in the screening of sarcopenia. When evaluating the functional parameters related to the foot and ankle, it has been noted: a reduced gait speed, sensitivity change in 25% of the sample, and, in relation to plantar pressure distribution, greater pressures in the right side and on hindfeet, highlighting the pre-fixed positioning of the center of gravity.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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The relationship between diabetes burden and successful ageing in diabetic elderly patients

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SUMMARY

OBJECTIVE: The aim of this study was to determine the diabetes burden in elderly individuals along with successful ageing, which defines how well individual ages contribute to coping with the disease and diabetes management. This study also aimed to evaluate the relationship between diabetes burden and successful ageing in elderly individuals with type 2 diabetes.

METHODS: The data for this descriptive study were collected from 526 individuals who were 65 years old patients diagnosed with type 2 diabetes in the diabetes polyclinic of a research and training hospital between January and June 2021.

RESULTS: It was found that the Successful Ageing Scale score was higher in women, those who had regular diabetes control, and those who had easy access to health services. Elderly Diabetes Burden Scale scores were found to be higher in men, those whose diabetes treatment was insulin, and those with poor perceived health status. No statistically significant relationship was determined between the Elderly Diabetes Burden Scale total score and the Successful Ageing Scale total score ($p>0.05$).

CONCLUSION: Accordingly, by enabling the elderly to have easy access to healthcare services, preventing complications, and providing elderly healthcare services, it will be possible to reduce the diabetes burden in the elderly and enable them to age successfully.

KEYWORDS: Disease burden. Diabetes mellitus, type 2. Healthy aging. Aging.

INTRODUCTION

Type 2 diabetes is a prevalent chronic disease that can be observed in all societies, especially in adulthood; it threatens the patient's ability to sustain an independent life, has a significant effect on the patient and the family, follows a course full of complications, can lead to organ damage when not treated well, severely reduces the quality of life, and has a relatively high cost[1-3]. According to the data of the International Diabetes Federation, 537 million adult individuals (20–79 years old) had diabetes worldwide in 2021, and this number is estimated to rise to 783 million by 2030[2]. The prevalence of diabetes in Turkey is similar to the world data, and its prevalence increased from 7.7 to 13.7%, with an increased rate of 90% between 1998 and 2010[4]. It has also been reported that type 2 diabetes makes up 90–95% of the total number of diabetes cases, and its prevalence in the elderly population is approximately 32%[1].

Along with the ageing process, both the burden brought about by chronic diseases and coping with several emotional, spiritual, and social problems are among the important issues in a successful ageing process[5,6]. Successful ageing is associated with factors such as avoiding diseases, physical and mental functionality, active participation in life, absence of disease, psychological well-being, life satisfaction, financial security, and having a positive perspective on life. Through activating a successful ageing process, it is aimed to minimise the biological, sociocultural, economic, and psychological losses and damages of the elderly individual[6-8].

In the literature review conducted, a limited number of studies that examined the relationship between diabetes burden in elderly individuals with type 2 diabetes and successful ageing were accessed[9-12]. It is believed that determining the diabetes burden in elderly individuals along with successful ageing, which defines how well an individual ages, will significantly contribute to coping with the disease and diabetes management. Besides, measuring diabetes burden and successful ageing levels in order to apply effective nursing care for elderly individuals with diabetes, increase patient satisfaction, and therefore reduce healthcare expenses will benefit the planning of diabetes treatment and care as well as the development of successful ageing policies for countries. Hence, the present study aimed to examine the relationship between diabetes burden in elderly individuals with type 2 diabetes and successful ageing.

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METHODS

Study design and participants

This descriptive study was conducted with the participation of elderly patients diagnosed with type 2 diabetes in the diabetes polyclinic of a research and training hospital between January and June 2021. An average of 200 elderly diabetes patients visit the diabetes polyclinic, and accepting this number as the population of the study, the minimum sample size was calculated as 520 with a 95% confidence interval and ±%5 sampling error. The inclusion criteria were determined as follows: (a) being voluntary to participate in the study and aged 65 years and above, (b) having been followed up with the diagnosis of type 2 diabetes for at least 6 months, (c) not having a medium or advanced level of dementia, and (d) being able to communicate verbally. In line with these criteria, the study sample consisted of 526 elderly individuals. The data were collected through face-to-face interviews with the patients who agreed to participate in the study.

Data collection tools

The Patient Information Form: This form consisted of 15 questions about the individual sociodemographic and disease characteristics of the elderly. The patient's weight and height values were obtained from the last measurements, and HbA1c values were retrieved from patient files that included the results of the last laboratory tests.

Elderly Diabetes Burden Scale (EDBS): The 23-item Likert-type scale developed by Araki and Ito in Japan in 2003 to measure the burden of diabetes in elderly diabetic patients consists of six subscales. Usta and Esen conducted the Turkish validity and reliability tests of the scale (Cronbach's alpha: 0.92). The total scale score ranges between 18 and 88, and an increase in the scale score indicates an increase in the burden in that area, while a decrease points to a decrease in the burden in that area[13,14]. The Cronbach's alpha coefficient of the scale was determined as 0.90 in the present study.

Successful Ageing Scale (SAS): The scale developed by Reker consists of 13 questions and two subscales[15]. The scale's validity and reliability study in Turkish was conducted by Hazer and Ozsungur (Cronbach's alpha: 0.85). The Turkish version of the scale consists of 10 questions and two subscales. The minimum and maximum scores to be obtained from the scale are 10 and 70, respectively; as the score increases, successful ageing status increases as well[16]. In the present study, Cronbach's alpha coefficient was 0.82.

Statistical analysis

The study's data were evaluated using SPSS 22.0 (IBM, Armonk, NY, USA) software. The measures of skewness and kurtosis were utilised to test whether the scores obtained from the measures were normally distributed, and in this regard, the acceptable range was set as (-1, +1). In the analysis of the data, descriptive statistics, Pearson correlation analysis, one-way analysis of variance (ANOVA), and Student's t-test were used. The results were evaluated at a 95% confidence interval, and the significance level was set at $p<0.05$.

Ethical considerations

The study was conducted with the ethics committee's approval (date: 08.12.2020; decision no.: E.42680) and in line with the principles of the Declaration of Helsinki. All participants included in the study were verbally informed about the purpose and procedures of the study. After written consent was taken from each participant, the data collection process was completed.

RESULTS

Of participants aged 65–74 years, 50.8% were male; the average body mass index (BMI) was calculated as 28.71±6.90 kg/m², and their HbA1c average value was found to be 9.92±1.11. The EDBS total mean score was 69.69±5.27, while the SAS score was 37.53±3.01 (Table 1).

Accordingly, it was determined that the SAS total scores were found to be statistically significantly higher in females compared to males, in those who had been diagnosed with

Table 1. Mean score of Elderly Diabetes Burden Scale and Successful Ageing Scale (n=526).

Scale and subscale	Mean±SD	Min–max
EDBS total score	69.69±5.27	26.00–84.00
Burden of symptoms	13.90±4.38	0.00–16.00
Social burden	15.43±1.87	5.00–20.00
Burden of dietary restrictions	14.14±1.42	8.00–16.00
Burden related to the worry about diabetes	13.46±1.19	8.00–16.00
Burden of therapy dissatisfaction	4.61±0.72	2.00–6.00
Burden related to oral antidiabetics and insulin	8.12±1.10	3.00–12.00
SAS total score	37.53±3.01	26.00–70.00
Healthy lifestyle	10.80±1.37	5.00–21.00
Layout	26.72±2.16	16.00–49.00

SD: standard deviation; min–max: minimum-maximum.

diabetes with a duration of 6–15 years compared to those diagnosed with a duration of 16 years and above (p<0.01), in those who had regular diabetes check-ups compared to those who did not (p<0.01), and in those who had easy access to health services compared to those who had partial access to health services (p<0.01) (Table 2).

The EDBS total scores were determined to be statistically significantly higher in males compared to females, in those who had insulin as a therapy type compared to those who had oral antidiabetic drug (OAD) and insulin, and in those whose perceived health status was poor compared to those whose perceived health status was moderate and good (p<0.05). It was also concluded that the EDBS Scale total score of those who had diabetes check-ups was statistically significantly higher in comparison to those who sometimes had diabetes check-ups (p<0.01) (Table 2).

A negative and significant relationship was found between the subscales of Burden of Dietary Restrictions and Burden

Table 2. Comparison of descriptive and diabetes-related characteristics of elderly individuals with the Elderly Diabetes Burden scale and Successful Aging Scale total scores (n=526).

Descriptive	n	%	EDBS		SAS	
			Mean±SD	Statistical analysis	Mean±SD	Statistical analysis
Age (year)						
65–74	410	77.9	69.60±5.37	t: -0.718	37.42±3.01	t: -1.522
≥75	116	22.1	70.01±4.92	p: 0.473	37.90±2.97	p: 0.129
Gender						
Female	259	49.2	69.14±5.13	t: -2.385	38.11±2.68	t: 4.475
Male	267	50.8	70.23±5.36	**p: 0.017**	36.96±3.19	**p: 0.000**
Education						
Literate	73	13.9	70.17±5.06	F: 2.306	37.50±3.24	F: 0.924
Elementary school	282	53.6	70.04±5.16	p: 0.057	37.70±2.94	p: 0.450
Middle school	150	28.5	69.10±5.47		37.19±3.07	
High school	21	4.0	68.01±5.19		37.75±2.35	
Perceived income status						
Good	32	6.1	70.78±5.35	F: 1.247	36.68±2.65	F: 1.889
Moderate	455	86.5	69.55±5.30	p: 0.288	37.62±3.03	p: 0.152
Not good	39	7.4	70.46±4.88		37.10±2.82	
Duration of diabetes diagnosis (year)						
6–10	28	5.3	70.57±4.34	F: 3.515	39.21±3.19[c]	F: 27.859
11–15	79	15	71.00±4.89[a]	**p: 0.030**	39.43±3.14[d]	**p: 0.000**
16 and over	419	79.7	**69.39±5.37[b]**		37.06±2.79[e]	
Diabetes treatment method						
OAD and insulin	448	85.2	69.59±5.24	t: 0.446	37.35±2.94	t: 0.040
Insulin	78	14.8	70.33±5.50	**p: 0.002**	38.56±3.17	p: 0.250
Regular diabetes control						
Yes	110	20.9	73.25±3.65	F: 9.897	38.30±2.90	F: 9.478
No	115	21.9	69.41±5.43	**p: 0.000**	37.02±3.58	**p: 0.000**
Partially	301	57.2	69.40±5.20		37.13±2.90	
Easy access to healthcare						
Yes	39	7.4	69.20±4.58	F: 2.832	39.17±2.70	F: 30.451
No	205	39	70.38±5.76	p: 0.060	36.36±3.17	**p: 0.000**
Partially	282	53.6	69.26±4.95		38.15±2.61	

F: one-way ANOVA test; t: t-test. Duration of diabetes diagnosis: between a–b, c–e, and between d–e. Bold values indicate statistical significance at the p<0.05 level.

Related to Oral Antidiabetics and Insulin and the SAS total score. In contrast, a positive, strong, and significant relationship was determined between the subscales of Burden Related to the Worry About Diabetes and Burden of Therapy Dissatisfaction and the SAS total score ($p<0.01$). No significant relationship was found between the EDBS total score and the SAS total score ($p>0.05$). In addition, it was determined that there was a positive and strong correlation between BMI and the EDBS subscale of Burden of Dietary Restrictions ($p<0.01$). Moreover, a negative and statistically significant relationship was found between the number of complications and the Burden of Therapy Dissatisfaction subscale score. In contrast, a positive and statistically significant correlation was determined between the Burden of Dietary Restrictions subscale score and the EDBS total score (Table 3).

DISCUSSION

In the present study, it was determined that, among the sociodemographic characteristics, being a women was an important factor in terms of successful ageing. Similar results have been obtained in studies conducted on the elderly population, which showed that females aged more successfully[5,10,17,18]. A study demonstrated that females had a more positive attitude towards life[11], and another study found that the rate of successful ageing was higher in females[10]. However, a study showed that gender has no effect on successful ageing[12].

As the duration of diabetes becomes longer, the rate of chronic complications developing in relation to diabetes increases[19]. These complications affect the individual physically and psychologically and lead to disabilities. In the present study, it was observed that the patients with shorter duration of diabetes diagnosis had better successful ageing rates and also higher care burden. It was thought that this could be because the development of diabetes-related chronic complications was less likely in patients with a shorter duration of a diabetes diagnosis.

According to the study's findings, elderly diabetic individuals who had regular diabetes check-ups and easy access to health services aged successfully. Successful ageing was generally studied in studies conducted as the absence of chronic diseases, absence of disabilities, good level of cognitive functions, and active life[3,10,20]. Individuals having regular check-ups and keeping their diabetes under control to maintain their health levels can be considered successful ageing[20]. This could be because these individuals have good cognitive functions, can meet their physical needs themselves, have their health checked to maintain their current health level, and have easy access to health services. Preventive health applications must be available, and individuals should be able to use them for successful ageing[18].

In the present study, it was determined that males had a higher diabetes burden. Diabetes burden can be higher in males compared to females as they experience comorbid diseases more intensely, have a lack of knowledge of diseases, have less awareness compared to females, and have deficiencies in managing diabetes on their own, and they cannot manage self-care. It is thought that future studies to be conducted on the relationship between gender and diabetes burden can contribute to the present study in terms of significance.

The primary principle in treating T2DM is nutrition and OAD. It is estimated in studies conducted that individuals with T2DM would become insulin dependent in 10 years following the diagnosis[4]. Administering insulin as an injection, especially in intense insulin therapies, brings heavier burdens and responsibilities for elderly individuals regarding both skill and adaptation. In the present study, the care burden of insulin patients was higher. Another study result showed that as the burden of the patient increased in relation to the medications used in diabetes therapy, their successful ageing was negatively affected[21].

Table 3. Relationship between Diabetes Burden Scale, Successful Aging Scale and descriptive variables (n=526).

EDBS	SAS			Age	HbA1c	BMI (kg/m²)	Number of complications
	Healthy lifestyle	Layout	Total score				
Burden of symptoms	0.040	0.034	0.043	0.057	0.001	0.016	0.218**
Social burden	0.007	0.042	0.033	0.005	0.040	0.044	0.022
Burden of dietary restrictions	**-0.418****	0.006	**-0.187****	0.026	0.081	**0.111***	**0.484****
Burden related to the worry about diabetes	**0.391****	**0.296****	**0.392****	0.056	0.025	-0.061	0.021
Burden of therapy dissatisfaction	**0.284****	**0.149****	**0.237****	0.036	0.027	-0.023	**-0.259****
Burden related to oral antidiabetics and insulin	**-0.132****	**-0.153****	**-0.170****	0.003	0.072	-0.010	0.081
Total score	0.022	**0.100***	0.082	0.075	0.061	0.040	**0.305****

Pearson correlation test. *$p<0.05$; **$p<0.001$. Bold values indicate statistical significance at the $p<0.05$ level.

The number of chronic cases the patients have and their little knowledge about the therapy are related to the care burden[22]. The present study determined that as the number of complications increased, the burden of diabetes also increased. Along with the increased complications, the number of patients presenting to the hospital for examination for the follow-up of these complications also increases[23]. In the current study, it was found that patients who had regular check-ups had a higher care burden. Besides having regular check-ups at the hospital, visiting the hospital for the follow-up of complications, or an increase in hospitalisation can bring a physical burden to the patients.

Obesity is a common comorbidity of diabetes[21]. In the study, the burden of dietary restriction is increased in people who do not age successfully, do not have healthy lifestyle behaviours, and have a high BMI. It is also known that regular physical activity, diet, and healthy lifestyle habits are highly effective in treating T2DM in terms of ensuring weight and metabolic control and preventing potential complications[7,24]. In addition, cardiovascular risks are associated with high BMI in metabolic disorders[7]. It is thought that getting diabetic individuals to gain healthy lifestyle habits, especially starting at an early age, and preventing obesity will ensure successful ageing[8,24].

In the present study, it was determined that as the diabetic patients' burden regarding their worries about diabetes and their dissatisfaction with the therapy increased, their successful ageing rate also increased. An increase in the diabetes burden in diabetic patients can help them struggle against problems in their successful ageing. The comorbidity of ageing and diabetes necessitates diabetic patients to develop new coping strategies and adopt lifestyle changes such as dietary restrictions, monitoring their blood sugar, exercise, and drug management. In published studies, successful ageing was mainly examined in the context of the absence of chronic disease and the ability to sustain physical activity. Nevertheless, elderly adults can consider their ageing successful even in the presence of a disease or a disability[17,25,26].

CONCLUSION

In the present study, it was determined that elderly type 2 diabetic patients who did not have regular check-ups and who could not access health services easily had low successful ageing scores and that those whose treatment type was insulin, who had poor perceived health status, and whose number of complications was high had more care burden. In this context, ensuring free and easy access to health services for the elderly, diagnosing diabetes at an early stage, planning suitable nutrition, exercise, and drug therapy for the individual, preventing complications, taking necessary precautions in time, and providing elderly care services in this regard will help reduce the diabetes burden and increase successful ageing.

AUTHORS' CONTRIBUTIONS

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

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ORIGINAL ARTICLE

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An enlarged fetal thymus may be the initial response to intrauterine inflammation in pregnant women at risk for preterm birth

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SUMMARY

OBJECTIVE: Fetal thymus involvement in prematurity has been studied, and this study aimed to evaluate its relationship with short cervix and amniotic fluid sludge in the second trimester of pregnancy.

METHODS: In this prospective cross-sectional study, 79 pregnant women (19+0 to 24+6 weeks) were included, and cervical length and the presence or absence of amniotic fluid sludge were evaluated. In the three-vessel view of the fetal thorax, the thymus was identified, and its perimeter and transverse diameter were measured and transformed to a zeta score based on gestational age.

RESULTS: Data from 22 women with short cervix (<25 mm) and 57 patients with normal cervix (≥25 mm) were analyzed. The transverse diameter of the fetal thymus was significantly greater in the short cervix group compared to that of the normal cervix group (z-score 2.708 vs. -0.043, p=0.003). There were no significant differences in the perimeter (z-score -0.039 vs. -0.071, p=0.890) or the transverse diameter (z-score 1.297 vs. -0.004, p=0.091) of the fetal thymus associated with the presence (n=21) or absence of sludge (n=58).

CONCLUSION: A short cervix is associated with an increased transverse diameter of the fetal thymus during the second trimester of gestation.

KEYWORDS: Thymus gland. Fetus. Premature birth. Cervix uteri.

INTRODUCTION

Prematurity is a global concern because it leads to high rates of short- and long-term disability and morbidity. In 2014, ~15 million babies were estimated to have been born at <37 weeks of gestation, and this statistics is probably underestimated[1].

Spontaneous preterm birth is considered a syndrome, and the multifactorial aspects involved, such as infection and inflammation, contribute to the difficulty in its prediction and prevention. When clinical chorioamnionitis is present, the diagnosis is clear. However, most cases of subclinical chorioamnionitis are diagnosed only by histological placental examination after premature birth or late miscarriage[2].

The presence of intraamniotic infection or microbial invasion of the amniotic cavity (MIAC) and the presence of pro-inflammatory cytokines in the amniotic fluid perhaps exist in most cases in the long preclinical stage of chorioamnionitis[2]. Some researchers have reported the ultrasonographic image of amniotic fluid sludge (AFS) as a signal of intra-amniotic microbial invasion and an independent risk factor for prematurity[3].

Fetal inflammatory response syndrome (FIRS) represents the involvement of the fetus in this infectious/inflammatory process[4]. Many fetal organs may undergo modifications, particularly organs related to immunity, such as the thymus, which develops early in gestation in humans. The fetal thymus can be visualized by ultrasound since the first trimester, and its size is associated with intrauterine infection in cases of preterm premature rupture of membranes (PROM) in the third trimester. Preterm labor with intact membranes and histological findings of funisitis were associated with a small fetal thymus[5].

Currently, the main strategy to prevent preterm birth is based on ultrasonographic evaluation of cervical length during the second trimester of pregnancy. It is known that the shorter the cervix, the higher the risk of prematurity, and intra-amniotic inflammation/infection has also been identified in these patients[6].

Traditionally, sonographic markers of preterm birth have primarily focused on maternal signs, and fetal involvement in response to the intrauterine infection/inflammation process is less investigated. This study aimed to evaluate fetal thymus size during the second trimester of pregnancy and determine its association with cervical length and the presence of AFS.

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METHODS

A total of 84 pregnant women referred to undergo ultrasound examination were invited to participate in a prospective cross-sectional study involving sonographic measurements of fetal thymus size and preterm birth predictors. Women with low- and high-risk pregnancies were included for ultrasound exams. The study was approved by the Local Ethics Committee, and written informed consent was obtained from each participant.

The inclusion criteria were as follows: gestational age from 19+0 to 24+6 weeks, singleton pregnancy, absence of malformations, absence of signs of PROM, and estimated fetal weight between the 10th and 90th centiles[7]. We analyzed the data of 79 patients and excluded 1 HIV-positive patient, 1 patient undergoing immunosuppressive therapy for kidney transplant, and 3 patients whose neonatal data were unavailable. Other exclusion criteria were suspected or confirmed congenital infections and the presence of malformations at birth.

Gestational age was estimated from the last menstrual period and confirmed by first-trimester ultrasound. The images were obtained using a Voluson 730 Expert (General Electric Medical Systems, Zipf, Austria) and Accuvix V20 (Samsung Corp., Seoul, South Korea). Fetal thymus evaluation was not used for clinical decisions. Patients with short cervixes and AFS received vaginal progesterone and/or cervical pessary and/or antibiotics, in accordance with local protocols.

Transvaginal ultrasound was used to measure the cervical length. Each pregnant woman with an empty bladder was subjected to this examination. A magnified image of the sagittal view of the cervix, including the cervical canal, internal and external os, and without excessive pressure applied over the transducer, was used to obtain the cervical length, excluding the lower uterine segment. Subsequently, three measurements of the cervical length were performed over a period of 3–5 min, and the lowest value was used for further evaluation. Short cervix was defined as a cervical length of <25 mm.

The transvaginal ultrasounds were carefully examined and actively searched for the presence of particulate material near the cervix. If this material was free-floating (confirmed by applying gentle pressure over the anterior uterine wall), it was defined as AFS.

The fetal thymus size was measured in the three-vessel view of the thorax, between the lungs, and in front of the vessels. The perimeter and transverse diameter (TD) of this organ were obtained, as shown in Figure 1. The maximum TD of the thymus was measured perpendicular to the line connecting the sternum and the spine, and the perimeter of the thymus is the line traced around the organ. The measurements were performed three times, and the mean values were taken and then transformed into the Z-scores, which are the standard deviations (SD) from the mean according to gestational age at measurement, based on the normative references[8]. All thymus measurements were performed by the same examiner (TENKH).

Fetal biometry, represented by head circumference (HC), biparietal diameter (BPD), occipito-frontal diameter, abdominal circumference, femur length (FL), and humerus length (HL), was also evaluated and used for fetal weight estimation. An assessment of the P/HC, P/FL, P/HL, TD/HC, TD/FL, and TD/HL ratios was performed.

Statistical analyses were performed using MedCalc® Statistical Software version 19.5.3 (MedCalc Software Ltd., Ostend, Belgium). Comparisons among groups were performed using the chi-square or Fisher's exact test for categorical variables, and the Mann-Whitney U test and Student's t-test for continuous variables. $p<0.05$ was considered statistically significant.

RESULTS

Of the 84 patients invited to participate in the study, 5 were excluded, and data from 79 patients were analyzed. In all, 57 participants presented with normal cervixes (≥25 mm) and 22 with short cervixes (<25 mm). AFS was not observed in 58 women but was present in 21 patients.

Table 1 shows that the between-group characteristics of women with normal and short cervical lengths are similar regarding maternal age, white skin color, parity, BMI, smoking, and high-risk pregnancy. Concerning the AFS, there was a statistically significant difference in maternal age and parity; however, this did not appear to impact the results.

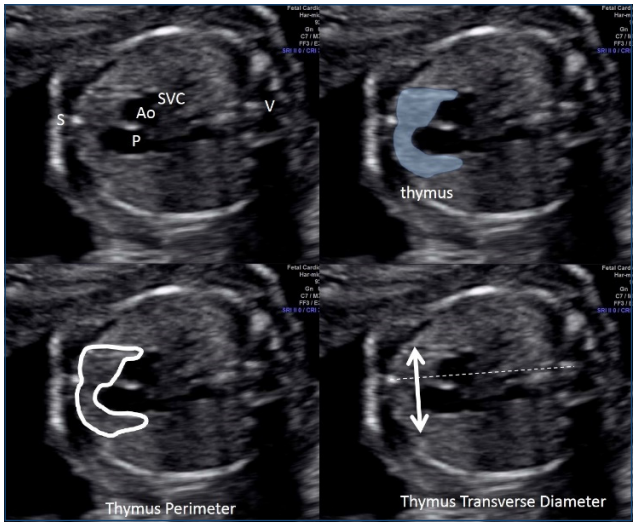

Figure 1. Sonographic fetal thymus measurement. S: sternum; P: pulmonary artery; Ao: aorta artery; SVC: superior vena cava; V: vertebra.

Of the 57 women with cervical length ≥25 mm, 16/57 (28%) presented with AFS, and this ultrasonographic sign was detected in 5/22 (22.7%) women with short cervixes. Of the 79 cases, 10 preterm births occurred (8 spontaneous preterm births, 10.3%). Table 1 also shows that women with short cervixes had births at lower gestational ages, compared to women with normal cervixes.

Table 2 presents the results of thymus measurements (TD and perimeter) and the presence of a short cervix and AFS. In women with a short cervix (<25 mm), the TD of the thymus was enlarged ($p<0.05$), with statistically significant higher TD/HC, TD/FL, and TD/HL ratios. Patients with AFS presented with increased thymus size; however, it was statistically significant only for the TD/HC and TD/FL ratios (Table 2).

DISCUSSION

In pregnant women at risk for preterm birth, if they have short cervixes, the TD of the fetal thymus is increased and is perhaps the initial response to intrauterine inflammation. A short cervix is related to MIAC, and it is believed that patients with this condition are more frequently subjected to inflammatory processes leading to fetal thymus involution, as reported in the literature[5,9]. Some studies have demonstrated that infectious and inflammatory processes involved in prematurity are responsible for FIRS, which is characterized by elevated interleukin 6 (IL-6) levels (>11 pg/mL) in fetal serum[4]. Following the insult, the thymus and adrenals are the first organs to undergo alterations. The endogenous corticosteroids increased by the activation of

Table 1. Population characterization according to the groups.

Characteristics	NC (n=57)	SC (n=22)	p-value	AAFS (n=58)	PAFS (n=21)	p-value
Maternal age, years	28.8 (6.1)	26.2 (8.8)	0.136	27.0 (7.2)	31.1 (5.6)	0.020
White	28 (49.1%)	8 (36.4%)	0.311	23 (39.7%)	13 (61.9%)	0.080
Nulliparous	24 (42.1%)	11 (50.0%)	0.970	31 (53.4%)	4 (19.1%)	0.02
BMI (kg/m^2)	26.3 (4.5)	26.7 (6.0)	0.739	26.3 (4.6)	26.6 (5.9)	0.781
Tobacco use	9 (15.8%)	3 (13.6%)	0.812	9 (15.5%)	3 (14.3%)	0.894
High-risk pregnancy	11 (19.3%)	2 (9.1%)	0.276	10 (17.2%)	3 (14.3%)	0.756
GA, weeks	22.2 (1.3)	22.7 (1.5)	0.204	22.6 (1.3)	21.8 (1.4)	0.035
CL, mm	33.0 (31.4–34.7)	21.1 (17.0–22.4)	<0.001	29.9 (26.8–31.9)	33.0 (27.0–36.5)	0.220
CL<25 mm	–	–	–	16 (27.6%)	6 (28.6%)	0.931
Absence of cervical gland area	0 (0.0%)	1 (4.5%)	0.279	1 (1.7%)	0 (0.0%)	1.0
Funneling presence	0 (0.0%)	8 (36.4%)	<0.001	5 (8.6%)	3 (14.3%)	0.432
Amniotic fluid sludge	1 (1.8%)	6 (27.3%)	0.932	–	–	–

Mean (SD), median (95%CI), n (%). NC: normal cervix; SC: short cervix; AAFS: absence of amniotic fluid sludge; PAFS: presence of amniotic fluid sludge; BMI: body mass index; GA: gestational age; CL: cervical length.

Table 2. Fetal thymus measurements according to groups short cervix versus normal cervix and absence of amniotic fluid sludge versus presence of amniotic fluid sludge.

	NC (n=57)	SC (n=22)	p-value	AAFS (n=58)	PAFS (n=21)	p-value
Perimeter						
P (Z-score)	-0.090 (-0.351 to -0.004)	0.056 (-0.119 to 0.248)	0.199	-0.071 (-0.268 to -0.058)	-0.039 (-0.371 to 0.254)	0.890
P/CC	0.235 (0.226 to 0.244)	0.244 (0.238 to 0.255)	0.238	0.239 (0.227 to 0.245)	0.235 (0.226 to 0.255)	0.965
P/FL	1.251 (1.178 to 1.314)	1.286 (1.180 to 1.334)	0.956	1.267 (1.183 to 1.313)	1.261 (1.136 to 1.386)	0.773
P/HL	1.322 (1.229 to 1.371)	1.371 (1.271 to 1.410)	0.638	1.334 (1.253 to 1.377)	1.325 (1.185 to 1.416)	0.641
Transverse diameter						
TD (Z-score)	-0.043 (-0.194 to 0.194)	2.708 (0.024 to 3.481)	0.003	-0.004 (-0.114 to 0.255)	1.297 (-0.135 to 3.060)	0.091
TD/CC	0.087 (0.084 to 0.092)	0.136 (0.089 to 0.155)	0.005	0.089 (0.084 to 0.094)	0.114 (0.085 to 0.155)	0.042
TD/FL	0.472 (0.444 to 0.501)	0.691 (0.459 to 0.795)	0.021	0.474 (0.446 to 0.507)	0.660 (0.443 to 0.834)	0.042
TD/HL	0.485 (0.469 to 0.539)	0.762 (0.505 to 0.862)	0.024	0.500 (0.475 to 0.543)	0.666 (0.464 to 0.865)	0.126

Median (95%CI). NC: normal cervix; SC: short cervix; AAFS: absence of amniotic fluid sludge; PAFS: presence of amniotic fluid sludge; P: perimeter; CC: cephalic circumference; FL: femur length; HL: humerus length.

the hypothalamus-pituitary-adrenal axis led to thymus involution, possibly due to lymphocyte depletion in the thymic cortex and medulla by apoptosis of the lymphoid tissue[9].

Based on these findings, and after reference ranges of fetal thymus size were established[8,10,11], many researchers have applied thymus involution as a marker in histological chorioamnionitis[5,12-15], in cases of preterm birth and PROM, since its sensitivity and specificity are higher than the classic markers, such as erythrocyte sedimentation rate and C-reactive protein levels[14]. However, in the present study, contrary to expectations, in patients with short cervixes, the fetal thymus was larger. In the short cervix group, the TD of the thymus was increased compared to that in women with normal cervical length. Women with AFS also had a tendency to have a larger thymus, and the TD/HC and TD/FL ratios were significantly greater in this group.

It is important to note that the majority of previous studies analyzed the fetal thymus in the third trimester of pregnancy and in cases of preterm PROM or preterm labor[5,12-14,16,17]. However, the present study was performed in the second trimester of pregnancy, before the occurrence of clinical manifestations, except for shrinkage of the cervix.

There are few studies on sonographic measurement of the fetal thymus in the first and second trimesters, and some studies have similar results as ours. Borgelt et al.[18] measured the anteroposterior diameter of the fetal thymus in the first trimester of pregnancy and found a positive relationship between fetal thymus and preterm birth ($p<0.001$). Brandt et al.[19] investigated the fetal thymus in pregnant women during the second trimester to predict prematurity. They did not observe a statistically significant association between small thymus and preterm birth. Nevertheless, the patients presenting with a smaller fetal thymus were more likely to have a greater cervical length. Their study focused on the involuted thymus, and they may not have observed an association with increased fetal thymus size in the cases of short cervix and preterm birth.

The thymus may be affected by various changes in the intrauterine environment. As an important organ in the human immune system, chronic inflammation associated with certain diseases and conditions may influence the size of this organ. One study analyzed HIV-exposed fetuses in the second trimester and observed fetal thymus enlargement[20]. Interestingly, the mean gestational age at examination was 21 weeks, similar to the present study. The authors also observed that HIV-exposed uninfected infants, when older, had reduced thymus sizes and lower CD4+ and CD8+ cell counts.

A large thymus could be caused by true hyperplasia (following recent stress, such as irradiation, corticosteroid therapy, chemotherapy, and infection) and lymphoid hyperplasia (usually related to immunologically mediated diseases, such as myasthenia gravis, Graves' disease, and systemic lupus erythematosus[21]). In true hyperplasia, the thymus becomes atrophic but can grow even larger after such stress – rebound hyperplasia[21]. However, rebound hyperplasia does not explain our findings because the evaluation of the fetuses occurred in the second trimester of pregnancy, during the initial phase of intraamniotic infection/inflammation.

In experimental studies, chorioamnionitis was induced by intraamniotic injection of lipopolysaccharide, and some authors described an increase in the size of the posterior mediastinal lymph nodes in sheep[22]. CD3, CD4, and CD8 T-cell counts increased 2–3 days after exposure. In Rhesus macaques infected with *Ureaplasma parvum* and *Mycoplasma hominis*, the fetal spleen had diffused hyperplasia, with an increase in T-cells after 15 days of exposure, the opposite of the splenic depletion reported in humans[23].

This splenic hyperplasia is a possible hypothesis to explain the thymus enlargement observed in the present study, as both the thymus and spleen are lymphoid organs. In this early period of fetal development, the regulatory mechanisms of the lymphoid response are poorly understood and may differ from the findings during the third trimester of pregnancy. The hypothesis of initial thymus edema is a more reasonable explanation for our findings than rebound hyperplasia[24].

Limitations of our study include the small sample size as well as interventions that could have influenced the outcomes. Furthermore, during the second trimester, the echogenicity of the thymus and lungs is very similar, impairing adequate measures in all cases. As suggested previously[16], we considered the TD of the thymus as the best parameter to evaluate this organ. The strength of this study was the performance of all fetal measurements by a single examiner, removing inter-rater variability.

CONCLUSION

A short cervix is associated with an increased TD diameter of the fetal thymus during the second trimester of gestation and can be the first signal of intra-amniotic inflammation and infection. Not all patients with short cervixes will experience spontaneous preterm birth; however, the association of short cervical length and an enlarged thymus could more accurately predict prematurity. Nevertheless, more studies are needed to elucidate the relationship between these sonographic prematurity markers and fetal thymus size during the second trimester of pregnancy.

AUTHORS' CONTRIBUTIONS

TENKH: Data curation, Visualization, Writing – original draft. **ARH:** Investigation, Visualization. **EAJ:** Visualization, Writing – review & editing. **MSF:** Formal Analysis, Visualization. **SGPS:** Methodology, Visualization. **TMH:** Investigation, Visualization. **RMYN:** Conceptualization, Validation, Visualization. **AFM:** Project administration, Supervision, Visualization.

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ORIGINAL ARTICLE

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Colic and sleep outcomes of nonpharmacological intervention in infants with infantile colic: systematic review and metaanalysis

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SUMMARY

OBJECTIVE: The aim of this study was to systematically review the colic and sleep outcomes of nonpharmacological intervention in infants with infantile colic and perform a meta-analysis of the available evidence.

METHODS: The literature review for this systematic review was conducted between December 2022 and January 2023 using five electronic databases, namely PubMed, CINAHL, Scopus, Web of Science, and ULAKBİM. Published articles were scanned using MeSH-based keywords. Only randomized controlled trials conducted in the past 5 years were included. The data were analyzed using the Review Manager computer program.

RESULTS: This meta-analysis included three studies involving a total of 386 infantile colic infants. After nonpharmacological treatment, it was found that infants with infantile colic reduced crying time (standardized mean difference: 0.61; 95%CI 0.29–0.92; Z=3.79; p=0.00002), improved sleep duration (standardized mean difference: 0.22; 95%CI -0.04 to 0.48; Z=1.64; p=0.10), and decreased crying intensity (mean difference: -17.24; 95%CI -20.11 to 14.37; Z=11.77; p<0.000001).

CONCLUSION: According to the meta-analysis findings, it was determined that the risk of bias was low in the studies included and that nonpharmacological chiropractic, craniosacral, and acupuncture treatments applied to infantile colic infants in the three included studies reduced crying time and intensity and increased sleep duration.

KEYWORDS: Crying. Chiropractic. Acupuncture therapy.

INTRODUCTION

Infantile colic (IC) is characterized by excessive crying and restlessness in babies who develop normally from all other directions[1-3]. IC is a common condition that occurs in 25% of infants[4]. Wessel et al.[5] were the first to describe the situation with his "rule of three," that is, the condition in which the infant cries at least 3 times a day for at least 3 days in the previous 3 weeks.

IC adversely affects the comfort and health of both the infants and the parents, and on the contrary, studies are carried out with different therapeutic perspectives because the exact etiology is unknown. In these studies, drug applications and probiotics[6-8] evaluated the efficacy of interventions involving parental behavior and counseling[9]. In addition, complementary medicine practices, which are rapidly gaining popularity, are also being studied. In these studies, acupuncture[10], reflexology[11], physiotherapy, and visceral osteopathy have been studied to treat IC such as vertebral manipulation[12]. In the literature, the evidence evaluating the effect of nonpharmacological applications on sleep in infants with IC is limited. For this reason, the aim of this study was to systematically review the colic and sleep outcomes of nonpharmacological intervention in infants with IC and to perform a meta-analysis of the available evidence.

METHODS

In this study, Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[13] was compiled in the preparation of the systematic review and meta-analysis.

Eligibility criteria

The following criteria (PICOS) were taken into account in the selection of the studies to be included in the study: participant (P): infants with IC. The infants included in the study had the following criteria for inclusion[1]: infants who have cried for at least 3 days for 3 h a day in the past week and[2] who have no health problems. Intervention (I): nonpharmacological methods. Nonpharmacological methods include the following[1]: chiropractic[2], craniosacral therapy[3], acupuncture[4], yoga[5], massage[6], swaddling, and[7] shaking. Comparison (C)[1]: placebo and[2] routine care. Results (O): sleep duration, crying intensity, and duration. Study design (S): randomized controlled trials and controlled groups were included. Articles that were not IC,

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but only had gas complaints, pharmacological and pre-probiotic interventions, and evaluated the effect of the interventions other than sleep and IC intensity were excluded from traditional and systematic reviews.

Search strategy

The literature review for this systematic review was conducted between December 2022 and January 2023 using five electronic databases (PubMed, CINAHL, Scopus, Web of Science, and ULAKBİM). The studies in which the efficacy of nonpharmacological interventions in infants with infants with IC were examined by selecting keywords. The keywords were "baby" OR "newborn" OR "infant" AND "therapy" OR "nonpharmacological" OR "alternative therapy" AND "infantile colic" OR "crying" AND "sleep" AND "crying intensity." The search strategy was changed according to the characteristics of each database. In addition, reviews on articles included in systematic reference lists and other previous systematic reviews were checked to reach further studies.

The data analysis

Meta-analysis was performed using Review Manager 5.4 (The Nordic Cochrane Center, Copenhagen, Denmark). The heterogeneity between the studies was evaluated using Cochran's Q test and Higgins' I^2, and it was accepted that I^2 greater than 50% showed significant heterogeneity. Accordingly, random effect results were taken into account when I^2 was greater than 50%, and fixed effect results were taken into account if it was less than the value. Odds ratio for categorical variables, mean difference (MD), and standardized mean difference (SMD) for continuous variables were calculated. MD or SMD, along with the corresponding 95% confidence interval (CI), is appropriately pooled for continuous variables based on whether the results are measured on the same scales. All tests were calculated from two-pronged tests, and a p-value of less than 0.05 was considered statistically significant. The quality of the articles in randomized controlled trials and the Version 2 of the Cochrane Risk-of-Bias (RoB-2) tool were used for randomized trials.

RESULTS

Literature review

The PRISMA flowchart is summarized for literature review and selection. A total of 15 studies were reached through electronic database search and manual search. All 15 articles whose full texts could be accessed were examined. Titles and abstracts were read to identify relevant articles; two articles were excluded because of review articles, protocols, replicates, different populations, and not meeting the inclusion criteria. The remaining 13 full texts were evaluated for suitability. Three randomized controlled trial (RCT) articles were included in the quantitative synthesis as they met the required criteria (Table 1). The three RCT articles included outline the study.

Study characteristics

This systematic review and meta-analysis included three studies involving a total of 386 IC infants to assess the impact of nonpharmacological interventions on IC intensity and sleep duration outcomes[10,14,15]. In the interventions for IC described in the articles included in the study, Holm et al.[14] applied chiropractic, Castejón et al.[15] applied craniosacral therapy, and Landgren et al.[10] applied acupuncture. While the intervention period of the studies in the review lasted 2 weeks, Castejón et al.[15] evaluated the first day and the first week of the intervention and ended the intervention if the symptoms regressed. In two of the articles, due to the placebo effect, infants in the control group were called to the clinic and stayed with the specialist for 5 min without their parents[10,14]. In the study by Castejón et al.[15], the control group was instructed to cope with IC. Nonpharmacological interventions to IC infants in the intervention group, Holm et al.[14] performed chiropractic twice a week for 2 weeks on IC infants in the intervention group. Castejón et al.[15] administered 1–3 sessions of craniosacral therapy to infants in the intervention group depending on the reduction of their symptoms. Landgren et al.[10] performed standard acupuncture at LI4 to a group twice a week for 2 weeks, while one group received traditional acupuncture points at LI4, ST36, or Sifeng, depending on the baby's symptoms, according to the diary. A crying diary and a sleep diary were used in all of the studies, and Castejón et al.[15] measured IC intensity with the Infant Colic Intensity Questionnaire.

Outcomes

The results of the meta-analysis were presented as Forest Pilot. In the included studies, IC was examined with improvement in crying duration, sleep duration, and crying intensity. The results of the research by Landgren et al.[10] were sleep duration and crying after the first and second interventions in the beginning. The Infant Pubic Intensity Per Day Questionnaire assessed sleep duration, crying time, sleep time, and being happy while awake before and after all interventions.

In three studies reviewed, the authors reported results on crying duration in the pre- and post-treatment periods.

Table 1. General features of the included studies.

Author (reference)\ Country	Study design	Population	The inclusion and exclusions criteria	Protocol	Comparisons	Drop out	Outcomes	Results
Holm et al. (2021)[14], Denmark	RCT	185 infant with IC (Interventions group n=96; control group n=89)	• Infants diagnosed with IC with excessive crying lasting at least 3 h a day for at least 3 days a week within 2 weeks. • No other health problems. • Born in time. • Growth and development normal. • 2–14 week old infants were included. Infants who had previously received chiropractic treatment and were treated for colic during the project period were not included.	• Infants evaluated for suitability. All parents were surveyed based on their infant's characteristics such as crying, feeding, and defecation. Parents recorded their infant's cries for 3 days. • In the second visit, infants who did not comply with the sample selection were excluded. Infants who were included in blockade randomization were randomized in a 1:1 ratio. All infants were called to the chiropractic clinic twice a week for 2 weeks. • Not all infants were taken to the practice room for their parents to go blind. The infants in the control group were taken to the intervention room for 5 min to mimic the treatment time in order to form a placebo. Infants in the intervention group underwent chiropractic during visits. • For all infants of 4 days and younger, it was practiced daily by his parents for 1–4 days after the visit.	Control group: Placebo	Interventions group (n=7), Control group (n=7)	Infant Crying, Sleep and Defecation Diary	Duration of crying in the treatment group was reduced by 1.5 h compared with 1 h in the control group, but when adjusted for baseline hours of crying, age, and chiropractic clinic, the difference was not significant. The proportion obtaining a clinically important reduction of 1 h of crying was 63% in the treatment group and 47% in the control group.
Castejón-Castejón et al. (2022)[15], Spain	RCT	54 Infant with IC (Interventions group n=29; control group n=25)	• Less than 90 days. • Infants diagnosed with IC with excessive crying lasting at least 3 h a day for at least 3 days a week within 1 week. • Born in time. • No other health problems. • Growth and development normal infants were included.	• Infants evaluated for suitability. • Infants in the intervention group received the first craniosacral treatment. If symptoms persist 7 and 14, the day was intervened again. If they did not show symptoms, the sessions were interrupted. • According to the craniosacral treatment sessions received by the infants, the infants were classified into two subgroups: maximum two sessions and maximum three sessions. Craniosacral treatment sessions lasted 30–40 min. • The control group was given instructions on how to deal with IC.	Control group: IC coping training	Interventions group (n=0), Control group (n=4)	Sleep Diary, Infant Colic Severity Questionnaire	Significant statistical differences were observed in favor of experimental group compared to the control group on day 24 in crying hours' primary outcome, and also in hours of sleep and colic severity secondary outcomes. Also, the differences between the groups of ≤2 CST sessions, three CST sessions and control were statistically significant on day 24 of the treatment for crying, sleep, and colic severity outcomes.
Landgren et al. (2020)[10], Sweden	RCT	147 Infant with IC (Minimal acupuncture n=48, Individual acupuncture n=49, control group n=48)	• Understand and read Swedish. • Infants diagnosed with IC with excessive crying lasting at least 3 h a day for at least 3 days a week within 2–8 weeks. • Born in time. • No other health problems infants were included.	• All infants were educated about breastfeeding. They were treated twice a week for 2 weeks. • In minimal acupuncture, LI4, unilaterally, in each second treatment, the needles in the right or left hand were advanced to a depth of about 3 mm and held for 2–5 s. • The individual acupuncturist was allowed to select a total of 1–5 placements at the traditional acupuncture points LI4, ST36 (unilateral or double-sided) and Sifeng (four placements), depending on the baby's symptoms, according to the diary. • The infants in the control group visited the clinic the same number of times and met with the specialist for 5 min, but acupuncture was not applied.	Control group: Placebo	Minimal acupuncture n=2, Individual acupuncture n=0, control group n=1	Infant Crying and Sleep Diary	There were no differences between groups for stooling, feeding, or sleeping at any time point according to data from the diaries. At the follow-up phone call, more parents in minimal acupuncture and individual acupuncture, control group perceived that feeding and sleep had changed and that the symptoms of colic had improved.

IC: infantile colic.

While the mean combined results of the studies did not differ in the post-treatment groups (SMD: 0.61, 95%CI 0.29–0.92, Z=3.79, p=0.00002), the effect of nonpharmacological treatment on crying time showed a significant difference between the groups (Figure 1A). In one study reviewed, the authors reported results on crying intensity in the pre- and post-treatment periods. The combined results of the studies showed that after treatment (MD: -17.24, 95%CI: -20.11 to 14.37, Z=11.77, p<0.000001), the effect of nonpharmacological treatment on crying intensity was significantly different between the groups and its intensity decreased (Figure 1B).

In the two studies examined, the authors reported results regarding sleep duration in the pre- and post-treatment periods. The combined results of the studies showed that while they did not differ in the post-treatment groups (SMD: 0.22, 95%CI: -0.04 to 0.48, Z=1.64, p=0.10), there was a significant difference in the effect of nonpharmacological treatment on sleep duration between the groups (Figure 2).

Risk of bias assessment

All the research has identified an adequate method for the random assignment of participants to treatment groups[10,14,15]. Therefore, there is a low risk of nepotism error. All studies reported adequate distribution secrecy using sequentially numbered and sealed opaque envelopes and rated them with a low risk of favoritism error [10,14,15]. In the two studies included in the meta-analysis, participants and researchers could not go blind to the study, so the two studies assessed participants and staff at risk of nepotism by blinding them, and this was taken into account when interpreting the findings[14,15]. Three studies assessed data at low risk as a result of blinding[10,14,15]. In three studies[10,14,15], the effect has been so small that it has been balanced or not affected by the intervention and control groups to stop working. For this reason, we concluded that the risk of attrition is low. Because they discussed the significant results reported in all study methods, including negative results, and matched those reported in their records, all studies included in

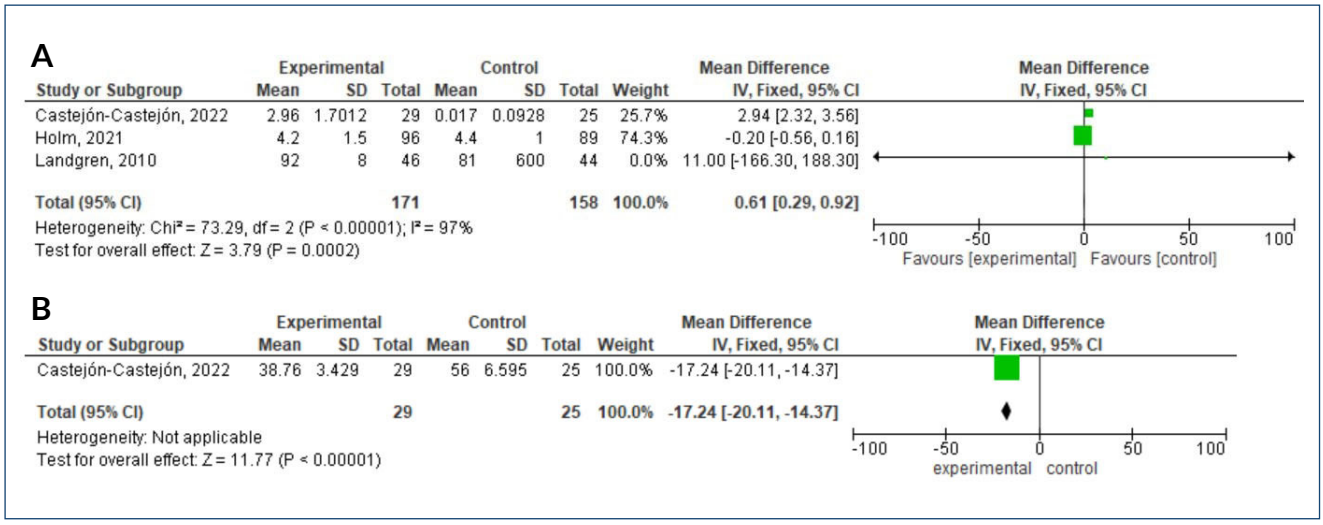

Figure 1. The results of the meta-analysis of the effect of nonpharmacological therapy on infantile colic crying time: (A) post-treatment and crying duration, and (B) post-treatment and crying intensity.

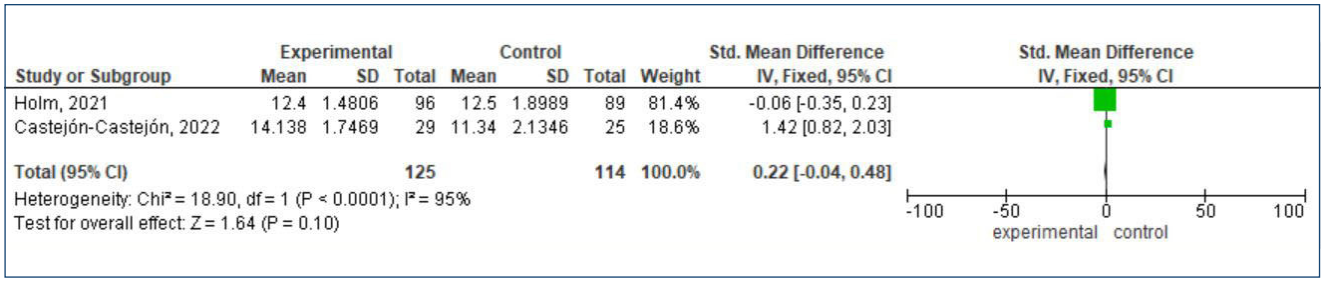

Figure 2. Results of the meta-analysis of the effect of nonpharmacological treatment on infantile colic sleep duration: post-treatment and sleep duration.

the meta-analysis were considered to have a low risk of reporting bias. For each study included, we described important concerns regarding other possible sources of bias that were not previously addressed in the above categories[10,14,15].

DISCUSSION

This study aimed to systematically review the colic and sleep outcomes of nonpharmacological intervention in infants with IC and make a meta-analysis of the available evidence. According to the meta-analysis findings, the studies included in the analysis were found to have a low risk of bias, and in all three included studies, nonpharmacological chiropractic, craniosacral, and acupuncture treatments applied to IC infants reduced crying time and intensity and increased sleep duration.

Infants with IC begin to cry at the same time every day, and these cries cannot be stopped[1,2]. In a meta-analysis study, prebiotic supplementation was reported to be strong in crying intensity of IC infants, while manual therapies were reported to have weak evidence[16]. In the three studies included in the analysis, crying time was assessed in infants with IC[10,14,15] and also evaluated the intensity of crying in a study[15]. In IC, in addition to the important crying time in infants, the intensity is also important. In this respect, it is thought that there is a weakness in measuring the effectiveness of interventions in two other studies that did not evaluate crying intensity. In addition, in the research, the sleep and crying times of the infants were evaluated by their parents, and they were asked to keep a diary. Parents of infants with IC often have increased levels of anxiety and burnout, and their perception of signs of crying and sleep may be altered[17]. Research suggests that this evaluation cannot be objective. In addition, two studies included the placebo effect in infants in the control group who were called to the clinic and stayed with the specialist for 5 min without their parents[10,14]. This suggests that it reduces bias in the evaluation process between groups.

In infants with IC, crying and attacks usually begin in the evening or at midnight and last for at least 3 h. This situation adversely affects the level and quality of sleep for both the baby and the parents[1,2]. It was found that the nonpharmacological methods used in the studies included in the analysis increased sleep duration. A study reported that IC is reduced in the infants of mothers who massage their infants[2]. In the three studies included in the analysis, IC asked parents to keep a sleep diary to assess sleep duration[10,14,15]. Keeping a stopwatch for the sleep duration of infants is considered quantitative and objective data and can be considered reliable. However, in the studies evaluating sleep in term infants, devices that record the sleep-wake status and duration are used[18]. A study reported that the majority of mothers used medical and complementary therapies together in the treatment of IC and that the rate of mothers benefiting from complementary therapies was higher than medical treatment[2]. In line with these findings, nonpharmacological treatments are preferred by the parents of IC infants, and it is seen that there is weak evidence in line with the results of this meta-analysis. In addition, studies examining the effect of interventions on sleep in infants with IC are very limited.

CONCLUSION

In three studies included in this analysis, chiropractic, craniosacral, and acupuncture treatments for IC infants were found to increase sleep duration while reducing crying duration and intensity. Although there are effective and available methods in IC, the levels of evidence obtained are weak. Randomized, placebo, and double-blind controlled trials with objective assessments such as measurement with more actigraphy to improve sleep in IC infants, which have an important place for infant development, may be recommended.

AUTHORS' CONTRIBUTIONS

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

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