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Swallowing interventions for older in-hospital patients: have we appropriately selected the desired outcomes?

Sérgio Renato da Rosa Decker[1*], Maiara Tomanchieviez[1], Luana Junges Lauxen[2], Cassiano Teixeira[1], Regis Goulart Rosa[1]

Pneumonia is a common condition that leads to hospitalization among older adults, with six times higher incidence of hospital admissions for individuals over 80 years of age versus the younger individuals[1]. As microaspiration is the primary pathogenic mechanism for most cases of pneumonia, aspiration pneumonia is better understood as a continuum that encompasses both community- and hospital-acquired pneumonia[1]. The lung microbiome is maintained through a delicate balance of bacterial migration from the oropharynx to the lungs, primarily through microaspiration, and elimination via ciliary clearance and coughing[1,2]. When this balance is disrupted by bacterial and viral virulence[1,2], or through macroaspiration, then infection, inflammation, and tissue damage result. Given the importance of aspiration in the pathophysiology of all pneumonia and the significant number of older adult patients hospitalized with pneumonia who have impaired swallowing, with studies indicating that nearly 92% of this population exhibit oropharyngeal dysphagia[1], it is critical to understand the preventive measures to reduce the risk of recurrence or to improve outcomes of hospitalized patients with pneumonia, such as the involvement of Speech Language Pathologists with a rehabilitation program, changing the viscosity of liquids, and the consideration of tube feeding[1,3]. Screening and specific measures aimed at preventing aspiration are recommended by international organizations for hospital quality and safety[4]. However, generally enteral feeding through nasogastric tube placement did not lead to a reduction in pneumonia risk[1]. Postpyloric or gastrostomy feeding methods are not superior to the nasogastric tube[1]. In a Cochrane meta-analysis, the use of percutaneous endoscopic gastrostomy compared to nasogastric tube feeding showed no significant difference in pneumonia risk (RR 0.7 [95%CI 0.46–1.06]) or mortality risk (RR 0.86 [95%CI 0.58–1.28]), regardless of the follow-up duration (evidence quality rated as low and very low, respectively)[5].

With respect to swallowing interventions with Speech Language Pathologists, the potential benefits can be significant[3], including a reduction in the length of hospital stay, involving compensatory techniques and exercises for muscle rehabilitation, thus improving food and fluid intake, preserving hydration and nutrition, and minimizing the risk of macroaspiration[3,6]. However, when we evaluate recurrence of pneumonia and mortality with behavioral swallowing interventions alone, we did not find significant differences in pneumonia risk (OR 0.56 [95%CI 0.31–1.0]), and for mortality outcomes, the results did not show any improvement with any type of swallowing interventions (OR 1 [95%CI 0.66–1.52]), with evidence quality rated as very low and moderate, respectively[7].

Similarly, the use of thickened liquids did not reduce mortality or pneumonia risk, but studies suggesting this may impact adherence and a tendency toward an increased risk of dehydration and weight loss[8]. Although there is an improvement in the physiology of swallowing with thick fluids, this is not necessarily linked to a reduction in respiratory complications, considering that the risk of developing aspiration pneumonia is possibly reduced by the aspiration of pure and thin water than by the aspiration of thick fluids because aquaporins allow the removal of water from the air spaces after laryngotracheal aspiration, reducing the risk of aspiration pneumonia if aspiration occurs[6].

These findings raise a crucial question regarding the selection of outcomes in clinical trials involving older adult patients: Are we appropriately choosing the outcomes to evaluate? These results suggest a trend toward unmodifiable risk factors among the oldest individuals with oropharyngeal dysphagia, highlighting the importance of gaining a better understanding of our current position and desired goals[9]. For instance, none of the clinical trials included in the Cochrane meta-analysis on

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swallowing therapist interventions assessed quality of life outcomes, which are highly relevant patient-centered outcomes for frail and older adults[3,7]. In the meta-analysis focusing on thickened liquids, worse quality of life scores were reported in the intervention group[8]. However, when evaluating protocols involving the provision of free water for oropharyngeal dysphagia, taking into account aspects such as oral hygiene, time of ingestion, type of liquid, and cognitive characteristics for ingestion safety[6,10], studies have shown that patients' perceptions of swallow-related quality of life improve (as assessed by standardized questionnaires like SWAL-QOL), without an increase in pulmonary complications[6,10].

Finally, it is crucial to comprehend the implications of these interventions particularly in middle- and low-income healthcare settings. Implementation of such interventions may potentially increase concerns among families during the discharge process, raising the length of hospital stay, thereby complicating matters. Consequently, there is an imperative for enhanced outcome selection in clinical trials aimed at interventions for dysphagia in hospitalized older patients. It is crucial that these trials prioritize patient-centered measures, including quality of life, length of hospital stays, and the duration of post-hospitalization survival. Additionally, it is essential to consider the viewpoints of family members and caregivers regarding these outcomes.

Recognizing the significance of a "core outcome set" is paramount in assessing the efficacy and impact of such interventions. This core setting, comprising a standardized selection of key outcomes, facilitates comparability and enables researchers to draw meaningful conclusions about the effectiveness of dysphagia interventions. By utilizing a core outcome set, we can ensure that future research in this field is robust, patient-focused, and capable of producing insights that genuinely benefit both patients and their caregivers. The emphasis should not solely be on pneumonia recurrence or mortality rates among older patients with multiple risk factors and frailty. We are certainly striving to do the right thing, but we may have chosen the wrong path.

AUTHORS' CONTRIBUTIONS

SRRD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MT:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. **LJL:** Conceptualization, Data curation, Formal Analysis, Investigation, Writing – original draft. **CT:** Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Writing – review & editing. **RGR:** Conceptualization, Supervision, Writing – review & editing.

REFERENCES

1. Mandell LA, Niederman MS. Aspiration pneumonia. N Engl J Med. 2019;380(7):651-63. https://doi.org/10.1056/NEJMra1714562
2. Torres A, Cilloniz C, Niederman MS, Menéndez R, Chalmers JD, Wunderink RG, et al. Pneumonia. Nat Rev Dis Primers. 2021;7(1):25. https://doi.org/10.1038/s41572-021-00259-0
3. Momosaki R. Rehabilitative management for aspiration pneumonia in elderly patients. J Gen Fam Med. 2017;18(1):12-5. https://doi.org/10.1002/jgf2.25
4. Munro SC, Baker D, Giuliano KK, Sullivan SC, Haber J, Jones BE, et al. Nonventilator hospital-acquired pneumonia: a call to action. Infect Control Hosp Epidemiol. 2021;42(8):991-6. https://doi.org/10.1017/ice.2021.239
5. Gomes CA, Andriolo RB, Bennett C, Lustosa SA, Matos D, Waisberg DR, et al. Percutaneous endoscopic gastrostomy versus nasogastric tube feeding for adults with swallowing disturbances. Cochrane Database Syst Rev. 2015;2015(5):CD008096. https://doi.org/10.1002/14651858.CD008096.pub4
6. Gaidos S, Hrdlicka HC, Corbett J. Implementation of a free water protocol at a long term acute care hospital. Sci Rep. 2023;13(1):2626. https://doi.org/10.1038/s41598-023-29448-5
7. Bath PM, Lee HS, Everton LF. Swallowing therapy for dysphagia in acute and subacute stroke. Cochrane Database Syst Rev. 2018;10(10):CD000323. https://doi.org/10.1002/14651858.CD000323.pub3
8. Hansen T, Beck AM, Kjaersgaard A, Poulsen I. Second update of a systematic review and evidence-based recommendations on texture modified foods and thickened liquids for adults (above 17 years) with oropharyngeal dysphagia. Clin Nutr ESPEN. 2022;49:551-5. https://doi.org/10.1016/j.clnesp.2022.03.039
9. Leipzig RM, Whitlock EP, Wolff TA, Barton MB, Michael YL, Harris R, et al. Reconsidering the approach to prevention recommendations for older adults. Ann Intern Med. 2010;153(12):809-14. https://doi.org/10.7326/0003-4819-153-12-201012210-00007
10. Gillman A, Winkler R, Taylor NF. Implementing the free water protocol does not result in aspiration pneumonia in carefully selected patients with dysphagia: a systematic review. Dysphagia. 2017;32(3):345-61. https://doi.org/10.1007/s00455-016-9761-3

https://doi.org/10.1590/1806-9282.2023D704

Renal denervation by radiofrequency in patients with hypertension: systematic review and meta-analysis

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

INTRODUCTION

Hypertension is a significant risk factor for cardiovascular morbidity and mortality[1]. Despite a wide array of pharmacological treatment options, many patients remain uncontrolled[2]. Medical inertia and patient non-adherence to medications are the main reasons for this lack of control. Hyperactivity of the sympathetic nervous system plays a crucial role in resistant hypertension. The sympathetic renal nerves primarily originate in the celiac and aortorenal ganglia around the aorta. The renal nerves run in the adventitia and perivascular adipose tissue around the renal arteries. At the renal level, the sympathetic efferent pathway (brain → kidney) directed to the kidneys results in increased production of noradrenaline, causing renal vasoconstriction and the release of renin, which in turn induces sodium retention. On the contrary, the afferent sympathetic fibers (kidney → brain) transmit signals to the brain, stimulating central sympathetic activity and contributing to neurogenic hypertension[3]. It is common to find an increase in sympathetic system activity in hypertension[4], especially in the presence of obesity[5]. In the past decade, renal denervation (RDN) has emerged as a treatment option for arterial hypertension. It is performed through the percutaneous insertion of the device catheter into the femoral artery, which is then advanced into the main renal arteries under fluoroscopic guidance[6]. RDN is a catheter-based ablation of the afferent and efferent sympathetic nerves within the wall of the renal arteries. Generally, the delivery of energy through radiofrequency or ultrasound heats up the surrounding adipose tissue of the renal arteries, where the renal nerves are located. Therefore, the renal nerves are destroyed as a result of a thermal injury[3]. The complete report of a trial [SPYRAL HTN-ON MED, 2023] (which constitutes 46% of the total data from second-generation placebo-controlled trials) has emerged recently. Therefore, we conducted an updated meta-analysis of RDN, by radiofrequency (RF-RDN), for hypertension, including the entirety of the data from second-generation randomized placebo-controlled trials, which are currently available.

OBJECTIVE

The objective of this study was to evaluate the benefits and harms of RF-RDN for the treatment of patients with uncontrolled hypertension, in the presence or absence of antihypertensive medications.

METHODOLOGY

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[7] and is supported by scientific information obtained through a systematic review of the literature (published).

Eligibility criteria

The eligibility criteria express the specific elements to answer the clinical question of this evaluation (objective). Considering the various arguments against combining first- and second-generation RDN trials in a single meta-analysis, we chose to include only "second-generation RF-RDN trials." These stand out not

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only for the strict control of medication intake but also for improvements in all other practical methods. This includes blood pressure (BP) measurements, patient selection, and the execution of the RF-RDN procedure itself. In addition, "second-generation trials" feature new catheter designs and a greater number of ablations in the arteries, thus being enhanced and distinct compared with "first-generation trials."

Criteria for study inclusion:

- Patients: Individuals with uncontrolled hypertension, whether or not they are using antihypertensive medications.
- Intervention: Renal sympathetic denervation through radiofrequency.
- Comparison: Placebo.
- Outcomes: Clinically relevant efficacy, and in the absence of such data, intermediate outcomes including reduction in BP (mmHg) and safety.
- Study design: Double-blind randomized controlled parallel trials.
- Language: No restrictions.
- Consultation period: No restrictions.
- Full text availability: Required.

Systematic reviews with or without meta-analysis, narrative reviews, observational and/or case series, first-generation RF-RDN trials or absence of extractable data (absolute numbers and/or averages), and Phase 2 study were excluded from this study.

Search for Evidence will be carried out in the virtual scientific information base Medline using the search strategy: #1 ((Blood Pressure OR Hypertension) AND Kidney AND (Catheter Ablation OR Catheters OR Catheterization)), #2 ((Blood Pressure OR Hypertension) AND (Kidney OR Renal Artery) AND (Sympathectomy* OR Denervation OR Endovascular Procedures)), ((#1) OR (#2)) AND (Random*); CENTRAL / Cochrane: ((Blood Pressure OR Hypertension) AND (renal denervation)); LILACS: Hypertension AND Renal AND Denervation AND (type_of_study:("clinical_trials")); and ClinicalTrials.gov: Hypertension AND Renal AND Denervation, Study Typ=Interventional (Clinical Trial). Additional manual searches were conducted in the reference list of the included studies and other relevant sources. The search in these databases was carried out until January 2024.

Study selection process and data extraction

The evidence retrieved from the consulted databases is initially selected based on the title and abstract, aiming to meet the eligibility criteria. The related studies in this first selection then have their full texts accessed to confirm their eligibility. The process of retrieving the studies, as well as the evaluation of the obtained titles and abstracts, was conducted by two researchers skilled in the development of systematic reviews (A.S. and I.F.) independently and blinded, following the inclusion and exclusion criteria. Subsequently, the selected articles were critically evaluated to be included or not in the review. When there was disagreement about the selection of studies among the investigators, a third reviewer was consulted (W.M.B.).

In the selected studies, we will extract the following data: author's name and year of publication, the studied population, intervention and comparison methods, and follow-up duration. For relevant outcomes, data extraction may include absolute event numbers or means and/or medians, along with corresponding standard deviations (SDs) or 95% confidence intervals (95%CIs), depending on the type of outcome.

Risk of bias and quality of evidence

Two independent reviewers assessed the risk of bias in the included studies using the items from the Cochrane Risk of Bias Tool for randomized trials (RoB 2)[8], supplemented with other fundamental elements, and expressed as high, moderate, and low. The levels of evidence will be extrapolated from the risk of bias obtained from the study/studies (if there is no meta-analysis) using the terminology of the Grading of Recommendations Assessment, Development, and Evaluation (GRADE)[9] as very low, low, and high, and through the GRADEpro software[10] (if there is a meta-analysis) as very low, low, moderate, and high. Two reviewers assessed the risk of bias, inconsistency, indirect evidence, imprecision, and publication bias for all reported outcomes.

Method of analysis and synthesis of results

The data were analyzed following the intention-to-treat (ITT) principle, and each trial included the most recent follow-up data. Categorical outcomes were reported as the risk difference (RD) between the intervention and control groups. If the RD was statistically significant (95% confidence), it would be presented with the 95%CI and the number needed to treat (NNT) or to produce harm (NNH).

For continuous measures, results were presented as mean differences (MDs) or standardized mean differences (SMDs) when different scales were reported, accompanied by corresponding 95%CIs. In instances where multiple studies with common outcomes were included, meta-analysis would be conducted using the Review Manager 5.4 software (The Nordic Cochrane Centre, The Cochrane Collaboration)[11]. The overall difference

in risk or mean, along with 95%CIs, served as the conclusive measure supporting the synthesis of evidence, addressing the clinical question (objective) of this assessment.

In cases where SD information was unavailable, we calculated SD from the sample size using either the standard error (SE) or the 95%CI. The estimation of combined effect sizes was carried out using either a fixed- or random-effect model after evaluating the results for heterogeneity. Statistical heterogeneity was assessed using the I^2 metric, which measures the percentage of variation related to heterogeneity between studies rather than randomness[12].

Evidence synthesis and conclusion

The synthesis of evidence directly present results from the analyses, carefully evaluating the benefits, harms, and absence of differences between the use of RF-RDN through parallel comparison with a placebo. Conclusions were primarily drawn from evidence of at least moderate quality, considering the presence of an effect, whether it was beneficial or harmful, and an overall favorable balance between benefits and harms. This was particularly crucial in patients with difficult-to-control or genuinely resistant hypertension.

RESULTS

In the quest for evidence regarding the use of RDN, we retrieved 943, 621, 486, and 146 studies from the MEDLINE, CENTRAL, LILACS, and CT.gov databases, respectively. No studies were obtained through manual and/or gray searches. Following the removal of duplicates and exclusion based on title and/or abstract, 20 studies remained, aligning with the previously established eligibility criteria (methodology). These 20 studies were further chosen for access to their full texts.

Upon a comprehensive review of the full texts, three randomized controlled trials conducted in parallel with a placebo[13-15] were included to substantiate the conclusions of this assessment. The exclusion of the other 17 studies was attributed to reasons such as the absence of a comparison of RF-RDN with Sham, involvement in "first-generation" studies, post-hoc analysis, or being classified as a Phase 2 study (refer to Figure 1 for details). The references and reasons for the exclusion of these studies are available in the "References" section. The flow diagram in Figure 1 illustrates the sequence from retrieval to the selection of evidence supporting this assessment.

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal. pmed1000097

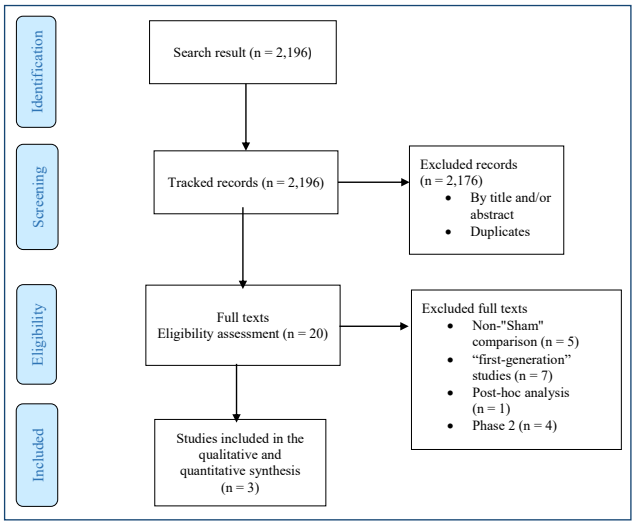

Figure 1. Flow diagram representing the study selection process.

Three "second-generation trials" that met the inclusion and exclusion criteria were identified[13-15]. The main baseline characteristics and details of each trial are outlined in Table 1 (ANNEXES). These trials collectively involved 719 participants, with 406 randomized to RF-RDN and 313 to the control group.

Risk of bias in studies

Regarding the risk of bias in the three included randomized clinical trials (RCTs)[13-15], one did not present an ITT analysis[13], and another terminated early with 51 patients included out of an expected total of 93, without performing an ITT analysis[14]. The risk of bias assessment for each individual study, conducted using the RoB 2 tool[8] supplemented with other essential elements, is provided in Table 2.

Outcomes

Efficacy

The data extracted from the three RCTs included changes (MDs [±SD]) in comparing BPs at follow-up time and baseline. These results facilitated the calculation of the MD (95%CI) in meta-analyses between the intervention (RF-RDN) and control (SHAM). Relevant clinical outcomes, such as hypertensive crises and strokes, were incorporated into adverse events, with a maximum follow-up of 6 months. The levels of evidence, as per the GRADE system, for each outcome can be found in Table 3 (ANNEXES).

The mean change in ambulatory (24 h) systolic and diastolic BP at 2–3-month follow-up was assessed in three RCTs[13-15] (total of 719 patients). Compared with SHAM, the RF-RDN procedure demonstrated a reduction of -2.50 mmHg [95%CI (-4.00, -1.00); p<0.001; I^2=72%] and -2.18 mmHg [95%CI (-3.17, -1.20);

Table 1. Key patient baseline characteristics and details of each trial.

First Author/ Trial, Year (Ref. No.)	Patients (N)	Intervention (N)	Control (N)	Denervation Method	Outcomes	Follow-Up Duration (Months)	Participating Centers
Böhm M, et al. SPYRAL HTN-OFF MED Pivotal, 2020 (13)	Untreated patients over the 3–4 weeks before randomization. Clinic BP 150–179/≥90 mmHg and average 24-h ambulatory BP 140–16 mmHg. Mean age: 53 years. (N = 331; 80 patients from randomized Pilot trial and 251 patients from randomized pivotal trial)	RF-RDN (N = 166)	SHAM: Renal angiography (N = 165)	Radiofrequency technique (Multielectrode Symplicity SpiralTM®)	Primary end point: change in mean 24-h systolic blood pressure Secondary end point: change in office SBP; changes in morning and nighttime BP Adverse events	3	44 centers in Australia, Austria, Canada, Germany, Greece, Ireland, UK, and USA
Kandzari DE, et al. SPYRAL HTN-ON MED, 2023 (14)	Patients with 1–3 drugs from ≥6 weeks, uncontrolled (clinic BP 150–180/≥90 mmHg, 24-h BP 140–170 mmHg). Mean age: 53-55 years. (N = 337; 80 patients from randomized Pilot trial and 257 patients from randomized Expansion trial).	RF-RDN (N = 206)	SHAM: Renal angiography (N = 131)	Radiofrequency technique (Multielectrode Symplicity SpiralTM®)	Primary end point: change in ambulatory blood pressure Secondary end point: office BP; changes in morning (daytime) and night-time BP Adverse Events	6	56 clinical centers worldwide (USA, Germany, Japan, UK, Australia, Austria, and Greece)
Weber MA, et al. REDUCE HTN: REINFORCE, 2020 (15)	Patients with office SBP of 150 to 180 mmHg and average 24-h ambulatory SBP of 135 to 170 mmHg after medication washout. Mean age: 58 years	RF-RDN (N = 34) 8 weeks: no antihypertensive medications (unless rescue) 8 weeks to 6 months: add medication if office SBP ≥140 mmHg	SHAM: Renal Angiography (N = 17) 8 weeks: no antihypertensive medications (unless rescue) 8 weeks to 6 months: add medication if office SBP ≥140 mmHg	Radiofrequency technique (Vessix Renal Denervation® system transmits radiofrequency energy via bipolar electrodes).	Primary end point: Mean reduction in average 24-h ambulatory BP. Secondary end point: Daytime ambulatory Office BP Adverse events	8 weeks 6 months: 12 months	12 centers in USA

Radiofrequency (RF); Renal denervation (RDN); Blood pressure (BP); systolic blood pressure (SBP); office blood pressure (OBP).

Table 2. Risk of bias in studies.

First Author/ Year (Ref. #)	Randomization	Blind allocation	Double-blind	Outcome researcher blind	Losses	Prognostic characteristics	Appropriate outcomes	Intention-to-treat analysis	Sample size calculation	Early interruption	Risk of Bias
Böhm M, 2020 [13]											High
Kandzari DE, 2023 [14]											Low
Weber MA, 2020 [14]											High
LEGEND	HIGH RISK		NOT INFORMED		LOW RISK						

Table 3. Levels of evidence – GRADE System.
Summary of results:
RF-RDN compared with SHAM in blood pressure change for hypertension
Patient or population: HYPERTENSION
Context: Efficacy and safety
Intervention: RF-RDN
Comparison: SHAM.

Outcomes Number of participants (studies)	Mean Difference	Certainty
24-h ambulatory systolic blood pressure – 2–3 months No. of participants: 719 (3 RCTs)	MD **2.5 lower** (4 lower to 1 lower)	⊕⊕○○ Low [a,b]
24-h ambulatory systolic blood pressure – 6 months No. of participants: 388 (2 RCTs)	MD **2.33 lower** (4.54 lower to 0.12 lower)	⊕⊕⊕○ Moderate [c]
24-h ambulatory diastolic blood pressure – 2–3 months No. of participants: 719 (3 RCTs)	MD **2.18 lower** (3.17 lower to 1.2 lower)	⊕⊕○○ Low [a,b]
24-h ambulatory diastolic blood pressure – 6 months No. of participants: 388 (2 RCTs)	MD **1.07 lower** (2.66 lower to 0.53 higher)	⊕⊕⊕○ Moderate [c]
In-office systolic blood pressure – 2–3 months No. of participants: 719 (3 RCTs)	MD **4.48 lower** (6.48 lower to 2.49 lower)	⊕⊕○○ Low [a,b]
In-office systolic blood pressure – 6 months No. of participants: 388 (2 RCTs)	MD **5.7 lower** (8.45 lower to 2.96 lower)	⊕⊕○○ Low [b,c]
In-office diastolic blood pressure – 2–3 months No. of participants: 719 (3 RCTs)	MD **2.63 lower** (3.86 lower to 1.4 lower)	⊕⊕○○ Low [a,b]
In-office diastolic blood pressure – 6 months No. of participants: 388 (2 RCTs)	MD **2.03 lower** (3.84 lower to 0.22 lower)	⊕⊕⊕○ Moderate [c]

RCTs: randomized controlled trials.

Explanations:

[a]Two studies were included, with a high risk of bias (no intention-to-treat analysis in both, and early termination in one), and one with low risk.

[b]Substantial heterogeneity.

[c]Two studies were included, with one not undergoing intention-to-treat analysis and experiencing early termination.

Outcome No. of participants (studies)	Relative effect (95%CI)	Potential absolute effects (95%CI)			Certainty
		RF-RDN	SHAM	Difference	
Serious adverse events No. of participants: 719 (3 RCTs)	**RR 0.87** (0.21–3.52)	1.0%	**0.8%** (0.2–3.4)	0.1% fewer (0.8 fewer to 2.4 more)	⊕⊕⊕○ Moderate [a]

*The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparator group and the relative effect of the intervention (and its 95%CI). CI: confidence interval; MD: mean difference; RR: risk ratio.

Explanations:

a. Two studies were included, with a high risk of bias (no intention-to-treat analysis in both, and early termination in one), and one with low risk.

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

p<0.0001; I^2=58%], respectively (Figures 2 and 3). According to the GRADE system, the level of evidence is considered low.

For the change in mean ambulatory (24 h) systolic and diastolic BP at 6-month follow-up, data were available from two RCTs[14,15] (a total of 388 patients). Compared with SHAM, the RF-RDN procedure exhibited a reduction of -2.33 mmHg [95%CI (-4.54, -0.12); p<0.04; I^2=10%] in systolic BP and no significant difference in diastolic BP [-1.07 mmHg [95%CI (-2.66, 0.53); p<0.19; I^2=0%] (Figures 2 and 3). Based on the GRADE system, the level of evidence is moderate.

The mean change in office systolic and diastolic BP at 2–3-month follow-up was analyzed across three RCTs[13-15] (a total of 719 patients). When compared with SHAM, the RF-RDN procedure demonstrated a reduction of -4.48 mmHg [95%CI

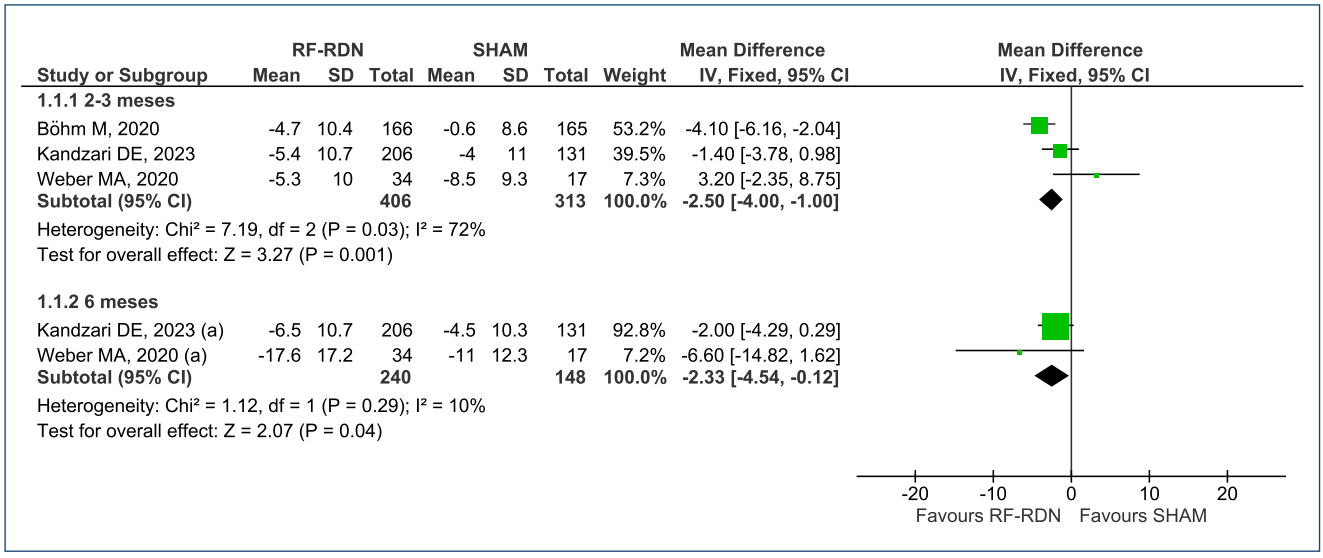

Figure 2. Forest plot of comparison: 1 RF-RDN versus SHAM, outcome: 1.1 Change in 24-h ambulatory systolic BP.

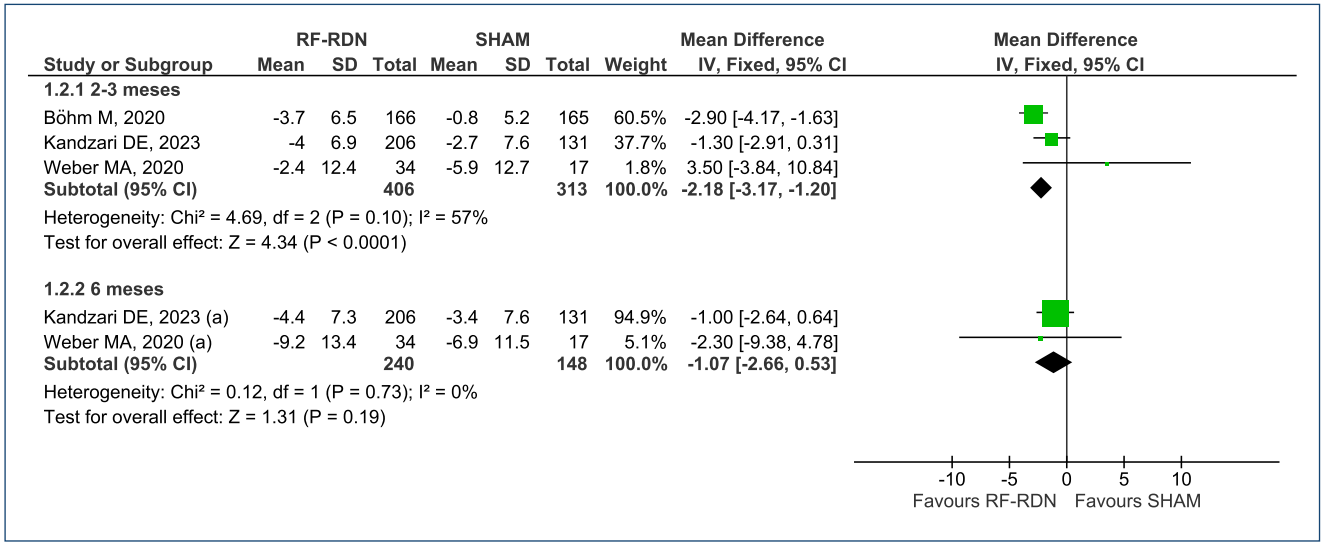

Figure 3. Forest plot of comparison: 1 RF-RDN versus SHAM, outcome: 1.2 Change in 24-h ambulatory diastolic BP.

(-6.48, -2.49); p<0.0001; I^2=58%] and -2.63 mmHg [95%CI (-3.86, -1.40); p<0.0001; I^2=66%], respectively (Figures 4 and 5). The level of evidence is considered low.

For the mean change in office systolic and diastolic BP at 6-month follow-up, data were available from two RCTs[14,15] (total of 388 patients). In comparison with SHAM, the RF-RDN procedure exhibited a reduction of -5.70 mmHg [95%CI (-8.45, -2.96); p<0.0001; I^2=62%] for systolic BP and -2.03 mmHg [95%CI (-3.84, -0.22); p<0.03; I^2=0%] for diastolic BP, respectively (Figures 4 and 5). The level of evidence is considered low for systolic BP and moderate for diastolic, in the office.

Safety

The assessed composite outcome is the occurrence of severe adverse events: hypertensive crisis requiring medical attention, new stroke, and/or vascular complications (necessitating surgical repair, thrombin intervention procedure, or blood transfusion). For this outcome, three RCTs[13-15] with a total of 719 evaluated patients were included in a follow-up of up to 6 months. In the comparison of RF-RDN with SHAM, no difference was observed between the two procedures (RD=-0.00 [95%CI -0.02, 0.01]; p=0.93; I^2=0%) (Figure 6). The level of evidence is considered moderate.

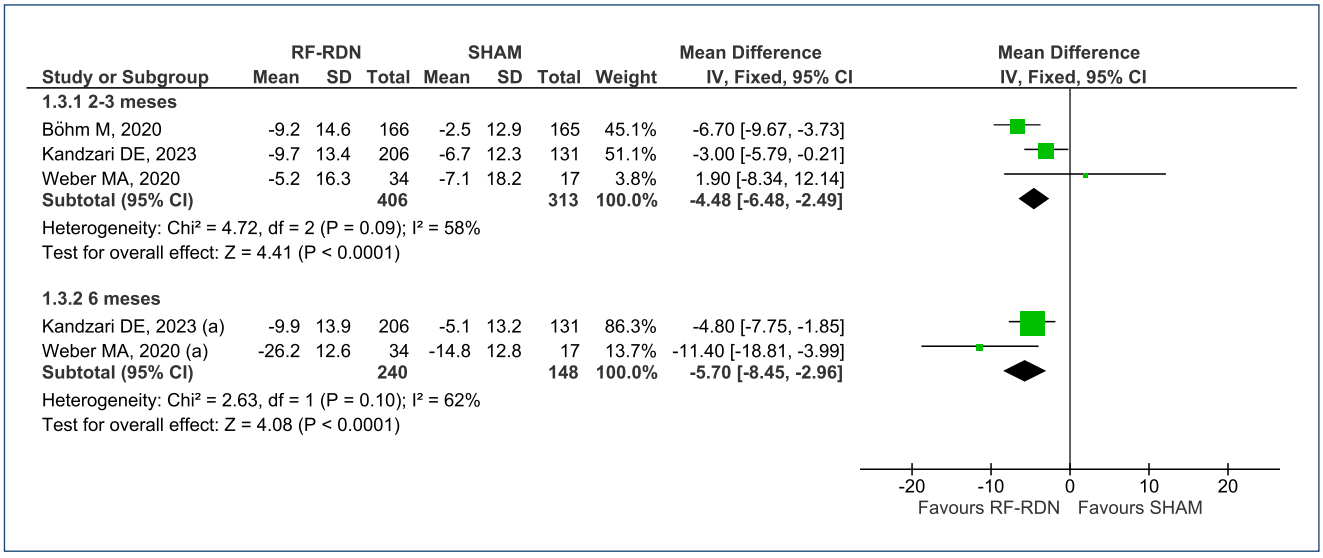

Figure 4. Forest plot of comparison: 1 RF-RDN versus SHAM, outcome: 1.3 Change in office systolic BP.

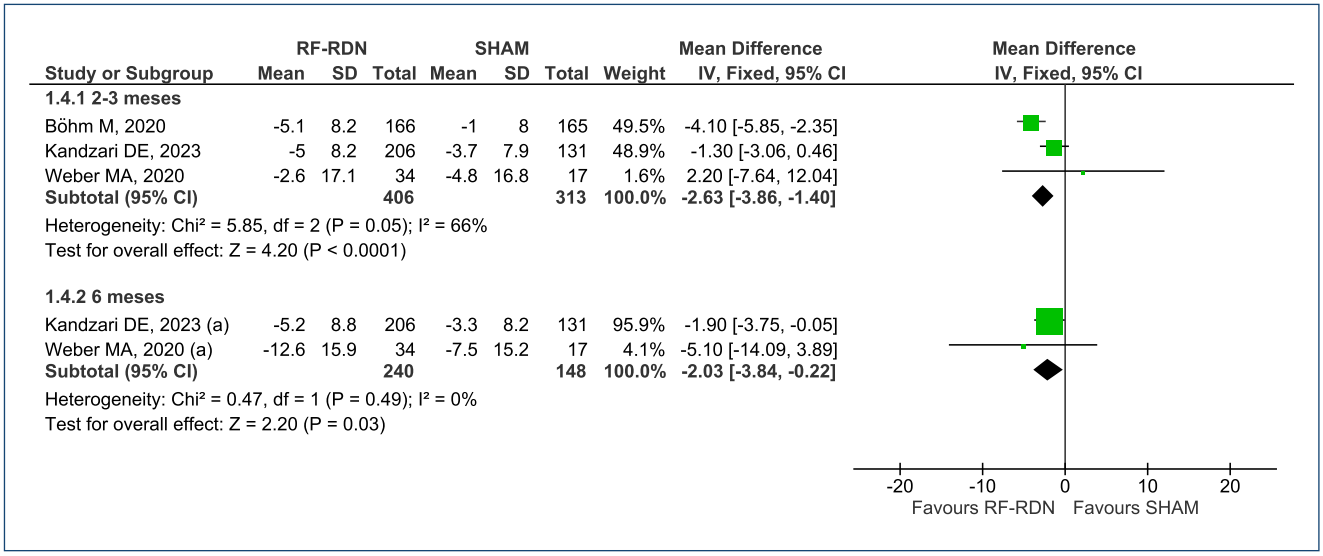

Figure 5. Forest plot of comparison: 1 RF-RDN versus SHAM, outcome: 1.4 Change in office diastolic blood pressure.

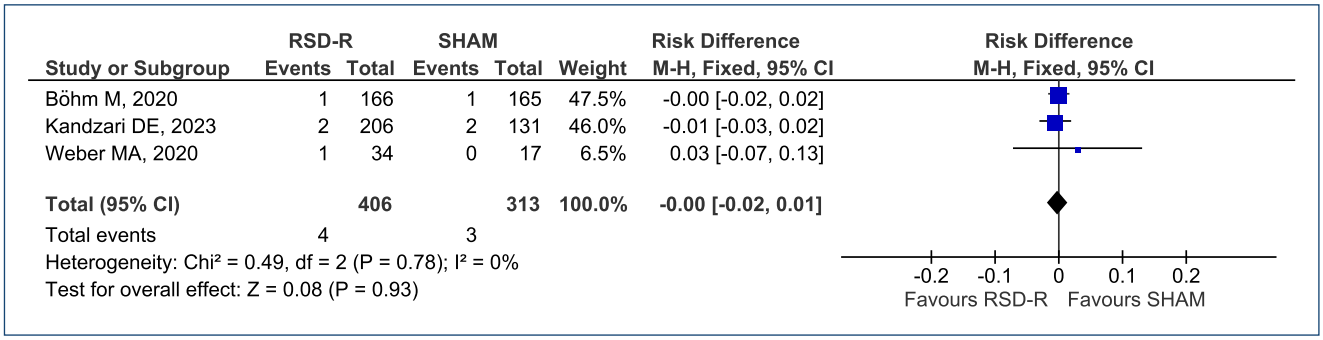

Figure 6. Forest plot of comparison: 1 RF-RDN versus SHAM in the change of blood pressure, outcome: 1.5 Severe Adverse Events.

Evidence synthesis

Radiofrequency renal denervation compared with SHAM:

- Reduces, in mean, ambulatory systolic BP (24 h) at 2–3 and 6 months (-2.5 and -2.3 mmHg, with low and moderate levels of evidence, respectively).
- Reduces, in mean, ambulatory diastolic BP (24 h) at 2–3 months (-2.18 mmHg; low level of evidence) and shows no difference at 6 months (moderate level of evidence).
- Reduces, in mean, office systolic and diastolic BPs at 2–3 and 6 months (approximately -5 and 2.6 mmHg, with low and moderate levels of evidence, respectively).
- There is no difference in this comparison for serious adverse events with moderate level of evidence.

DISCUSSION

Unlike previous meta-analyses, this analysis offers new details and information. In addition to the usual analysis for changes in office BP and changes in ambulatory BP, we conducted analyses including only the "second-generation studies," considering the various arguments against combining "first- and second-generation" RDN trials in a single meta-analysis. Among these arguments, we highlight not only the strict control of medication intake but also the improvement in all other practical methods. This includes BP measurements, patient selection, and the execution of the ablation procedure itself. These "second-generation" trials feature new catheter designs and a greater number of ablations in the arteries, thus being enhanced and distinct compared with the "first-generation trials." This meta-analysis is the first to exclusively incorporate studies assessing sympathetic denervation through radiofrequency in comparison with a placebo. Additionally, it includes comprehensive results from the SPYRAL HTN ON MED trial, constituting 46% of the total data from "second-generation" placebo-controlled trials. As a follow-up to the primary analysis, we gathered data from both the Pilot and Expansion phases. The longest follow-up time presented thus far is 6 months. In this meta-analysis, we present the results separately for the 2–3- and 6-month follow-ups.

In this review, we incorporated a study involving hypertensive patients not treated for 3–4 weeks before randomization and another study involving patients using 1–3 medications for ≥6 weeks and having uncontrolled hypertension. The systematic review conducted by Ahmad et al.[16] assessed the impact of RDN on ambulatory and office BP in hypertensive patients. They utilized a metaregression with mixed-effects models to explore any significant interaction between the characteristics of the clinical trial and the effect size on ambulatory systolic BP.

The results of Ahmad et al.'s metaregression revealed no significant interaction between the presence of baseline antihypertensive medications and the effect size. This indicates a consistent effect size of RDN, irrespective of whether used in patients not yet on medication or in those already taking medications but with inadequate control. The difference observed was -1.10 mmHg for trials without medications (95%CI -4.40 to -2.2 mmHg; p=0.514). Our current analysis reveals a reduction in ambulatory systolic BP (24 h) at 2–3 and 6 months with RF-RDN, showing decreases of -2.5 and -2.3 mmHg, supported by low and moderate levels of evidence, respectively. Furthermore, in "second-generation" trials, RF-RDN resulted in a modest yet significant reduction in ambulatory diastolic BP (24 h) at 2–3 months (-2.18 mmHg; low level of evidence) and exhibited no significant difference at 6 months (moderate level of evidence).

Another noteworthy finding from this meta-analysis is the reduction in office systolic and diastolic BPs at 2–3 and 6 months with RF-RDN, amounting to approximately -5 and 2.6 mmHg, supported by low and moderate levels of evidence, respectively. The results suggest the safety of procedures in "second-generation" studies, showing no evidence of a difference in the occurrence of serious adverse events such as hypertensive crises requiring medical attention, new stroke, and/or vascular complications between RF-RDN and the Sham group.

Study limitations

Our primary (intermediate) outcomes display either zero or quite acceptable heterogeneity. Nonetheless, the primary limitations of this meta-analysis encompass the small number of included RCTs (N=3), a relatively modest overall sample size (n=719 [406 randomized to RF-RDN and 313 to the control group]), and a short follow-up period (up to 6 months). Undoubtedly, more extensive and larger RCTs are warranted, providing sufficient power to yield more precise information (GRADE system level of evidence: low/moderate) regarding the role of renal sympathetic denervation by radiofrequency in treating primary hypertension and evaluating clinical outcomes.

CONCLUSION

This meta-analysis suggests that RDN shows positive short-term results, offering a potential contribution to the enhanced management of uncontrolled hypertension in an ideal

population. However, the observed effect seems relatively modest. Additionally, the results indicate that when compared with RF-RDN, the sham intervention also significantly influences BP reduction, in both office and ambulatory settings (24 h), highlighting the substantial impact of its effect. Further research is warranted to demonstrate the clinical benefits associated with this reduction.

AUTHORS' CONTRIBUTIONS

AS, **IF**, and **WMB** conceived and designed the study. **AS** and **IF** conducted the scientific literature search, screened the studies, and extracted the data. **AS** and **IF** also assessed the quality of the included studies. **AS** performed the analyses and wrote the first draft of the review. All authors contributed to the interpretation and edited the preliminary review.

REFERENCES

1. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002;360(9349):1903-13. https://doi.org/10.1016/s0140-6736(02)11911-8
2. Lloyd-Jones DM, Evans JC, Levy D. Hypertension in adults across the age spectrum: current outcomes and control in the community. JAMA. 2005;294(4):466-72. https://doi.org/10.1001/jama.294.4.466
3. Barbato E, Azizi M, Schmieder RE, Lauder L, Böhm M, Brouwers S, et al. Renal denervation in the management of hypertension in adults. A clinical consensus statement of the ESC Council on Hypertension and the European Association of Percutaneous Cardiovascular Interventions (EAPCI). EuroIntervention. 2023;18(15):1227-243. https://doi.org/10.4244/EIJ-D-22-00723
4. Smith PA, Graham LN, Mackintosh AF, Stoker JB, Mary DA. Relationship between central sympathetic activity and stages of human hypertension. Am J Hypertens. 2004;17(3):217-22. https://doi.org/10.1016/j.amjhyper.2003.10.010
5. Lohmeier TE, Iliescu R, Liu B, Henegar JR, Maric-Bilkan C, Irwin ED. Systemic and renal-specific sympathoinhibition in obesity hypertension. Hypertension. 2012;59(2):331-8. https://doi.org/10.1161/HYPERTENSIONAHA.111.185074
6. Denker MG, Cohen DL. Resistant Hypertension and Renal Nerve Denervation. Methodist Debakey Cardiovasc J. 2015;11(4):240-4. https://doi.org/10.14797/mdcj-11-4-240
7. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. https://doi.org/10.1136/bmj.n71
8. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898. https://doi.org/10.1136/bmj.l4898
9. Grade Working Group. 2024. [cited on 2024 Jan]. Available from:http://gradeworkinggroup.org/society/index.htm.
10. GRADEpro GDT. 2020. GRADEpro guideline development tool [software]. McMaster University, 2020 (developed by Evidence Prime, Inc.). Available from: https://www.gradepro.org/.
11. Review Manager (RevMan). 2020.[Computer program]. Version 5.4. The Cochrane Collaboration, 2020.
12. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315(7109):629-34. https://doi.org/10.1136/bmj.315.7109.629
13. Böhm M, Kario K, Kandzari DE, Mahfoud F, Weber MA, Schmieder RE, et al. Efficacy of catheter-based renal denervation in the absence of antihypertensive medications (SPYRAL HTN-OFF MED Pivotal): a multicentre, randomised, sham-controlled trial. Lancet. 2020;395(10234):1444-51. https://doi.org/10.1016/S0140-6736(20)30554-7
14. Kandzari DE, Townsend RR, Kario K, Mahfoud F, Weber MA, Schmieder RE, et al., SPYRAL HTN-ON MED Investigators. Safety and efficacy of renal denervation in patients taking antihypertensive medications. J Am Coll Cardiol. 2023;82:1809-23. https://doi.org/10.1016/j.jacc.2023.08.045
15. Weber MA, Kirtane AJ, Weir MR, Radhakrishnan J, Das T, Berk M, et al. The REDUCE HTN: REINFORCE: randomized, sham-controlled trial of bipolar radiofrequency renal denervation for the treatment of hypertension. JACC Cardiovasc Interv. 2020;13(4):461-70. https://doi.org/10.1016/j.jcin.2019.10.061
16. Ahmad Y, Kane C, Arnold AD, Cook CM, Keene D, Shun-Shin M, et al. Randomized blinded placebo-controlled trials of renal sympathetic denervation for hypertension: a meta-analysis. Cardiovasc Revasc Med. 2022;34:112-8. https://doi.org/10.1016/j.carrev.2021.01.031

https://doi.org/10.1590/1806-9282.20231367

Sleep quality depends not only on radicular pain but also on other factors

Fulvio Alexandre Scorza[1], Antonio-Carlos Guimaraes de Almeida[2], Josef Finsterer[3*]

Dear Editor,

We read with interest the article by Ay and Tuna, which is a case-control study involving 42 patients with lumbar radicular pain due to a herniated disc and its influence on sleep quality and lower limb functionality[1]. All patients underwent needle electromyography, visual analog scale (VAS) test, Pittsburgh Sleep Quality Index (PSQI), and lower extremity functional scale (LEFS) assessment to identify the nerve roots that were affected[1]. It was found that radiculopathy did not affect sleep quality and lower extremity functionality but it was hypothesized that the degree of disc herniation might influence the two outcome measures. The study is excellent but it raises concerns that should be discussed.

The first limitation of the study is that the current pain medications such as orthopedic therapy and physical therapy were not included in the analysis. Since the suppression of pain depends largely on the type and intensity of treatment and pain greatly affects sleep quality, it is important to know all types of treatments that the patients have received for their radicular pain before inclusion. How many patients received hives therapy, infiltration, or CT-guided infiltration in addition to oral or intravenous pain killers before inclusion? It is also important to know the details of medications that were regularly taken by patients in addition to painkillers.

The second limitation is that the inclusion and exclusion criteria were not well defined. Previous spinal surgery, radiculitis due to Borreliosis or Elsberg syndrome, varicositas spinalis, myelitis, vertebrostenosis, subdural hematoma, and syringomyelia were not mentioned as exclusion criteria. Did any of the patients undergo previous spinal surgery before inclusion?

The third limitation is that the included patients did not undergo assessment of depression and anxiety. As depression can result from chronic pain and can strongly influence pain perception and processing, it is crucial to know the number of the included patients who also suffered from depression in addition to their radicular pain. People with depression may aggravate their pain syndrome compared to people without depression.

The fourth limitation is that, in addition to pain, factors that determine sleep quality and intensity were not included in the assessment. We should know the number of patients who had alcohol addiction, regularly took tetra-hydro-cannabinol (THC), or smoked. In addition, we should know the number of patients who regularly consumed stimulating substances such as coffee, cola, red bull, and other adrenergic drugs. Unless these stimulating substances are included in the evaluation, the results are not reliable.

We disagree with the notion in the introduction that neuropathic pain is generally associated with weakness, wasting, decreased tendon reflexes, allodynia, hyperalgesia, burning, tingling, and sudomotor dysfunction[1]. Neuropathic pain can occur without additional neurological symptoms or signs such as those occuring in small fiber neuropathy. Whether or not neuropathic pain is associated with additional neurological symptoms or signs depends largely on the underlying etiology of the neuropathic pain.

The finding that patients with radicular pain had less nocturnal pain than patients without radicular pain is contradictory to previous findings and requires a plausible explanation.

Overall, the interesting study has limitations that call into question the results and their interpretations. Clarifying these limitations would strengthen the conclusions and could add value to the study.

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AUTHORS' CONTRIBUTIONS

JF: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing. **FAS:** Formal Analysis, Methodology, Writing – review & editing. **ACGA:** Formal Analysis, Methodology, Writing – review & editing.

REFERENCE

1. Ay BK, Tuna M. Does the presence of radiculopathy affect sleep quality and lower extremity functionality in neuropathic low back pain? Rev Assoc Med Bras (1992). 2023;69(9):e20230459. https://doi.org/10.1590/1806-9282.20230459

https://doi.org/10.1590/1806-9282.20231543

Comment on "Relationship between platelet indices and red cell distribution width and short-term mortality in traumatic brain injury with 30-day mortality"

Changjiu Tang[1], Rong Huang[2*]

Dear Editor,

Palabiyik et al.[1] explored the relationship between platelet indices, red cell distribution width (RDW), and short-term mortality in patients with traumatic brain injury. The study aimed to uncover potential associations among these factors, employing a comprehensive analysis with a substantial sample size to bolster the study's reliability. Results indicated no significant differences in mean platelet volume and platelet distribution width values between survivors and non-survivors. Although the platelet count-to-total lymphocyte count ratio values were lower in those who did not survive, this discrepancy did not reach statistical significance. However, within the first 30 days post-traumatic brain injury, deceased patients displayed a notable increase in RDW compared to their living counterparts. Notably, the inclusion of RDW as a parameter in the analysis brought a fresh perspective to the investigation. These findings provide important insights into specific prognostic markers for short-term mortality in patients with traumatic brain injury, emphasizing the significance of considering the RDW levels in the clinical assessment of patients with traumatic brain injury over a 30-day period. However, we note that some concerns require further clarification and elaboration.

First, traumatic brain injury is a diverse condition that may result in significant corticosteroid insufficiency linked to life-threatening illness, contributing to elevated rates of mortality and morbidity. Thus, the clinical management of traumatic brain injury has historically involved the extensive use of glucocorticoids. A meta-analysis[2], after combining data from 16 studies, shows an increased likelihood of death in patients with traumatic brain injury who were administered substantial doses of glucocorticoids for a brief duration compared to those who received smaller doses over a prolonged period, wherein a tendency toward clinical improvement was noted. Additionally, the application of stress doses of glucocorticoids was associated with a further reduction in pneumonia incidence among patients with traumatic brain injury grappling with critical corticosteroid insufficiency related to life-threatening illness (CIRCI). Evidently, this study[1] encompasses individuals with traumatic brain injury, a cohort potentially subjected to glucocorticoid exposure. Should these patients indeed have encountered glucocorticoids, the potential repercussions on platelet indices, and red cell distribution width, particularly the platelet count-to-total lymphocyte count ratio, poses a concern. Such an influence could introduce a notable bias in the conclusions drawn from this study. In addition, the information regarding the continuation of glucocorticoid usage post-admission is equally crucial. Hence, it is recommended to furnish a comprehensive account of whether the enrolled patients have utilized glucocorticoids, a measure that would markedly mitigate confounding biases in the analysis and interpretation of the study findings.

Second, it is evident that the major outcomes of this study are the 7- or 30-day mortality rates in patients with traumatic brain injury. However, it is crucial to emphasize that the short-term mortality in patients with traumatic brain injury is not solely linked to platelet indices and red cell distribution width, but it is also influenced by numerous other factors, such as early tracheostomy, the occurrence of acute lung injury (ALI), and acute respiratory distress syndrome (ARDS). Research by Dunham et al.[3] suggests that, for patients with severe brain injuries, early tracheostomy may not reduce the incidence of ventilator-associated pneumonia, but it can decrease the duration of mechanical ventilation. Another study by Rincon et al.[4] shows that during a 20-year study period, the prevalence of ARDS/ALI increased from 2% in 1988 to 22% in 2008, and it was associated with a higher risk of in-hospital mortality. However, this present study[1] only describes mean platelet volume, platelet distribution width, platelet count-to-total lymphocyte count ratio, or RDW, neglecting other factors significantly associated with 7- or 30-day mortality. Therefore, it is imperative

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to further incorporate factors related to short-term mortality and make additional adjustments in the logistic regression model to accurately identify risk factors closely associated with short-term mortality in patients with traumatic brain injury.

AUTHORS' CONTRIBUTIONS

CT: Conceptualization, Investigation, Supervision, Writing – original draft. **RH**: Conceptualization, Investigation, Supervision, Writing – review & editing.

REFERENCES

1. Palabiyik O, Tomak Y, Acar M, Erkorkmaz U, Tuna AT, Suner KO, et al. Relationship between platelet indices and red cell distribution width and short-term mortality in traumatic brain injury with 30-day mortality. Rev Assoc Med Bras (1992). 2023;69(1):18-23. https://doi.org/10.1590/1806-9282.00210889
2. Zhao ZL, Chen X, Zhu H, Zhang BL, Chai Y, Li XY, et al. Effects of glucocorticoids on traumatic brain injury related critical illness-related corticosteroid insufficiency. Chin Med J (Engl). 2013;126(19):3754-61. PMID: 24112177
3. Dunham CM, Cutrona AF, Gruber BS, Calderon JE, Ransom KJ, Flowers LL. Early tracheostomy in severe traumatic brain injury: evidence for decreased mechanical ventilation and increased hospital mortality. Int J Burns Trauma. 2014;4(1):14-24. PMID: 24624310
4. Rincon F, Ghosh S, Dey S, Maltenfort M, Vibbert M, Urtecho J, et al. Impact of acute lung injury and acute respiratory distress syndrome after traumatic brain injury in the United States. Neurosurgery. 2012;71(4):795-803. https://doi.org/10.1227/NEU.0b013e3182672ae5

https://doi.org/10.1590/1806-9282.20231580

Reply to the letter: "Postintervention pain levels after elective coronary angiography"

Raif Kılıç[1*], Tuncay Güzel[2], Adem Aktan[3]

Dear editor,

We read with great appreciation the comment made by Engin et al.[1] for our original article entitled "Comparison of pain levels of traditional radial, distal radial, and transfemoral coronary catheterization[2]". We appreciate the authors for their interest toward our article and for their time to share their concerns.

In our study, the pain levels were evaluated according to different intervention points in patients who underwent coronary angiography. Our patients were enrolled from 3 centers, and a total of 540 patients were included, with 180 patients in each group. The interventions were performed by the same physician in each center. Angiography was performed through three access points at each center. In all the three centers, femoral access is mostly preferred and distal radial access (DRA) is less commonly used. In approximately 1 year, 180 patients who underwent distal radial access were reached. This number of patients was reached earlier in other patient groups. Since the groups being equal would make the study more valuable, we determined the number of patients to be 180 for each group. During this period, coronary angiography was performed on approximately 6,500 people in 3 centers. The choice of method is left to the patient's preference and the physician's discretion. For example, if the patient is extremely obese, radial access may be preferred, while a weak radial pulse or the presence of an arteriovenous fistula may cause a deviation from radial access.

It is known that the use of analgesics and sedation in patients before the procedure reduces the pain levels[3]. We did not perform any such procedure on our study patients. In the anamnesis we obtained from the patients, we found that they did not routinely use any analgesic or antipsychotic medication. We did not include patients using these medications in the study. The level of anxiety before the procedure is directly proportional to the pain. There are various studies on this subject[4,5]. However, in our study, we focused more on the differences in pain levels at access sites. Therefore, we did not assess the level of anxiety before the procedure.

Doppler ultrasonography (DUSG) can play a pivotal role in improving the success rate of the intervention[6]. Since access to DUSG was not easy enough in the centers where the study was conducted, it was not used routinely in our study. This is stated in the limitations section of the study.

Table 1. Independent determinants of severe pain in patients undergoing coronary angiography in univariate and multivariate logistic regression analysis model.

	Univariate analysis			Multivariate analysis		
	OR	95%CI	p	OR	95%CI	p
Body mass index	0.820	0.752–0.894	**<0.001**	0.814	0.718–0.923	**0.001**
Processing time	1.095	1.068–1.124	**<0.001**	1.097	1.064–1.131	**<0.001**
Access time	1.080	1.056–1.103	**<0.001**	1.022	0.986–1.059	0.237
Number of punctures	5.570	3.415–9.084	**<0.001**	3.232	1.345–7.768	**0.009**
Access zone (DRA)	0.105	0.047–0.234	**<0.001**	0.114	0.040–0.328	**<0.001**

OR: odds ratio; CI: confident interval; DRA: distal radial access.

In our study, there were 40 patients in the severe pain group, and 32 of these patients underwent coronary angiography via distal radial artery access. The average number of punctures was approximately two times higher in this patient group than the other groups. However, there was also a higher body mass index and longer processing time and access time in the severe pain group. Therefore, it may not be correct to attribute severe pain only to the number of punctures. To clarify this, we performed univariate and multivariate logistic regression analysis and presented it in Table 1. Accordingly, in multivariate logistic regression analysis, body mass index, processing time, number of punctures and access zone (DRA) were found to be predictors of severe pain.

AUTHORS' CONTRIBUTIONS

RK: Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **TG:** Supervision, Validation, Writing – original draft. **AA:** Data curation, Investigation, Methodology, Writing – review & editing.

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REFERENCES

1. Engin M, Kagan As A, Aydın U, Ata Y, Yavuz S. Postintervention pain levels after elective coronary angiography. Rev Assoc Med Bras (1992). 2023;69(11):e20231014. https://doi.org/10.1590/1806-9282.20231014
2. Kılıç R, Güzel T, Aktan A, Arslan B, Aslan M, Günlü S, et al. Comparison of pain levels of traditional radial, distal radial, and transfemoral coronary catheterization. Rev Assoc Med Bras (1992). 2023;69(7):e20230198. https://doi.org/10.1590/1806-9282.20230198
3. Whitehead NJ, Clark AL, Williams TD, Collins NJ, Boyle AJ. Sedation and analgesia for cardiac catheterisation and coronary intervention. Heart Lung Circ. 2020;29(2):169-77. https://doi.org/10.1016/j.hlc.2019.08.015
4. Atik D, Atik C, Karatepe H, Karatepe C, Cinar S. The effect on anxiety level of coronary angiographic result and the perception of pain. Int J Res Med Sci. 2015;3(2):391-8.
5. Vaughn F, Wichowski H, Bosworth G. Does preoperative anxiety level predict postoperative pain? AORN J. 2007;85(3):589-604. https://doi.org/10.1016/S0001-2092(07)60130-6
6. Nguyen P, Makris A, Hennessy A, Jayanti S, Wang A, Park K, et al. Standard versus ultrasound-guided radial and femoral access in coronary angiography and intervention (SURF): a randomised controlled trial. EuroIntervention. 2019;15(6):e522-30. https://doi.org/10.4244/EIJ-D-19-00336

https://doi.org/10.1590/1806-9282.20231584

Re: "American Thyroid Association and Thyroid Imaging Reporting and Data System developed by the American College of Radiology: which one is better at predicting malignancy risk?" in thyroidology

Demet Sengul[1*], Ilker Sengul[2,3], Tugrul Kesicioglu[3], Esma Cinar[1]

Dear Editor,

We read with a great deal and interest the research article entitled "American Thyroid Association and Thyroid Imaging Reporting and Data System developed by the American College of Radiology: which one is better at predicting malignancy risk?" by Andreda and colleagues[1]. This beneficial research of high quality seems to demand determining in order of comparing the capacity of the 2015 American Thyroid Association (ATA)[2] and the 2017 American College of Radiology Thyroid Imaging Reporting and Data System (ACR-TIRADS)[3] in predicting malignancy risk of thyroid nodules. We postulate that Andreda et al.[1] performed a worthy comparison of ATA and ACR-TIRADS in terms of avoiding unnecessary fine needle aspiration (FNA) application, a valued and crucial issue in the thyroid lexicon, which has recently been published in the 67th volume of Rev Assoc Med Bras. However, we would like to emphasize some issues in thyroidology for the aforementioned study. First of all, thyroid nodules should be stated as a common clinical diagnostic challenge instead of a common clinical diagnosis (probably, a misspelling). Second, the authors reported the details of the ultrasound (a B-mode sonography) but not the size(s) and applying method(s) of the fine needles which had been used for the sampling procedures from the mentioned nodules to present them to the Department of (Cyto) Pathology. A wide range of (20–27 gauge in size) needles have been used for the procedure in different geographical regions (e.g., 25–27 gauge in most Western countries and 21–22 gauge in Japan)[4]. Debate is still ongoing on an optimal needle size for thyroid FNA cytology in thyroidology. In this sense, we reported a favorable non-diagnostic cytology rate on a sum of 500 nodules in 425 eligible consecutive outpatients during 38 months, involving ultrasonography (US)-guided FNA with a surgeon-performed US (SUS) in thyroid nodules with 27-G fine needles[5-12]. Therefore, would the outcomes of the study at that point be altered as they had harnessed significantly (i) finer or (ii) larger needle sizes? Herein, is it essential, at least, to state the relevant gauge(s) in order to design this deducing valued educational and technical study? Third, the authors emphasized that "the nodules with Category I, III, and IV, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC)[13], were not included in the analysis due to the impossibility of assigning its behavior." Nevertheless, they enunciated their purpose of comparing the capacity of the 2015 ATA[2] and the 2017 ACR-TIRADS[3] in predicting the malignancy risk of thyroid nodules[1]. Today, it has been widely accepted by thyroidologists that non-diagnostic (Category I) and indeterminate cytology (Categories III, IV, and V), in particular, TBSRTC, mostly deserve to be able to estimate an optimal and accurate malignancy risk. In addition, indeterminate cytology is constituted by Categories III, IV, and V[2] but not "III and IV[1]", TBSRTC[13]. Furthermore, Category V, *per se*, is not "malign" and even cannot "be considered malign". Furthermore, mutational testing for BRAF or the seven-gene mutation marker panel (BRAF, RAS, RET/PTC, PAX8/PPARc) is recommended today in nodules suspicious for malignancy, Category V, cytology after consideration of clinical and sonographic features if such data would be expected to alter surgical decision-making[2]. *Breviter*, disorders with their diagnostic options of this papilionaceous and delicate endocrine gland remain their significance in tellurian[1-23]. As such, this work published in the 67th volume of Rev Assoc Med Bras[1] is crucial for endocrine surgeons, endocrine pathologists, endocrinologists, head & neck surgeons and radiologists, otorhinolaryngologists, and thyroidologists, who stay informed of the growing spectrum of clinical management

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for challenging nodules for these thyroid providers and thyroid health as different peas in a pod[24-29]. This issue merits further investigation. We thank Andreda et al.[1] for their valuable study.

AUTHORS' CONTRIBUTIONS

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REFERENCES

1. Andrade MN, Costa JR, Sousa LM, Moreira LFGG, Oliveira RF, Álvares MCB, et al. American Thyroid Association and Thyroid Imaging Reporting and Data System developed by the American College of Radiology: which one is better at predicting malignancy risk? Rev Assoc Med Bras (1992). 2023;69(10):e20221694. https://doi.org/10.1590/1806-9282.20221694
2. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26(1):1-33. https://doi.org/10.1089/thy.2015.0020
3. Tessler FN, Middleton WD, Grant EG, Hoang JK, Berland LL, Teefey SA, et al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): white paper of the ACR TI-RADS committee. J Am Coll Radiol. 2017;14(5):587-95. https://doi.org/10.1016/j.jacr.2017.01.046
4. Maeda H, Kutomi G, Satomi F, Shima H, Mori M, Hirata K, et al. Clinicopathological characteristics of thyroid cancer misdiagnosed by fine needle aspiration. Exp Ther Med. 2016;12(4):2766-72. https://doi.org/10.3892/etm.2016.3651
5. Sengul I, Sengul D, Veiga ECA. Revisiting optimal needle size for thyroid fine-needle aspiration cytology: not much finer, less non-diagnostic? Rev Assoc Med Bras (1992). 2021;67(9):1213-4. https://doi.org/10.1590/1806-9282.20210671
6. Sengul I, Sengul D. Apropos of quality for fine-needle aspiration cytology of thyroid nodules with 22-, 23-, 25-, even 27-gauge needles and indeterminate cytology in thyroidology: an aide memory. Rev Assoc Med Bras (1992). 2022;68(8):987-8. https://doi.org/10.1590/1806-9282.20220498
7. Sengul D, Sengul I. Association between Tsukuba elasticity scores 4 and 5 on elastography and Bethesda undetermined cytology on US-guided FNA with 27-G needle, verified by histopathology: a cut-off point of 20 mm of diameter designated for thyroid nodules. J BUON. 2019;24(1):382-90. PMID: 30941995
8. Sengul D, Sengul I. A vignette epexegesis of a model for training sonography-guided fine-needle aspirations in thyroidology and thyroidologists: think twice with needle size? Rev Assoc Med Bras (1992). 2023;69(8):e20230465. https://doi.org/10.1590/1806-9282.20230465
9. Sengul D, Sengul I. Minimum minimorum: thyroid minimally invasive FNA, less is more concept? Volens nolens? Rev Assoc Med Bras (1992). 2022;68(3):275-6. https://doi.org/10.1590/1806-9282.20211181
10. Sengul I, Sengul D. Delicate needle with the finest gauge for a butterfly gland, the thyroid: is it worth mentioning? Sanamed. 2021;16(2):173-4. https://doi.org/10.24125/sanamed.v16i2.515
11. Sengul I, Sengul D. Proposal of a novel terminology: minimally invasive FNA and thyroid minimally invasive FNA; MIFNA and Thyroid MIFNA. Ann Ital Chir. 2021;92:330-1. PMID: 34312332
12. Sengul I, Sengul D. Big gain, no pain: thyroid minimally invasive FNA (Thy MIFNA): proposal of novelty in terminology. Rev Assoc Med Bras (1992). 2021;67(12):1749-50. https://doi.org/10.1590/1806-9282.20210922
13. Cibas ES, Ali SZ. The 2017 Bethesda system for reporting thyroid cytopathology. Thyroid. 2017;27(11):1341-6. https://doi.org/10.1089/thy.2017.0500
14. Sengul I, Sengul D. Hermeneutics for evaluation of the diagnostic value of ultrasound elastography in TIRADS 4 categories of thyroid nodules. Am J Med Case Rep. 2021;9(11):538-9. https://doi.org/10.12691/ajmcr-9-11-5
15. Sengul D, Sengul I. Reassessing combining real-time elastography with fine-needle aspiration biopsy to identify malignant thyroid nodules. Am J Med Case Rep. 2021;9(11):552-3. https://doi.org/10.12691/ajmcr-9-11-9
16. Sengul I, Sengul D. Comment on: "Evaluating treatment options in managing thyroid nodules with indeterminate cytology of TBSRTC in thyroidology: addendum aut non?" Rev Assoc Med Bras (1992). 2022;68(7):973-4. https://doi.org/10.1590/1806-9282.20220383
17. Sengul I, Sengul D. Emphasis on the novel age cutoff, 55 years, for postsurgical adjuvant radioiodine as consideration for American Thyroid Association ¾ low-intermediate risk differentiated thyroid carcinoma. Rev Assoc Med Bras (1992). 2021;67(4):485-6. https://doi.org/10.1590/1806-9282.20201013
18. Sengul I, Sengul D. Notes on "elastography for the diagnosis of high-suspicion thyroid nodules based on the 2015 American Thyroid Association guidelines: a multicenter study". North Clin Istanb. 2020;8(1):109-10. https://doi.org/10.14744/nci.2020.74240
19. Sengul D, Sengul I. Is there any link between a kind of thyrocyte dysfunction, hypothyroidism, and inflammatory hematologic parameters in the cases having the benign thyroid nodules?: a 5-year single-centre experience. Sanamed. 2018;13(1):35-40. https://doi.org/10.24125/sanamed.v13i1.211
20. Sengul D, Sengul I. Are there any variation in neutrophil lymphocyte ratio, mean platelet volume, and platelet count between papillary thyroid cancer and benign nodular thyroid diseases? Sanamed. 2018;13(1):11-6. https://doi.org/10.24125/sanamed.v13i1.209
21. Sengul I, Sengul D. Focusing on thyroid nodules in suspense: 10-15 mm with repeat cytology, category III, the Bethesda system for reporting thyroid cytopathology, TBSRTC. Rev Assoc Med Bras (1992). 2021;67(2):166-7. https://doi.org/10.1590/1806-9282.67.02.20200828

22. Sengul I, Sengul D. Blurred lines for management of thyroid nodules in the era of atypia of undetermined significance/ follicular lesion of undetermined significance: novel subdivisions of categories IIIA and IIIB in a possible forthcoming the Bethesda system for reporting thyroid cytopathology, 3rd edition; amending versus unnecessary? Rev Assoc Med Bras (1992). 2021;67(10):1385-6. https://doi.org/10.1590/1806-9282.20210763

23. Sengul D, Sengul I, Soares Junior JM. Repercussion of thyroid dysfunctions in thyroidology on the reproductive system: conditio sine qua non? Rev Assoc Med Bras (1992). 2022;68(6):721-2. https://doi.org/10.1590/1806-9282.20220255

24. Sengul I, Sengul D. May 25-31, International Thyroid Awareness week & May 25, World Thyroid Day, 2022: indetermination of indeterminate cytology, AUS/FLUS, FN, SUSP, in thyroidology? Sanamed. 2022;17(2):109-10. https://doi.org/10.5937/sanamed.17-38153

25. Sengul D, Sengul I. World Thyroid Day 2023 in thyroidology: no overlook thyroid dis-eases to opt for "thyroid health" purposes. Rev Assoc Med Bras (1992). 2023;69(10):e20230864. https://doi.org/10.1590/1806-9282.20230864

26. Sengul D, Sengul I. Subdivision of intermediate suspicion, the 2021 K-TIRADS, and category III, indeterminate cytology, the 2017 TBSRTC, 2nd edition, in thyroidology: let bygones be bygones? Ultrasonography. 2023;42(4):600-1. https://doi.org/10.14366/usg.23113

27. Sengul I, Sengul D. The 2023 Bethesda system for reporting thyroid cytopathology: novi sub sole, subdivision is no more debatable, in thyroidology. Rev Assoc Med Bras (1992). 2023;69(12):e20231124. https://doi.org/10.1590/1806-9282.20231124

28. Sengul I, Sengul D. Evangely, the subcategorization has been announced in the 2023 Bethesda System for Reporting Thyroid Cytopathology: let bygones be bygones in Thyroidology! Rev Assoc Med Bras (1992). 2023;e20231511. [Epub ahead of print].

29. Ali SZ, Baloch ZW, Cochand-Priollet B, Schmitt FC, Vielh P, VanderLaan PA. The 2023 Bethesda system for reporting thyroid cytopathology. Thyroid. 2023;33(9):1039-44. https://doi.org/10.1089/thy.2023.0141

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Comment on "Role of increased plasminogen activator inhibitor-1 and vitronectin in gestational diabetes mellitus"

Hejia Yin[1], Rou Shi[1*]

Dear Editor,

Ozgen et al[1]. recently conducted a study that focused on exploring the role of increased plasminogen activator inhibitor-1 (PAI-1) and vitronectin in gestational diabetes mellitus (GDM). GDM is a condition characterized by elevated blood sugar levels during pregnancy and is associated with various health risks. The study aimed to investigate the involvement of two key factors, namely, PAI-1 and vitronectin, in the pathophysiology of GDM. The research involved the analysis of blood samples and clinical data from pregnant women with and without GDM to compare the levels of PAI-1 and vitronectin. In this study, it was found that levels of vitronectin and PAI-1 were significantly elevated in the GDM group when compared to the control group. The findings of the study may have provided insights into the mechanisms underlying GDM, particularly the potential contribution of these two factors to the development and progression of the condition. Understanding the role of increased PAI-1 and vitronectin in GDM could have significant implications for the diagnosis, treatment, and management of this condition. It might open up avenues for targeted therapies or interventions aimed at mitigating the adverse effects of GDM and improving maternal and fetal health during pregnancy. However, some of the concerns outlined below require further clarification and explanation.

First, it is important to emphasize that this study[1] lacks a completely healthy-control group. Although this study included 60 women between 24 and 27/6 weeks of gestation as a control group, this evidence is insufficient because it is unclear how PAI-1 and vitronectin levels vary in a completely healthy-control group. Possible hypotheses are that in a completely healthy-control group, PAI-1 and vitronectin levels are at extremely high levels, and pregnancy or pregnancy combined with GDM results in a significant decrease in PAI-1 and vitronectin. Alternatively, PAI-1 and vitronectin levels may be extremely low in a completely healthy-control group, and pregnancy or pregnancy combined with GDM may lead to a significant increase in PAI-1 and vitronectin. Therefore, in the absence of a completely healthy-control group, the trends in changes in PAI-1 and vitronectin are not yet clear. Therefore, it is recommended to include a group of individuals who are age and body mass index-matched and do not have diabetes or pregnancy as a healthy-control group to further elucidate the trends in PAI-1 and vitronectin changes.

Second, the broad application of antenatal corticosteroids (ACS)[2,3] in obstetrics is primarily driven by their proven efficacy in reducing the incidence and severity of critical conditions such as respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis, and neonatal mortality associated with preterm births. Adding to this, emerging evidence[4] also points to potential advantages of ACS administration for pregnant individuals diagnosed with GDM. It is worth highlighting that the study consisted of GDM patients, and among them, there exists the possibility that some received ACS treatment, leading to a substantial elevation in PAI-1 and vitronectin levels. Nevertheless, the study omitted any specific details regarding whether ACS therapy was administered to the participants. In the event that ACS was indeed given to the study subjects, the conspicuous increase in PAI-1 and vitronectin levels might be more attributable to ACS use rather than the presence of GDM itself. Hence, it remains imperative to furnish a comprehensive account of ACS utilization within the study population. Therefore, it is advisable to provide a detailed account of whether the included patients received ACS to mitigate potential confounding bias.

AUTHORS' CONTRIBUTIONS

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REFERENCES

1. Ozgen L, Ozgen G, Dincgez B, Bayram F. Role of increased plasminogen activator inhibitor-1 and vitronectin in gestational diabetes mellitus. Rev Assoc Med Bras (1992). 2023;69(9):e20230563. https://doi.org/10.1590/1806-9282.20230563
2. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2006;(3):CD004454. https://doi.org/10.1002/14651858.CD004454.pub2
3. Gyamfi-Bannerman C, Thom EA, Blackwell SC, Tita AT, Reddy UM, Saade GR, et al. Antenatal betamethasone for women at risk for late preterm delivery. N Engl J Med. 2016;374(14):1311-20. https://doi.org/10.1056/NEJMoa1516783
4. Sukarna N, Tan PC, Hong JGS, Sulaiman S, Omar SZ. Glycemic control following two regimens of antenatal corticosteroids in mild gestational diabetes: a randomized controlled trial. Arch Gynecol Obstet. 2021;304(2):345-53. https://doi.org/10.1007/s00404-020-05950-3

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The role of cognitive restraint savings and the safety of ketogenic weight loss interventions

Jônatas de Oliveira[1*]

Achieving a caloric deficit through diets and meal plans requires various actions, including planning, consistency, and effective execution. These actions necessitate cognitive processing of information, decision-making, and choice[1]. Additionally, they require skills like inhibiting impulses, managing cravings and conflicts that arise with the desire to lose weight, challenging emotions associated with the body, and responding to environmental cues about food[2,3]. Many studies have identified factors that improve or impede adherence to dietary guidelines. The general consensus is that high adherence is crucial[4,5]. However, there are noteworthy dietary interventions, such as the ketogenic diet, that possess distinct characteristics.

The term "low-carb and ketogenic" encompasses various dietary possibilities, but I want to emphasize three distinctive nuances that are contradictory to the current discussion: (i) professionally prescribed low-carb and ketogenic diets; (ii) professionally prescribed food substitute options marketed as ketogenic diets; and (iii) self-imposed low-carb and ketogenic diets[6,7]. It is crucial to consider that studies that provide meals to participants might not reflect actual eating behavior, and their outcomes are based on highly regulated conditions. Thus, it appears that food substitutes may enhance both clinical studies and regular nutrition practice by offering a more practical solution. In particular, the inquiry surrounding these diets, despite their limitations and high cost, involves an aspect that is not often studied but is applied to all nutritional interventions: how much cognitive restraint individuals require to adhere to dietary recommendations[8-11]?

THE ROLE OF COGNITIVE RESTRAINT

Cognitive restraint is defined as an effort made to eat less, but the way it is evaluated using the subscale "cognitive restraint" of the three factor eating questionnaire considers some cognitive distortions that subsidize the restrictions such as "I do not eat some foods because they make me fat"[12]. In nutritional treatment and future research, it is essential to consider how more prudent thinking that values nutrient importance can help make good choices and even impose food restrictions if required, without any cognitive distortions involved. We recently published a case study demonstrating that the utilization of food substitutes aided in the restoration of the resting metabolic rate in a woman who had a history of bulimia nervosa[13]. In this case study, a 36-year-old white woman with a history of obesity and bulimia nervosa who has had difficulty in losing and maintaining weight despite numerous dietary and pharmacological treatments was considered. There was a loss of 12 kg in 115 days, reaching 13.4 kg, with 11.4 kg of fat mass. The resting metabolic rate showed an increase of 79% in relation to the initial rate, reaching normal levels for the predictive equations and maintaining this level in the first-year follow-up[13].

Opting for substitutes seems to eliminate several stages of food choice, wherein the notion of control over carbohydrate source foods is omnipresent during every meal and this control is solely in the hands of the individual[5]. The intervention successfully preserved lean mass and restored the basal metabolic rate to high levels. Prior to the intervention, efforts were directed toward behavioral nutrition to prevent the use of cognitive restriction as a solution. Simply lowering calorie intake was inadequate and it actually reinforced beliefs related to bulimia nervosa psychopathology[13]. Although it may seem to be an open question, the utilization of food substitutes offers additional safety measures when taking into account the stress that comes with self-imposed restrictions brought about by cognitive restraint[7,12].

IMPLEMENTATION OF KETOGENIC DIETS

It is essential not to overlook these factors as these individuals may need obesity treatments mostly due to reduced resting

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metabolic rates or high-fat mass that requires significant intervention time. Another issue neglected in many studies is the lack of pre-intervention eating behavior preparation. It is not worth to assume that a safe intervention will be effective for any individual. However, there will likely be an interaction between (i) eating history, (ii) cognitive-behavioral changes (such as disinhibition of eating behavior, increased fatphobic attitudes, intense food cravings, disconnection with internal signals, and changes in interoceptivity after many diets)[14-16], and (iii) metabolic changes resulting from the length of time the individual follows the diet[16-19].

While interventions using food substitutes may provide safer outcomes, they may not necessarily affect the behavioral and cognitive aspects reinforced by personal beliefs and the environment. Thus, further studies should concentrate on comprehensive screenings for an individual's history of eating disorders and behaviors that go beyond by simply identifying their risk or the presence of an eating disorder[20]. Successful screening should identify individuals who require behavioral therapy, specifically in the field of behavioral nutrition, to prepare them for dietary intervention and enable them to maintain a diet that aligns with the recommendations given post-intervention. As professionals, do we just recommend and track weight loss outcomes or do we also have an obligation to ensure the progress and involvement of individuals even after the completion of the diet? A weight-loss strategy that focuses solely on simplification or improved biochemical markers is inadequate for ensuring long-term outcomes for individuals struggling with obesity.

REFERENCES

1. Rangel A. Regulation of dietary choice by the decision-making circuitry. Nat Neurosci. 2013;16(12):1717-24. https://doi.org/10.1038/nn.3561
2. Oliveira J. The body asks and the mind judges : the episode of food craving, its triggers and nutritional treatment. Einstein (Sao Paulo). 2022;20:eMD6705. https://doi.org/10.31744/einstein_journal/2022MD6705
3. Reents J, Pedersen A. Differences in food craving in individuals with obesity with and without binge eating disorder. Front Psychol. 2021;12:660880. https://doi.org/10.3389/fpsyg.2021.660880
4. Johnston BC, Kanters S, Bandayrel K, Wu P, Naji F, Siemieniuk RA, et al. Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. JAMA. 2014;312(9):923-33. https://doi.org/10.1001/jama.2014.10397
5. Oliveira J, Colombarolli MS, Figueredo LS, Cordás TA. Cognitive restraint directed at carbohydrates in individuals on low-carb diet with binge eating: the role of guilt about food cravings. Einstein (São Paulo). 2021;19:eAO5599. https://doi.org/10.31744/einstein_journal/2021AO5599
6. Goday A, Bellido D, Sajoux I, Crujeiras AB, Burguera B, García-Luna PP, et al. Short-term safety, tolerability and efficacy of a very low-calorie-ketogenic diet interventional weight loss program versus hypocaloric diet in patients with type 2 diabetes mellitus. Nutr Diabetes. 2016;6:e230. https://doi.org/10.1038/nutd.2016.36
7. Pandurevic S, Mancini I, Mitselman D, Magagnoli M, Teglia R, Fazzeri R, et al. Efficacy of very low-calorie ketogenic diet with the Pronokal® method in obese women with polycystic ovary syndrome: a 16-week randomized controlled trial. Endocr Connect. 2023;12:(7):e220536. https://doi.org/10.1530/EC-22-0536
8. Polk SE, Schulte EM, Furman CR, Gearhardt AN. Wanting and liking: separable components in problematic eating behavior? Appetite. 2017;115:45-53. https://doi.org/10.1016/j.appet.2016.11.015
9. Boyce JA, Gleaves DH, Kuijer RG. Measuring dietary restraint status: comparisons between the dietary intent scale and the restraint scale. Front Nutr. 2015;2:8. https://doi.org/10.3389/fnut.2015.00008
10. Vartanian LR, Herman CP, Polivy J. What does it mean to eat an appropriate amount of food? Eat Behav. 2016;23:24-7. https://doi.org/10.1016/j.eatbeh.2016.07.002
11. Mills JS, Weinheimer L, Polivy J, Herman CP. Are there different types of dieters? A review of personality and dietary restraint. Appetite. 2018;125:380-400. https://doi.org/10.1016/j.appet.2018.02.014
12. Oliveira J. Cognitive carbohydrate restriction: a new proposal for the diet mentality in the era of low-carb diets. Rev Assoc Med Bras. 2023;69(8):e20230601. https://doi.org/10.1590/1806-9282.20230601
13. Oliveira J, Carvalho Stelmo I, Figueredo LS, Freitas CC. Very low-calorie ketogenic diet in the treatment of adaptive thermogenesis: a case report. Nutrition. 2023;117:112252. https://doi.org/10.1016/j.nut.2023.112252
14. Colombarolli MS, Oliveira J, Cordás TA. Craving for carbs: food craving and disordered eating in low-carb dieters and its association with intermittent fasting. Eat Weight Disord. 2022;27(8):3109-17. https://doi.org/10.1007/s40519-022-01437-z
15. Hall KD. Energy compensation and metabolic adaptation: "The Biggest Loser" study reinterpreted. Obesity. 2022;30:11-3. https://doi.org/10.1002/oby.23308
16. Fothergill E, Guo J, Howard L, Kerns JC, Knuth ND, Brychta R, et al. Persistent metabolic adaptation 6 years after "The Biggest Loser" competition. Obesity (Silver Spring). 2016;24(8):1612-9. https://doi.org/10.1002/oby.21538
17. Obarzanek E, Lesem MD, Goldstein DS, Jimerson DC. Reduced resting metabolic rate in patients with bulimia nervosa. Arch Gen Psychiatry. 1991;48(5):456-62. https://doi.org/10.1001/archpsyc.1991.01810290068013
18. Sender K, Sullivan M. Epidemics of will, failures of self-esteem: responding to fat bodies in the biggest loser and what not to wear. Continuum (N Y). 2008;22(4):57384.
19. Yoo JH. No clear winner: effects of the biggest loser on the stigmatization of obese persons. Health Commun. 2013;28:294-303. https://doi.org/10.1080/10410236.2012.684143
20. Sobal J, Bisogni CA. Constructing food choice decisions. Ann Behav Med. 2009;38(Suppl 1):S37-46. https://doi.org/10.1007/s12160-009-9124-5

SHORT COMMUNICATION

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"Homeopathy is not placebo effect": proof of the scientific evidence for homeopathy

Marcus Zulian Teixeira[1*]

Homeopathy has been a medical practice recognized worldwide for more than two centuries, providing care, teaching, and research activities in several health institutions and medical schools. It employs a clinical approach based on non-conventional and complementary scientific principles (principle of therapeutic similitude, homeopathic pathogenetic experimentation, and use of dynamized doses and individualized medicines), with the aim of awakening a curative response from the body against its own disorders or diseases[1].

Homeopathy proposes to understand and treat the sick-disease binomial according to a vitalist, globalizing, and humanist anthropological approach, valuing the different aspects of the sick individuality (mental, general, and physical) and contributes to maintain health and organic homeostasis, acting as a therapeutic alternative for various health disorders[2,3].

However, to achieve this objective, homeopathic therapy must be well conducted and follow the epistemological premises of the homeopathic model[1], among which include applying therapeutic similitude/similarity between the set of signs and symptoms of the sick individual (characteristic symptomatic totality of the sick-disease binomial) and the set of pathogenetic signs and symptoms caused by the medicine in the healthy individual (homeopathic pathogenetic experimentation), meaning individualized homeopathic treatment.

Several double-blind and placebo-controlled randomized clinical trials (RCTs) and their systematic reviews with meta-analyses which disrespected this therapeutic individualization by administering the same medication to different individuals with the same disease did not show significant results compared with placebo, as they violated scientific rationality of the homeopathic model[1,4,5].

On the contrary, as homeopathy is based on premises different from those used by conventional medical practice, it is often the target of criticism and attacks by individuals who systematically disregard homeopathic assumptions and any scientific evidence that proves them, as they have a denialist and biased stance which prevents a correct and prejudice-free analysis. In reality, they are pseudoskeptics masquerading as pseudoscientists[6,7].

To enlighten doctors, researchers, health professionals, and the general public, demystifying culturally ingrained dogmatic positions and the pseudoskeptical fallacies that "there is no scientific evidence for homeopathy" and that "homeopathy is placebo effect," the Technical Chamber for Homeopathy (TC-Homeopathy) of the Regional Medical Council of the State of São Paulo (Cremesp), in 2017, developed the *Special Dossier: "Scientific Evidence for Homeopathy,"* available in three independent editions (online in Portuguese and English; printed in Portuguese) in the scientific journal *Revista de Homeopatia* (*São Paulo*). In 2023, the dossier was published in Spanish in the *La Homeopatía de México* journal in an edition commemorating its 90th anniversary[8-10].

The respective dossier was composed of nine narrative reviews of research on several fields of medical science (historical, social, medical education, pharmacological, basic, clinical, patient safety, and pathogenetic experimentation) and two randomized clinical trials developed by TC-Homeopathy members, encompassing hundreds of scientific articles describing experimental and clinical studies, and seeks to highlight the state of the art of homeopathic research[8-10].

To prove and expand this scientific evidence for homeopathy, on September 25, 2023[11], we published an electronic book (e-book) in Portuguese "*Homeopatia não é efeito placebo*": *comprovação das evidências científicas da homeopatia* ("*Homeopathy is not placebo effect*": *proof of the scientific evidence for homeopathy*), indexed and made available in the Virtual Health Library (VHL-LILACS-BIREME)[12,13], updating knowledge in the area in 13 interactive chapters. In addition to elucidating the epistemological premises of the homeopathic model in detail, the study describes data and bibliographic references in the information continuum, as well as the different areas of basic and clinical research in homeopathy which endorse homeopathic practice and treatment.

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The study discusses various topics related to research in homeopathy, covering everything from homeopathic clinical epidemiology to the pseudoskeptical and pseudoscientific strategies used in attacks on homeopathy, including the panorama of research in homeopathy (databases), the pharmacological basis of the principle of similitude, experimental studies in biological models, randomized controlled clinical trials, systematic reviews and meta-analyses, and observational studies, among others[12,13].

In the chapter "Homeopatia" ("Homeopathy"), the scientific evidence of homeopathic assumptions is described in general databases, discussing the epistemological premises of the homeopathic model in detail (principle of therapeutic similitude, homeopathic pathogenetic experimentation, and use of dynamized doses and individualized medicines)[1] and providing the reader with an overview of treatment and clinical practice in homeopathy.

In "Epidemiologia clínica em homeopatia" ("Clinical epidemiology in homeopathy"), after a general review of the principles of clinical epidemiology and the types of epidemiological studies used to evaluate the efficacy and clinical effectiveness of conventional treatments, the premises and principles of homeopathic clinical epidemiology are described, as well as the types of epidemiological studies in homeopathy[14]. As we initially emphasized, the epistemological premise of individualized homeopathic treatment in the face of the characteristic symptomatic totality of the patient–disease binomial is a *sine qua non* condition for the ultra-diluted homeopathic medicine to be able to stimulate a significant curative response against its own disorders[1,4,5]. Failure to do so is a serious flaw in the design of homeopathic clinical trials of high methodological quality[14].

In addition to the general databases, the various databases that group homeopathic experimental studies into biological and physicochemical models are described ("Homeopathy Basic Research Experiments database," "HomVetCR database," and "PROVINGS.INFO database") in the chapter "Panorama da pesquisa em homeopatia—Bancos de dados" ("Overview of homeopathy research—Databases"), as well as homeopathic clinical epidemiological studies of all types ("Clinical Outcome Research in Homeopathy," "Homeopathic Intervention Studies," and "CAM-QUEST databases"). In these databases, readers will be able to see the wide range of studies indexed in the areas of basic and clinical research in homeopathy, with proposals for bibliographic surveys exemplified in each chapter of the study.

Next, the principle of therapeutic similitude is approached according to the homeopathic model and modern pharmacology in the chapter "Fundamentação farmacológica do princípio da similitude" ("Pharmacological basis of the principle of similitude"), describing hundreds of experimental and clinical studies that substantiate the curative response (vital reaction) of homeopathic treatment in accordance with the rebound effect of modern drugs (paradoxical reaction of the organism). Furthermore, it describes the proposal to use modern drugs according to the therapeutic similarity, using the rebound effect of drugs in a therapeutic way[15-17].

In the field of basic research in homeopathy, the chapter "Estudos experimentais em modelos biológicos (in vitro, em vegetais e em animais)" ["Experimental studies in biological models (in vitro, in plants and animals)"] describes hundreds of controlled experimental studies in cells, plants, and animals, demonstrating the superiority of the effect of homeopathic medicine compared with control groups and showing through systematic reviews and meta-analyses that "homeopathy is not placebo effect"[18-20].

In the field of clinical research in homeopathy, the chapter "Ensaios clínicos controlados randomizados (RCTs)" ["Randomized controlled clinical trials (RCTs)"] describes dozens of randomized, double-blind, placebo-controlled clinical trials (level of evidence 1B) with good methodological quality, which demonstrate the effectiveness of homeopathic treatment compared with placebo. Increasing the level of evidence of the clinical effectiveness of homeopathy (1A), four chapters address systematic reviews of RCTs, global (any clinical indication) and specific (specific clinical indication), with and without meta-analyses.

Then in the chapter "Revisões sistemáticas e relatórios globais com resultados positivos da homeopatia perante placebo" ("Systematic reviews and global reports with positive results of homeopathy compared to placebo"), five global systematic reviews with meta-analyses (and one global report) that demonstrated the superiority of homeopathic treatment over placebo are described. On the contrary, the studies that brought negative results of homeopathy compared with placebo are presented in the chapter "Revisões sistemáticas e relatórios globais com resultados negativos da homeopatia perante placebo (Falhas metodológicas)" ["Systematic reviews and global reports with negative results of homeopathy compared to placebo (Methodological flaws)"], including two global systematic reviews, one with a meta-analysis and the other without, and a global report, highlighting their numerous biases and methodological flaws, presented in several reanalyses published later (post hoc analyses).

Confirming these post hoc analyses, on October 7, 2023, a systematic review of global meta-analyses of RCTs was published demonstrating that "the quality of evidence for positive effects of homeopathy beyond placebo was high

for individualised homeopathy and moderate for non individualised homeopathy" and that "there was no support for the alternative hypothesis of no outcome difference between homeopathy and placebo"[21].

The chapter "Revisões sistemáticas para condições clínicas específicas" ("Systematic reviews for specific clinical conditions") presents a description of specific systematic reviews that demonstrated the superiority of homeopathy over placebo, in various clinical conditions, with meta-analyses (allergic rhinitis, acute childhood diarrhea, postoperative ileus, and attention deficit hyperactivity disorder) and without meta-analyses (otitis acute media, postoperative inflammation, psychiatric disorders, and rheumatic diseases).

Then, in the chapter "Estudos observacionais" ("Observational studies"), we mainly discuss analytical observational studies (level of evidence 2B), describing robust cohort studies that present important information about the effectiveness and cost-effectiveness of homeopathic treatment in thousands of patients, both in the long term and in different clinical conditions[22-25].

The final chapter "Estratégias pseudocéticas e pseudocientíficas usadas em ataques à homeopatia" ("Pseudoskeptical and pseudoscientific strategies used in attacks on homeopathy") discusses pseudoskepticism and pseudoscience, describing the indicative signs of pseudoskepticism (false skepticism or pathological skepticism) in detail, which are topics of fundamental importance to unmask individuals who systematically maintain a denialist and dogmatic stance against homeopathy (pseudoskeptics and pseudoscientists), disregarding the countless existing scientific evidence which was presented in detail in the various chapters of this e-book[6,7].

As we reiterate throughout the study, despite the difficulties and limitations that exist in developing research in homeopathy, both due to methodological aspects and the lack of institutional and financial support, the set of experimental and clinical studies described is indisputable proof that "there is scientific evidence for homeopathy" and that "homeopathy is not placebo effect," contrary to falsely disseminated prejudice[6,7]. However, new studies must continue to be developed to improve clinical practice and elucidate characteristic aspects of the homeopathic paradigm.

Acting as an integrative and complementary therapy to other specialties, homeopathy can add efficacy, effectiveness, efficiency, and safety to medical practice, acting in a curative and preventive manner, reducing symptomatic manifestations and the predisposition to falling ill, with low cost and minimal adverse events, and helping physicians to fulfill their "high and *only* mission, which is to restore the sick to healthy, to cure, as it is termed" (Samuel Hahnemann, *Organon of medicine*, § 1).

REFERENCES

1. Teixeira MZ. Scientific evidence of the homeopathic epistemological model. Int J High Dilution Res. 2011;10(34):46-64. https://doi.org/10.51910/ijhdr.v10i34.421
2. Teixeira MZ. [Homeopathy: a humanistic approach to medical practice]. Rev Assoc Med Bras (1992). 2007;53(6):547-9. https://doi.org/10.1590/s0104-42302007000600026
3. Teixeira MZ. [Homeopathy: coadjutant medical practice]. Rev Assoc Med Bras (1992). 2007;53(4):374-6. https://doi.org/10.1590/s0104-42302007000400027
4. Mathie RT, Lloyd SM, Legg LA, Clausen J, Moss S, Davidson JR, et al. Randomised placebo-controlled trials of individualised homeopathic treatment: systematic review and meta-analysis. Syst Rev. 2014;3(1):142. https://doi.org/10.1186/2046-4053-3-142
5. Mathie RT, Ramparsad N, Legg LA, Clausen J, Moss S, Davidson JR, et al. Randomised, double-blind, placebo-controlled trials of non-individualised homeopathic treatment: systematic review and meta-analysis. Syst Rev. 2017;6(1):63. https://doi.org/10.1186/s13643-017-0445-3
6. Teixeira MZ. Falácias pseudocéticas e pseudocientíficas do "Contradossiê das Evidências sobre a Homeopatia" [Pseudoskeptic and pseudoscientific fallacies of the "Counter-dossier of Evidence on Homeopathy"]. São Paulo (SP): Marcus Zulian Teixeira; 2020. [cited on 2021 Oct 15]; p. 43 Available from: https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1145551
7. Teixeira MZ. Pseudoskeptical and pseudoscientific strategies used in attacks on homeopathy. Rev Assoc Med Bras (1992). 2021;67(6):777-80. https://doi.org/10.1590/1806-9282.20210367
8. Teixeira MZ. Special Dossier: "Scientific Evidence for Homeopathy". Rev Assoc Med Bras (1992). 2018;64(2):93-4. https://doi.org/10.1590/1806-9282.64.02.93
9. Teixeira MZ. Proofs that Homeopathic Medicine Works: Dossier "Scientific Evidence for Homeopathy" (Revista de Homeopatia, São Paulo Homeopathic Medical Association). Homeopathy. 2018;107(1):45. https://doi.org/10.1055/s-0037-1613677
10. Teixeira MZ. "Scientific Evidence for Homeopathy". Clinics (Sao Paulo). 2023;78:100255. https://doi.org/10.1016/j.clinsp.2023.100255
11. Associação Médica Homeopática Brasileira (AMHB). AMB informa: lançado o livro digital "HOMEOPATIA NÃO É EFEITO PLACEBO", de Marcus Zulian Teixeira. São Paulo (SP): AMHB; 2023. [cited on 2023 Oct 15]. Available from: https://amb.org.br/noticias/amb-informa-lancado-o-livro-digital-homeopatia-nao-e-efeito-placebo-de-marcus-zulian-teixeira/
12. Teixeira MZ. "Homeopatia não é efeito placebo": comprovação das evidências científicas da homeopatia ["Homeopathy is not placebo effect": proof of the scientific evidence for homeopathy] [e-book, PDF editing]. São Paulo (SP): Marcus Zulian Teixeira; 2023. [cited on 2023 Oct 15]; p. 223. Available from: https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1451677?lang=en
13. Teixeira MZ. "Homeopatia não é efeito placebo": comprovação das evidências científicas da homeopatia ["Homeopathy is not placebo effect": proof of the scientific evidence for homeopathy] [e-book, KINDLE editing]. São Paulo (SP): Marcus Zulian Teixeira; 2023. [cited on 2023 Oct 15]; p. 357. Available from: https://www.amazon.com.br/dp/B0CK6JDWTJ
14. Teixeira MZ. Epidemiologia clínica homeopática: premissas e princípios para a elaboração da pesquisa clínica em homeopatia

[Homeopathic clinical epidemiology: premises and principles for elaboration of clinical research in homeopathy]. Rev Homeopatia (São Paulo). 2022. [cited on 2023 Oct 15]; 84(3-4):4-24. Available from: https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1402361

15. Teixeira MZ. "Similitude in Modern Pharmacology": two decades of studies contributing to the scientific basis of the homeopathic healing principle. Rev Assoc Med Bras (1992). 2022;68(3):303-7. https://doi.org/10.1590/1806-9282.20211362
16. Teixeira MZ. "New Homeopathic Medicines" proposal: a database made available in three free-access bilingual digital books. Rev Assoc Med Bras (1992). 2021;67(10):1387-91. https://doi.org/10.1590/1806-9282.20210482
17. Teixeira MZ. "Similia Similibus Curentur": The scientific grounding of the homeopathic therapeutic principle through the systematic study of the rebound effect of modern drugs. Clinics (Sao Paulo). 2022;77:100091. https://doi.org/10.1016/j.clinsp.2022.100091
18. Witt CM, Bluth M, Albrecht H, Weisshuhn TE, Baumgartner S, Willich SN. The in vitro evidence for an effect of high homeopathic potencies--a systematic review of the literature. Complement Ther Med. 2007;15(2):128-38. https://doi.org/10.1016/j.ctim.2007.01.011
19. Ücker A, Baumgartner S, Sokol A, Huber R, Doesburg P, Jäger T. Systematic review of plant-based homeopathic basic research: an update. Homeopathy. 2018;107(2):115-29. https://doi.org/10.1055/s-0038-1639580
20. Bonamin LV, Cardoso TN, Carvalho AC, Amaral JG. The use of animal models in homeopathic research--a review of 2010-2014 PubMed indexed papers. Homeopathy. 2015;104(4):283-91. https://doi.org/10.1016/j.homp.2015.06.002
21. Hamre HJ, Glockmann A, Ammon K, Riley DS, Kiene H. Efficacy of homoeopathic treatment: systematic review of meta-analyses of randomised placebo-controlled homoeopathy trials for any indication. Syst Rev. 2023;12(1):191. https://doi.org/10.1186/s13643-023-02313-2
22. Grimaldi-Bensouda L, Begaud B, Lert F, Rouillon F, Massol J, Guillemot D, et al. Benchmarking the burden of 100 diseases: results of a nationwide representative survey within general practices. BMJ Open. 2011;1(2):e000215. https://doi.org/10.1136/bmjopen-2011-000215
23. Rossi E, Stefano M, Picchi M, Panozzo MA, Noberasco C, Nurra L, et al. Integration of homeopathy and complementary medicine in the Tuscan Public Health System and the experience of the homeopathic clinic of the Lucca Hospital. Homeopathy. 2018;107(2):90-8. https://doi.org/10.1055/s-0038-1636839
24. Witt CM, Lüdtke R, Mengler N, Willich SN. How healthy are chronically ill patients after eight years of homeopathic treatment?--Results from a long term observational study. BMC Public Health. 2008;8:413. https://doi.org/10.1186/1471-2458-8-413
25. Kass B, Icke K, Witt CM, Reinhold T. Effectiveness and cost-effectiveness of treatment with additional enrollment to a homeopathic integrated care contract in Germany. BMC Health Serv Res. 2020;20(1):872. https://doi.org/10.1186/s12913-020-05706-4

ORIGINAL ARTICLE

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Is having a moderate or low history, electrocardiogram, age, risk factors, troponin risk score a handicap for long-term mortality?

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SUMMARY

OBJECTIVE: History, electrocardiogram, age, risk factors, troponin risk score and troponin level follow-up are used to safely discharge low-risk patients with suspected non-ST elevation acute coronary syndrome from the emergency department for a 1-month period. We aimed to comprehensively investigate the 6-month mortality of patients with the history, electrocardiogram, age, risk factors, troponin risk score.

METHODS: A total of 949 non-ST elevation acute coronary syndrome patients admitted to the emergency department from 01.01.2019 to 01.10.2019 were included in this retrospective study. History, electrocardiogram, age, risk factors, troponin scores of all patients were calculated by two emergency clinicians and a cardiologist. We compared the 6-month mortality of the groups.

RESULTS: The mean age of the patients was 67.9 (56.4–79) years; 57.3% were male and 42.7% were female. Six-month mortality was significantly lower in the high-risk history, electrocardiogram, age, risk factors, troponin score group than in the low- and moderate-risk groups: 11/80 (12.1%), 58/206 (22%), and 150/444 (25.3%), respectively (p=0.019).

CONCLUSION: Patients with high history, electrocardiogram, age, risk factors, troponin risk scores are generally treated with coronary angioplasty as soon as possible. We found that the mortality rate of this group of patients was lower in the long term compared with others. Efforts are also needed to reduce the mortality of moderate and low-risk patients. Further studies are needed on the factors affecting the 6-month mortality of moderate and low-risk acute coronary syndrome patients.

KEYWORDS: Heart disease. Acute coronary syndrome.

INTRODUCTION

Many studies have investigated the strategies that can be developed to prevent acute coronary syndrome (ACS) from being overlooked in patients admitted to the emergency department (ED) with chest pain or cardiac symptoms[1,2]. Testing of high-sensitivity troponin I (hs-TnI) levels and the use of certain risk scores [TIMI, GRACE, history, electrocardiogram, age, risk factors, troponin (HEART), EDACS] greatly reduced this outcome[2]. Patients with a low-risk score are safely discharged from the hospital early, while those with a high-risk score are usually treated with appropriate treatment methods during coroner angiography[3,4].

Major adverse cardiac event (MACE) was defined as definite or probable nonfatal myocardial infarction (MI), nonfatal stroke, or mortality caused by cardiovascular diseases. MACE is increasingly used in randomized controlled trials and observational studies[5]. MACE rates in the first 1–1.5 months after discharge are <2%[6-8] and <3.3%[9], which is a satisfactory level. Risk scores have been extensively studied using the 1-month MACE rates[7,9].

We did not encounter any studies comparing the high-risk group with other groups in terms of their 6-month mortality. In this study, we wanted to investigate the positive effects of interventional treatments on mortality in patients with high HEART risk scores and the long-term outcomes of moderate- and low-risk patients who were discharged safely in the short term.

METHODS

This is a retrospective cohort study conducted with data collected in the ED of a tertiary university hospital with a monthly admission of 24,000–30,000 patients. The study was approved by the Bezmialem Vakif University Ethics Committee (number E-54022451-050.05.04-42848 and decision number 2021/386). The data were obtained from the hospital's patient

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clinical information system (Nucleus MBS). In this study, 949 patients admitted to the ED from 01.01.2019 to 01.10.2019 were diagnosed with non-ST elevation ACS (NSTE-ACS). The strengthening the reporting of observational studies in epidemiology (STROBE) checklist was used to form this study[10].

Definitions and variables

Clinical presentation of NSTE-ACS was anginal pain [with prolonged (0.20 min) anginal pain at rest, new onset (de novo) angina (class II or III of the Canadian Cardiovascular Society classification), recent destabilization of previously stable angina with at least Canadian Cardiovascular Society Class III angina characteristics (crescendo angina), and post-MI angina][11].

We evaluated the outcome retrospectively by examining the citizen information system (E-DEVLET) and evaluated them for significant differences in 6-month mortality. The data were classified according to the outcome of the patients: diagnosed with NSTE-ACS, discharged from the ED [emergency outpatient (EO)], treated in the intensive care unit (ICU), or treated in other clinics (OC). The HEART scores of all patients were calculated by two emergency clinicians and a cardiologist. The HEART score classifies NSTE-ACS as low (0–3), moderate (4–6), or high (7–10) risk (Table 1). We analyzed patients diagnosed as NSTE-ACS checking mortality at 6 months. All assessments were of troponin I and were performed by the hospital laboratory using a chemiluminescent microparticle immunoassay (ARCHITECT STAT High Sensitivity Troponin-I Assay, Abbot Laboratories, USA) (99th percentile normal concentration <34.2 pg/mL).

Table 1. The history, electrocardiogram, age, risk factors, troponin score.

HEART score		
History	Highly suspicious	2
	Moderately suspicious	1
	Slightly suspicious	0
ECG	Significant ST depression	2
	Non-specific repolarization disturbance	1
	Normal	0
Age	≥65 years	2
	>45 to <65 years	1
	≤45 years	0
Risk factors	≥3 risk factors,[a] or history of atherosclerotic disease[b]	2
	1 or 2 risk factors	1
	No risk factors known	0
Troponin	≥3× normal limit	2
	>1 to <3× normal limit	1
	≤normal limit	0

[a]Risk factors: diabetes mellitus, smoking, hypertension, hypercholesterolemia, family history of coronary artery disease, and obesity (body mass index >30). [b]History of atherosclerotic disease: coronary revascularization, myocardial infarction, stroke, and peripheral arterial disease.

Statistical analysis

The sample size was calculated with a two-tailed alpha of 0.05, a two-tailed beta of 0.1, an estimated AUC of 0.860, and a ratio of patients with a negative–positive outcome of 0.0526. Accordingly, it was determined that approximately 88 patients, with at least 83 living and 5 died, needed to be collected. The Shapiro-Wilk test was used in the analysis of the normality of data. The data did not follow a normal distribution. Categorical data were presented as numbers (%) and compared with the chi-square test. Quantitative variables were presented as median and interquartile range (25–75th percentile) values and then compared for the three groups using the Kruskal-Wallis test. To compare subgroups, we conducted pairwise comparisons using the Dwass-Steel-Critchlow-Fligner method. Statistical significance was determined as p=0.05 for all cases. To investigate mechanisms that potentially underlie the relationship between HEART score and mortality, age, diagnosis of chronic diseases, ECG, complaint analysis, and risk factors were considered. All data used for the calculation of statistical values were anonymized before being provided to the researchers.

RESULTS

As the records of all patients are registered in the E-DEVLET and death notification is mandatory, no case is excluded due to death information. The mean age of the patients was 67.9 (56.4–79) years, with 57.3% male and 42.7% female. The complaints of the patients were as follows: cardiac chest pain 211 (22.2%), non-cardiac chest pain 259 (27.3%), and ACS equivalent symptoms 479 (50.5%) (i.e., fatigue, sweating, and fainting). The HEART score distribution was 264 (27.8%) in the low-risk group, 594 (62.6%) in the moderate-risk group, and 91 (9.6%) in the high-risk group (Table 2).

We classified the patients into four categories according to the outcome: 500 (52.7%) were EO, 251 (26.4%) were treated in the coronary unit (CU), 100 (10.5%) were treated in the ICU, and 98 (10.3%) were treated in OC. The distribution of 6-month mortality according to outcome was as follows. Of the 251 patients treated at the CU, 22 (2%) died. Of the 100 patients whose troponin elevation was associated with Type 2 MI in the ICU, 67 (7%) died. A total of 98 patients whose troponin levels were associated with other causes were treated in OC with various diagnoses, of whom 36 (4%) died.

Patients who were not diagnosed with MI or other serious diseases and were not treated by hospitalization (patients who did not show an increase or decrease in troponin follow-up and had chronic diseases) were EO. Of these 500 patients, 94 (10%) died (Table 3).

When we compared the mortality rates of all patients between the HEART groups, we found that at least two groups had statistically significant differences (p=0.019). There were 58 (22%) mortalities in the low-risk group, 150 (25.3%) mortalities in the moderate-risk group, and 11 (12.1%) mortalities in the high-risk group. Post hoc analysis found that the moderate- and high-risk HEART groups' mortality is different (p=0.016) (Table 2).

DISCUSSION

In this study, we showed the relationship of HEART risk score with the 6-month mortality of patients.

Patients who are considered low risk in heart score have low MACE (1–1.5 months) rates[7-9]. When used in combination with serial troponin measurements, the HEART score allows more patients to be discharged early and safely, limits heart test rates, and reduces hospital stays[12]. Most studies have used classical troponin when calculating the HEART score[13-15]. However, serial measurement of conventional troponin provides limited benefit in low-risk HEART score patients[16]. The HEART score consists of age, risk factors, history, ECG, and troponin level. Low-risk patients, defined by a score of 0–3, show a low MACE rate (<2%). This score decreases admission for chest pain by at least 20%, with a negative predictive value for MACE (>99%)[17]. Moderate-risk patients (scoring 4–6) show a 12–16.6% risk of MACE, and high-risk patients (scoring ≥7) have a 50–65% risk of MACE[18]. According to these MACE rates, more interventional treatments and bypasses should be performed in high-risk patients, fewer in moderate-risk patients, and a few in low-risk patients.

We compared 6-month mortality for HEART risk score groups, and the results were interesting. The mortality rate was highest in the moderate-risk group (25.3%) and lowest in the high-risk group (12.1%). There was a significant difference between the medium and high-risk groups (p=0.016) (Table 2). One reason for this may be that there is less invasive examination and treatment in the moderate-risk group. For these,

Table 3. Six-month mortality of patients according to outcomes.

Outcome of patient groups			
	n (groups) (%)	Mortality	n (%)
Emergency outpatient	500 (52.7)	No	406 (43)
		Yes	94 (10)
Coronary unit	251 (26.4)	No	229 (24)
		Yes	22 (2)
Intensive care	100 (10.5)	No	33 (3)
		Yes	67 (7)
Other clinics	98 (10.3)	No	62 (7)
		Yes	36 (4)
Total	949 (100)		949 (100)

Table 2. Distribution of history, electrocardiogram, age, risk factors, troponin risk groups of patients by age, gender, complaints, and 6-month mortality.

HEART groups						
		Low	Moderate	High	Total	p
		n (%)	n (%)	n (%)	n (%)	
Age (years)	Median (IQR)	59 (45.2–71.6)	70.8 (59.4–80.6)	70.2 (61.4–76.9)	67.9 (56.4–79.0)	<0.001
Sex	Male	160 (60.6)	332 (55.9)	52 (57.1)	544 (57.3)	0.436
	Female	104 (39.4)	262 (44.1)	39 (42.9)	405 (42.7)	
Complaints	Cardiac chest pain	11 (4.2)	147 (24.7)	53 (58.2)	211 (22.2)	<0.001
	Noncardiac chest pain	58 (22.0)	174 (29.3)	27 (29.7)	259 (27.3)	
	ACS equivalent symptoms	195 (73.9)	273 (46.0)	11 (12.1)	479 (50.5)	
Mortality	No	206 (78.0)	444 (74.7)	80 (87.9)	730 (76.9)	0.019
	Yes	58 (22.0)	150 (25.3)	11 (12.1)	219 (23.1)	
	p*	0.100[a]	0.555[b]	0.016[c]		
	Total	264 (27.8)	594 (62.6)	91 (9.6)		

ACS: acute coronary syndromes; IQR: interquartile range. *Mann-Whitney U test; Kruskal-Wallis test; chi-square test; Dwass-Steel-Critchlow-Fligner pairwise comparisons test. [a]Low and high comparison; [b]low and moderate comparison; [c]moderate and high comparison.

we may recommend a further heart examination. Patients in the moderate- and low-risk groups are usually discharged after being called for a follow-up examination for tests such as outpatient exercise tests, cardiac scintigraphy, and echocardiography. However, a significant portion of these patients do not undergo invasive tests because they do not come to their follow-up examinations on time or because the sensitivity of the tests predicts negative risks in the near future. In this study, we showed that these patients face a significant increase in mortality within 6 months.

Clinicians have difficulty diagnosing NSTE-ACS patients who do not have cardiac chest pain but have ACS-equivalent symptoms if their troponin is high. In these patients, in addition to important diagnoses such as diabetic ketoacidosis, sepsis, pneumonia, shock, acute pancreatitis, and acute renal failure, simpler diagnoses such as minor infection, mild electrolyte disorder, and mild dehydration can be considered. This is observed in the type 2 MI group treated in the ICU, whose troponin elevation is attributed to other important underlying diseases. While the 6-month mortality of type 1 MI patients treated in CU was low (2%), the mortality of type 2 MI patients treated in ICU was high (7%). Additionally, the mortality of patients treated in OC with any diagnosis was also high (4%) (Table 3). According to these results, the mortality of those who receive interventional treatment is better than that of the other groups. The HEART risk score does not score ACS patients in terms of comorbid diseases. In this case, even if the patient's score is calculated as low or moderate risk, their mortality may be high. However, studies are needed to answer the question of how cardiac invasive diagnoses and treatments may contribute to mortality in these patients.

A multicenter prospective study recommends designating a HEART score of 2 or less as the cutoff point not to miss MI in patients considered low risk[19]. The fact that the mortality of EO patients (10%) is higher than that of inpatients (2, 7, and 4%) may be related to this result (Table 3). Given our results, we think that some NSTE-ACS patients may not be given the advanced cardiac evaluation they need. We recommend that these patients undergo further cardiac evaluation after other treatments are completed. In a review written on non-coronary troponin elevation, cardiac examination is recommended if ECG or ischemic findings persist in patients when other pathologies that increase troponin have been treated and eliminated[20].

LIMITATIONS

The limitation of the study was that cardiac controls of those with moderate- and low-risk heart scores were performed as outpatients, and we did not know what kind of controls they had.

CONCLUSION

Invasive treatments are mostly applied to patients in the HEART high-risk group. Mortality rates between risk groups as a result of these treatments have not been compared before. The high-risk group benefited greatly from the heart treatments they received and had low 6-month mortality rates. We believe that studies that will reduce mortality in moderate- and low-risk groups are needed.

ETHICAL APPROVAL

This study was approved by the Bezmialem Vakif University Ethics Committee under the approval number 2021/386 (05.04.2021).

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients to be included in the study.

AUTHORS' CONTRIBUTIONS

ES: Conceptualization, Data curation, Formal Analysis, Supervision, Writing – original draft, Writing – review & editing. **BT:** Formal Analysis, Funding acquisition. **AO:** Software, Visualization. **MAD:** Project administration, Resources. **HK:** Methodology. **BG:** Validation, Visualization.

REFERENCES

1. Gibbs J, McCord J. Chest pain evaluation in the emergency department: risk scores and high-sensitivity cardiac troponin. Curr Cardiol Rep. 2020;22(7):49. https://doi.org/10.1007/s11886-020-01294-1

2. Carlton EW, Khattab A, Greaves K. Identifying patients suitable for discharge after a single-presentation high-sensitivity troponin result: a comparison of five established risk scores and two high-sensitivity assays. Ann Emerg Med. 2015;66(6):635-45.e1. https://doi.org/10.1016/j.annemergmed.2015.07.006

3. Nallet O, Gouffran G, Lavie Badie Y. [Troponin elevation in the absence of acute coronary syndrome]. Ann Cardiol Angeiol (Paris). 2016;65(5):340-5. https://doi.org/10.1016/j.ancard.2016.09.006
4. Gulati M, Levy PD, Mukherjee D, Amsterdam E, Bhatt DL, Birtcher KK, et al. 2021 AHA/ACC/ASE/CHEST/SAEM/SCCT/SCMR guideline for the evaluation and diagnosis of chest pain: a report of the American College of Cardiology/American Heart Association Joint Committee on clinical practice guidelines. J Am Coll Cardiol. 2021;78(22):e187-285. https://doi.org/10.1016/j.jacc.2021.07.053
5. Bosco E, Hsueh L, McConeghy KW, Gravenstein S, Saade E. Major adverse cardiovascular event definitions used in observational analysis of administrative databases: a systematic review. BMC Med Res Methodol. 2021;21(1):241. https://doi.org/10.1186/s12874-021-01440-5
6. Long B, Oliver J, Streitz M, Koyfman A. An end-user's guide to the HEART score and pathway. Am J Emerg Med. 2017;35(9):1350-5. https://doi.org/10.1016/j.ajem.2017.03.047
7. Salimi A, Zolghadrasli A, Jahangiri S, Hatamnejad MR, Bazrafshan M, Izadpanah P, et al. The potential of HEART score to detect the severity of coronary artery disease according to SYNTAX score. Sci Rep. 2023;13(1):7228. https://doi.org/10.1038/s41598-023-34213-9
8. Laureano-Phillips J, Robinson RD, Aryal S, Blair S, Wilson D, Boyd K, et al. HEART score risk stratification of low-risk chest pain patients in the emergency department: a systematic review and meta-analysis. Ann Emerg Med. 2019;74(2):187-203. https://doi.org/10.1016/j.annemergmed.2018.12.010
9. Berg P, Body R. The HEART score for early rule out of acute coronary syndromes in the emergency department: a systematic review and meta-analysis. Eur Heart J Acute Cardiovasc Care. 2018;7(2):111-9. https://doi.org/10.1177/2048872617710788
10. Cuschieri S. The STROBE guidelines. Saudi J Anaesth. 2019;13(Suppl 1):S31-4. https://doi.org/10.4103/sja.SJA_543_18
11. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: task force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European society of cardiology (ESC). Eur Heart J. 2016;37(3):267-315. https://doi.org/10.1093/eurheartj/ehv320
12. Gomez R. HEART score for predicting adverse outcomes in patients with chest pain. Am Fam Physician. 2018;98(2):72-5. PMID: 30215999
13. Wassie M, Lee MS, Sun BC, Wu YL, Baecker AS, Redberg RF, et al. Single vs serial measurements of cardiac troponin level in the evaluation of patients in the emergency department with suspected acute myocardial infarction. JAMA Netw Open. 2021;4(2):e2037930. https://doi.org/10.1001/jamanetworkopen.2020.37930
14. Mahler SA, Miller CD, Hollander JE, Nagurney JT, Birkhahn R, Singer AJ, et al. Identifying patients for early discharge: performance of decision rules among patients with acute chest pain. Int J Cardiol. 2013;168(2):795-802. https://doi.org/10.1016/j.ijcard.2012.10.010
15. Mahler SA, Hiestand BC, Goff DC, Hoekstra JW, Miller CD. Can the HEART score safely reduce stress testing and cardiac imaging in patients at low risk for major adverse cardiac events? Crit Pathw Cardiol. 2011;10(3):128-33. https://doi.org/10.1097/HPC.0b013e3182315a85
16. Reyes J, Becker BA, D'Angelo J, Golden B, Stahlman BA, Miraoui M, et al. Utility of serial conventional troponin testing for emergency department patients stratified by HEART score and symptom timing. Am J Emerg Med. 2023;69:173-9. https://doi.org/10.1016/j.ajem.2023.04.044
17. Backus BE, Six AJ, Kelder JC, Bosschaert MA, Mast EG, Mosterd A, et al. A prospective validation of the HEART score for chest pain patients at the emergency department. Int J Cardiol. 2013;168(3):2153-8. https://doi.org/10.1016/j.ijcard.2013.01.255
18. Sun BC, Laurie A, Fu R, Ferencik M, Shapiro M, Lindsell CJ, et al. Comparison of the HEART and TIMI risk scores for suspected acute coronary syndrome in the emergency department. Crit Pathw Cardiol. 2016;15(1):1-5. https://doi.org/10.1097/HPC.0000000000000066
19. Singer AJ, Than MP, Smith S, McCullough P, Barrett TW, Birkhahn R, et al. Missed myocardial infarctions in ED patients prospectively categorized as low risk by established risk scores. Am J Emerg Med. 2017;35(5):704-9. https://doi.org/10.1016/j.ajem.2017.01.003
20. Long B, Long DA, Tannenbaum L, Koyfman A. An emergency medicine approach to troponin elevation due to causes other than occlusion myocardial infarction. Am J Emerg Med. 2020;38(5):998-1006. https://doi.org/10.1016/j.ajem.2019.12.007

ORIGINAL ARTICLE

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Evaluation of some immunological markers in co-infection of COVID-19 with thrush candidiasis

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SUMMARY

OBJECTIVE: COVID-19 infection poses significant risks, including life-threatening consequences and fungus synchronization, making it a significant concern. This study seeks to assess the effect of concurrent infection of COVID-19 with Thrush *Candida albicans* on the patient's health state by measuring the proportion of immune cells and certain interleukins such as IL-8, -10, -17, and -33.

METHODS: The study involved 70 patients (30 patients with COVID-19, 17 patients with thrush candidiasis, and 23 patients with Thrush *Candida albicans*) and 50 healthy individuals as a control group. COVID-19 was identified using RT-PCR, while *C. albicans* were identified through culture media, biochemical testing, and oral swabs. Ruby equipment and ELISA kits were used for blood counts and interleukin detection.

RESULTS: COVID-19, thrush candidiasis, and Thrush *Candida albicans* infections occur in a wide range of age groups (4–80 years), with no significant differences between sexes ($p>0.05$). Immunologically, our study found that Thrush *Candida albicans* patients had the highest rate of neutrophils (89.6%) and basophils (2.01%), while corona patients had the highest percentage of lymphocytes (70.12%) and eosinophils (7.11%), and patients with thrush candidiasis had the highest percentage of monocytes. Thrush *Candida albicans* patients showed increased IL-8 (56.7 pg/mL) and IL-17 (101.1 pg/mL) concentrations, with the greatest concentration of IL-33 (200.5 pg/mL) in COVID-19, and a decrease in the level of IL-10 in patient groups compared with controls.

CONCLUSION: Patient groups showed increased neutrophils, lymphocytes, monocytes, and IL-8 levels, with a significant linear association between proinflammatory interleukins and these cells.

KEYWORDS: Biomarkers. COVID-19. Candidiasis. Co-infection. IL-8. IL-10.

INTRODUCTION

The coronavirus disease, which is known as SARS-CoV-2, is a threat to public health. It can cause severe illness and require hospitalization[1]. Individuals with weak immune systems or medical conditions are more susceptible to severe COVID-19 and respiratory distress syndrome[2]. Critically ill patients with ARDS admitted to the ICU and need artificial ventilation are more susceptible to nosocomial fungal infections, with COVID-19 patients experiencing bacterial and fungal co-infections[3]. The prevalence of diseases has increased due to increased disease transmission and pesticide use[4].

COVID-19 patients face candidiasis due to compromised immune systems, zinc and iron deficiencies, and iatrogenic and nosocomial transmissions, which are not fully understood[5]. COVID-19-related mucormycosis is the most common contagious infection in COVID-19 patients, while candidiasis, a less concentrated parasitic disease, has emerged in countries such as India, Iran, China, and the United Kingdom[6].

Serious COVID-19 can cause severe cytokine delivery, immune weakness, and mortality in established patient populations. While superinfections were initially uncommon, reports of optional contagious diseases as potential entanglements are increasing[7]. COVID-19 patients with acute respiratory distress syndrome (ARDS) face challenges in clinical training due to aspiratory Aspergillosis and candidiasis, but immunological tools can improve infection control[8].

Thrush candidiasis, caused by *Candida albicans*, is a severe parasitic disease causing clinical issues and COVID-19-related complications. The exact pathophysiology of candidiasis in COVID-19 patients is not fully understood, and there is limited information on contagious co-contaminations. Deficient understanding and lack of preventive measures may lead to misdiagnosis and potentially destroy COVID-19 outcomes[9]. The meaning of the sole impacts of the safe reaction on parasitic contaminations needs further examination[10]. Accordingly, this study meant to decide the job of a few safe cells as neutrophils, lymphocytes, monocytes, eosinophils, and basophils in extra to certain interleukins such as IL-8, -10, -17, and -33 in co-disease of COVID-19 with thrush candidiasis (CTC).

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METHODS

Study design and sample collection

Before the start of the research project, this study was approved by the ethical committee of the Faculty of Medicine, University of AL-Qadisiyah, and informed consent was obtained from all individuals, and permission was obtained from the Al-Diwaniaya Teaching Hospital/Quarantine unit. A case-control study was conducted with individuals recruited from the Al-Diwaniaya Teaching Hospital/Quarantine unit in the Al-Qadisiyah governorate. The study was carried out during the period from December 1, 2021 to the end of January 2022. The age ranged between 4 and 80 years. The study involved 70 patients (30 patients with COVID-19, 17 patients with thrush candidiasis, and 23 patients with CTC), and 50 healthy individuals as a control group. Assent was taken from all members while gathering the survey and tests.

Immunological study

Absolute IL-8, IL-10, IL-17, and IL-33 ELISA units (Demeditec/Germany) were utilized for the quantitative assurance of complete IL-8, IL-10, IL-17, and IL-33 in human serum. The reagents readiness and examination technique were done by the producer's depiction, an enzyme-linked immunosorbent assay (ELISA). The plate had been pre-coated with human IL antibody. ILs present in the sample were added and they bound to antibodies coated on the wells. Then, biotinylated human IL antibody was added and it bound to ILs in the sample. Then, Streptavidin-HRP was added and it bound to the biotinylated IL antibody. After incubation, unbound Streptavidin-HRP was removed during washing. The substrate solution was then added, and a color was developed in proportion to the amount of human ILs. The reaction was terminated by the addition of an acidic stop solution, and the absorbance was measured at 450 nm.

A complete blood count (CBC) was performed using the RUBY framework, using EDTA tubes and natural testing. After 1–5 min, a complete count of neutrophils, lymphocytes, monocytes, eosinophils, and basophils was displayed on a PC screen, with each patient's name and number printed.

Isolation and identification of C. albicans on Sabouraud dextrose agar were performed using brooding at 37°C for 48 h, revealing cream, smooth, pale curved states with minimal separation. Germ-tube test: The test showed *C. albicans* hyphal outgrowths in horse serum, followed by a biochemical test. Direct microbial examination: Morphological highlights of *C. albicans* were identified using a smear, revealing non-pigmented septate hyphae with distinctive dichotomous fanning at 45°. COVID-19 identification: Positive RT-PCR SARS-CoV-2 nasopharyngeal swab test confirms COVID-19. To confirm the infection with SARS-COV2, especially in mild infection, the individuals underwent rapid tests for the detection of IgG and IgM Abs.

Statistical analysis

Data were converted into the modern database, cleaned using range and legitimate methods, and analyzed using expert guidance for factual examinations using SPSS 20 and Microsoft Excel 2010.

RESULTS

Demographic characteristics of patients and controls are shown in Table 1 and Figure 1. The ages of patients varied from 4 to 80 years, with an average age of 36.11, 38.7, and 40.92 years for COVID-19, CTC, and TC patients, respectively. Results found no significant differences in age or gender distribution in fungi or virus infection rates between groups.

Total blood cell count shows immune cells increased in COVID-19 patients, with some increasing in both the virus and fungus simultaneously. Neutrophils and lymphocytes were highest in COVID-19 patients, followed by CTC and TC patients.

Individuals with TC and CTC had the highest percentages of monocytes (25.69 and 23.67%), while patients with COVID-19

Table 1. Demographic characteristics of patients and controls.

Age statistic	COVID-19 patients	CTC patients	CT patients	Control	p-value
Age range (years)	4–80	4–80	4–80	4–80 years	
Mean	36.11	38.7	40.92	38.3	0.155
SD	9.85	11.61	13.4	11.77	
SE	1.80	2.42	3.25	1.66	
Number	30	23	17	50	
Females	17	11	10	23	0.085
Males	13	12	7	27	0.052

CTC: COVID-19 and thrush candidiasis; CT: thrush candidiasis.

and CTC had the largest percentages of eosinophils (7.11%) and basophils (2.01%). Significant differences were observed in immune cell proportions compared with healthy individuals (Table 2).

The study found increased proinflammatory interleukins IL-8, -17, and -33 in patient groups compared with healthy controls. Anti-inflammatory IL-10 concentrations were low in patients but not statistically significant. IL-8 and IL-17 concentrations were highest in CTC patients, while IL-33 was higher in COVID-19 and CTC. The lowest levels of IL-10 were found in CT and CTC serum (Table 3).

The study found a strong positive relationship between proinflammatory interleukins (IL-8, -17, and -33) and immune cells, particularly neutrophils and lymphocytes, in patients with viral and fungal infections. The relationship was weakly positive in fungal infections. High IL-33 concentrations are significantly linked to lymphocytes and neutrophils, as well as IL-17 and IL-8.

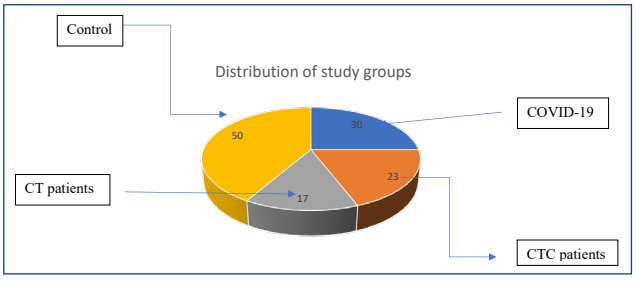

Figure 1. Frequency distribution of study groups.

DISCUSSION

Candida albicans co-infections with COVID-19 can occur in people of all ages, and they are associated with higher levels of immune cells and proinflammatory interleukins. Also, COVID-19 can cause immunosuppression or cytokine release, which can increase the risk of fungal infections with increased IL-18, IL-17, IL-33, neutrophils, monocytes, and basophils. According to Song et al.'s[11] research, severe COVID-19 patients with better access to antibiotics, nutrition, and tests, as well as persistent neutropenia, are more likely to contract *Candida* species infections. The results of this study agree with that of Remy et al.[10] who found that COVID-19 often causes immunological reactions affecting innate and adaptive immune responses, impacting clinical course and prognosis. Severe COVID-19 may cause immunosuppression or cytokine release, leading to morbidity and death, especially in elderly patients. COVID-19 increases the risk of fungal infections due to immune system influence and potential reduction in defenses from COVID-19 therapies[12]. The exact mechanism by which COVID-19 increases the risk of fungal superinfections is unknown[13]. *C. albicans*, a polymorphic commensal fungus, is immunocompetent and unaffected by the human microbiota. However, when disrupted, it can cause superficial skin and mucous membrane infections, leading to systemic infections[14].

Glucans and -mannans recognize surface pattern recognition receptors on monocytes and macrophages, triggering an adequate immune response against *C. albicans*, including TLR2/4, NOD-like

Table 2. Evaluation of immune cells in studied groups.

Immune cells count	COVID-19 patients	CTC patients	CT patients	Control	X^2	p-value
Neutrophils %	85.3*	89.6*	77.9*	39.44	1.071	0.089
Lymphocytes %	70.12*	67.3"	67.26*	19.32	0.853	0.100
Monocytes %	20.75*	23.67*	25.69"	4.99	0.555	0.114
Eosinophils %	7.11*	3.61*	1.68*	0.76	0.304	0.261
Basophils %	1.66*	2.01*	0.77*	0.27	0.300	0.329

*Significant differences in comparison with control ($p<0.05$); "nonsignificant differences in comparison with control ($p<0.114$); X^2: chi square; CTC: COVID-19 and thrush candidiasis; CT: thrush candidiasis.

Table 3. Evaluation of some interleukins in studied groups.

Interleukins	COVID-19 patients	CTC patients	CT patients	Control	X^2	p-value
IL-8 (pg/mL)	25.3*	56.7*	45.6*	3.17	8.07	0.041
IL-10 (ng/L)	4.31	2.81	2.44	4.73	0.52	0.093
IL-17 (pg/mL)	98.91*	101.1*	52.34*	9.61	9.84	0.029
IL-33 (pg/mL)	200.5*	193.5*	88.76*	10.31	17.11	0.002

*Significant differences in comparison with control ($p<0.05$); X^2: chi square; CTC: COVID-19 and thrush candidiasis; CT: thrush candidiasis.

receptors, and C-type lectin receptors[15]. NLRP3 inflammasome activates receptors, producing proinflammatory cytokines such as TNF, IL-8, IL-17, IL-33, and IL-18 with fungicidal effects[16]. Moser et al.[17] found that monocyte activation markers and cytokines IL-6, IL-8, TNF, IL-10, and sIL2R were enhanced in COVID-19 patients with *C. albicans* infection. This suggests that these patients may be more susceptible to *C. albicans* infection.

IL-33 triggers an innate defensive response after systemic CTC infection, increasing neutrophil phagocytosis and NK cell production of GM-CSF. It induces IL-17, which enhances protection against fungi and bacteria by recruiting neutrophils, synthesis of antimicrobial peptides, and barrier function[18]. Leukocytes struggle to phagocytize biofilm-associated cells, potentially causing COVID-19 patients to continue having *Candida* spp. infections during a cytokine storm, leading to increased immune cell count[19]. CTC lacks greater phagocytosis and lower fungal multiplication due to reduced T-cell count and ineffective phagocytosis, potentially due to T-cell reduction[20]. Severe COVID-19 increases secondary fungal infections, worsening clinical course in ARDS patients with pulmonary aspergillosis and candidiasis[21].

Chen et al.[22] discovered that five fungal infections were found in 99 COVID-19 patients at admission, including *Aspergillus flavus*, *Candida glabrata*, and *C. albicans*. Yang et al.[23] discovered that fungal co-infections were found in 52 critically ill patients (3/52, 5.8%), including *A. flavus*, *A. fumigatus*, and *C. albicans*, according to the research.

Our findings provide initial evidence showing a weakened immune response to *C. albicans* and COVID-19 in Iraq, highlighting the need for attention and a good overview of the current understanding of COVID-19 and *C. albicans* co-infection. It also highlights the importance of further research in this area.

CONCLUSION

Thrush candidiasis, an opportunistic fungal illness, is becoming more common as COVID-19. COVID-19 and *C. albicans* co-infection is associated with increased levels of proinflammatory interleukins and immune cells. Concurrent infection with COVID-19 and *C. albicans* (CTC) further increases these markers. There is a positive relationship between humoral and cellular immunity in patients with CTC. COVID-19 and CTC can lead to a hyperinflammatory state, which can increase the risk of complications and death. Patients with CTC may be more susceptible to other infections, including bacterial infections. Both humoral and cellular immunity are important for protecting against COVID-19 and *C. albicans* infection.

ACKNOWLEDGMENTS

This study provides valuable insights into the immunological mechanisms underlying co-infection with COVID-19 and *Candida albicans*. Our research sheds light on the potential role of IL-8, IL-10, IL-17, and IL-33 in this complex interaction. These findings suggest that COVID-19 patients with candidal colonization (CTC) may be at increased risk for adverse outcomes. We would like to express our sincere gratitude to all friends and everyone who has supported and helped us in all our steps. We are also grateful to our colleagues for their constructive feedback throughout the research process. The authors acknowledge the members of the Community in Al-Diwaniyah Teaching Hospital, Al-Diwaniyah, Iraq, for their support during the preparation of the manuscript.

AUTHORS' CONTRIBUTIONS

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

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. https://doi.org/10.1016/S0140-6736(20)30183-5
2. Freeman TL, Swartz TH. Targeting the NLRP3 inflammasome in severe COVID-19. Front Immunol. 2020;11:1518. https://doi.org/10.3389/fimmu.2020.01518
3. Didbaridze T, Ratiani L, Labadze N, Maziashvili T. Prevalence and prognosis of candidiasis among Covid-19 patients: data from ICU department. Int J Prog Sci Technol. 2021;1:36-9.
4. Zuo T, Zhan H, Zhang F, Liu Q, Tso EYK, Lui GCY, et al. Alterations in fecal fungal microbiome of patients with COVID-19 during time of hospitalization until discharge. Gastroenterology. 2020;159(4):1302-10.e5. https://doi.org/10.1053/j.gastro.2020.06.048
5. Al-Hatmi AMS, Mohsin J, Al-Huraizi A, Khamis F. COVID-19 associated invasive candidiasis. J Infect. 2021;82(2):e45-6. https://doi.org/10.1016/j.jinf.2020.08.005
6. Heard KL, Hughes S, Mughal N, Moore LSP. COVID-19 and fungal superinfection. Lancet Microbe. 2020;1(3):e107. https://doi.org/10.1016/S2666-5247(20)30065-3
7. Chowdhary A, Tarai B, Singh A, Sharma A. Multidrug-resistant *Candida auris* infections in critically Ill coronavirus disease patients, India, April-July 2020. Emerg Infect Dis. 2020;26(11):2694-6. https://doi.org/10.3201/eid2611.203504

8. White PL, Dhillon R, Cordey A, Hughes H, Faggian F, Soni S, et al. A national strategy to diagnose coronavirus disease 2019-associated invasive fungal disease in the intensive care unit. Clin Infect Dis. 2021;73(7):e1634-44. https://doi.org/10.1093/cid/ciaa1298
9. Uwamahoro N, Verma-Gaur J, Shen HH, Qu Y, Lewis R, Lu J, et al. The pathogen *Candida albicans* hijacks pyroptosis for escape from macrophages. mBio. 2014;5(2):e00003-14. https://doi.org/10.1128/mBio.00003-14
10. Remy KE, Mazer M, Striker DA, Ellebedy AH, Walton AH, Unsinger J, et al. Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. JCI Insight. 2020;5(17):e140329. https://doi.org/10.1172/jci.insight.140329
11. Song G, Liang G, Liu W. Fungal co-infections associated with global COVID-19 pandemic: a clinical and diagnostic perspective from China. Mycopathologia. 2020;185(4):599-606. https://doi.org/10.1007/s11046-020-00462-9
12. Segrelles-Calvo G, Araújo GR, Llopis-Pastor E, Carrillo J, Hernández-Hernández M, Rey L, et al. *Candida* spp. co-infection in COVID-19 patients with severe pneumonia: prevalence study and associated risk factors. Respir Med. 2021;188:106619. https://doi.org/10.1016/j.rmed.2021.106619
13. Mastrangelo A, Germinario BN, Ferrante M, Frangi C, Voti R, Muccini C, et al. Candidemia in coronavirus disease 2019 (COVID-19) patients: incidence and characteristics in a prospective cohort compared with historical non-COVID-19 controls. Clin Infect Dis. 2021;73(9):e2838-9. https://doi.org/10.1093/cid/ciaa1594
14. Gozalbo D, Maneu V, Gil ML. Role of IFN-gamma in immune responses to *Candida albicans* infections. Front Biosci (Landmark Ed). 2014;19(8):1279-90. https://doi.org/10.2741/4281
15. Silva LN, Mello TP, Souza Ramos L, Branquinha MH, Roudbary M, Santos ALS. Fungal infections in COVID-19-positive patients: a lack of optimal treatment options. Curr Top Med Chem. 2020;20(22):1951-7. https://doi.org/10.2174/1568026620222009171101O2
16. Heung LJ. Monocytes and the host response to fungal pathogens. Front Cell Infect Microbiol. 2020;10:34. https://doi.org/10.3389/fcimb.2020.00034
17. Moser D, Biere K, Han B, Hoerl M, Schelling G, Choukér A, et al. COVID-19 impairs immune response to *Candida albicans*. Front Immunol. 2021;12:640644. https://doi.org/10.3389/fimmu.2021.640644
18. Ahmed N, Mahmood MS, Ullah MA, Araf Y, Rahaman TI, Moin AT, et al. COVID-19-associated candidiasis: possible patho-mechanism, predisposing factors, and prevention strategies. Curr Microbiol. 2022;79(5):127. https://doi.org/10.1007/s00284-022-02824-6
19. Ombrello MJ, Schulert GS. COVID-19 and cytokine storm syndrome: are there lessons from macrophage activation syndrome? Transl Res. 2021;232:1-12. https://doi.org/10.1016/j.trsl.2021.03.002
20. Rocha FAC, Alves AMCV, Rocha MFG, Cordeiro RA, Brilhante RSN, Pinto ACMD, et al. Tumor necrosis factor prevents *Candida albicans* biofilm formation. Sci Rep. 2017;7(1):1206. https://doi.org/10.1038/s41598-017-01400-4
21. White PL, Dhillon R, Cordey A, Hughes H, Faggian F, Soni S, et al. A national strategy to diagnose coronavirus disease 2019-associated invasive fungal disease in the intensive care unit. Clin Infect Dis. 2021;73:e1634-44. https://doi.org/10.1093/cid/ciaa1298
22. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395(10223):507-13. https://doi.org/10.1016/S0140-6736(20)30211-7
23. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020;8(5):475-81. https://doi.org/10.1016/S2213-2600(20)30079-5

ORIGINAL ARTICLE

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Aggressive treatment may be needed for idiopathic membranous nephropathy with focal segmental glomerulosclerosis lesions

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SUMMARY

OBJECTIVE: The purpose of this study was to analyze the clinical, pathological, prognostic features and treatment response of the coexistence of focal segmental glomerulosclerosis lesions with idiopathic membranous nephropathy.

METHODS: This is a two-center retrospective cohort study. Patients of idiopathic membranous nephropathy were enrolled and divided into two groups with or without focal segmental glomerulosclerosis lesions according to the renal biopsy. Laboratory data and pathological manifestation were compared. Renal phospholipase A_2 receptor was detected by immunofluorescence. During the follow-up, the effects of different therapies and renal function were estimated.

RESULTS: A total of 236 patients were finally enrolled in this study, of which 60 and 176 idiopathic membranous nephropathy patients were enrolled in the FSGS+ and FSGS- groups, respectively. The FSGS+ group showed a higher percentage of hypertension history (38.3 vs. 20.0%, p=0.004), with a significantly higher level of systolic pressure [137 (120, 160) mmHg vs. 130 (120, 140) mmHg, p=0.009]. Main laboratory findings, including serial albumin (20.4±7.8 g/L vs. 24.5±6.7 g/L, p<0.001), 24-h proteinuria [5.61 (3.10, 7.87) g/day vs. 3.82 (2.31, 5.79) g/day, p=0.002], serial creatinine [80.8 (65.8, 97.9) μmol/L vs. 72.0 (58.7, 84.9) μmol/L, p=0.003], and estimated glomerular filtration rate [86 (66, 101) mL/min/1.73 m^2 vs. 95 (81, 108) mL/min/1.73 m^2, p=0.007] showed significant differences between the two groups. Pathologically, patients with focal segmental glomerulosclerosis lesions appeared with a higher percentage of crescents, a more severe degree of interstitial fibrosis, and a higher level of membranous nephropathy stage. Renal phospholipase A_2 receptor showed a relatively lower positive rate of only 75.0% in the FSGS+ group in comparison with the positive rate of 90.3% in the FSGS- group (p=0.031). The prognosis was generally similar between the two groups. Among patients who were given non-immunosuppression treatment, those with focal segmental glomerulosclerosis lesions took a relatively longer period of time to achieve complete remission (29.3±7.0 m vs. 15.4±8.9 m, p=0.025) and experienced a higher rate of renal function deterioration (37.5 vs. 5.4%, p=0.033) compared with the other ones. While among those receiving immunosuppression treatment, both groups received similar remission rates.

CONCLUSION: Compared with FSGS- group, idiopathic membranous nephropathy with focal segmental glomerulosclerosis lesions represented more severe nephrotic syndrome and worse renal function. In view of the renal function decline during the follow-up, more aggressive treatment with the use of immunosuppressants should be considered for idiopathic membranous nephropathy patients with focal segmental glomerulosclerosis lesions.

KEYWORDS: Idiopathic membranous nephropathy (IMN). Focal segmental glomerulosclerosis (FSGS). Treatment. Phospholipase A_2 receptor (PLA_2R).

INTRODUCTION

Idiopathic membranous nephropathy (IMN), which is one of the leading causes of nephrotic syndrome in adults, continued to update during the past two decades[1], especially after M-type phospholipase A_2 receptor (PLA_2R) was identified as a major target antigen in 70% of IMN[2]. Among patients who have been diagnosed with IMN, different situations may occur: spontaneous remission, persistent nephrotic syndrome with preserved renal function, or refractory proteinuria with worsening renal function. Some may even develop end-stage renal disease (ESRD). Various features have been shown to predict unfavorable course in IMN patients, including male sex, heavy proteinuria, renal insufficiency at presentation, hypertension, age, and degree of interstitial fibrosis[3-7]. The prognostic indicators of IMN are still needed to be studied to predict the outcomes, help choose individual therapy, and weigh the advantages and disadvantages of different therapies. With the in-depth study of the pathogenesis and clinical features of membranous nephropathy, the correlation between pathological features and prognosis has been found gradually.

Since the first report of IMN with focal segmental glomerulosclerosis (FSGS) lesions pathologically by Churg and Ehrenreich in 1973[8], the clinical and pathological features of these patients have received increasing attention. The incidence

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rate is 10–43% according to the current reports[9-16]. A few studies have stressed the poor prognostic meaning of FSGS lesions with IMN[10,11,13,16,17]. According to the previous reports, patients with FSGS lesions showed higher serum creatinine levels and more severe nephrotic syndrome. While some other research and meta-analysis did not support this conclusion[3,14,18], some research demonstrated the relationship between the specific FSGS lesions and the renal outcome, such as non-atypical lesions (pure synechia, segmental hyperplasia of podocytes or thickening of the GBM accompanied by proliferation of the mesangial matrix, and absence of typical FSGS) and non-glomerular tip lesion[19,20]. In addition, the previous studies were less focused on the treatment efficiency of those with FSGS lesions. Because of the heterogeneity of the study design and the small sample size among those studies, there was still no uniform conclusion, and more research is still required. In this study, we aimed to ascertain the clinical and pathological characteristics of IMN with and without FSGS lesions and analyze the outcomes and treatment efficiency of the two groups.

METHODS

Study design

This study was designed as a retrospective cohort study. We used the data who were diagnosed as IMN at Huashan Hospital affiliated with Fudan University and Wuxi People's Hospital. The data underlying this study were collected from the medical record system of the two centers. The study received local ethics committee approval (approval number and date: KY2016-394, February 6, 2017), and all patients gave written informed consent.

Patients selection

Briefly, patients who were diagnosed as IMN pathologically by renal biopsy between January 2008 and December 2014 with ages above 18 years and gender unlimited were enrolled. Exclusion criteria included secondary MN, such as V-type lupus nephritis, hepatitis B, hepatitis C, malignancy, syphilis, autoimmune conditions such as Sjogren syndrome, rheumatoid arthritis, ankylosing spondylitis, and anti-glomerular basement membrane disease. Patients without FSGS lesions, if the number of glomeruli obtained by renal biopsy was less than 8, were also excluded to avoid bias due to missed diagnosis. Patients who were biopsy-proven superimposed FSGS lesions were selected as a study group. Those without FSGS lesions were selected for comparison.

Clinical and laboratory data

Data such as gender, age, medical history, medications, serum creatinine, serum albumin, cholesterol, 24-h urinary protein excretion, and systolic and diastolic blood pressure were collected retrospectively according to the medical records at the time of biopsy. The estimated glomerular filtration rate (eGFR) was calculated with the CKD-EPI creatinine equation. In patients who were followed up at the two centers, treatment was divided into two classes: (1) non-immunosuppressive therapy with symptomatic approaches included angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) and (2) immunosuppressive therapy included glucocorticoid combined with immunosuppressant (cyclophosphamide or calcineurin inhibitors). Serum creatinine, serum albumin, and 24-h urinary protein excretion were measured at least every 6 months.

Pathological evaluation

All biopsy specimens were processed with light microscopy, immunohistochemistry, and electron microscopy using standardized techniques. The biopsies were evaluated in detail for the following features: total number of glomeruli, crescents, global sclerosis, FSGS lesion, the extent of interstitial fibrosis, and the degree of arteriosclerosis. Focal glomerulosclerosis was defined as a focal lesion with mesangial matrix expansion leading to the collapse of the glomerular capillary loops. Criteria of Columbia classification of FSGS were used for classification.

Renal PLA_2R staining

Renal tissues from 163 of the patients were stained for the PLA_2R antigen. PLA_2R was detected in 2-μm frozen sections using rabbit polyclonal anti-PLA_2R antibodies (Sigma, America, 295631) at a dilution of 1:500 followed by donkey anti-rabbit IgG (Millipore, America, AP182F) at a dilution of 1:100. PLA_2R staining was considered positive if there was positive granular staining along the capillary loops of glomeruli, and negative if there was no staining in the glomeruli.

Outcomes

Follow-up was started at the time of biopsy and continued until July 2016. We analyzed the remission, relapse, and renal function of those who were followed up for over 12 months. CR was defined by proteinuria <0.3 g/day, with normal serum albumin level and renal function. Partial remission (PR) was defined by proteinuria <3.5 g/day or descending over half the peak level with normal renal function. Otherwise, no remission (NR) was diagnosed. Relapse of proteinuria was defined as recurrent proteinuria within the nephrotic range

or over half the peak level. Renal function deterioration was defined as the last eGFR descending over 30% compared with eGFR at baseline.

Statistical analysis

Statistical analysis was performed with SPSS version 13.0 for Mac. For continuous variables, normal distribution was examined by the Kolmogorov-Smirnov (K-S) test. T-test and Mann-Whitney U test were applied for normal distribution variables and non-normal distribution variables, respectively. For categorical variables, correlations were calculated using the chi-square test and Fisher exact test, if appropriate. For all analyses, $p<0.05$ was considered significant.

RESULTS

Clinical and laboratory characteristics

Finally, 236 patients were included. A total of 60 cases were accompanied by FSGS lesions (FSGS+ group), and 176 cases were not (FSGS- group). Table 1 shows the baseline characteristics of the two study groups. There is no difference in gender distribution and mean age between the two groups. FSGS+ group shows a much higher percentage of the history of hypertension and a higher median systolic blood pressure level at the time of renal biopsy. The two groups were similar with respect to diastolic blood pressure. Patients in the FSGS+ group represented more severe nephrotic syndrome with a higher 24-h proteinuria of a median level of 5.61 g/24 h and a lower serum albumin of a median level of 20.4 g/L than the FSGS- group. Also, the cholesterol level showed the same trend. Meanwhile, there was a significant decline in serum creatinine and eGFR levels in FSGS+ cases compared with FSGS- ones.

Pathological characteristics

With respect to pathological findings, there was no difference in the number of glomeruli between the two groups. Patients in the FSGS+ group presented a higher frequency of crescents, higher interstitial fibrosis and tubular level, and a higher proportion of obsolescent glomeruli, although the latter was not statistically significant. In the FSGS- group, the most common stage of MN was early stage I (48.3%). In comparison, the FSGS group showed a higher level of stage II to stage III (Table 1). According to Columbia's classification of FSGS, the most common cases were classified as no otherwise specified (NOS) seen in 66.67% of the FSGS+ group. Notably, 28.33% were Tip and 5.00% were perihilar (PH). No collapsing or cellular lesion was noted.

Prognostic characteristics

A total of 25 patients and 104 patients were followed, respectively. No difference was observed between the two groups neither in the remission rate nor in the relapse rate. Notably, 6 patients in the FSGS+ group (24.0%) and 10 in the FSGS- group (9.6%) had their eGFR descending over 30% during follow-up ($p=0.050$). eGFR at the latest follow-up was also lower in the FSGS+ group, although there was no statistical difference (Table 2).

Prognosis situations with different treatments

A total of 8 patients in the FSGS+ group and 37 patients in the FSGS- group received non-immunosuppressive therapy. These patients presented a relatively lower level of 24-h urinary protein of mean 4.6±4.3 and 3.3±2.5 g/day. Both groups showed more than 60% of spontaneous remission. However, in the FSGS+ group, an additional 13.3 months on average was needed to achieve CR, and an obvious decline in eGFR was presented at the latest follow-up. Two patients in the FSGS+ group even progressed to ESRD. In comparison, patients with no combined FSGS lesion presented a relatively lower eGFR descending rate and a well-preserved renal function, which was similar to the eGFR level at baseline. This result indicated that renal function deterioration was more likely to occur in IMN patients combined with FSGS lesions if non-immunosuppression therapy was accepted; also, it would take much longer for them to achieve PR or CR. For those who achieved immunosuppressive therapy, no difference was observed in remission or renal function (Table 3).

DISCUSSION

Patients with IMN would greatly benefit if some clinical or pathological characteristics could predict disease prognosis with high accuracy. It is still insufficient for making a decision on what therapy should be chosen and when to start immunosuppressive therapy.

Idiopathic membranous nephropathy with FSGS lesions was first reported by Churg and Ehrenreich[8]. Dumoulin et al., mentioned in his study that FSGS lesions on IMN portended a significantly worse outcome in terms of nephrotic syndrome and renal insufficiency[16]. A recent study published in 2014 also concluded that FSGS lesions predict renal outcomes independently of clinical data in nephrotic IMN patients with decreased renal function[17]. However, there has been no consensus yet. According to another two studies[3,14], there is no significant difference between patients with or without FSGS lesions on remission, renal insufficiency, and ESRD. FSGS is not an accurate prognostic marker in IMN.

Table 1. Comparison of the medical history, laboratory parameters, and pathological characteristics between the FSGS+ and FSGS- groups.

		FSGS+ (n=60)	FSGS- (n=176)	p
Gender (M%)		65.0%	56.3%	NS
Age (years)		52.4±16.3	51.8±15.1	NS
History of DM (%)		15.3%	17.0%	NS
History of HBP (%)		38.3%	20.0%	0.004
Systolic BP (mmHg)		137 (120, 160)	130 (120, 140)	0.009
Diastolic BP (mmHg)		80 (78, 90)	80 (75, 90)	NS
Albumin (g/L)		20.4±7.8	24.5±6.7	<0.001
24-h proteinuria (g/day)		5.61 (3.10, 7.87)	3.82 (2.31, 5.79)	0.002
BUN (mmol/L)		4.5 (3.6, 6.0)	4.3 (3.5, 5.7)	NS
Serum creatinine (μmol/L)		80.8 (65.8, 97.9)	72.0 (58.7, 84.9)	0.003
eGFR (mL/min/1.73 m^2)		86 (66, 101)	95 (81, 108)	0.007
UA (mmol/L)		0.345±0.086	0.364±0.092	NS
Glomeruli (n)		15.5 (12.0, 22.0)	18.5 (13.0, 25.8)	NS
Obsolescent glomeruli (%)		6.5% (4.5%, 8.3%)	5.4% (3.9%, 7.7%)	NS
Crescent (%)		13.3%	2.8%	0.005
Tubular atrophy	0	17 (28.3%)	61 (34.7%)	
	1	28 (46.7%)	89 (50.6%)	
	2	13 (21.7%)	24 (13.6%)	
	3	2 (3.3%)	2 (1.1%)	NS
Interstitial fibrosis	0	14 (23.3%)	59 (33.5%)	
	1	28 (46.7%)	89 (50.6%)	
	2–3	18 (30.0%)	28 (15.9%)	0.045
Cast	0	5 (8.3%)	43 (24.4%)	
	1	37 (61.7%)	87 (49.4%)	
	2	18 (30.0%)	46 (26.1%)	0.022
MN stage	Early stage and stage I	19 (31.7%)	85 (48.3%)	
	Stage I–II	10 (16.7%)	43 (24.4%)	
	Stage II	18 (30.0%)	42 (23.9%)	
	Stage II–III	8 (13.3%)	3 (1.7%)	
	≥Stage III	1 (1.7%)	1 (0.5%)	0.001
Renal PLA_2R +(%)		45 (75.0%)	159 (90.3%)	0.031

Values are n (%), mean (SD), and median (interquartile ranges). M: male; DM: diabetes mellitus; HBP: high blood pressure; BP: blood pressure; BUN: blood urea nitrogen; eGFR: estimated glomerular filtration rate; UA: uric acid; CHO: cholesterol; PLA_2R: phospholipase A_2 receptor; NS: no significant.

We reviewed the medical history of 236 IMN patients in two medical centers and found that patients with FSGS lesions presented heavier nephrotic syndrome and relatively lower eGFR. Pathologically, the FSGS+ group presented a higher frequency of crescents and a greater degree of interstitial fibrosis and tubular atrophy. The eGFR level declined more severely in the FSGS group than the other, which indicates that IMN combined with FSGS lesion may portend poorer renal outcome.

In our study, we observed a relatively higher remission rate with a relatively lower dose of proteinuria. According to KDIGO clinical practice guideline for glomerulonephritis in 2012, on the basis of antihypertensive and antiproteinuric therapy during an observation period of 6 months, for patients with nephrotic syndrome and urinary protein excretion persistently NR, initial immunosuppressive therapy may be started. However, our study points out that for

Table 2. Comparison of prognostic characteristics between the FSGS+ and FSGS- groups.

	FSGS+ (n=25)	FSGS- (n=104)	p
Period of follow-up (months)	25.0 (20.0, 39.5)	24.0 (19.0, 36.0)	NS
Outcome-NR	4 (16.0%)	27 (26.0%)	
Outcome-PR	10 (40.0%)	35 (33.7%)	
Outcome-CR	11 (44.0%)	42 (40.3%)	NS
Time between biopsy and PR (months)	7.4 (5.4)	10.2 (6.6)	NS
Time between biopsy and CR (months)	16.6 (9.8)	12.3 (6.4)	NS
Relapse (%)	6 (28.6%)	14 (24.7%)	NS
eGFR at the latest follow-up	75.9 (33.3)	85.9 (26.4)	NS
eGFR descending>30%	6 (24.0%)	10 (9.6%)	0.050

Values are n (%), mean (SD), and median (interquartile ranges). PR: partial remission; CR: complete remission; eGFR: estimated glomerular filtration rate; NS: no significant.

Table 3. Prognostic characteristics between the FSGS+ and FSGS- groups with non-immunosuppressive and immunosuppressive therapy.

		Non-immunosuppressive therapy			Immunosuppressive therapy		
		FFSGS+ (n=8)	FSGS- (n=37)	p	FSGS+ (n=17)	FSGS- (n=67)	p
Period of follow-up (months)		39.6±25.3	30.1±16.1	NS	23.0 (19.5, 33.0)	24.0 (18.0, 36.0)	NS
Outcome (%)	NR	3 (37.5%)	13 (35.1%)		1 (5.9%)	14 (20.9%)	
	PR	12 (25.0%)	11 (29.7%)		8 (47.1%)	24 (35.8%)	
	CR	3 (37.5%)	13 (35.1%)	NS	8 (47.1%)	29 (43.2%)	NS
Time from biopsy to PR (months)		11.3±5.0	15.5±7.7	NS	6.4±5.2	8.1±4.8	NS
Time from biopsy to CR (months)		29.3±7.0	15.4±8.9	0.025	11.8±5.2	11.0±4.5	NS
Relapse (%)		0 (0.0%)	1 (4.2%)	–	6 (37.5%)	18 (34.0%)	NS
eGFR at the latest follow-up		66.3±45.0	91.2±28.8	NS	81.9±27.4	83.0±24.7	NS
eGFR descending>30%		3 (37.5%)	2 (5.4%)	0.033	3 (17.6%)	8 (11.9%)	NS

Values are n (%), mean (SD), and median (interquartile ranges). Alb: albumin; NR: no remission; PR: partial remission; CR: complete remission; NS: no significant.

IMN patients with proteinuria levels of 3–5 g/L, the observation period could be extended to 1 year and an additional 30% remission might be observed. The FSGS+ group took an average of 29.3 months to achieve CR, which meant those patients might have had more exposure to a series of potential complications caused by nephrotic syndrome. At the latest follow-up, renal function decreased significantly in FSGS+ IMN. It inferred that the poor renal outcome may be correlated to the delayed remission of proteinuria, which may be inclined to more aggressive therapy for patients superimposed with FSGS lesions to achieve remission and protect renal function. However, physicians must have to weigh the pros and cons of different therapies to make a rational decision.

Phospholipase A_2 receptor was characterized as a major target antigen of idiopathic MN. It may be a biomarker for identifying whether it is idiopathic or not. Serum anti-PLA_2R antibodies (PLA_2R-Ab) are detected in a majority of patients with IMN, and the antibody titer is associated with disease activity and prognosis. Those who had low levels of anti-PLA_2R antibodies were prone to developing remission within a shorter period[21-24]. There are few studies on PLA_2R and FSGS combined IMN. According to our study, both groups had a high positive rate. While 75.0% of FSGS+ patients showed PLA_2R positive, which was significantly lower compared with the FSGS- group. The result may indicate that PLA_2R perhaps may not completely explain the pathogenesis of the FSGS lesion.

Our study has several limitations. First, as it is a retrospective study, some patients were lost during the follow-up, which may cause a selective bias. The follow-up sample size of the FSGS+ group receiving non-immunosuppressive therapy was too small to draw stronger conclusions, so our analyses should be treated with caution, and further work with larger sample sizes is warranted. Second, in our study, we describe a more severe disease status of IMN with FSGS lesions, yet we did not have enough samples to further analyze the relationship between prognosis and different types of FSGS lesions, and the

mechanism underlying the FSGS lesion remains unclear. More research is still needed.

CONCLUSION

Idiopathic membranous nephropathy with FSGS lesions presents more severe nephrotic syndrome and worse renal function at baseline. Renal function may decline faster, especially for those who receive non-immunosuppressive therapy, which may indicate that more aggressive treatment to use immunosuppressants to achieve proteinuria remission and protect renal function should be considered for IMN patients with FSGS lesions.

AUTHORS' CONTRIBUTIONS

PC: Conceptualization, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **QX:** Investigation, Methodology, Resources, Writing – original draft. **SL:** Writing – review & editing. **XL**: Methodology. **CMH:** Project administration, Supervision. **LW**: Project administration, Supervision**.**

REFERENCES

1. Pan X, Xu J, Ren H, Zhang W, Xu Y, Shen P, et al. Changing spectrum of biopsy-proven primary glomerular diseases over the past 15 years: a single-center study in China. Contrib Nephrol. 2013;181:22-30. https://doi.org/10.1159/000348638
2. Beck LH, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(1):11-21. https://doi.org/10.1056/NEJMoa0810457
3. Troyanov S, Roasio L, Pandes M, Herzenberg AM, Cattran DC. Renal pathology in idiopathic membranous nephropathy: a new perspective. Kidney Int. 2006;69(9):1641-8. https://doi.org/10.1038/sj.ki.5000289
4. Shiiki H, Saito T, Nishitani Y, Mitarai T, Yorioka N, Yoshimura A, et al. Prognosis and risk factors for idiopathic membranous nephropathy with nephrotic syndrome in Japan. Kidney Int. 2004;65(4):1400-7. https://doi.org/10.1111/j.1523-1755.2004.00518.x
5. Cattran DC, Pei Y, Greenwood CM, Ponticelli C, Passerini P, Honkanen E. Validation of a predictive model of idiopathic membranous nephropathy: its clinical and research implications. Kidney Int. 1997;51(3):901-7. https://doi.org/10.1038/ki.1997.127
6. Cattran DC, Pei Y, Greenwood C. Predicting progression in membranous glomerulonephritis. Nephrol Dial Transplant. 1992;7(Suppl 1):48-52. PMID: 1337182
7. Horvatic I, Ljubanovic DG, Bulimbasic S, Knotek M, Prkacin I, Tisljar M, et al. Prognostic significance of glomerular and tubulointerstitial morphometry in idiopathic membranous nephropathy. Pathol Res Pract. 2012;208(11):662-7. https://doi.org/10.1016/j.prp.2012.08.004
8. Churg J, Ehrenreich T. Membranous nephropathy. Perspect Nephrol Hypertens. 1973;1(Pt 1):443-8. PMID: 4536436
9. Yamamoto S, Inaba S, Yoshida R, Takahashi T, Ishihara S, Sakai Y, et al. Clinicopathological characteristics of the focal and segmental form of idiopathic membranous nephropathy: comparison with the typical form of this disease. Acta Paediatr Jpn. 1997;39(3):349-53. https://doi.org/10.1111/j.1442-200x.1997.tb03751.x
10. Wakai S, Magil AB. Focal glomerulosclerosis in idiopathic membranous glomerulonephritis. Kidney Int. 1992;41(2):428-34. https://doi.org/10.1038/ki.1992.59
11. Damme B, Tardanico R, Vanrenterghem Y, Desmet V. Adhesions, focal sclerosis, protein crescents, and capsular lesions in membranous nephropathy. J Pathol. 1990;161(1):47-56. https://doi.org/10.1002/path.1711610109
12. Morita M, Mii A, Shimizu A, Yasuda F, Shoji J, Masuda Y, et al. Glomerular endothelial cell injury and focal segmental glomerulosclerosis lesion in idiopathic membranous nephropathy. PLoS One. 2015;10(4):e0116700. https://doi.org/10.1371/journal.pone.0116700
13. Lee HS, Koh HI. Nature of progressive glomerulosclerosis in human membranous nephropathy. Clin Nephrol. 1993;39(1):7-16. PMID: 8428410
14. Heeringa SF, Branten AJ, Deegens JK, Steenbergen E, Wetzels JF. Focal segmental glomerulosclerosis is not a sufficient predictor of renal outcome in patients with membranous nephropathy. Nephrol Dial Transplant. 2007;22(8):2201-7. https://doi.org/10.1093/ndt/gfm188
15. Gupta R, Sharma A, Mahanta PJ, Jacob TG, Agarwal SK, Roy TS, et al. Focal segmental glomerulosclerosis in idiopathic membranous glomerulonephritis: a clinico-pathological and stereological study. Nephrol Dial Transplant. 2010;25(2):444-9. https://doi.org/10.1093/ndt/gfp521
16. Dumoulin A, Hill GS, Montseny JJ, Meyrier A. Clinical and morphological prognostic factors in membranous nephropathy: significance of focal segmental glomerulosclerosis. Am J Kidney Dis. 2003;41(1):38-48. https://doi.org/10.1053/ajkd.2003.50015
17. Chen Y, Tang L, Feng Z, Cao X, Sun X, Liu M, et al. Pathological predictors of renal outcomes in nephrotic idiopathic membranous nephropathy with decreased renal function. J Nephrol. 2014;27(3):307-16. https://doi.org/10.1007/s40620-014-0057-0
18. Li J, Chen B, Gao C, Huang J, Wang Y, Zhang S, et al. Clinical and pathological features of idiopathic membranous nephropathy with focal segmental sclerosis. BMC Nephrol. 2019;20(1):467. https://doi.org/10.1186/s12882-019-1641-2
19. He HG, Wu CQ, Ye K, Zeng C, Huang YY, Luo SW, et al. Focal segmental glomerulosclerosis, excluding atypical lesion, is a predictor of renal outcome in patients with membranous nephropathy: a retrospective analysis of 716 cases. BMC Nephrol. 2019;20(1):328. https://doi.org/10.1186/s12882-019-1498-4
20. Wang H, Wan C, Jiang M, Zhang C. Non-glomerular tip lesion focal segmental glomerulosclerosis as a negative predictor in idiopathic membranous nephropathy. Curr Med Sci. 2022;42(5):1007-14. https://doi.org/10.1007/s11596-022-2628-y

21. Thokhonelidze I, Maglakelidze N, Sarishvili N, Kasradze T, Dalakishvili K. Association of anti-phospholipasea2-receptor antibodies with clinical course of idiopathic membranous nephropathy. Georgian Med News. 2015;(241):(241):49-53. PMID: 25953939

22. Hofstra JM, Debiec H, Short CD, Pellé T, Kleta R, Mathieson PW, et al. Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. J Am Soc Nephrol. 2012;23(10):1735-43. https://doi.org/10.1681/ASN.2012030242

23. Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, Nikam M, et al. Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. Kidney Int. 2013;83(5):940-8. https://doi.org/10.1038/ki.2012.486

24. Hoxha E, Thiele I, Zahner G, Panzer U, Harendza S, Stahl RA. Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. J Am Soc Nephrol. 2014;25(6):1357-66. https://doi.org/10.1681/ASN.2013040430

ORIGINAL ARTICLE

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Prevalence of anticipatory nausea and vomiting in breast cancer patients undergoing highly emetogenic chemotherapy

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SUMMARY

OBJECTIVE: Anticipatory nausea and vomiting are unpleasant symptoms observed before undergoing chemotherapy sessions. Less is known about the occurrence of symptoms since the advent of the new neurokinin-1 antagonist.

METHODS: This prospective cohort study was performed at a single Brazilian Institution. This study included breast cancer patients who received doxorubicin and cyclophosphamide chemotherapy and an appropriate antiemetic regimen (dexamethasone 10 mg, palonosetron 0.56 mg, and netupitant 300 mg in the D1 followed by dexamethasone 10 mg 12/12 h in D2 and D4). Patients used a diary to record nausea, vomiting, and use of rescue medication in the first two cycles of treatment. The prevalence of anticipatory nausea and vomiting was assessed before chemotherapy on day 1 of C2.

RESULTS: From August 4, 2020, to August 12, 2021, 60 patients were screened, and 52 patients were enrolled. The mean age was 50.8 (28–69) years, most had stage III (53.8%), and most received chemotherapy with curative intent (94%). During the first cycle, the frequency of overall nausea and vomiting was 67.31%, and that of severe nausea and vomiting (defined as grade>4 on a 10-point visual scale or use of rescue medication) was 55.77%. Ten patients had anticipatory nausea and vomiting (19.23%). The occurrence of nausea and vomiting during C1 was the only statistically significant predictor of anticipatory nausea and vomiting (OR=16, 95%CI 2.4–670.9, p=0.0003).

CONCLUSION: The prevalence of anticipatory nausea is still high in the era of neurokinin-1 antagonists, and failure of antiemetic control in C1 remains the main risk factor. All efforts should be made to control chemotherapy-induced nausea or nausea and vomiting on C1 to avoid anticipatory nausea.

KEYWORDS: Nausea. Vomiting. Antineoplastic protocols.

INTRODUCTION

Chemotherapy-induced nausea and vomiting (CINV) are common treatment-related side effects that worsen the quality of life and adherence and may lead to dose reductions or discontinuation[1,2]. Approximately 70–80% of patients receiving chemotherapy are at risk of developing nausea and vomiting[3]. In the past, they were unavoidable side effects, leading patients to postpone or refuse potentially curative treatments. Since the late 1980s, drugs such as dopamine and 5-HT3 receptor antagonists, and later neurokinin-1 antagonists, have enabled greater control of CINV[1,2,4,5].

The main risk factor for CINV is the emetogenic potential of the chemotherapeutic agent. Chemotherapy regimens are classified as having high (>90% chance of nausea and vomiting), moderate (30–90%), low (10–30%), and minimal emetogenic potential (<10%)[2,4,6]. Other risk factors related to CINV are young age, female sex, history of nausea and vomiting during pregnancy, and vomiting in previous chemotherapy, while alcohol consumption is a protective factor[1,2,7]. Multiple mechanisms are involved in the appearance of CINV[8], which differ according to when the condition manifests: acute, late, and anticipatory. The acute period is the first 24 h of antineoplastic drug administration, while the late period begins after 24 h after chemotherapy administration, usually 2–3 days after infusion. Anticipatory nausea and/or vomiting occurs when an adverse memory triggers nausea and/or vomiting before chemotherapy administration[1,2].

Anticipatory nausea and vomiting (ANV), also referred to as conditioned (learned) nausea and vomiting to chemotherapy, are described in approximately 25% of chemotherapy patients. The risk tends to increase with the number of cycles received and may persist after the end of chemotherapy. Most studies on ANV were performed before the introduction of neurokinin-1 inhibitors.

The primary objective of this study was to evaluate the prevalence of ANV in cancer patients undergoing highly emetogenic chemotherapy who received adequate antiemetic

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prophylaxis, including corticosteroids, 5-HT3 antagonists, and neurokinin-1 inhibitors. The secondary objective was to find predictors of ANV.

METHODS

A prospective cohort study was conducted in an oncology center located in the city of São Paulo, where patients were recruited from August 2020 to August 2021. Eligible patients were adults (≥18 years old) with breast cancer who had never received highly emetogenic chemotherapy and were scheduled to receive at least two cycles. We excluded patients who were unable to complete the diary or made incorrect use of emetic prophylaxis, patients who reported symptoms of nausea or vomiting before the first cycle of treatment, and patients who presented with a pathology or condition that caused emesis (central nervous system metastasis, gastrointestinal obstruction, metabolic or electrolyte disorders, alcohol abuse, or opioid use).

For data collection, a questionnaire prepared by the researchers was used with information containing sociodemographic characteristics, clinical data, and data referring to chemotherapy treatment, in addition to the regimen used to prevent nausea and vomiting. The occurrence of nausea and vomiting at home was assessed using a diary in which the patients recorded each episode and the use of rescue antiemetic medications, in addition to grading the intensity of symptoms according to the visual analog scale (VAS), to be completed after each cycle of chemotherapy, for two consecutive cycles. Immediately before the second cycle of chemotherapy, patients were evaluated for the occurrence of ANV, defined as the occurrence of nausea and/or vomiting up to 24 h before the infusion of chemotherapy.

The study was conducted in accordance with the ethical principles of international guidelines such as the Declaration of Helsinki and the ICH-GCPC Guideline and was approved by the research ethics committee of the institution (Opinion No. 4128120/ClinicalTrials.gov number, NCT04785495). All patients signed an informed consent form.

Categorical variables were described according to frequency distribution, and continuous variables were described with summary measures (mean, standard deviation, median, minimum, and maximum). Fisher's test was used to evaluate the association of clinical characteristics with the occurrence of ANV. The McNemar test was used to evaluate the association between nausea and vomiting in the first cycle and the occurrence of ANV. The analyses were performed using the Stata 17 software, and a significance level of 5% was considered.

To calculate the sample size needed, we estimated that the prevalence of ANV would be 20%. We estimated that the inclusion of 50 patients in the triage phase (first cycle) would lead to the occurrence of nausea and vomiting in the second cycle in 10 patients, which would allow us to run univariate analyses to find predictive factors of ANV. Assuming a 20% loss to follow-up, we screened 60 patients.

RESULTS

Between August 2020 and August 2021, 60 patients were recruited, of whom 52 were considered eligible and were included in the study. All patients received the same chemotherapy regimen, doxorubicin (60 mg/m²) and cyclophosphamide (600 mg/m²). The regimen used to prevent nausea and vomiting was composed of dexamethasone 10 mg, palonosetron 0.56 mg, and netupitant 300 mg in the D1 followed by dexamethasone 10 mg 12/12 h in D2 and D4.

The sociodemographic and clinical characteristics of the patients are shown in Table 1. The median age was 50.2 years (interval 28–69), and all patients were female. Regarding the risk factors for nausea and vomiting, 43.48% reported a history of nausea or vomiting during pregnancy.

Acute nausea and vomiting (in the first 24 h after infusion) in the first cycle were reported by 30 patients (57.69%), with rescue medication use in 40.38% of the sample. Delayed nausea and vomiting, after 24 h, were recorded in 57.69% of patients, with the need for rescue agents in 42.31% of patients. Overall, 35 patients (67.31%) reported acute or delayed nausea and vomiting, and 55.77% of the patients rated the symptoms as ≥4 (moderate) or took a rescue drug (Table 2).

The prevalence of ANV was 19.23% (n=10). The occurrence of nausea and vomiting during the first cycle was the only factor statistically associated with the onset of ANV symptoms (OR=16, 95%CI 2.4–670.9, p=0.0003; Table 3). We did not observe an association between age, history of nausea and vomiting in previous pregnancies, treatment intention, or regimen, and the occurrence of ANV.

DISCUSSION

The objective of this study was to measure the prevalence of ANV in patients undergoing highly emetogenic chemotherapy and who used optimal prophylaxis. We also sought the factors associated with the onset of symptoms.

Our results suggest that ANV remains a prevalent problem, as it was reported by approximately 20% of the patients in the study. The data presented indicate that despite antiemetic prophylaxis with 5-HT3 antagonists and NK1 inhibitors, considered the gold standard by international protocols[4,9,10],

Table 1. Sociodemographic and clinical characteristics.

	No.	%		No.	%
Gender			**Age (years)**		
Woman	60	100	Median	50.2	–
			Range	28–69	
Number of children			**Marital status**		
None	6	11.54	Single	11	21.15
At least 1	46	88.46	Married/stable union	28	53.85
			Separated/widowed	13	25
Molecular characteristics			**Staging**		
RE positive	22	42.31	I	1	1.92
RE negative	30	57.69	II	20	38.46
HER 2 positive	38	73.08	III	28	53.85
HER 2 negative	14	26.92	IV	3	5.77
Treatment			**Chemotherapy regime**		
Neoadjuvant/adjuvant	49	94.23	AC*	31	59.62
Palliative	3	5.77	Dense dose AC**	21	40.38

RE: estrogen receptor; HER2: human epidermal growth factor receptor type 2; RE: estrogen receptor; HER2: human epidermal growth factor receptor type 2; *doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2), every 3 weeks; **doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2), every 2 weeks.

Table 2. Occurrence of acute and delayed nausea and vomiting in the first cycle.

	No.	%
Acute		
Yes	30	57.69
No	22	42.31
Use of rescue drug		
Yes	21	40.38
No	31	59.62
Late		
Yes	30	57.69
No	22	42.31
Use of rescued rug		
Yes	22	42.31
No	30	57.69
Acute or late		
Yes	35	67.31
No	17	32.69
≥4 or rescue use		
Yes	29	55.77
No	23	44.23

Table 3. Relationship between the occurrence of nausea/vomiting in C1 and anticipatory nausea/vomiting before C2.

	Pre-C2 anticipatory nausea/vomiting		OR	p-value
Nausea/ vomiting in C1	**Yes**	**No**		
Yes	9	16	16	0.0003
No	1	26	95%CI 2.4–670.9	

the overall management of these events remains a challenge that deserves attention. The prevalence of ANV found in the study was slightly higher than that reported in some studies[9,11].

Overall, women and young age are both risk factors for this symptomology[1,2,6]. However, such variations in the prevalence of ANV have already been found in previous studies, which is explained by the differences between the populations and the chemotherapy regimens evaluated in each study[9-12].

In this study, nausea and vomiting in the first cycle were the only significant predictors of ANV before the second cycle (p-value 0.0003). Thus, the control of nausea and vomiting from the first cycle is essential to reduce the prevalence of ANV before the second cycle and possibly in later cycles.

There is a well-established relationship between the non-control of CINV in early cycles and the onset of anticipatory symptoms in later cycles. This relationship is explained by the conditioning component and is particularly linked to psychological processes or previous experiences with the symptoms of ANV[13-15]. The use of adjuvant therapies based on behavioral or psychological interventions, such as music therapy, mindfulness,

acupuncture, inhaled aromatherapy, and hypnosis, can help control anticipatory symptoms[9,10,16-19], which reinforces the role of psychological processes in the emergence of these symptoms. It is believed that drug therapy, then, may occupy an adjuvant position and that when it is used in combination with behavioral therapies for the management of CIVN, we may achieve higher rates of control of ANV.

Historically, nausea and vomiting have been studied concomitantly considering the same physiological mechanisms. However, vomiting, when compared with nausea, has been better controlled and the evolution of new therapies suggests that mechanisms for the development of symptoms are different[4].

Other epidemiological and clinical variables were not associated with ANV here. A history of nausea and vomiting during pregnancy was associated as a risk factor for the onset of ANV in previous studies. It could be that the improvement of antiemetic therapy, including for symptom control during pregnancy may explain this finding in our study. Intention-to-treat approach, education level, and race are no longer considered predictive factors of symptoms, and indeed, we observed no relationship between these factors and the onset of ANV.

Another relevant aspect identified during this study is the way in which patients who initiated treatment understood the information we gave them. Professionals and cancer centers should strengthen surveillance for the identification of potential flaws that contribute to the emergence of CINV. For example, lack of knowledge of rescue antiemetic therapy, lack of access to drugs, and inappropriate use of therapies are relatively simple improvement points for controlling such symptoms.

Guideline recommendations for the management of anticipatory NV focus on its prevention through the use of optimal antiemetic therapy for each cycle of chemotherapy. Therefore, it is concluded that nausea and vomiting are more easily prevented than treated. One of the limitations of this study was the small number of patients analyzed, all of whom took the same chemotherapy regimen. We also considered a limiting factor the fact that the symptoms were analyzed only before cycle 2 and were not studied in later cycles, which may have led us to underestimate the occurrence of nausea and vomiting in our population.

CONCLUSION

The prevalence of ANV is still high even in the era of neurokinin-1 inhibitors. Failure of antiemetic control in the first cycle remains the main risk factor associated with the onset of symptoms. Therefore, every effort should be made to control nausea or CINV in the first cycle to prevent the symptoms prophylactically. More research is needed to evaluate other risk factors in the emergence of ANV and the impact of ANV on patients' quality of life.

AUTHORS' CONTRIBUTIONS

RBA: Data curation, Investigation, Project administration, Writing – original draft. **CVR:** Data curation, Investigation, Writing – original draft. **AMTDY:** Conceptualization, Methodology, Project administration, Supervision. **FJSMC:** Formal Analysis, Methodology, Supervision, Resources, Writing – original draft.

REFERENCES

1. Adel N. Overview of chemotherapy-induced nausea and vomiting and evidence-based therapies. Am J Manag Care. 2017;23(14 Suppl):S259-65. PMID: 28978206
2. Navari RM, Aapro M. Antiemetic prophylaxis for chemotherapy-induced nausea and vomiting. N Engl J Med. 2016;374(14):1356-67. https://doi.org/10.1056/NEJMra1515442
3. Henson LA, Maddocks M, Evans C, Davidson M, Hicks S, Higginson IJ. Palliative care and the management of common distressing symptoms in advanced cancer: pain, breathlessness, nausea and vomiting, and fatigue. J Clin Oncol. 2020;38(9):905-14. https://doi.org/10.1200/JCO.19.00470
4. Razvi Y, Chan S, McFarlane T, McKenzie E, Zaki P, Angelis C, et al. ASCO, NCCN, MASCC/ESMO: a comparison of antiemetic guidelines for the treatment of chemotherapy-induced nausea and vomiting in adult patients. Support Care Cancer. 2019;27(1):87-95. https://doi.org/10.1007/s00520-018-4464-y
5. Heckroth M, Luckett RT, Moser C, Parajuli D, Abell TL. Nausea and vomiting in 2021: a comprehensive update. J Clin Gastroenterol. 2021;55(4):279-99. https://doi.org/10.1097/MCG.0000000000001485
6. Lindley CM, Hirsch JD, O'Neill CV, Transau MC, Gilbert CS, Osterhaus JT. Quality of life consequences of chemotherapy-induced emesis. Qual Life Res. 1992;1(5):331-40. https://doi.org/10.1007/BF00434947
7. Aapro M, Jordan K, Feyer P. Pathophysiology of chemotherapy-induced nausea and vomiting. In: Prevention of nausea and vomiting in cancer patients. London: Springer Healthcare Ltd; 2015.
8. Wickham RJ. Revisiting the physiology of nausea and vomiting-challenging the paradigm. Support Care Cancer. 2020;28(1):13-21. https://doi.org/10.1007/s00520-019-05012-8
9. Qureshi F, Shafi A, Ali S, Siddiqui N. Clinical predictors of anticipatory emesis in patients treated with chemotherapy at a tertiary care cancer hospital. Pak J Med Sci. 2016;32(2):337-40. https://doi.org/10.12669/pjms.322.9493
10. Hesketh PJ, Bohlke K, Lyman GH, Basch E, Chesney M, Clark-Snow RA, et al. Antiemetics: American society of clinical oncology guideline update. J Clin Oncol. 2020;38:2782-97. https://doi.org/10.1200/JCO.2015.64.3635
11. Roscoe JA, Morrow GR, Aapro MS, Molassiotis A, Olver I. Anticipatory nausea and vomiting. Support Care Cancer. 2011;19(10):1533-8. https://doi.org/10.1007/s00520-010-0980-0

12. Chan A, Kim HK, Hsieh RK, Yu S, Lima Lopes G, Su WC, et al. Incidence and predictors of anticipatory nausea and vomiting in Asia Pacific clinical practice--a longitudinal analysis. Support Care Cancer. 2015;23(1):283-91. https://doi.org/10.1007/s00520-014-2375-0

13. Gupta K, Walton R, Kataria SP. Chemotherapy-induced nausea and vomiting: pathogenesis, recommendations, and new trends. Cancer Treat Res Commun. 2021;26:100278. https://doi.org/10.1016/j.ctarc.2020.100278

14. Kamen C, Tejani MA, Chandwani K, Janelsins M, Peoples AR, Roscoe JA, et al. Anticipatory nausea and vomiting due to chemotherapy. Eur J Pharmacol. 2014;722:172-9. https://doi.org/10.1016/j.ejphar.2013.09.071

15. Stockhorst U, Spennes-Saleh S, Körholz D, Göbel U, Schneider ME, Steingrüber HJ, et al. Anticipatory symptoms and anticipatory immune responses in pediatric cancer patients receiving chemotherapy: features of a classically conditioned response? Brain Behav Immun. 2000;14(3):198-218. https://doi.org/10.1006/brbi.1999.0581

16. Hunter JJ, Maunder RG, Sui D, Esplen MJ, Chaoul A, Fisch MJ, et al. A randomized trial of nurse-administered behavioral interventions to manage anticipatory nausea and vomiting in chemotherapy. Cancer Med. 2020;9(5):1733-40. https://doi.org/10.1002/cam4.2863

17. Marchioro G, Azzarello G, Viviani F, Barbato F, Pavanetto M, Rosetti F, et al. Hypnosis in the treatment of anticipatory nausea and vomiting in patients receiving cancer chemotherapy. Oncology. 2000;59(2):100-4. https://doi.org/10.1159/000012144

18. Jing S, Zhang A, Chen Y, Shen C, Currin-McCulloch J, Zhu C. Mindfulness-based interventions for breast cancer patients in China across outcome domains: a systematic review and meta-analysis of the Chinese literature. Support Care Cancer. 2021;29(10):5611-21. https://doi.org/10.1007/s00520-021-06166-0

19. Toniolo J, Delaide V, Beloni P. Effectiveness of inhaled aromatherapy on chemotherapy-induced nausea and vomiting: a systematic review. J Altern Complement Med. 2021;27(12):1058-69. https://doi.org/10.1089/acm.2021.0067

ORIGINAL ARTICLE

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Fecal leukocyte frequency in children with acute viral gastroenteritis: a single-center experience

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SUMMARY

OBJECTIVE: Our objective was to determine the frequency of rotavirus, adenovirus, and rota-adenovirus co-infections and investigate the fecal leukocyte rate associated with these infections in patients with gastroenteritis.

METHODS: This is a retrospective study. We identified patients who were admitted to the pediatric emergency department with acute gastroenteritis and had their stool samples tested for rotavirus and/or adenovirus antigens. Among them, we determined the individuals who underwent stool microscopy tests on the same day and recorded their results.

RESULTS: A total of 1,577 patients who underwent testing for rotavirus and/or adenovirus antigens in their stool samples were identified. Among these patients, 583 individuals had concurrent fecal microscopy results. The prevalence of solely rotavirus antigen positivity was 16.4%, solely adenovirus antigen positivity was 2.9%, and rota-adenovirus co-infections were detected in 1.8% of the children. The fecal leukocyte rates in children infected with rotavirus, adenovirus, and rota-adenovirus co-infections were 4.8, 13.3, and 88.9%, respectively.

CONCLUSION: The presence of fecal leukocytes was detected at a high rate in cases of viral gastroenteritis, especially in rota-adenovirus co-infections. Therefore, clinicians should not consider only bacterial pathogens in the presence of fecal leukocytes.

KEYWORDS: Rotavirus. Adenovirus. Virus. Leukocyte. Children.

INTRODUCTION

Acute gastroenteritis is a common disease affecting all age groups, especially children. It is mostly caused by viral agents[1,2]. Every year, viral gastroenteritis is the cause of death in more than 200,000 children worldwide[3]. Rotavirus and adenovirus are common causes of viral gastroenteritis in children[4].

Rotavirus infection is confirmed as the important cause of severe diarrhea in children under 5 years of age. However, it affects all age groups worldwide. According to the reports, 45% of rotavirus deaths in 2016 occurred in people aged 5 years and older[5]. It is estimated that rotavirus caused the deaths of about 128,500 children under the age of 5 years and more than 258 million episodes of diarrhea worldwide in the same year[6].

Rotavirus is known to cause noninflammatory and secretory, malabsorptive diarrhea by infecting intestinal villus enterocytes and enteroendocrine cells[2]. Despite widespread tissue damage and cell death in rotavirus infection, it is noteworthy that the inflammatory reaction is very limited. It has been reported that this may be due to the anti-inflammatory effects of rotavirus-induced cholinergic stimulation. Thus, tissue damage is also limited in this infection[7].

Adenovirus is another important cause of gastroenteritis in children. It causes approximately 2–15% of acute diarrheal episodes in children[8]. Adenoviruses are subdivided into over a hundred types based on their biological and genetic characteristics. Adenovirus subgroups 40 and 41 are frequently responsible for gastroenteritis. The virus most commonly affects children younger than 2 years in low- and middle-income countries[9].

Infectious diarrheas are classified as inflammatory and non-inflammatory according to their pathogenesis and clinical findings. One of the differences in this distinction is the presence of leukocytes in the stool accompanying inflammatory diarrhea[10]. Both rotavirus and adenovirus are non-invasive pathogens. Therefore, they are considered non-inflammatory agents of diarrhea. Nevertheless, they can interact with the enteric cells of the host to remove an inflammatory response so mild fecal leukocytes may be detected in feces[11]. However, there is limited data on the level of fecal leukocytes as inflammatory response markers in children infected with these agents.

The diarrhea management guidelines of the Infectious Diseases Society of America (IDSA)[12] do not recommend fecal leukocyte tests for diagnosis and treatment planning.

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However, the detection of leukocytes in the fecal analysis still influences physicians' decisions regarding antibiotic prescription[13].

Therefore, this study was conducted to investigate the presence and quantity of fecal leukocytes in children infected with rotavirus, adenovirus, and co-infections of rotavirus and adenovirus. Additionally, the frequency of fecal leukocytes was compared among these viral agents. This study aimed to bring clinicians' attention to the potential occurrence of fecal leukocytes in viral infections.

METHODS

This study was approved by the local Ethics Committee in accordance with the Declaration of Helsinki (No. E-20/370).

We conducted a retrospective, cross-sectional study in the pediatric emergency department of a tertiary care hospital. We reviewed the medical records of children under the age of 18 years who were diagnosed with acute gastroenteritis between April 01, 2017, and December 31, 2020. Patients with rotavirus and adenovirus antigen (AdVA) test results on the same day were included in the study. Their age, gender, stool antigen test results, and application seasons were recorded.

In the second stage of the study, patients who underwent stool microscopy simultaneously with rotavirus and AdVA tests were identified and their results were recorded. Based on the results of stool antigen tests, the children were divided into four groups. Group 1: negative for both rotavirus antigen (RVA) and AdVA, group 2: negative for RVA and positive for AdV, group 3: positive for RVA and negative for AdVA, and group 4: positive for both RVA and AdVA.

The presence of rotavirus and AdVAs in fresh stool samples was investigated using a commercial immunochromatographic test kit (AV-RV Combo test, Rapid Diagnostic Test, Turkey). The test kit utilized a nitrocellulose membrane coated with monoclonal anti-rotavirus and anti-adenovirus antibodies as the scanning element. The test method is a rapid, qualitative approach based on the principle of the formation of antigen-antibody complexes on the test region (T1–T2) of the test card, resulting in a visible pink line after the binding of RVA and/or AdVA to the antibody-coated membrane following a 10–15-min incubation period, according to the manufacturer's recommendations. The presence of ≥5/mL leukocytes in stool samples was accepted as a sign of inflammatory diarrhea[14].

Statistical analysis

Categorical variables were expressed as numbers and percentages. The chi-square test for categorical variables was used to compare the distributions of age, gender, and season characteristics between the rotavirus and AdVA positivity groups. The chi-square test was also used to compare the distributions of age, gender, and season in the rotavirus, adenovirus, and coinfection antigen positivity groups. Here, the Bonferroni method was used to compare column proportions, adjusted p-value were obtained, and the findings are presented with letters in the relevant table. All analyses were performed using the IBM SPSS Statistics Version 20.0 statistical software package. All tests were two-sided, and the statistical level of significance for all tests was considered to be 0.05.

RESULTS

A total of 1,577 cases were included in the study. RVA positivity was detected in 288 (18.3%) patients, and AdVA positivity was detected in 74 (4.7%) patients. Gender, age, and seasonal distribution according to adenovirus and rotavirus positivity are given in Table 1. Adenovirus positivity is higher in females (p=0.003), but it does not show a statistically significant difference according to age and season (p=0.884 and p=0.624, respectively). Rotavirus positivity did not differ according to gender (p=0.192), but it showed statistically significant differences according to age and season (both p<0.001). While rotavirus positivity is high in the 13–24-month age group, it is low in children older than 12 years. In addition, the rotavirus positivity is higher in spring, followed by winter, autumn, and summer.

In our study, only adenovirus or rotavirus positivity and both antigen positivity and negativity were also evaluated. Only RVA positivity was detected in 259 (16.4%), only AdVA positivity was detected in 45 (2.9%), and rota-adenovirus co-infections were detected in 29 (1.8%) of the children. The distribution of characteristics according to these groups is given in Table 2.

In addition, stool microscopy evaluation was also performed in 583 (36.9%) of these patients, and the distribution of erythrocytes and leukocytes is summarized in Table 3. Fecal leukocyte rates in children infected with rotavirus, adenovirus, and rota-adenovirus co-infections were 4.8, 13.3, and 88.9%, respectively.

DISCUSSION

In this study, we compared the fecal leukocyte rates in children diagnosed with acute gastroenteritis in the pediatric emergency clinic who had rotavirus, adenovirus, and rota-adenovirus co-infections as part of the disease etiology. The detection rates of rotavirus, adenovirus, and rota-adenovirus coinfections in children with acute gastroenteritis for whom antigen testing was requested were 16.4, 2.9, and 1.8%, respectively. Fecal leukocyte detection rates were determined most frequently in rota-adenovirus coinfections and least in rotavirus infections, inversely proportional to the frequency of gastroenteritis agents.

In line with previous studies, we also observed a higher prevalence of rotavirus compared with adenovirus in patients

with acute gastroenteritis in our study[1,15,16]. The frequency of acute gastroenteritis caused by rotavirus and adenovirus varies across different countries. We believe that this variability may be attributed to differences in detection methods, the age distribution of the tested populations, hygiene and sanitation conditions, and the inclusion or exclusion of rotavirus in the vaccination schedule.

In a study conducted in Turkey, the frequency of rotavirus, adenovirus, and rota-adenovirus co-infection was found to be 12.6, 2.6, and 0.14%, respectively[4]. These results were lower than ours. We think that this is because the age group in this study covers the 0–76 years age group. However, some studies report that the frequency of rotavirus and adenovirus is lower than this study, although it includes younger age groups[17]. We suppose that it may

Table 1. Distribution of characteristics by adenovirus and rotavirus antigen positivity.

Characteristics		Adenovirus		p	Rotavirus		p
		Negative	Positive		Negative	Positive	
		n (%)	n (%)		n (%)	n (%)	
Gender	Female	630 (41.9)	44 (59.5)	0.003	541 (42.0)	133 (46.2)	0.192
	Male	873 (58.1)	30 (40.5)		748 (58.0)	155 (53.8)	
Age	0–12 months	113 (7.5)	5 (6.8)	0.884	97 (7.5)	21 (7.3)	<0.001
	13–24 months	621 (41.3)	29 (39.2)		498 (38.6)	152 (52.8)	
	25–72 months	284 (18.9)	14 (18.9)		243 (18.9)	55 (19.1)	
	6–12 years	333 (22.2)	20 (27.0)		304 (23.6)	49 (17.0)	
	13–18 years	152 (10.1)	6 (8.1)		147 (11.45)	11 (3.8)	
Season	Spring	387 (25.7)	15 (20.3)	0.624	265 (20.6)	137 (47.6)	<0.001
	Summer	471 (31.3)	23 (31.1)		465 (36.1)	29 (10.1)	
	Autumn	380 (25.3)	23 (31.1)		357 (27.7)	46 (16.0)	
	Winter	265 (17.6)	13 (71.6)		202 (15.7)	76 (26.4)	
Total		1503 (95.3)	74 (4.7)		1289 (81.7)	288 (18.3)	

Table 2. Distribution of characteristics according to rotavirus, adenovirus, and coinfection antigen positivity.

Characteristics	Group				p
	RVA-/–/AdV-–	RVA-/–/AdV+	RVA+/AdV-–	RVA+/AdV+	
	(n=1244)	(n=45)	(n=259)	(n=29)	
Gender, n (%)					
Female	514 (41.3)[a]	27 (60.0)[b]	126 (45.3)[a]	17 (58.6)[b]	0.019
Male	730 (58.7)[a]	18 (40.0)[b]	152 (54.7)[a]	12 (41.4)[b]	
Age, n (%)					
0-–12 months	96 (7.7)[a]	1 (2.2)[a]	17 (6.6)[a]	4 (13.8)[a]	<0.001
13-–24 months	471 (37.9)[a]	27 (60.0)[b]	150 (57.9)[b]	2 (6.9)[c]	
25-–72 months	231 (18.6)[a]	12 (26.7)[a]	53 (20.5)[a]	2 (6.9)[a]	
6-–12 years	299 (24.0)[a]	5 (11.1)[a,b]	34 (13.1)[b]	15 (51.7)[c]	
13-–18 years	147 (11.8)[a,b]	0 (0.0)[b,c]	5 (1.9)[c]	6 (20.7)[a]	
Season, n (%)					
Spring	261 (21.0)[a,b]	4 (8.9)[b]	126 (48.6)[c]	11 (37.9)[a,c]	<0.001
Summer	447 (35.9)[a]	18 (40.0)[a]	24 (9.3)[b]	5 (17.2)[a,b]	
Autumn	340 (27.3)[a]	17 (37.8)[a]	40 (15.4)[b]	6 (20.7)[a,b]	
Winter	196 (15.8)[a]	6 (13.3)[a,b]	69 (26.6)[b]	7 (24.1)[a,b]	

Each subscript letter denotes a subset of groups whose column proportions do not differ significantly from each other at the 0.05 level.

Table 3. Erythrocyte and leukocyte distribution in patients with stool microscopy evaluation.

	RVA−/AdVA− (n=475)	RVA−/AdVA+ (n=15)	RVA+/AdVA− (n=84)	RVA+/AdVA+ (n=9)	p
Erythrocyte, n (%)					
No	425 (89.5)[a]	15 (100.0)[a]	81 (96.4)[a]	3 (33.3)[b]	<0.001
<5	19 (4.0)[a]	–[a,b]	1 (1.2)[a]	2 (22.2)[b]	
>5	31 (6.5)[a]	–[a]	2 (2.4)[a]	4 (44.4)[b]	
Leukocyte, n (%)					
No	324 (68.2)[a]	12 (80.0)[a,b]	75 (89.3)[b]	1 (11.1)[c]	<0.001
<5	43 (9.1)[a]	1 (6.7)[a]	5 (6.0)[a]	–[a]	
>5	108 (22.7)[a]	2 (13.3)[a,b]	4 (4.8)[b]	8 (88.9)[c]	

AdVA: adenovirus antigen; RVA: rotavirus antigen. Each subscript letter denotes a subset of groups whose column proportions do not differ significantly from each other at the 0.05 level.

be related to hygiene conditions or vaccination. In the other study, rotavirus, adenovirus, and rota-adenovirus co-infection frequencies were detected at 22, 10.3, and 1.1%, respectively. This study was conducted with children under 5 years of age with acute gastroenteritis[18]. We think that the more frequent detection of viral antigen positivity compared with our study is due to the greater susceptibility to viral infections in this age group. However, the frequency of co-infection rates was higher in our study. However, when we evaluated the frequency of co-infection rates under 6 years of age, we detected the frequency as 0.5% in our study, which was lower compared with the other study.

Viral gastroenteritis can lead to a wide range of clinical conditions, ranging from asymptomatic infections to severe dehydration. Clinically differentiating between viral and bacterial etiology in patients with gastroenteritis is not always possible. However, the treatment plan varies depending on the viral or bacterial etiology. For instance, in cases of invasive gastroenteritis infections caused by bacteria, appropriate antibiotic therapy is crucial to minimize mortality and morbidity. Stool cultures are commonly utilized to identify bacterial agents. However, this method typically yields results after several days and has limited sensitivity in detecting bacterial pathogens. One of the debated methods for identifying intestinal inflammation in infectious gastroenteritis is the presence of fecal leukocytes. Fecal leukocytes can increase enteroinvasive gastrointestinal infections. However, fecal leucocytes may be observed in some cases of viral diarrhea[19]. In our study, fecal leukocyte rates detected in children infected with rotavirus, adenovirus, and rota-adenovirus co-infections were 4.8, 13.3, and 88.9%, respectively. There was a remarkable increase in fecal leukocyte rates in rota-adenovirus co-infections. In a study, it was reported that the frequency of rotavirus was 10.7%, adenovirus was 5%, and rota-adenovirus co-infections were 1.4% among children under 10 years of age. In the same study, the frequency of fecal leukocytes was detected at 3.3% in patients with only rotavirus, 22% in those with only adenovirus, and 48.4% in those with co-infection with rota-adenovirus[20]. In this study, similar to our findings, fecal leukocytes were most frequently detected in rota-adenovirus coinfections and least frequently in rotavirus infections.

The mechanisms underlying viral coinfection have not yet been fully elucidated[21]. We propose a hypothesis that rotavirus infections may exhibit specific mechanisms that increase the likelihood of secondary infections, as supported by the observed higher frequency of coinfections during periods of elevated rotavirus prevalence. When we evaluated the seasonal variability for the caused agent, rotavirus positivity was found most frequently in the spring, adenovirus positivity in the autumn, and rota-adenovirus co-infection most often in the spring. Xiao et al., found that rotavirus positivity was most common in winter, adenovirus in summer, and rota-adenovirus co-infection most commonly in winter. In both Xiao's[21] study and our own, the season characterized by a high prevalence of rota-adenovirus coinfection coincided with the season when rotavirus was frequently detected.

Zaraket et al., investigated the frequencies of rotavirus, adenovirus, and rota-adenovirus co-infections and found them to be 17, 6.5, and 2.1%, respectively. Furthermore, they observed elevated levels of inflammatory markers such as white blood cells, absolute neutrophil count, and C-reactive protein in the co-infection group compared with the groups with rotavirus or adenovirus alone[15]. In another study, the detection rates of fecal leukocytes in gastroenteritis were found to be 19% in the bacterial pathogens and 7.9% in the virus agents, and this difference was not found to be significant in detecting bacterial and virus differentiation in the etiology of gastroenteritis[22].

This study has several limitations. First, it is not possible to definitively exclude bacterial infections in children diagnosed with inflammatory diarrhea due to the lack of stool culture results. Second, as is known, the presence of leukocytes

in feces may be also an indicator of antibiotic-associated colitis, pseudomembranous colitis, and idiopathic inflammatory bowel diseases. Although patient files are examined in detail in terms of these diseases, we cannot be sure that they have been completely ruled out because our study is retrospective. Third, this study is single-centered, so its generalizability is low.

CONCLUSION

This study showed that viruses, especially rota-adenovirus co-infections, might play a role in inflammatory diarrhea. Therefore, antimicrobials should not be routinely recommended when fecal leukocytes are detected in patients with gastroenteritis.

AUTHORS' CONTRIBUTIONS

DK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. **SPYK**: Methodology, Project administration, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

REFERENCES

1. Li W, Zhu Y, Lou J, Chen J, Xie X, Mao J. Rotavirus and adenovirus infections in children during COVID-19 outbreak in Hangzhou, China. Transl Pediatr. 2021;10(9):2281-6. https://doi.org/10.21037/tp-21-150
2. Bányai K, Estes MK, Martella V, Parashar UD. Viral gastroenteritis. Lancet. 2018;392(10142):175-86. https://doi.org/10.1016/S0140-6736(18)31128-0
3. Stuempfig ND, Seroy J. Viral gastroenteritis. In: StatPearls [Internet]. StatPearls Publishing; 2022.
4. Arabaci C, Kenan AK, Dag H. Investigation of rotavirus and enteric adenovirus antigen frequency in patients with acute gastroenteritis by immunochromatographic method: a single center experience of seven years. Acta Medica Mediterranea. 2022;38(2).
5. Arakaki L, Tollefson D, Kharono B, Drain PK. Prevalence of rotavirus among older children and adults with diarrhea: a systematic review and meta-analysis. Vaccine. 2021;39(33):4577-90. https://doi.org/10.1016/j.vaccine.2021.06.073
6. Troeger C, Khalil IA, Rao PC, Cao S, Blacker BF, Ahmed T, et al. Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years. JAMA Pediatr. 2018;172(10):958-65. https://doi.org/10.1001/jamapediatrics.2018.1960
7. Marie H. Rotavirus disease mechanisms diarrhea, vomiting and inflammation: how and why. Linköping: Linköping University Electronic Press; 2015.
8. Shieh WJ. Human adenovirus infections in pediatric population - An update on clinico-pathologic correlation. Biomed J. 2022;45(1):38-49. https://doi.org/10.1016/j.bj.2021.08.009
9. Lee B, Damon CF, Platts-Mills JA. Pediatric acute gastroenteritis associated with adenovirus 40/41 in low-income and middle-income countries. Curr Opin Infect Dis. 2020;33(5):398-403. https://doi.org/10.1097/QCO.0000000000000663
10. Navaneethan U, Giannella RA. Mechanisms of infectious diarrhea. Nat Clin Pract Gastroenterol Hepatol. 2008;5(11):637-47. https://doi.org/10.1038/ncpgasthep1264
11. Mercado EH, Ochoa TJ, Ecker L, Cabello M, Durand D, Barletta F, et al. Fecal leukocytes in children infected with diarrheagenic *Escherichia coli*. J Clin Microbiol. 2011;49(4):1376-81. https://doi.org/10.1128/JCM.02199-10
12. Shane AL, Mody RK, Crump JA, Tarr PI, Steiner TS, Kotloff K, et al. 2017 Infectious diseases society of america clinical practice guidelines for the diagnosis and management of infectious diarrhea. Clin Infect Dis. 2017;65(12):e45-80. https://doi.org/10.1093/cid/cix669
13. Abdulhadi AH, Almugheerbi SI, Alamri MA, Alloqmani MM, Almohammadi GS, Bazarbay AA, et al. Prevalence of antibiotic use for pediatric acute viral gastroenteritis in medinah medicine Almunwarah, KSA. Pharmacophore. 2019;10(6):37.
14. Gardezi SAA, Niazi NK, Tirmizi SH, Rafique I, Sadiq R, Rehman MU, et al. Frequency of inflammatory diarrhoea (faecal leukocyte count≥ 5/Ml or faecal cal-protetctin positive) in young patients presenting with acute gastroenteritis. Pakistan J Med Health Sci. 2022;16(06):144. https://doi.org/10.53350/pjmhs22166144
15. Zaraket R, Salami A, Bahmad M, Roz A, Khalaf B, Ghssein G, et al. Prevalence, risk factors, and clinical characteristics of rotavirus and adenovirus among Lebanese hospitalized children with acute gastroenteritis. Heliyon. 2020;6(6):e04248. https://doi.org/10.1016/j.heliyon.2020.e04248
16. Öner SZ, Kaleli İ, Demi RM, Mete E, Çalişkan A. Rotavirus and adenovirus prevalence in patients with acute viral gastroenteritis in Denizli, Turkey, 2017-2021. J Med Virol. 2022;94(8):3857-62. https://doi.org/10.1002/jmv.27834
17. Dinç HÖ, Taner Z, Özbey D, Gareayaghi N, Sirekbasan S, Kocazeybek BS. The prevalence of rotavirus and adenovirus childhood gastroenteritis: data of the university hospital of cerrahpaşa medical faculty between January 2013 and December 2018. Türk Mikrobiyoloji Cem Derg. 2019;49:206-11.
18. Liu L, Qian Y, Zhang Y, Zhao L, Jia L, Dong H. Epidemiological aspects of rotavirus and adenovirus in hospitalized children with diarrhea: a 5-year survey in Beijing. BMC Infect Dis. 2016;16(1):508. https://doi.org/10.1186/s12879-016-1829-z
19. Gonzalez MD, Wilen CB, Burnham CA. Markers of intestinal inflammation for the diagnosis of infectious gastroenteritis. Clin Lab Med. 2015;35(2):333-44. https://doi.org/10.1016/j.cll.2015.02.001
20. Dong JX, Li AF, Li RQ, Chao S, Yang S, Zhao XY. Epidemiological and clinical features of rotavirus and adenovirus related gastroenteritis in Beijing: a retrospective case-control study in pediatric patients. Biomed Environ Sci. 2020;33(3):196-200. https://doi.org/10.3967/bes2020.027
21. Makimaa H, Ingle H, Baldridge MT. Enteric viral co-infections: pathogenesis and perspective. Viruses. 2020;12(8):904. https://doi.org/10.3390/v12080904
22. Shim DH, Kim DY, Cho KY. Diagnostic value of the vesikari scoring system for predicting the viral or bacterial pathogens in pediatric gastroenteritis. Korean J Pediatr. 2016;59(3):126-31. https://doi.org/10.3345/kjp.2016.59.3.126

ORIGINAL ARTICLE

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Protective effect of coenzyme Q10 in cyclophosphamide-induced kidney damage in rats

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SUMMARY

OBJECTIVE: We aimed to investigate the effect of coenzyme q10 on cyclophosphamide-induced kidney damage in rats.

METHODS: A total of 30 female Wistar-Albino rats were utilized to form three groups. In group 1 (control group) (n=10), no drugs were given. In group 2 (cyclophosphamide group) (n=10), 30 mg/kg intraperitoneal cyclophosphamide was administered for 7 days. In group 3 (cyclophosphamide+coenzyme q10 group) (n=10), 30 mg/kg cyclophosphamide and 10 mg/kg coenzyme q10 were given for 7 days via intraperitoneal route. Right kidneys were removed in all groups. Blood malondialdehyde levels and activities of catalase and superoxide dismutase were measured. Histopathological damage was evaluated by examining the slides prepared from kidney tissue using a light microscope.

RESULTS: Tissue damage was significantly higher in the cyclophosphamide group than in the cyclophosphamide+coenzyme q10 group ($p<0.05$). The malondialdehyde levels were significantly higher and the activities of superoxide dismutase and catalase were lower in the cyclophosphamide group than in the cyclophosphamide+coenzyme q10 group ($p<0.05$).

CONCLUSION: Coenzyme q10 may be a good option to prevent cyclophosphamide-induced kidney damage.

KEYWORDS: Cyclophosphamide. Coenzyme q10. Rat. Kidney. Toxicity.

INTRODUCTION

Cyclophosphamide is a nitrogen mustard-type alkylating agent. It has been utilized for the treatment of malignancies such as lymphoma, solid tumors, and autoimmune disorders[1]. It turns into phosphoramide mustard, which is its active metabolite, in the liver and gains effectiveness. Cyclophosphamide is hydroxylated in the liver and turns into a metabolite, acrolein, and side effects occur when acrolein is excreted by the kidney[2]. Numerous mechanisms could lead to kidney damage. Stankiewicz et al., reported that oxidative stress and elevated reactive oxygen species (ROS) could play an important role in cyclophosphamide-induced kidney damage[3]. Furthermore, prior studies demonstrated that cyclophosphamide could inhibit the activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT)[4,5]. Other theories related to nephrotoxicity due to cyclophosphamide were imbalance of the oxidants–antioxidants system, increase of the inflammatory cytokines, and apoptosis[6], although there is no consensus that, to prevent the toxicity due to cyclophosphamide, a potent antioxidant agent could be useful.

Coenzyme q10 is a vitamin-like substance with antioxidant, anti-inflammatory, and anti-apoptotic activity, which is essential for the proper functioning of many organs and chemical reactions in the body, especially in the heart, liver, kidney, and pancreas[7]. Coenzyme q10 is in charge of the electron transport chain and controls redox reaction and metabolism[8]. Yousef et al., indicated that coenzyme q10 decreases ROS production and free radicals and reverses oxidative stress[9]. Therefore, it was thought that an antioxidant chemical such as coenzyme q10 might enhance the adverse effects of cyclophosphamide. In this study, we aimed to investigate whether coenzyme q10 has a protective effect against cyclophosphamide-induced damage to the kidney in rats.

METHODS

Cyclophosphamide and coenzyme q10 were bought from a pharmacy (Kirsehir, Turkey). The study was reviewed and approved by the local ethical committee with the approval number 23/115 and date 07.06.2023. The study was carried out in Erciyes University Faculty of Medicine, Department of Histology and Embryology. A total of 30 female Wistar-Albino rats of 10–12 weeks old were included in the study. The animals were fed *ad libitum* feeding method with free access to water and food. All the rats were exposed to a temperature between 20 and 22°C under a 12-h light/12-h dark cycle.

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Study design

We planned an experimental animal study. In animal studies, groups are universally planned to be 6–8 rats on the basis of minimal animal use according to the 3R principle. The use of more rats is not ethically approved. When the literature is examined, it will be seen that animal studies are carried out according to this principle. In our study, we determined the number of animals in the study group according to these basic principles. As it is not possible to exceed 10 in a group due to the possible loss of rats, the sample size was planned to be 10 in each group.

A total of three groups were created. In group 1 (control group), neither any drugs were given nor anything was performed. In group 2 (cyclophosphamide group) (n=10), 30 mg/kg intraperitoneal cyclophosphamide was given for 7 days, and nothing was done. In group 3 (cyclophosphamide+coenzyme q10 group) (n=10), 30 mg/kg cyclophosphamide and 10 mg/kg coenzyme q10 were administered for 7 days intraperitoneally.

Anesthesia procedure was performed by utilizing ketamine hydrochloride (45 mg/kg, Ketalar, Eczacibasi, Istanbul, Turkey) and xylazine hydrochloride (5 mg/kg, Rompun, Bayer, Leverkusen, Germany). Blood samples were obtained from the animals by cardiac puncturing. The right kidney tissues were surgically extirpated. All rats were sacrificed via cervical dislocation.

Biochemistry

Malondialdehyde (MDA) levels and SOD and CAT activities were measured by calculating absorbance in a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). The thiobarbituric acid test was used to calculate the MDA levels[10]. SOD enzyme activity was determined by Marklund et al. It was calculated according to the method reported by Marklund S and Marklund G[11]. CAT activity was measured as stated by Aebi et al[12].

Histopathological examination

Tissues were stored in 10% formaldehyde. Then, paraffin embedding was performed. The tissues were cut at 5 µm and stained with hematoxylin-eosin. Additionally, immunohistochemical p53 staining was performed. Histopathological assessment was performed by the same clinician via light microscopy (Olympus® Inc., Tokyo, Japan). The tissue damage was scored by determining the highest area. A modified semi-quantitative scoring was performed. Four categories were described (0: Absent, 1: Minimal, 2: Mild, 3: Moderate, and 4: Severe). Tubular dilatation, hemorrhage, necrosis, edema, inflammation, and glomerular atrophy were used to determine the degree of kidney damage. The histopathological assessment was performed according to the study reported by Neto et al[13].

Immunohistochemistry

p53 expression was graded using the 0–3+range (p53; 0: no staining, 1: less than 10% nuclear staining in renal tubular epithelial cells, 2: 10–30% nuclear staining, and 3: more than 30% nuclear staining).

Statistical analysis

Statistical Package for the Social Sciences (22.00 SPSS Inc., Chicago, IL) was used for statistical analysis. Power analysis was used, and the sample size was calculated as at least 8 for each group with 80% accuracy. The chi-square test for categorical variables and the independent t-test for numerical values were used. A $p<0.05$ was considered statistically significant.

RESULTS

Blood MDA levels and SOD and CAT enzyme activities are shown in Table 1. The MDA level was significantly higher in the cyclophosphamide group than in the cyclophosphamide+coenzyme q10 group ($p<0.05$). SOD and CAT activities were found to be significantly lower in the cyclophosphamide group than in the cyclophosphamide+coenzyme q10 group ($p<0.05$).

There was no difference between the groups in terms of the macroscopic appearance of the kidney tissue. Markers showing histopathological damage such as hemorrhage, edema, tubular dilatation, glomerular atrophy, and inflammation were more prominent in the cyclophosphamide group than the cyclophosphamide+coenzyme q10 group, and the differences were statistically significant ($p<0.05$) (Table 2).

When kidney tissues were evaluated microscopically, parenchyma structure, glomeruli, and tubules were normal in the control group (Figure 1A). In the cyclophosphamide group, hemorrhage, edema, inflammation, and glomerular and tubular injury were observed (Figure 1B). In the cyclophosphamide+coenzyme q10 group, it was observed that the damage in the renal parenchyma, tubular, and glomerular structures regressed (Figure 1C).

Sections made with the p53 immunostain were similar to the evaluations made with hematoxylin-eosin. It was observed that the histopathological damage, which was more prominent in the cyclophosphamide group, was reversed with the addition of coenzyme q 10 (Figures 2A–C).

DISCUSSION

In this prospective randomized trial, we found significantly lower MDA levels and higher SOD and CAT enzyme activities in the cyclophosphamide+coenzyme q10 group than in the cyclophosphamide group. Also, tissue damage was common in

the cyclophosphamide group and the addition of coenzyme q10 reversed the harmful effect of cyclophosphamide. We aimed to assess the effect of coenzyme q10 on cyclophosphamide-induced nephrotoxicity. To the best of our knowledge, this is the first experimental trial to investigate the protective effect of coenzyme q10 on renal toxicity due to cyclophosphamide.

Table 1. Blood levels of malondialdehyde, superoxide dismutase, and catalase in serum samples of the groups.

	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)
(Control group) (n=10)	16.59±7.08	29.4±10.81	69.58±17.72
(Cyclophosphamide group) (n=10)	35.12±13.85*	11.27±3.14*	27.56±10.41*
(Cyclophosphamide+coenzyme q10 group) (n=10)	23.66±10.27*	19.31±8.76*	40.67±16.70*

*Significant difference ($p<0.05$) between groups 2 and 3. Data are presented as mean±SD.

Table 2. Distribution of histological damage according to the groups.

	(Control group) (n=10)	(Cyclophosphamide group) (n=10)	(Cyclophosphamide+coenzyme q10 group) (n=10)
Hemorrhage	0.00	2.00*	1.00*
Necrosis	0.00	1.00	1.00
Edema	0.00	2.00*	1.00*
Inflammation	0.00	2.00*	1.00*
Tubular dilatation	0.00	2.76*	1.56*
Glomerular atrophy	0.00	2.37*	1.03*

*Significant difference ($p<0.05$) between groups 2 and 3. Histopathological scoring was done by determining the highest area. Four categories (0: None, 1: Minimal, 2: Mild, 3: Moderate, and 4: Severe) were determined by making a semi-quantitative analysis, and the parameters were scored accordingly.

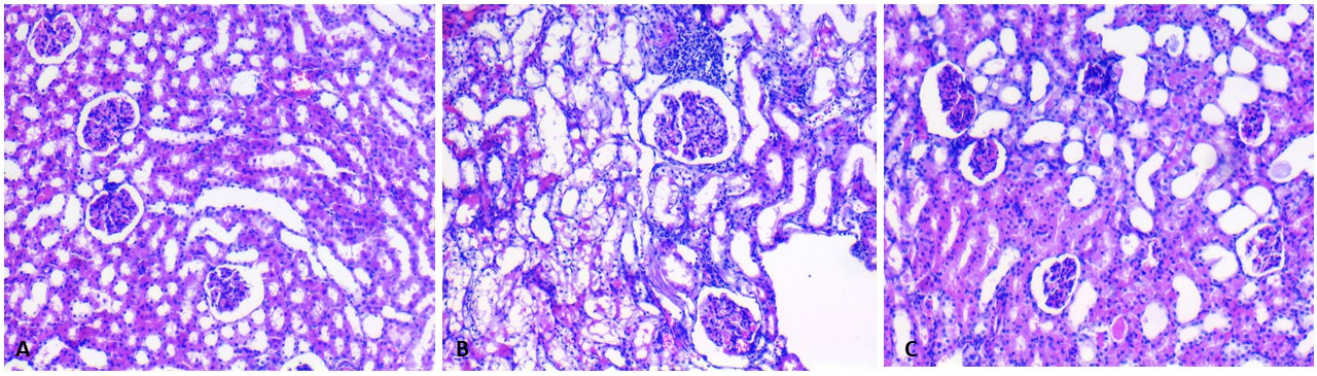

Figure 1. Demonstration of the histopathological examination by light microscopy. (A) View of renal parenchymal tissue from the control group (H&E, 200×). (B) Histopathological damage in the rats from the cyclophosphamide group. There was inflammation, minimal hemorrhage, and glomerular and tubular damage (H&E, 200×). (C) Renal parenchyma view of rats in the cyclophosphamide+coenzyme q10 group. Inflammation, hemorrhage, and tubulo-glomerular damage were improved (H&E, 200×).

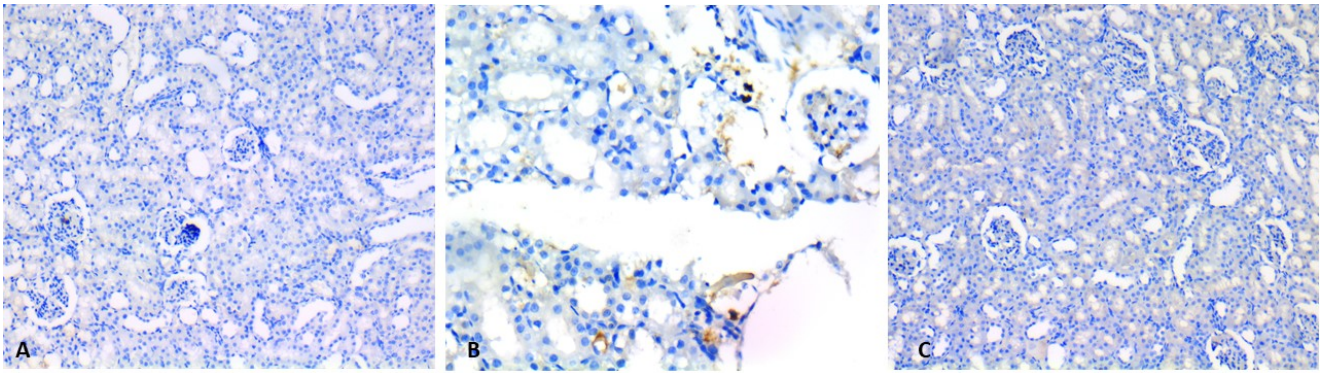

Figure 2. Evaluation of kidney with p53 immunostain. (A) Renal parenchyma view of rats in the control group (200×). (B) Renal parenchyma view of rats in the cyclophosphamide group. (200×). (C) Renal parenchyma view of rats in the cyclophosphamide+coenzyme q10 group (200×).

Even though cyclophosphamide has been utilized in the treatment of malignancies, its toxicity due to cumulative dose is the main limiting factor[14]. Ahlmann et al., reported that, in addition to the gastrointestinal system, bone marrow, and cardiac toxicity, nephrotoxicity and hepatotoxicity can occur due to cyclophosphamide[15]. The main underlying mechanism of cyclophosphamide-induced kidney damage is oxidative stress. It leads to an increase in the levels of hydrogen peroxide, ROS, and hydroxyl radicals[16]. Antioxidant enzymes such as SOD and CAT tend to be lower in the rats given cyclophosphamide, and the addition of antioxidants such as amifostine reverses the whole picture and preserves the cell[17]. In our study, we observed that coenzyme q10, an antioxidant chemical, enhanced the biochemical and histological results in rats administered cyclophosphamide.

Coenzyme q10 acts through the benzoquinone ring in its structure and is involved in many reactions and processes in the cell, especially the electron transport chain[18]. It protects the cellular membranes from oxidative stress by reducing the levels of free radicals and ROS. Moreover, it also shows a direct antioxidant effect by increasing the effect of vitamins C and E[19,20]. It has been reported that coenzyme q10 levels are low in patients with chronic kidney disease. The addition of coenzyme q10 has been shown to improve kidney function and reduce the need for dialysis[21]. Kuang et al., demonstrated that SOD and CAT are major protective enzymes and prevent injury due to oxidative stress[22]. In our study, the tissue damage was more in the cyclophosphamide group than in other groups. Therefore, we assessed the effect of coenzyme q10 in rats given cyclophosphamide. In addition to biochemical and histological examinations, we strengthened our study by measuring the level of p53, which is closely related to oxidative stress and apoptosis. There are many publications in the literature showing the relationship between immunohistochemical expression and cell damage[13,23]. In these publications, it has been shown that p53 expression increases when cell damage increases after oxidative stress.

In our study, coenzyme q10 supplementation increased the activities of SOD and CAT and decreased the MDA levels. These findings were also confirmed histologically. The parameters demonstrating the histopathological damage such as tubular dilatation, hemorrhage, necrosis, edema, inflammation, and glomerular atrophy were significantly lower in the cyclophosphamide+coenzyme q10 group than in the cyclophosphamide group. In the examinations performed with p53 dye, it was shown that the addition of coenzyme q10 reduced the damage. Our negative aspects are that the findings obtained from the study are short-lived and the difficulty to adapt the data obtained from animal experiments to humans.

In conclusion, coenzyme q10 appears to be a promising and unique molecule in the prevention and treatment of cyclophosphamide-induced kidney injury.

LEARNING POINT

We aimed to assess the effects of coenzyme q10 on cyclophosphamide-induced kidney damage in rats.

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AUTHORS' CONTRIBUTIONS

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

REFERENCES

1. Iqubal A, Iqubal MK, Sharma S, Ansari MA, Najmi AK, Ali SM, et al. Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: old drug with a new vision. Life Sci. 2019;218:112-31. https://doi.org/10.1016/j.lfs.2018.12.018
2. Alaqeel NK, Hariri MT. Naringenin ameliorates cyclophosphamide-induced nephrotoxicity in experimental model. Saudi J Biol Sci. 2023;30(6):103674. https://doi.org/10.1016/j.sjbs.2023.103674
3. Stankiewicz A, Skrzydlewska E, Makieła M. Effects of amifostine on liver oxidative stress caused by cyclophosphamide administration to rats. Drug Metabol Drug Interact. 2002;19(2):67-82. https://doi.org/10.1515/dmdi.2002.19.2.67
4. Mohamed MA, Mleeh AA, Hamad RT, Abu-Alya IS, Hewaity MH, Elbestawy AR, et al. Immunostimulant potential of moringa oleifera leaves alcoholic extract versus oregano essential oil (OEO) against cyclophosphamide-induced immunosuppression in broilers chicks. Trop Anim Health Prod. 2023;55(3):209. https://doi.org/10.1007/s11250-023-03620-5
5. Ye Y, Fang C, Li L, Liu D, Wang Y, Huang F, et al. Protective effect of l-theanine on cyclophosphamide-induced testicular toxicity in mice. J Agric Food Chem. 2023;71(21):8050-60. https://doi.org/10.1021/acs.jafc.3c01010
6. Xie A, Wan H, Feng L, Yang B, Wan Y. Protective effect of anoectochilus formosanus polysaccharide against cyclophosphamide-induced immunosuppression in BALB/c mice. Foods. 2023;12(9):1910. https://doi.org/10.3390/foods12091910

7. Dunning BJ, Bourgonje AR, Bulthuis MLC, Alexander J, Aaseth JO, Larsson A, et al. Selenium and coenzyme Q10 improve the systemic redox status while reducing cardiovascular mortality in elderly population-based individuals. Free Radic Biol Med. 2023;204:207-14. https://doi.org/10.1016/j.freeradbiomed.2023.04.024
8. Dimitriadis F, Borgmann H, Struck JP, Salem J, Kuru TH. Antioxidant supplementation on male fertility-a systematic review. Antioxidants (Basel). 2023;12(4):836. https://doi.org/10.3390/antiox12040836
9. Yousef AOS, Fahad AA, Abdel Moneim AE, Metwally DM, Khadragy MF, Kassab RB. The neuroprotective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities. Int J Environ Res Public Health. 2019;16(16):2895. https://doi.org/10.3390/ijerph16162895
10. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. https://doi.org/10.1016/0003-2697(79)90738-3
11. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x
12. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6. https://doi.org/10.1016/s0076-6879(84)05016-3
13. Neto LC, Soares JM, Giusa-Chiferi MG, Gonçalves WJ, Baracat EC. Expression of p53 protein in the endometrial polyp in postmenopausal women. Eur J Gynaecol Oncol. 2013;34(6):509-12. PMID: 24601040
14. Emadi A, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. Nat Rev Clin Oncol. 2009;6(11):638-47. https://doi.org/10.1038/nrclinonc.2009.146
15. Ahlmann M, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer Chemother Pharmacol. 2016;78(4):661-71. https://doi.org/10.1007/s00280-016-3152-1
16. Khairnar SI, Mahajan UB, Patil KR, Patel HM, Shinde SD, Goyal SN, et al. Disulfiram and its copper chelate attenuate cisplatin-induced acute nephrotoxicity in rats via reduction of oxidative stress and inflammation. Biol Trace Elem Res. 2020;193(1):174-84. https://doi.org/10.1007/s12011-019-01683-w
17. Stankiewicz A, Skrzydlewska E. Protection against cyclophosphamide-induced renal oxidative stress by amifostine: the role of antioxidative mechanisms. Toxicol Mech Methods. 2003;13(4):301-8. https://doi.org/10.1080/713857191
18. Hargreaves I, Heaton RA, Mantle D. Disorders of human coenzyme Q10 metabolism: an overview. Int J Mol Sci. 2020;21(18):6695. https://doi.org/10.3390/ijms21186695
19. Acosta MJ, Vazquez Fonseca L, Desbats MA, Cerqua C, Zordan R, Trevisson E, et al. Coenzyme Q biosynthesis in health and disease. Biochim Biophys Acta. 2016;1857(8):1079-85. https://doi.org/10.1016/j.bbabio.2016.03.036
20. Rodick TC, Seibels DR, Babu JR, Huggins KW, Ren G, Mathews ST. Potential role of coenzyme Q10 in health and disease conditions. Nutr Diet. 2018;10:1-11. https://doi.org/10.2147/NDS.S112119
21. Singh RB, Khanna HK, Niaz MA. Randomized, double-blind placebo-controlled trial of coenzyme Q10 in chronic renal failure: discovery of a new role. J Nutr Environ Med. 2000;10:281-8. https://doi.org/10.1080/13590840020013266
22. Kuang H, Jiao Y, Wang W, Wang F, Chen Q. Characterization and antioxidant activities of intracellular polysaccharides from agaricus bitorquis (QuéL.) Sacc. Chaidam ZJU-CDMA-12. Int J Biol Macromol. 2020;156:1112-25. https://doi.org/10.1016/j.ijbiomac.2019.11.142
23. Panzan MQ, Mattar R, Maganhin CC, Simões Rdos S, Rossi AG, Motta EL, et al. Evaluation of FAS and caspase-3 in the endometrial tissue of patients with idiopathic infertility and recurrent pregnancy loss. Eur J Obstet Gynecol Reprod Biol. 2013;167(1):47-52. https://doi.org/10.1016/j.ejogrb.2012.10.021

ORIGINAL ARTICLE

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Factors of mortality in patients with cardiac implantable electronic device: 5-year experience

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SUMMARY

OBJECTIVE: The use of cardiac implantable electronic devices has increased in recent years. It has also brought some issues. Among these, the complications of cardiac implantable electronic devices infection and pocket hematoma are difficult to manage. It can be fatal with the contribution of patient-related risk factors. In this study, we aimed to find mortality rates in patients who developed cardiac implantable electronic devices infection and pocket hematoma over 5 years. We also investigated the risk factors affecting mortality in patients with cardiac implantable electronic devices.

METHODS: A total of 288 cardiac implantable electronic devices patients were evaluated. Demographic details, history, and clinical data of all patients were recorded. Cardiac implantable electronic devices infection was defined according to the modified Duke criteria. The national registry was used to ascertain the mortality status of the patients. The patients were divided into two groups (exitus and survival groups). In addition, the pocket hematoma was defined as significant bleeding at the pocket site after cardiac implantable electronic devices placement.

RESULTS: The cardiac implantable electronic devices infection was similar in both groups (p=0.919), and the pocket hematoma was higher in the exitus group (p=0.019). The exitus group had higher usage of P2Y12 inhibitors (p≤0.001) and novel oral anticoagulants (p=0.031). The Cox regression analysis, including mortality-related factors, revealed that renal failure is the most significant risk factor for mortality. Renal failure was linked to a 2.78-fold higher risk of death.

CONCLUSION: No correlation was observed between cardiac implantable electronic devices infection and mortality, whereas pocket hematoma was associated with mortality. Furthermore, renal failure was the cause of the highest mortality rate in patients with cardiac implantable electronic devices.

KEYWORDS: Defibrillators, implantable. Cardiac pacing, artificial. Hematoma. Mortality. Infection.

INTRODUCTION

Cardiac implantable electronic devices (CIED) have become more widespread recently. However, this rise has also brought about issues related to CIED, which can cause severe morbidity and mortality. The primary concerns with a CIED are infection and hematoma at the insertion site. Treating and managing CIED infection is a challenging task. Although its incidence is 0.13–19.9%, mortality due to CIED infection has been reported as 27–65%. CIED infections may result from patient, operator, or device-related factors[1].

The development of a pocket hematoma often accompanies the CIED. A report indicates that a hematoma at the pocket site can act as a nutrient medium for microorganisms, multiplying the risk of CIED infection up to 20 times. Many factors, ranging from the use of antiplatelets and anticoagulants in the patients to surgical techniques, may contribute to the hematoma. It is important to determine the risk factors and establish treatment strategies to prevent CIED and pocket hematoma[2]. Research on this topic is still going on. The high death rates associated with CIED infections and the worries about the cost have prompted investigations into the research of risk factors. We examined cases of CIED infection and pocket site hematoma over a 5-year timeframe. In addition, we investigated patient-related risk factors that influence mortality in patients with CIED.

METHODS

In this retrospective analysis, a total of 288 CIED patients who were admitted to our institution from January 2016 to December 2020 were evaluated. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval has been granted from the ethics committee of Kahramanmaraş Sütçü İmam

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Demographic details, history, and clinical data of all patients were recorded. The details of CIED [PPM (permanent pacemaker), ICD (intracardiac cardioverter defibrillator), cardiac resynchronization therapy (CRT)], the count of battery replacements, and the number of leads were registered. CIED infection was defined according to the modified Duke criteria. CIED infections developed within 60 days were categorized as early-stage CIED infection, whereas those developed after 60 days were considered late-stage CIED infection. Patients were segregated into two groups based on whether or not they developed a significant hematoma at the pocket site after the CIED. The significant pocket hematoma was identified as a swelling requiring drainage. Follow-up contacts were made by outpatient visits or telephone. Our hospital and national databases revealed the patient's admission information and whether they were alive.

The dates and causes of death of individuals were documented. The endpoint was accepted as all-cause death. In addition, the patients were divided into exitus and survival groups during the follow-up period from January 2016 to January 2023. Variables were compared between the two groups. The internationally accepted renal failure definition was a glomerular filtration rate below 60 mL/min.

The procedure of the cardiac implantable electronic devices

Cardiac device implantation was performed in the catheter laboratory. A single gram of cefazolin antibiotic was given intravenously to all patients 30 min before the procedure. During the procedure, 10% povidone-iodine was administered to the patient's CIED pocket area. The skin was opened with cautery or a surgical scalpel. The axillary vein, or subclavian vein, was punctured. Direct skin-to-vein puncture was left to the operator's preference. A subcuticular suture was used for skin closure. Intravenous sedation and nasal oxygen were administered to all patients during the procedure. Oxygen saturation and blood pressure were monitored. All patients underwent anti-biotherapy for 1 week post-procedure.

Statistical analysis

Data were analyzed using SPSS 22 (SPSS Inc., Chicago, IL, USA). The normality of the variables was tested according to the Kolmogorov-Smirnov test. The numerical data were given as the mean and standard deviation for the exitus and survival groups. The median interquartile range was used to express numerical data that were not normally distributed. Continuous variables were compared using an independent-sample t-test or a Mann-Whitney U test. Categorical variables were shown as percentages. The chi-square test was utilized to analyze categorical data. Kaplan-Meier analysis was performed for those who developed CIED infection and hematoma for survival analysis. The effects of variables on mortality were examined using Cox regression analysis. A $p<0.05$ was considered statistically significant.

RESULTS

A total of 288 patients were included in the study, and 38.2% of these patients were females and 61.8% were males. The mean age of the patients with CIED was 65.64±15.24 years. Regarding CIED type, patients had 37.8% PPM, 37.2% ICD, and 25% CRT, respectively. The mean total lead count was 2.00±0.70. CIED patients were split into two groups: exitus and survival groups. Table 1 indicates the clinical and demographic comparisons between the two groups.

While CIED infection was similar in both groups (p=0.919), pocket hematoma was higher in the exitus group (p=0.019). The acetylsalicylic acid (ASA) (p=0.094) and Warfarin usage rates (p=0.684) were similar in both groups. Additionally, the percentages of ASA and warfarin usage were equal in both groups. In contrast, the exitus group had higher usage of P2Y12 inhibitors ($p\leq0.001$) and novel oral anticoagulants (NOACs) (p=0.031).

The CIED infection was detected in 17 (5.9%) patients in our study. No bacterial growth was detected in the blood culture. However, coagulase-negative *Staphylococcus* (CNS) had the highest incidence rate in the pocket site or device material swab. The microorganisms obtained from the pocket site or device material are elaborated. Early infection was observed in 11 (64.7%) patients and late infection in 6 (35.3%) patients. Six (100%) late infections were CNS-detected. Of those who developed CIED infections, 12 were removed by simple traction and five by the lead extraction procedure (using locking stylets and mechanical non-powered telescoping sheaths). Hematoma drainage was required in seven of the patients with CIED. In addition, the survival tables of those with pocket hematoma and CIED infections are illustrated in Figure 1.

The Cox regression analysis, including mortality-related factors, revealed that renal failure is the most significant risk factor for mortality. Renal failure was linked to a 2.78-fold higher risk of death. Diabetes mellitus (p=0.215) and hypertension (p=0.977) did not affect mortality. The remaining variables are denoted in Table 2.

Table 1. Comparison of the demographic and clinical characteristics of the patients with cardiac implantable electronic devices who died and those who survived after 60 months of follow-up.

	Exitus group (n=166)	Survival group (n=122)	p-value
Age (years)	61.83±16.07	70.82±12.31	**<0.001**
Male, n (%)	98 (59)	80 (65.6)	0.259
Heart failure, n (%)	88 (53)	94 (77)	**<0.001**
Diabetes mellitus, n (%)	43 (25.9)	46 (37.7)	**0.032**
Atrial fibrillation, n (%)	40 (24.1)	31 (25.4)	0.798
Hypertension, n (%)	144 (86.7)	117 (95.9)	**0.008**
Malignancy, n (%)	4 (2.4)	4 (3.3)	0.657
CODP, n (%)	12 (7.2)	25 (20.5)	**0.001**
CAD, n (%)	119 (71.7)	103 (84.4)	**0.011**
Renal failure, n (%)	10 (6)	28 (23)	**<0.001**
Antiplatelet use, n (%)			
ASA	110 (66.3)	92 (75.4)	0.094
P2Y12 inhibitors	54 (32.5)	69 (56.6)	**<0.001**
Anticoagulation use, %			
Warfarin	26 (15.7)	17 (13.9)	0.684
NOAK	23 (13.9)	29 (23.8)	**0.031**
Steroid use, n (%)	3 (1.8)	3 (2.5)	0.702
Statin use, n (%)	95 (57.2)	66 (54.1)	0.597
Generator replacement, n (%)	23 (13.9)	16 (13.1)	0.856
Number of leads	1.95±0.66	2.08±0.75	0.113
Pocket hematoma, n (%)	1 (0.6)	6 (4.9)	**0.019**
CIED infection, n (%)	10 (6)	7 (5.7)	0.919

Bold indicates statistically significant p-value. ASA: acetylsalicylic acid; CAD: coronary artery disease; CIED: cardiac implantable electronic device; COPD: chronic obstructive pulmonary disease; NOAC: novel oral anticoagulant.

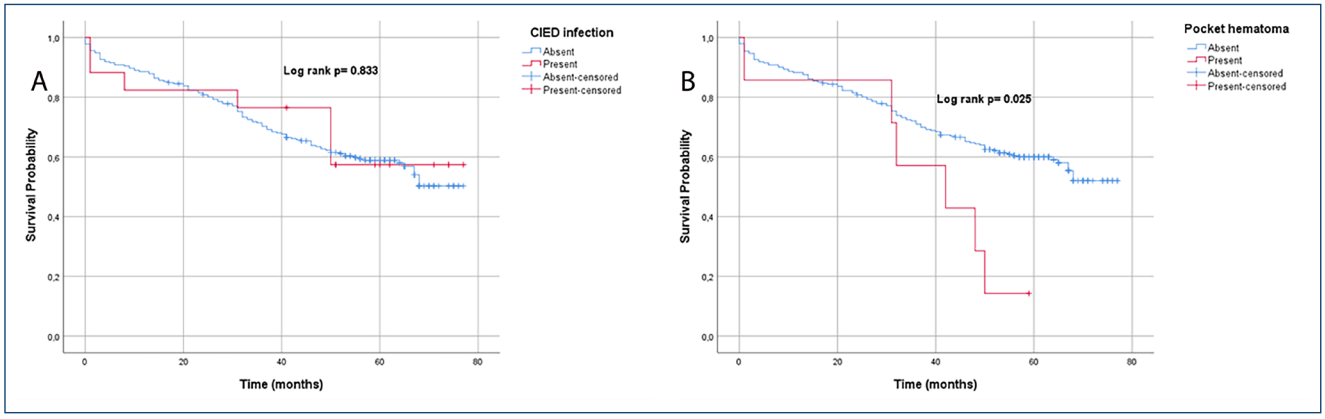

Figure 1. Survival tables of patients with pocket hematoma and cardiac implantable electronic device infection.

DISCUSSION

In recent years, the prevalence of CIED infection has risen due to population growth, technological advancement, and the widespread usage of CIED in cardiac conditions. Examining indicators of CIED infection that result in severe illness and death can help us tackle the problem. Our goal with this study was

Table 2. Cox regression analysis showing the effects of variables on mortality.

	B	OR	p-value	95%CI	
				Lower	Upper
Age	0.033	1.034	**<0.001**	1.017	1.051
Heart failure	0.715	2.044	**0.005**	1.240	3.369
Diabetes mellitus	0.247	1.281	0.215	0.867	1.892
Hypertension	0.015	1.015	0.977	0.369	2.793
COPD	0.804	2.234	**0.001**	1.417	3.522
CAD	-0.258	0.773	0.422	0.412	1.450
Renal failure	1.025	2.786	**<0.001**	1.787	4.345
Developing pocket hematoma	0.633	1.884	0.152	0.792	4.483
Developing CIED infection	-0.334	0.716	0.397	0.331	1.551
Use of P2Y12 inhibitors	-0.267	0.766	0.196	0.511	1.148
Use of NOAC	-0.172	0.842	0.441	0.543	1.305

Bold indicates statistically significant p-value. CAD: coronary artery disease; CIED: cardiac implantable electronic device; COPD: chronic obstructive pulmonary disease; NOAC: novel oral anticoagulant.

to evaluate risk factors and introduce new treatments by analyzing the patients in our clinic who had CIED infection and hematoma over 5 years[3].

The percentage of CIED infections in our study was 5.9%. Research conducted on 1326 CIED patients found that 2.4% had a CIED infection and 1.2% had a pocket hematoma[2]. Polyzos et al., conducted a meta-analysis of 60 studies, revealing a 1–1.3% CIED infection rate[4]. Mela et al., observed that only 1.2 out of 1700 CIED caused infection[5]. The incidence of infection was relatively low; however, the mortality rates were quite high. In a study, the 1-year mortality rate was 15–30% in patients who developed CIED infection[6]. In our study, the high rate of CIED infection may be due to poor sterilization conditions. However, due to the low mortality rate in the long-term follow-up of those who develop CIED infection, we can state that the infection was treated with an effective anti-biotherapy regimen without causing infective endocarditis.

Our findings indicate CNS as the predominant microorganism causing CIED infection. Results from a study conducted by Goya et al., showed that 181 CIED infections contained 30.1% CNS and 37.1% *S. aureus*[7]. In a study by Bongiorni et al., on CIED, 69% of the microorganisms responsible for the infection were CNS, and 13.8% were *S. aureus*[8]. Tarakji et al., reported that 44.4% of CIED infections were caused by CNS, 20.1% by methicillin-susceptible *S. aureus*, and 15.8% by methicillin-resistant *S. aureus*[9]. Staff are commonly located in the skin flora. During the CIED procedure, it may penetrate through the open skin. Unsuitable antiseptic regulations augment the rate of staph infection. Administering beta-lactam antibiotics before and after the procedure decreases the risk of infection. Nevertheless, beta-lactam antibiotics are ineffective against methylene-resistant microorganisms present in 5–10%. Therefore, a single dose of vancomycin has been reported to be effective in prophylaxis for CIED infections[10].

We found no relationship between CIED infection and the type of CIED. A retrospective meta-analysis study involving 78.267 French participants revealed that PPM, ICD, cardiac resynchronization therapy with a defibrillator (CRT-D), and cardiac resynchronization therapy with a pacemaker (CRT-P) infection rates were 0.5, 1.6, 1.6, and 1%, respectively. Of those who had a device replacement, the infection rates were 2.9, 2.9, 1.3, and 3.9% for PM, ICD, CRT-P, and CRT-D, respectively[11]. Harper et al., reported that the rate of CIED was between 0.3 and 1.1%, whereas the infection rate in those who experienced lead revision and upgrade was 2.1%[12]. Previous studies suggest the infection rate is linked to the number of leads, replacements, and device type. The magnitude of the device, the lead cable's thickness, and the presence of the coil may raise the risk of CIED infections. The vast majority of our patients had active fixation leads.

One of the most common complications of CIED is the presence of a hematoma. Patients with CIED are prescribed anticoagulants and antiaggregates due to their high risk of cardiovascular disease and atrial fibrillation. Previously, 0.9% of the participants in the unmedicated group had hematoma, while 5.5 and 5.6% of the ASA and NOAC groups had hematoma, respectively. In addition, with dual antiplatelet treatment, the incidence of hematoma increased up to five times[13]. Warfarin users have discontinued using heparin-bridging strategies because of the heightened danger of hemorrhage. An investigation revealed that the

utilization of heparin for bridging treatment caused a 20% boost in a pocket hematoma. Therefore, uninterrupted NOAC treatments have been applied recently. Our research demonstrated that mortality rates were higher among NOAC and P2Y12 inhibitor users. The fact that P2Y12 inhibitors are more potent agents than ASA and the difficulty in finding the antidote for NOACs may have caused an increase in all-cause mortality[14].

Cardiac conditions commonly co-occur with chronic diseases. There is a correlation between chronic diseases and CIED infections. A meta-analysis showed that diabetes mellitus, kidney disease, chronic obstructive pulmonary disease, malignancy, and heart failure were risk factors for CIED infection[15]. No relationship could be established between age and infection. A total of 2792 patients with CIED were analyzed by Qintar et al. Diabetes, young age, and heart failure were independent precursors of CIED infection[16]. Conflicting studies have been reported between age and CIED infection. Duval et al., described an increase in CIED infections in older individuals. Da Costa et al., revealed that CIED infections had increased among diabetes and dialysis patients[17]. The same study did not suggest that lack of antibiotic therapy, age, and cardiomyopathy were risk factors for CIED infection. A comparative analysis was not conducted since the number of CIED-infected patients was limited. In the CIED infection group, male gender, hypertension, coronary artery disease, and heart failure were particularly prevalent. Among those with CIED infection, renal failure was rare[18].

Our study has some limitations. First, it included a retrospective collection of patients. Second, there were differences between CIED manufacturers in terms of battery sizes and thickness of leads. This can affect the CIED infection.

CONCLUSION

No correlation was observed between CIED infection and mortality, whereas pocket hematoma was associated with mortality. Furthermore, renal failure was the cause of the highest mortality rate in patients with CIED.

INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all participants.

INSTITUTIONAL REVIEW BOARD APPROVAL

Ethics committee approval was granted from our institution on November 1, 2022, with decision number 02.

AUTHORS' CONTRIBUTIONS

KG: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **AÇA:** Formal Analysis, Methodology, Software, Supervision. **ASB:** Conceptualization, Supervision, Writing – review & editing. **EA:** Data curation, Formal Analysis, Writing – review & editing. **MK:** Data curation, Resources. **NSG:** Formal Analysis, Investigation, Writing – review & editing. **MD:** Data curation, Resources. **NSG:** Formal Analysis, Investigation, Writing – review & editing.

REFERENCES

1. Phillips P, Krahn AD, Andrade JG, Chakrabarti S, Thompson CR, Harris DJ, et al. Treatment and prevention of cardiovascular implantable electronic device (CIED) infections. CJC Open. 2022;4(11):946-58. https://doi.org/10.1016/j.cjco.2022.07.010
2. Kewcharoen J, Kanitsoraphan C, Thangjui S, Leesutipornchai T, Saowapa S, Pokawattana A, et al. Postimplantation pocket hematoma increases risk of cardiac implantable electronic device infection: a meta-analysis. J Arrhythm. 2021;37(3):635-44. https://doi.org/10.1002/joa3.12516
3. Alyesh D, Sundaram S. A call for large-scale CIED recycling. J Interv Card Electrophysiol. 2023;66(5):1033-4. https://doi.org/10.1007/s10840-022-01389-5
4. Polyzos KA, Konstantelias AA, Falagas ME. Risk factors for cardiac implantable electronic device infection: a systematic review and meta-analysis. Europace. 2015;17(5):767-77. https://doi.org/10.1093/europace/euv053
5. Mela T, McGovern BA, Garan H, Vlahakes GJ, Torchiana DF, Ruskin J, et al. Long-term infection rates associated with the pectoral versus abdominal approach to cardioverter-defibrillator implants. Am J Cardiol. 2001;88(7):750-3. https://doi.org/10.1016/s0002-9149(01)01845-8
6. Imberti JF, Mei DA, Fontanesi R, Gerra L, Bonini N, Vitolo M, et al. Low occurrence of infections and death in a real-world cohort of patients with cardiac implantable electronic devices. J Clin Med. 2023;12(7):2599. https://doi.org/10.3390/jcm12072599
7. Goya M, Nagashima M, Hiroshima K, Hayashi K, Makihara Y, Fukunaga M, et al. Lead extractions in patients with cardiac implantable electronic device infections: single center experience. J Arrhythm. 2016;32(4):308-12. https://doi.org/10.1016/j.joa.2016.02.004
8. Bongiorni MG, Tascini C, Tagliaferri E, Cori A, Soldati E, Leonildi A, et al. Microbiology of cardiac implantable electronic device infections. Europace. 2012;14(9):1334-9. https://doi.org/10.1093/europace/eus044

9. Tarakji KG, Chan EJ, Cantillon DJ, Doonan AL, Hu T, Schmitt S, et al. Cardiac implantable electronic device infections: presentation, management, and patient outcomes. Heart Rhythm. 2010;7(8):1043-7. https://doi.org/10.1016/j.hrthm.2010.05.016
10. Baker M, Bhattarai B, Johnson PJ, Wade C, Micetic B, Mody K. The effect of a single dose of prophylactic vancomycin prior to peripherally inserted central catheter removal on sepsis. J Pediatr Pharmacol Ther. 2022;27(8):715-9. https://doi.org/10.5863/1551-6776-27.8.715
11. Barra S, Providência R, Tang A, Heck P, Virdee M, Agarwal S. Importance of implantable cardioverter-defibrillator back-up in cardiac resynchronization therapy recipients: a systematic review and meta-analysis. J Am Heart Assoc. 2015;4(11):e002539. https://doi.org/10.1161/JAHA.115.002539
12. Harper MW, Uslan DZ, Greenspon AJ, Baddour LM, Carrillo RG, Danik SB, et al. Clinical presentation of CIED infection following initial implant versus reoperation for generator change or lead addition. Open Heart. 2018;5(1):e00068z1. https://doi.org/10.1136/openhrt-2017-000681
13. Daubaraitė A, Marinskis G, Račkauskas G. Anticoagulant therapy during cardiovascular implantable electronic device procedures. Postepy Kardiol Interwencyjnej. 2023;19(2):99-112. https://doi.org/10.5114/aic.2023.129207
14. Fei YP, Wang L, Zhu CY, Sun JC, Hu HL, Zhai CL, et al. Effect of a novel pocket compression device on hematomas following cardiac electronic device implantation in patients receiving direct oral anticoagulants. Front Cardiovasc Med. 2022;9:9:817453. https://doi.org/10.3389/fcvm.2022.817453
15. McGee MJ, Ray M, Brienesse SC, Sritharan S, Boyle AJ, Jackson N, et al. Remote monitoring in patients with heart failure with cardiac implantable electronic devices: a systematic review and meta-analysis. Open Heart. 2022;9(2):e002096. https://doi.org/10.1136/openhrt-2022-002096
16. Qintar M, Zardkoohi O, Hammadah M, Hsu A, Wazni O, Wilkoff BL, et al. The impact of changing antiseptic skin preparation agent used for cardiac implantable electronic device (CIED) procedures on the risk of infection. Pacing Clin Electrophysiol. 2015;38(2):240-6. https://doi.org/10.1111/pace.12514
17. Costa A, Tulane C, Dauphinot V, Terreaux J, Romeyer-Bouchard C, Gate-Martinet A, et al. Preoperative skin antiseptics for prevention of cardiac implantable electronic device infections: a historical-controlled interventional trial comparing aqueous against alcoholic povidone-iodine solutions. Europace. 2015;17(7):1092-8. https://doi.org/10.1093/europace/euu293
18. Casorelli E, Pescatori I, Ruocco G, Bonnemeier H, Assadian O, Bui F. [Pacemaker infection in fragile patients]. Herzschrittmacherther Elektrophysiol. 2023;34(2):161-4. https://doi.org/10.1007/s00399-023-00940-9

ORIGINAL ARTICLE

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Effect of mode of delivery on postpartum health-related quality of life

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SUMMARY

OBJECTIVE: The aim of the study was to explore the impact of mode of delivery on health-related quality of life in mothers.

METHODS: This cross-sectional study was conducted between May and August 2022 on healthy singleton pregnant women aged between 18 and 45 years. Data on socio-demographic variables, clinic features, pregnancy and birth characteristics, and neonatal outcomes were collected. Health-related quality of life was assessed by using EQ-5D-5L questionnaire.

RESULTS: A total of 1,015 healthy pregnant women were included. The EQ-5D-5L index score was higher in those with regular sleep patterns ($p<0.001$), those who did physical activity (PA) during pregnancy ($p<0.001$), those who received spousal support ($p<0.001$), and those with very good and good perceived health ($p<0.001$). EQ-5D-5L index and EQ-5D-5L-VAS scores were lower in those with unplanned pregnancy, those who preferred cesarean section, those who had cesarean section, those who underwent episiotomy, and those who admitted to the intensive care unit ($p<0.001$). Emergency cesarean section and elective cesarean section had the lowest and second lowest health-related quality of life mean scores, while normal vaginal deliveries had the highest health-related quality of life mean scores, respectively ($p<0.001$).

CONCLUSION: This study showed that health-related quality of life was higher after vaginal delivery than after cesarean section. In addition, spousal support, regular sleep pattern, and PA during pregnancy play an important role in maternal health-related quality of life.

KEYWORDS: Cesarean section. Episiotomy. Delivery, obstetric. Pregnancy. Quality of life.

INTRODUCTION

Pregnancy, delivery, and puerperium are important periods that affect women physically, mentally, and socially and cause considerable changes in their quality of life (QoL). During postpartum period, the mother needs to recover and get used to her new roles and responsibilities[1]. While puerperal changes usually resolve within 6 weeks following delivery, many women suffer from postpartum complications for a prolonged time[2]. Postpartum recovery is of paramount importance as it affects the QoL of both the mother and the newborn[3].

In recent years, the rate of cesarean sections (CS) has increased globally. By 2030, there will be some countries with this rate over 60%. World Health Organization (WHO) has warned about the growing trend in CS and recommends countries to maintain a 10–15% rate[4]. In 2017, the overall delivery rate of CS in Turkey was 51.2%[5]. The majority of mothers still prefer CS over vaginal deliveries, despite studies demonstrating that cesareans can result in a number of complications. It appears that pregnant women lack awareness regarding the consequences of delivery methods[6]. Thus, it is imperative to apprise them regarding the advantages and disadvantages of cesarean and vaginal deliveries.

Health-related quality of life (HRQoL) has been accepted as a valid indicator of maternal health[7]. A thorough understanding of the impact of delivery methods on pregnant women's HRQoL is critical in order to design and implement effective health interventions for this unique group. Despite the widespread use of the EQ-5D-5L questionnaire in different populations and diseases, there is an inadequate understanding of HRQoL assessment in pregnant women in Turkey.

The number of CS is escalating as more women are electing to have the procedure. To the best of our knowledge, there is a lack of data regarding the effects of delivery mode on HRQoL among Turkish pregnant women. Therefore, this study aims to fill this research gap in the literature by investigating the impact of the mode of delivery on HRQoL in postpartum women using a preference-based HRQoL measure.

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METHODS

This cross-sectional study was carried out in the obstetric unit of a tertiary health facility, between May and August 2022. The institution is a public hospital that has received accreditation under the International Baby Friendly Hospital Initiative, developed by WHO and United Nations Children's Fund. It provides maternity and child health services at no cost and is the largest tertiary healthcare facility in Istanbul. The present study obtained permission from the EuroQol Research Foundation and approval from the Research Ethics Committee (Approval date: 06.04.2022 number: 49). The study adheres to the principles of the Helsinki Declaration. All subjects provided informed consent prior to data collection.

Participants were healthy singleton pregnant women aged between 18 and 45 years, greater than 28 weeks of gestation, literate, and willing to participate in the study. Exclusion criteria were (1) mothers with chronic medical conditions (pre-eclampsia, diabetes, chronic hypertension, asthma, gestational diabetes mellitus, cholestasis); (2) with risk of preterm birth, placenta previa, myoma uteri, polyhydramnios, oligohydramnios, multiple gestations; (3) under 18 years old or over 45 years old; (4) had a depression/psychiatric disease; (5) had given birth to a baby with anomalies; (6) had given birth to a baby with health problems (intrauterine growth restriction, etc.); and (7) had issues that were stressful such as death of a loved one, divorce, or family disruptions.

Data on socio-demographic variables, clinic features, pregnancy and birth characteristics, and neonatal outcomes were collected. HRQoL was assessed by using EQ-5D-5L questionnaire. The EQ-5D-5L is a two-part instrument. In the first part, the EQ-5D-5L instrument includes five different health dimensions: mobility, self-care, usual activities, pain or discomfort, and anxiety or depression. The severity levels of each dimension are rated on a scale of 1 (no problems) to 5 (extreme problems). The second part of the questionnaire includes EQ-VAS, a self-rating on a 20-cm vertical scale in which 0 and 100 indicate the worst and best imaginable health statutes. A higher score indicates lower quality of life. As recommended by the EuroQol Research Foundation, the EQ-5D-5L utility values presented were derived from the United Kingdom (UK) value sets, due to the lack of country-specific data for Turkey[8].

Statistical analysis

The data collected in the study were transferred to the Epi info 7.2 program and analyzed. Numbers, percentages, median values, and minimum and maximum values are used to describe descriptive characteristics. The data were tested for normality using Kolmogorov-Smirnov tests. Chi-square test for two categorical variables, Mann Whitney U test for pairwise comparisons, and Kruskal-Wallis test for continuous variables were performed. The relationship between two continuous variables was evaluated with the Spearman correlation test. A p-value was set at 0.05 in order to determine the level of statistical significance.

RESULTS

Out of total deliveries conducted, 588 (57.9%) were vaginal deliveries, 193 (19.0%) were elective CS, 201 (19.8%) were emergency CS, and 33 (3.3%) were instrumental deliveries. It was found that 178 (17.5%) pregnant women participated in vigorous-intensity, 235 (23.2%) moderate-intensity, and 602 (59.3%) light-intensity physical activity (PA).

In total, 902 babies (88.9%) did not receive noninvasive respiratory support, while 113 babies (11.1%) received. Notably, 60 (5.9%) babies were admitted to neonatal intensive care unit (NICU), and 955 (94.1%) were not admitted to NICU.

The EQ-5D-5L index and VAS scores were higher in those who had a regular sleep pattern ($p<0.001$), those who did PA during pregnancy ($p<0.001$), those who received spousal support ($p<0.001$), and those with very good and good perceived health ($p<0.001$) (Table 1).

EQ-5D-5L index and EQ-5D-5L-VAS scores were lower in those with unplanned pregnancy, those who preferred CS, those who had CS, those who underwent episiotomy, and those who were admitted to the intensive care unit (ICU) ($p<0.001$) (Table 2).

Mothers whose newborns required respiratory support or who were hospitalized in the ICU had lower EQ-5D-5L index and EQ-5D-5L-VAS scores.

Emergency CS and elective CS had the lowest and second lowest HRQoL mean scores, while normal vaginal deliveries had the highest HRQoL mean scores, respectively ($p<0.001$) (Table 3).

DISCUSSION

This study found that PA during pregnancy, sleeping regularly, receiving spousal support, and having good perceived health were associated with higher HRQoL scores. Significant poorer EQ-5D-5L index scores were found in women who had unplanned pregnancies, those who preferred CS, those who had a CS, those who underwent episiotomy, and those who were admitted to ICU. In addition, having a meconium-contaminated newborn, the newborn being admitted to the ICU, and

Table 1. Association of EQ-VAS and EQ5D index score with demographic, social, and clinical variables.

		EQ5D Index Score		EQ-VAS	
		Median (min–max)	p-value*	Median (min–max)	p-value*
Cigarette smoking	Yes	-0.59 (-0.59–1.00)	0.112	80 (75–85)	0.570
	No	0.57 (-0.59–1.00)		75 (35–100)	
Sleep pattern	Regular	0.59 (-0.59–1.00)	**<0.001**	80 (35–100)	**<0.001**
	Irregular	0.03 (-0.59–1.00)		70 (40–100)	
Physical exercise during pregnancy	Regular	0.59 (-0.59–1.00)	**<0.001**§	85 (50–100)	**<0.001**§
	Irregular	0.59 (-0.59–1.00)		80 (40–100)	
	None	0.08 (-0.59–1.00)		70 (35–100)	
Spousal support during pregnancy and birth	Yes	0.59 (-0.59–1.00)	**<0.001**	80 (35–100)	**<0.001**
	No	-0.08 (-0.59–1.00)		65 (40–90)	
Perceived status of health	Very Good	0.65 (-0.59–1.00)	**<0.001**§	90 (80–100)	**<0.001**§
	Good	0.59 (-0.59–1.00)		80 (40–100)	
	Fair	0.04 (-0.59–1.00)		65 (40–90)	
	Poor	-0.59 (-0.59–1.00)		55 (35–85)	
	Very Poor	0.52 (-0.59–1.00)		65 (40–100)	

*Mann-Whitney U–test. §Kruskal-Wallis test. Statistically significant values are denoted in bold.

Table 2. Association of EQ-VAS and EQ5D index score with obstetric and reproductive health-related characteristics.

		EQ5D Index Score		EQ-VAS	
		Median (min–max)	p-value*	Median (min–max)	p-value*
Intention of pregnancy	Planned	0.58 (-0.59–1.00)	**0.004**	80 (40–100)	**<0.001**
	Unplanned	0.04 (-0.59–1.00)		70 (35–95)	
Mode of delivery Preferences	Normal vaginal delivery	0.59 (-0.59–1.00)	**<0.001**	80 (35–100)	**<0.001**
	Cesarean section	0.04 (-0.59–1.00)		70 (40–100)	
Mode of delivery	Normal vaginal delivery	0.88(-0.59–1.00)	**<0.001**§	85 (40–100)	**<0.001**§
	Instrumental NVD	0.30 (0.04–0.85)		80 (55–90)	
	Elective cesarean	0.04 (-0.59–0.52)		60 (35–85)	
	Emergency cesarean	-0.59 (-0.59–1.00)		60 (40–90)	
Episiotomy during birth	Yes	0.59 (-0.59–1.00)	**<0.001**	85 (50–100)	**<0.001**
	No	0.10 (-0.59–1.00)		70 (35–100)	
Perineal tear during birth	Yes	0.36 (0.04–0.59)	**<0.001**	80 (55–90)	0.648
	No	1.00 (-0.59–1.00)		75 (35–100)	
Degree of perineal tear	No	1.00 (-0.59–1.00)	**<0.001**§	75 (35–100)	0.890§
	1st	–		–	
	2nd	0.35 (0.04–0.59)		80 (55–90)	
	3rd	0.54 (0.54–0.54)		80 (80–80)	
Need of blood transfusion	Yes	0.59 (-0.59–0.65)	0.976	60 (50–75)	**0.001**
	No	0.56 (-0.59–1.00)		80 (35–100)	
Maternal admission to ICU	Yes	0.04 (-0.59–1.00)	**0.014**	50 (40–70)	**<0.001**
	No	0.58 (-0.59–1.00)		80 (35–100)	

*Mann-Whitney U–test. §Kruskal-Wallis test. NICU: Neonatal intensive care unit; NVD: normal vaginal delivery. Statistically significant values are denoted in bold.

Table 3. Association of EQ5D health dimensions with mode of delivery.

	Mode of delivery								p-value*
	NVD		Instrumental NVD		Elective CS		Emergency CS		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
EQ5D-mobility	1.59	0.74	2.82	0.58	4.19	0.68	4.78	0.54	**<0.001**
EQ5D-self care	1.59	0.74	2.82	0.64	4.20	0.67	4.79	0.53	**<0.001**
EQ5D-usual activities	1.61	0.77	2.97	0.68	4.20	0.67	4.82	0.52	**<0.001**
EQ5D-pain/discomfort	1.62	0.78	2.79	0.60	4.20	0.67	4.80	0.53	**<0.001**
EQ5D-anxiety/depression	1.76	1.08	3.82	0.92	4.19	0.70	4.77	0.66	**<0.001**

*Kruskal-Wallis test. NVD: normal vaginal delivery; CS: cesarean section. Statistically significant values are denoted in bold.

noninvasive respiratory support for the newborn were linked to a lower EQ5DL index score.

This study indicated a considerable difference in HRQoL by birth mode. According to the HRQoL scores, spontaneous vaginal births were the highest, followed by instrument-assisted vaginal births, elective cesareans, and emergency cesareans, respectively. The study findings are in accordance with several studies that show HRQoL improved after vaginal delivery in the early postpartum period and 5 years after delivery[9]. In addition, they concur with a recent review indicating that a CS negatively affected HRQoL[10]. However, not all studies agreed, some showed that CS does not contribute to poor QoL, and others showed no significant difference between delivery methods[11]. The discrepancy between the literature can be attributed to the different study methodologies, such as the instruments used for measuring QoL and the location of studies.

Our study revealed that gestational age serves as a predisposing factor for improved HRQoL, which is contrary to Martínez-Galiano et al.'s findings[7] that gestational age was a risk factor associated with reduced HRQoL.

Our findings were similar to those of Martínez-Galiano et al.[7], which showed that perineal tears and episiotomies were related to poor postpartum HRQoL, whereas other studies failed to demonstrate such an association[12]. Nevertheless, their studies did not differentiate between different types of perineal lesions as our study did, but did take into account more severe perineal lesions that cause more discomfort[13].

Regular exercise during pregnancy has positive effects on physical and mental health of mothers. Comparison of our findings with those of other studies confirmed that PA during pregnancy is associated with improved HRQoL[14]. On the contrary, a study conducted in Iran found no association between PA in pregnancy and HRQoL[15]. A possible explanation for this might be the high prevalence of physical inactivity among Iranian pregnant women.

Following a regular sleep pattern was observed to have a positive effect on postpartum QoL in our research, which is congruent with other studies[16]. In the same vein, a recent review has provided evidence that poor sleep quality was linked to a lower HRQoL during pregnancy[17].

Spousal support was ascertained as a factor that augmented the QoL of pregnant women, which is in agreement with other studies[18]. Therefore, it can be inferred that partner support may have a positive effect on gestational HRQoL.

Maternal preference for CS was another factor contibuting to a worse postpartum QoL in our study, which overlapped with earlier studies, which found that compared with women who plan to give birth vaginally, those who request a CS reported less perceived postpartum HRQoL[19]. According to a previously published study[20], women opting for CS have difficulty in preparing themselves for motherhood before deciding on such a procedure, which may explain why their health is poor during pregnancy.

Admission of newborn to NICU was identified as a contributor to reduced QoL among mothers, which is in line with the study by Rai and Rani[21]. In a longitudinal study, it was shown that admission of newborn to NICU may be related to poor maternal QoL up to 12 months[22].

Limitations

There are several caveats that must be borne in mind. First, we were unable to examine the impact of factors that influence the relationship between mode of delivery and postpartum HRQoL in the long term. Second, since the study was conducted in a developing country, the results may not be applicable to all settings. Notwithstanding these limitations, this study has advantages, including large sample size and utilization of a widely used preference-based HRQoL measure. To the best of our knowledge, the present study is one of the most comprehensive assessments of HRQoL and modes of delivery in Turkish pregnant women.

CONCLUSION

This study showed that HRQoL was higher after vaginal delivery than after CS. In addition, spousal support, regular sleep pattern, and PA during pregnancy play an important role in maternal HRQoL. Policymakers must translate this information into healthcare policies to improve maternal HRQoL.

AVAILABILITY OF DATA AND MATERIALS

The dataset used and/or analyzed in the study is available from the corresponding author on reasonable request.

ETHICS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of University of Health Sciences Turkey, Zeynep Kamil Women and Children's Diseases Training and Research Hospital (Approval date: 06.04.2022 number: 49). The present study obtained permission from the EuroQol Research Foundation.

AUTHORS' CONTRIBUTIONS

EK: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. **ZK:** Data curation, Writing – original draft, Writing – review & editing. **LK:** Conceptualization, Data curation, Writing – original draft, Writing – review & eiting. **NY:** Formal Analysis, Writing – original draft, Writing – review & editing.

REFERENCES

1. Boutib A, Chergaoui S, Marfak A, Hilali A, Youlyouz-Marfak I. Quality of life during pregnancy from 2011 to 2021: systematic review. Int J Womens Health. 2022;14:975-005. https://doi.org/10.2147/IJWH.S361643
2. Rezaei N, Tavalaee Z, Sayehmiri K, Sharifi N, Daliri S. The relationship between quality of life and methods of delivery: a systematic review and meta-analysis. Electron Physician. 2018;10(4):6596-607. https://doi.org/10.19082/6596
3. Galle A, Moran AC, Bonet M, Graham K, Muzigaba M, Portela A, et al. Measures to assess quality of postnatal care: a scoping review. PLoS Glob Public Health. 2023;3(2):e0001384. https://doi.org/10.1371/journal.pgph.0001384
4. Betran AP, Ye J, Moller AB, Souza JP, Zhang J. Trends and projections of caesarean section rates: global and regional estimates. BMJ Glob Health. 2021;6(6):e005671. https://doi.org/10.1136/bmjgh-2021-005671
5. Eyi EGY, Mollamahmutoglu L. An analysis of the high cesarean section rates in Turkey by Robson classification. J Matern Fetal Neonatal Med. 2021;34(16):2682-92. https://doi.org/10.1080/14767058.2019.1670806
6. Suwanrath C, Chunuan S, Matemanosak P, Pinjaroen S. Why do pregnant women prefer cesarean birth? A qualitative study in a tertiary care center in Southern Thailand. BMC Pregnancy Childbirth. 2021;21(1):23. https://doi.org/10.1186/s12884-020-03525-3
7. Martínez-Galiano JM, Hernández-Martínez A, Rodríguez-Almagro J, Delgado-Rodríguez M. Quality of life of women after giving birth: associated factors related with the birth process. J Clin Med. 2019;8(3):324. https://doi.org/10.3390/jcm8030324
8. Hernandez Alava M, Pudney S, Wailoo A. The EQ-5D-5L value set for England: findings of a quality assurance program. Value Health. 2020;23(5):642-8. https://doi.org/10.1016/j.jval.2019.10.017
9. Angelini CR, Pacagnella RC, Parpinelli MA, Silveira C, Andreucci CB, Ferreira EC, et al. Quality of life after an episode of severe maternal morbidity: evidence from a cohort study in Brazil. Biomed Res Int. 2018;2018:9348647. https://doi.org/10.1155/2018/9348647
10. Evans K, Fraser H, Uthman O, Osokogu O, Johnson S, Al-Khudairy L. The effect of mode of delivery on health-related quality-of-life in mothers: a systematic review and meta-analysis. BMC Pregnancy Childbirth. 2022;22(1):149. https://doi.org/10.1186/s12884-022-04473-w
11. Triviño-Juárez JM, Romero-Ayuso D, Nieto-Pereda B, Forjaz MJ, Criado-Álvarez JJ, Arruti-Sevilla B, et al. Health related quality of life of women at the sixth week and sixth month postpartum by mode of birth. Women Birth. 2017;30(1):29-39. https://doi.org/10.1016/j.wombi.2016.06.005
12. Prick BW, Bijlenga D, Jansen AJ, Boers KE, Scherjon SA, Koopmans CM, et al. Determinants of health-related quality of life in the postpartum period after obstetric complications. Eur J Obstet Gynecol Reprod Biol. 2015;185:88-95. https://doi.org/10.1016/j.ejogrb.2014.11.038
13. Priddis H, Schmied V, Dahlen H. Women's experiences following severe perineal trauma: a qualitative study. BMC Womens Health. 2014;14(1):32. https://doi.org/10.1186/1472-6874-14-32
14. Kolu P, Raitanen J, Luoto R. Physical activity and health-related quality of life during pregnancy: a secondary analysis of a cluster-randomised trial. Matern Child Health J. 2014;18(9):2098-105. https://doi.org/10.1007/s10995-014-1457-4
15. Davoud A, Abazari M. The relationship between quality of life and physical activity, worry, depression, and insomnia in pregnant women. Iran J Psychiatry. 2020;15(2):159-68. PMID: 32426012
16. Lagadec N, Steinecker M, Kapassi A, Magnier AM, Chastang J, Robert S, et al. Factors influencing the quality of life of pregnant women: a systematic review. BMC Pregnancy Childbirth. 2018;18(1):455. https://doi.org/10.1186/s12884-018-2087-4
17. Peters AEJ, Verspeek LB, Nieuwenhuijze M, Harskamp-van Ginkel MW, Meertens RM. The relation between sleep quality during pregnancy and health-related quality of life-a systematic review. J Matern Fetal Neonatal Med. 2023;36(1):2212829. https://doi.org/10.1080/14767058.2023.2212829
18. Calou CGP, Oliveira MF, Carvalho FHC, Soares PRAL, Bezerra RA, Lima SKM, et al. Maternal predictors related to quality of life in pregnant women in the Northeast of Brazil. Health Qual Life Outcomes. 2018;16(1):109. https://doi.org/10.1186/s12955-018-0917-8

19. Wiklund I, Edman G, Andolf E. Cesarean section on maternal request: reasons for the request, self-estimated health, expectations, experience of birth and signs of depression among first-time mothers. Acta Obstet Gynecol Scand. 2007;86(4):451-6. https://doi.org/10.1080/00016340701217913

20. Khatony A, Soroush A, Andayeshgar B, Saedpanah N, Abdi A. Attitude of primiparous women towards their preference for delivery method: a qualitative content analysis. Arch Public Health. 2019;77:38. https://doi.org/10.1186/s13690-019-0364-y

21. Rai P, Rani U. Effect of newborn's admission to intensive care unit on quality of life of mother: an Indian perspective. J Matern Fetal Neonatal Med. 2019;32(13):2188-93. https://doi.org/10.1080/14767058.2018.1428550

22. Santos Oliveira SJG, Tavares CSS, Vaez AC, Santos VS, Santos Estevam E, Leite DCF, et al. Anxiety, depression, and quality of life in mothers of newborns admitted to the neonatal intensive care unit: a follow-up study from birth to the first two years of life. J Neonatal Nurs. 2023;29(4):645-51. https://doi.org/10.1016/j.jnn.2022.11.017

ORIGINAL ARTICLE

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Independent predictors for non-alcoholic fatty liver disease in patients with treatment-naïve chronic hepatitis B

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SUMMARY

OBJECTIVE: There are limited data on non-alcoholic fatty liver disease in chronic hepatitis B virus infection. We aimed to determine the predictors for non-alcoholic fatty liver disease in patients with treatment-naïve chronic hepatitis B virus infection.

METHODS: All consecutive treatment-naïve patients with chronic hepatitis B virus infection at the Haseki Training and Research Hospital between October 1, 2021, and September 31, 2022, were retrospectively enrolled. Chronic hepatitis B virus infection is defined by positive serum hepatitis B surface antigen for 6 months or more. Patients with significant alcohol consumption, prolonged steatogenic drug use, malignancy, monogenic hereditary disorders, patients co-infected with hepatitis D virus, hepatitis C virus infection, or human immunodeficiency virus were excluded. Demographic characteristics, anthropometric determinants, laboratory findings, and virological parameters were retrospectively collected from patients' charts and electronic medical records.

RESULTS: A total of 457 patients with treatment-naïve chronic hepatitis B virus infection were included in the study. The three multivariate regression models revealed that age (p<0.028), body mass index (p=0.046), diabetes mellitus (p=0.030), hemoglobin (p=0.008), platelet (p=0.012), and triglyceride (p=0.002) in Model 1; body mass index (p=0.033), diabetes mellitus (p<0.001), hemoglobin (p=0.008), platelet (p=0.004), LDL (p=0.023), and HDL (p=0.020) in Model 2; and age (p<0.001), body mass index (p=0.033), hemoglobin (p=0.004), platelet (p=0.004), and HDL (p=0.007) in Model 3 were independent predictors.

CONCLUSION: Non-alcoholic fatty liver disease was observed in about one-third of patients with chronic hepatitis B virus infection and was positively associated with older age, higher body mass index, presence of comorbid conditions including diabetes mellitus, increased levels of metabolic laboratory parameters, especially serum triglyceride and LDL, and decreased HDL.

KEYWORDS: NAFLD. Hepatitis B. BMI. Hyperlipidemia. Diabetes.

INTRODUCTION

Both chronic hepatitis B and non-alcoholic fatty liver disease (NAFLD) have caused chronic liver diseases and resulted in poor clinical outcomes[1]. Currently, chronic hepatitis B has affected 296 million patients all around the world[2]. In addition, about a quarter of the global population and one-third of both Western and Asian populations suffer from NAFLD[3-5]. Despite the lower rate of NAFLD in patients with chronic hepatitis B compared with community, NAFLD is still a major public health issue[6,7]. In addition, NAFLD is associated with an increased risk for cardiovascular disease[8].

Rastogi et al., reported that advanced age, male gender, obesity, lower viral load, and elevated levels of triglycerides, cholesterol, and insulin were associated with hepatic steatosis among patients with chronic hepatitis B virus (HBV). In their study, only serum triglyceride level was detected as an independent predictor for hepatic steatosis[9]. Similarly, Machado et al., showed that male gender, alcohol consumption, body mass index (BMI), obesity, diabetes mellitus, triglycerides, and cholesterol were associated with hepatic steatosis[10]. Although some studies have revealed factors associated with hepatic steatosis in chronic hepatitis B patients, there is still limited data on NAFLD in chronic HBV infection[11-13]. Therefore, in this study, we aimed to determine the predictors for NAFLD in patients with treatment-naïve chronic HBV infection.

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PATIENTS AND METHODS

Ethical statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Declaration of Helsinki. This study was approved by the Ethics Committee of Haseki Training and Research Hospital (approval no: 2022-200, date: November 9, 2022). Written informed consent was waived due to the retrospective nature of this study.

Study design

All consecutive treatment-naïve patients with chronic HBV infection at the Haseki Training and Research Hospital between October 1, 2021, and September 31, 2022, were retrospectively enrolled. Demographic characteristics (sex, age, and underlying diseases), anthropometric determinants (body mass index), laboratory findings (hemoglobin, platelet, aspartate aminotransferase, alanine aminotransferase, total bilirubin, LDL, HDL, triglyceride, fasting blood glucose, INR, and alpha fetoprotein), and virological parameters (HBV DNA) were retrospectively collected from patients' charts and electronic medical records. NAFLD was defined as the presence of hepatic steatosis by ultrasonography and the absence of secondary causes of hepatic fat accumulation. The presence of steatosis was evaluated by ultrasonography as grades 1–3.

A total of 472 patients with treatment-naïve patients with chronic HBV infection aged ≥18 years were included. Patients with significant alcohol consumption (n=2, 0.4%), prolonged steatogenic drug use (n=1, 0.2%), malignancy (n=3, 0.6%), monogenic hereditary disorders (n=1, 0.2%), patients co-infected with hepatitis D virus (n=5, 1.1%), hepatitis C virus infection (n=1, 0.21%), or human immunodeficiency virus (n=2, 0.4%) were excluded.

Definitions

Chronic HBV infection is defined by positive serum hepatitis B surface antigen (HBsAg) for 6 months or more in accordance with AASLD 2018 Hepatitis B Guidance[14]. NAFLD was defined as the presence of hepatic steatosis detected by radiologic imaging or histologic evaluation and the absence of significant alcohol consumption, prolonged use of a steatogenic drug, or other secondary causes of hepatic fat accumulation. Diagnosis criteria of NAFLD were based on NAFLD Practice Guidance from the AASLD[15]. Ultrasonography was used to diagnose NAFLD.

Statistical analysis

Categorical variables were expressed as frequencies (n) and percentages (%), while numerical variables were expressed as medians (interquartile range). Chi-square and Fisher's exact tests were used for categorical variables. The Mann-Whitney U test was used for continuous variables. Univariate and multivariate logistic regression analyses were performed to identify independent predictors for NAFLD. A p-value less than 0.05 was considered statistically significant. IBM SPSS Statistics for Windows was used for statistics.

RESULTS

A total of 457 patients with treatment-naïve chronic HBV infection/hepatitis were included in the study. Of those, 244 (53.4%) were male and the median age was 43 (36–52) years. The median BMI was 26.3 (23.4–29.3). The most common underlying diseases were hypertension (n=75, 16.4%), diabetes mellitus (n=44, 9.6%), hyperlipidemia (n=14, 3.1%), and coronary artery disease (n=12, 2.6%) (Table 1). Twelve (2.6%) patients were HBeAg positive. The median value of HBV DNA was 892 (131–5920) IU/mL (Table 2).

Non-alcoholic fatty liver disease was observed in 162 (35.4%) patients. Patients with NAFLD were older than patients without NAFLD (47 years vs. 42 years, p<0.001). Presence of underlying diseases (at least one or more) (39.5% vs. 21.4%, p<0.001), diabetes mellitus (16% vs. 6.1%, p=0.001), hypertension (22.2% vs. 13.2%, p=0.013), and hyperlipidemia (5.6% vs. 1.7%, p=0.022) were more common in patients with NAFLD than without NAFLD (Table 1).

The median values of HBV DNA (p=0.021) and HDL levels (p<0.001) were lower in patients with NAFLD than those without NAFLD. However, BMI (p<0.001), hemoglobin (p=0.014), platelet count, LDL (110 mg/dL vs. 100 mg/dL, p=0.003), HDL (p<0.001), triglyceride (p<0.001), fasting blood glucose (p=0.003), and INR (p=0.004) were higher in patients with NAFLD (Table 2).

In univariate analysis, age (p<0.001), BMI (p=0.001), hypertension (p=0.014), diabetes mellitus (p=0.001), hyperlipidemia (p<0.030), hemoglobin (p=0.009), platelet (p=0.032), LDL (p=0.001), HDL (p<0.001), and triglyceride (p<0.001) were predictors for NAFLD in patients with chronic hepatitis B (Table 3).

The three multivariate regression models revealed that age (p<0.028), BMI (p=0.046), diabetes mellitus (p=0.030), hemoglobin (p=0.008), platelet (p=0.012), and triglyceride (p=0.002) in Model 1; BMI (p=0.033), diabetes mellitus (p<0.001), hemoglobin (p=0.008), platelet (p=0.004), LDL (p=0.023), and HDL (p=0.020) in Model 2; and age (p<0.001), BMI (p=0.033), hemoglobin (p=0.004), platelet (p=0.004), and HDL (p=0.007) in Model 3 were independent predictors (Table 3).

Table 1. Comparison of demographic characteristics and underlying diseases in patients with non-alcoholic fatty liver disease and without non-alcoholic fatty liver disease.

Parameters		In total		Patients with NAFLD (n=162)		Patients without NAFLD (n=295)		OR	CI	p-value
		n	%	n	%	n	%			
Sex, n (%)	Male	244	53.4	94	58.0	150	50.8	0.748	0.508–1.102	0.141
	Female	213	46.6	68	42.0	145	49.2			
Underlying diseases, n (%)	Yes	127	27.8	64	39.5	63	21.4	2.405	1.579–3.662	**<0.001**
	No	330	72.2	98	60.5	232	78.6			
Diabetes mellitus, n (%)	Yes	44	9.6	26	16	18	6.1	2.942	1.559–5.552	**0.001**
	No	413	90.4	136	84	256	93.9			
Hypertension, n (%)	Yes	75	16.4	36	22.2	39	13.2	1.875	1.137–3.094	**0.013**
	No	382	83.6	126	77.8	256	86.8			
Chronic artery diseases, n (%)	Yes	12	2.6	7	4.3	5	1.7	2.636	0.823–8.445	0.091
	No	444	97.4	154	95.7	290	98.3			
Chronic kidney disease, n (%)	Yes	8	1.8	2	1.2	6	2	0.602	0.120–3.018	0.533
	No	449	98.2	160	98.8	289	98			
Chronic obstructive pulmonary disease, n (%)	Yes	7	1.5	3	1.9	4	1.4	1.373	0.303–6.210	0.702
	No	450	98.5	159	98.1	291	98.6			
Neurological disease, n (%)	Yes	8	1.8	2	1.2	6	2	0.602	0.120–3.018	0.533
	No	449	98.2	160	98.8	289	98			
Hyperlipidemia, n (%)	Yes	14	3.1	9	5.6	5	1.7	3.412	1.124–10.359	**0.022**
	No	443	96.9	153	94.4	290	98.3			
HBeAg positive, n (%)	Yes	12	2.6	4	2.5	8	2.7	0.908	0.269–3.064	0.877
	No	445	97.4	158	97.5	287	97.3			

Statistically significant values are indicated in bold.

DISCUSSION

In this study, the prevalence of NAFLD among patients with treatment-naïve chronic HBV infection was 35.4% (n=162). We found that age, BMI, diabetes mellitus, hemoglobin, serum triglyceride, LDL, and HDL were independent predictors for NAFLD.

Non-alcoholic fatty liver disease is commonly associated with obesity, diabetes mellitus, and elevated cholesterol[8]. In the study of Zhu et al., obesity and diabetes mellitus were associated with 8.5-fold and 2-fold increased risk for NAFLD among patients with chronic hepatitis B, respectively[16]. In this study, we observed a 2–3.5-fold increased risk for NAFLD in patients with hypertension, diabetes mellitus, and hyperlipidemia. Furthermore, the presence of diabetes mellitus was independently associated with about 3.5-fold increased risk for NAFLD among patients with treatment-naïve chronic HBV infection in multivariate regression analysis.

The association between HBV replication and hepatic steatosis is also unclear[17]. While some studies demonstrated that there is a negative association between hepatic steatosis and HBV DNA[18], others have reported no associations between viral load and hepatic steatosis[19]. In a recent study, Wang et al., demonstrated that HBV DNA level was negatively and independently associated with NAFLD in the pediatric population with chronic hepatitis B[20]. Similar to our study, Zhu et al., reported that viral load or other viral factors were not independently associated with NAFLD[16]. Similarly, the negative association between NAFLD and HBV seromarkers was also supported by studies in animal models. In one animal model of NAFLD-CHB comorbidity, HBeAg, HBsAg, hepatitis B core antigen, and HBV DNA levels were higher in mice without NAFLD than those with NAFLD, although the mechanism was not explored[21]. In our study, a significant association between HBV DNA and NAFLD was not detected. This could be because the majority of our study group consisted of grade-1 steatosis and the rate of advanced steatosis was low.

Table 2. Comparison of age, body mass index, viral load, laboratory parameters, and liver histopathology scores in patients with non-alcoholic fatty liver disease and without non-alcoholic fatty liver disease.

Parameters	In total	Patients with NAFLD (n=162)	Patients without NAFLD (n=295)	p-value
	Median (IQR 25–75)	Median (IQR 25–75)	Median (IQR 25–75)	
Age, years, median (IQR)	43 (36–52)	47 (40–55)	42 (34–51)	**<0.001**
BMI, median (IQR)	26.3 (23.4–29.3)	28.1 (25.8–31.5)	25.5 (22.9–28.5)	**<0.001**
HBV-DNA, IU/mL, median (IQR)	892 (131–5920)	572 (88–3730)	1030 (187–7200)	**0.021**
Hemoglobin, g/dL, median (IQR)	14 (13–15.3)	14.6 (13.2–15.4)	14 (12.6–15.3)	**0.014**
Platelet /mm^3, median (IQR)	232 (196–269)	237 (204–278)	227 (194–259)	**0.017**
Aspartate aminotransferase (AST), IU/mL, median (IQR)	20 (17–24)	20 (17–25)	20 (17–24)	0.607
Alanine aminotransferase (ALT), IU/mL median (IQR)	20 (15–29)	20 (15–32)	19 (15–28)	0.074
Total bilirubin, mg/dL, median (IQR)	0.44 (0.33–0.64)	0.44 (0.34–0.61)	0.46 (0.33–0.65)	0.538
LDL, mg/dL, median (IQR)	103 (84–128)	110 (86–136)	100 (82–122)	**0.003**
HDL, mg/dL, median (IQR)	47 (40–56)	45 (36–52)	49 (41–58)	**<0.001**
Triglyceride, mg/dL, median (IQR)	109 (77–160)	148 (104–193)	95 (71–135)	**<0.001**
Fasting blood glucose, mg/dL, median (IQR)	94 (88–105)	99 (89–110)	93 (87–103)	**0.003**
INR, median (IQR)	1.0 (1.0–1.1)	1.0 (1.0–1.0)	1.0 (1.0–1.1)	**0.004**
Alfa fetoprotein, ng/mL, median (IQR)	2.5 (1.8–3.6)	2.5 (1.8–3.4)	2.6 (1.8–3.6)	0.78
FIB-4 score, median (IQR)	0.8 (0.7–1.2)	0.9 (0.7–1.1)	0.8 (0.6–1.2)	0.967
Fibrosis, median (IQR)	1 (0–1)	0 (0–1)	1 (0–1)	0.647
Hepatic activity index (HAI), median (IQR)	4 (3–5)	4 (3–5)	4 (3–6)	0.636

Statistically significant values are indicated in bold.

Table 3. Univariate and multivariate analyses for predicting non-alcoholic fatty liver disease in patients with chronic hepatitis B.

Parameters	Univariate analysis			Multivariate Model 1			Multivariate Model 2			Multivariate Model 3		
	OR	CI	p	OR	CI	p	OR	CI	p	OR	CI	p
Age	1.029	1.013–1.046	**<0.001**	1.034	1.004–1.065	**0.028**	–	–	–	1.048	1.023–1.074	**<0.001**
Body mass index	1.072	1.028–1.117	**0.001**	1.049	1.001–1.100	**0.046**	1.051	1.004–1.101	**0.033**	1.048	1.004–1.095	**0.033**
Hypertension	1.875	1.137–3.094	**0.014**	1.009	0.425–2.394	0.983	–	–	–	–	–	–
Diabetes mellitus	2.942	1.559–5.552	**0.001**	3.446	1.130–10.515	**0.030**	5.711	2.075–15.722	**<0.001**	–	–	–
Hyperlipidemia	3.412	1.124–10.359	**0.030**	1.248	0.241–6.470	0.792	–	–	–	–	–	–
Hemoglobin	1.165	1.039–1.306	**0.009**	1.315	1.076–1.608	**0.008**	1.292	1.068–1.564	**0.008**	1.336	1.098–1.625	**0.004**
Platelet count	1.004	1.000–1.007	**0.032**	1.008	1.002–1.014	**0.012**	1.008	1.003–1.014	**0.004**	1.009	1.003–1.015	**0.004**
Triglyceride	1.011	1.007–1.014	**<0.001**	1.009	1.003–1.014	**0.002**	–	–	–	–	–	–
LDL	1.010	1.004–1.016	**0.001**	1.008	0.998–1.017	0.130	1.010	1.001–1.019	**0.023**	–	–	–
HDL	0.967	0.952–0.983	**<0.001**	0.987	0.957–1.017	0.384	0.969	0.943–0.995	**0.020**	0.963	0.937–0.990	**0.007**

Model 1: All significant variables in univariate analysis were included. Model 2: BMI, diabetes mellitus, hemoglobin, platelet, and LDL were included. Model 3: Age, BMI, hemoglobin, platelet, and HDL were included. Statistically significant values are indicated in bold.

Minakari et al., evaluated 132 treatment-naïve patients. Of those, 35 (26.5%) were HBeAg positive and 56 (42.4%) had NAFLD[12]. In univariate analysis, patients without steatosis were significantly older than those with steatosis. HBV DNA levels were lower in those with steatosis, but no statistically significant difference was found. BMI, serum triglyceride, fasting blood glucose, and GGT were found as predictors for NAFLD in univariate analysis. However, only serum triglyceride was an independent predictor in multivariate analysis. In the study of Yun et al., among untreated young males with chronic hepatitis

B, serum insulin, total cholesterol, and triglyceride were significantly higher in patients with steatosis than in patients without steatosis[22]. The researchers reported that homeostatic model assessment for insulin resistance and triglyceride was found to be significant in the multivariate analysis. In a study conducted by Vigano et al., the severity of steatosis was significantly associated with advanced age, male gender, and higher BMI[23]. In their study, a higher prevalence of hyperglycemia was observed in patients with mild steatosis, while triglyceride levels increased progressively with the severity of steatosis. Nau et al., included 83 patients with an HbeAg-positive rate of 9.1%[24]. Fatty liver was observed in 11.3% of patients. They reported that total cholesterol was higher and prothrombin time was longer in patients with steatosis on ultrasound. Higher fasting insulin levels and higher BMI were found in patients with steatosis. AST levels were lower in patients with steatosis.

Our study had several strengths. First, the sample size was relatively high. Second, we could add various variables in the multivariate regression models. This study had some limitations. First, this study was conducted in a single center. Second, we used ultrasonography to identify NAFLD. Histopathological examination was not evaluated. Third, because the prevalence of patients with grade-3 steatosis in our study group was rare, this might affect the generalizability of our results. Therefore, large-scale studies are needed to identify associated factors for NAFLD in patients with advanced hepatic steatosis.

CONCLUSION

Non-alcoholic fatty liver disease was observed in about one-third of patients with chronic HBV infection and was positively associated with older age, higher BMI, presence of comorbid conditions including diabetes mellitus, increased levels of metabolic laboratory parameters, especially serum triglyceride and LDL, and decreased HDL. However, neither HBV DNA levels nor HBeAg positivity were independent predictors for NAFLD.

AUTHORS' CONTRIBUTIONS

GT: Conceptualization, Data curation, Methodology, Validation. CGG: Conceptualization, Data curation, Methodology. OFB: Formal Analysis, Software. BAB: Writing – review & editing. CY: Data curation, Writing – review & editing. BC: Writing – review & editing. KGG: Writing – review & editing. MY: Methodology, Writing – review & editing. GS: Methodology, Writing – review & editing. FP: Methodology, Writing – review & editing.

REFERENCES

1. Zhang J, Lin S, Jiang D, Li M, Chen Y, Li J, et al. Chronic hepatitis B and non-alcoholic fatty liver disease: conspirators or competitors? Liver Int. 2020;40(3):496-508. https://doi.org/10.1111/liv.14369
2. World Health Organization. Hepatitis B. 2023. [cited on 2023 Aug 07]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b
3. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023;77(5):1797-835. https://doi.org/10.1097/HEP.0000000000000323
4. Farrell GC, Wong VW, Chitturi S. NAFLD in Asia--as common and important as in the West. Nat Rev Gastroenterol Hepatol. 2013;10(5):307-18. https://doi.org/10.1038/nrgastro.2013.34
5. Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. J Hepatol. 2017;67(4):862-73. https://doi.org/10.1016/j.jhep.2017.06.003
6. Yang M, Wei L. Impact of NAFLD on the outcome of patients with chronic hepatitis B in Asia. Liver Int. 2022;42(9):1981-90. https://doi.org/10.1111/liv.15252
7. Hui RWH, Seto WK, Cheung KS, Mak LY, Liu KSH, Fung J, et al. Inverse relationship between hepatic steatosis and hepatitis B viremia: results of a large case-control study. J Viral Hepat. 2018;25(1):97-104. https://doi.org/10.1111/jvh.12766
8. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, et al. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023;77(5):1797-835. https://doi.org/10.1097/HEP.0000000000000323
9. Rastogi A, Sakhuja P, Kumar A, Hissar S, Jain A, Gondal R, et al. Steatosis in chronic hepatitis B: prevalence and correlation with biochemical, histologic, viral, and metabolic parameters. Indian J Pathol Microbiol. 2011;54(3):454-9. https://doi.org/10.4103/0377-4929.85074
10. Machado MV, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. J Gastroenterol Hepatol. 2011;26(9):1361-7. https://doi.org/10.1111/j.1440-1746.2011.06801.x
11. Tsochatzis E, Papatheodoridis GV, Manesis EK, Kafiri G, Tiniakos DG, Archimandritis AJ. Metabolic syndrome is associated with severe fibrosis in chronic viral hepatitis and non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2008;27(1):80-9. https://doi.org/10.1111/j.1365-2036.2007.03538.x
12. Minakari M, Molaei M, Shalmani HM, Mohammad Alizadeh AH, Jazi AH, Naderi N, et al. Liver steatosis in patients with chronic hepatitis B infection: host and viral risk factors. Eur J Gastroenterol Hepatol. 2009;21(5):512-6. https://doi.org/10.1097/MEG.0b013e328326792e
13. Yang M, Wei L. Impact of NAFLD on the outcome of patients with chronic hepatitis B in Asia. Liver Int. 2022;42(9):1981-90. https://doi.org/10.1111/liv.15252
14. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67(4):1560-99. https://doi.org/10.1002/hep.29800
15. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American association for the study of liver diseases. Hepatology. 2018;67(1):328-57. https://doi.org/10.1002/hep.29367

16. Zhu L, Jiang J, Zhai X, Baecker A, Peng H, Qian J, et al. Hepatitis B virus infection and risk of non-alcoholic fatty liver disease: a population-based cohort study. Liver Int. 2019;39(1):70-80. https://doi.org/10.1111/liv.13933

17. Hui RWH, Seto WK, Cheung KS, Mak LY, Liu KSH, Fung J, et al. Inverse relationship between hepatic steatosis and hepatitis B viremia: results of a large case-control study. J Viral Hepat. 2018;25(1):97-104. https://doi.org/10.1111/jvh.12766

18. Machado MV, Oliveira AG, Cortez-Pinto H. Hepatic steatosis in hepatitis B virus infected patients: meta-analysis of risk factors and comparison with hepatitis C infected patients. J Gastroenterol Hepatol. 2011;26(9):1361-7.https://doi.org/10.1111/j.1440-1746.2011.06801.x

19. Sasso M, Beaugrand M, Ledinghen V, Douvin C, Marcellin P, Poupon R, et al. Controlled attenuation parameter (CAP): a novel VCTE™ guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. Ultrasound Med Biol. 2010;36(11):1825-35. https://doi.org/10.1016/j.ultrasmedbio.2010.07.005

20. Wang L, Wang Y, Liu S, Zhai X, Zhou G, Lu F, et al. Nonalcoholic fatty liver disease is associated with lower hepatitis B viral load and antiviral response in pediatric population. J Gastroenterol. 2019;54(12):1096-105. https://doi.org/10.1007/s00535-019-01594-6

21. Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010;139(2):474-82. https://doi.org/10.1053/j.gastro.2010.04.048

22. Yun JW, Cho YK, Park JH, Kim HJ, Park DI, Sohn CI, et al. Hepatic steatosis and fibrosis in young men with treatment-naïve chronic hepatitis B. Liver Int. 2009;29(6):878-83. https://doi.org/10.1111/j.1478-3231.2009.01976.x

23. Viganò M, Valenti L, Lampertico P, Facchetti F, Motta BM, D'Ambrosio R, et al. Patatin-like phospholipase domain-containing 3 I148M affects liver steatosis in patients with chronic hepatitis B. Hepatology. 2013;58(4):1245-52. https://doi.org/10.1002/hep.26445

24. Nau AL, Soares JC, Shiozawa MB, Dantas-Corrêa EB, Schiavon LL, Narciso-Schiavon JL. Clinical and laboratory characteristics associated with dyslipidemia and liver steatosis in chronic HBV carriers. Rev Soc Bras Med Trop. 2014;47(2):158-64. https://doi.org/10.1590/0037-8682-0009-2014

ORIGINAL ARTICLE

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Impact of anthro-metabolic indices and gestational weight gain on maternal and neonatal outcomes: a prospective observational study

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SUMMARY

OBJECTIVE: The aim of this study was to examine the relationship of anthro-metabolic indices on maternal and neonatal outcomes.

METHODS: This prospective observational study was conducted on healthy mother–baby pairs between January 1, 2023 and July 1, 2023. Detailed sociodemographic information was collected through an interview with the mother. Clinical, biochemical, obstetric, fetal, and neonatal outcomes were abstracted from hospital medical records. Anthropometric measurements were obtained from the examination of mother–baby pairs.

RESULTS: A total of 336 healthy mothers–children pairs were included. Mothers of newborn ≥4000 g had higher gestational age (p=0.003), body mass index (p=0.003), gestational weight gain (p=0.016), waist circumferences (p=0.002), and hip circumferences (p=0.001). gestational weight gain was associated with the mode of delivery (p=0.023). waist-to-hip ratio (p=0.005), gestational weight gain (p=0.013), and a body shape ındex (p<0.001) were associated with longer length of hospital stay. Age (p<0.001) and inter-pregnancy interval (p=0.004) were higher in pre-pregnancy underweight/obese mothers. Receiver operating characteristic analysis revealed that maternal waist circumferences (AUC: 0.708, p=0.005), maternal weight (AUC: 0.690, p=0.010), and hip circumferences (AUC: 0.680, p=0.015) were sufficient to predict macrosomia (p<0.05).

CONCLUSION: The study demonstrated a significant association between gestational weight gain and cesarean delivery, prolonged hospital stay, and macrosomia. It was also found that maternal body mass index, waist circumferences, and hip circumferences during pregnancy were associated with macrosomia. On the contrary, no significant relationship was found between maternal anthro-metabolic characteristics and maternal–fetal and birth outcomes.

KEYWORDS: Waist-to-hip ratio. Body mass index. Cesarean section. Macrosomia. Weight gain.

INTRODUCTION

Overweight and obesity have reached epidemic proportions, causing more than 4 million deaths worldwide annually[1]. World Health Organization (WHO) notes that worlwide prevalence of obesity almost tripled over the past four decades[2]. In the United States, more than 50% of pregnant women are suffering from obesity, while in England, 21.3% of pregnant women are living with obesity[3,4]. Previous research has established that a high prepregnancy body mass index (BMI) and gestational weight gain (GWG) are linked to unfavorable maternal and neonatal outcomes, including gestational diabetes, preeclamsia, cesarean delivery, and fetal macrosomia[5,6].

Body mass index is a commonly used risk stratification tool in pregnancy. However, a disadvantage of BMI is that it does not differentiate fat and lean mass, or reflect fat distribution[7]. It is assumed that all women with obesity are at equal risk of having a poor pregnancy outcome. However, a study involving 5,628 women with uncomplicated pregnancies found that 47% of women with obesity did not experience any adverse pregnancy outcome, whereas 42% of overweight women did[8]. Consequently, BMI has been questioned because it does not accurately predict which women are at high risk of an obesity-related adverse outcome of pregnancy. Therefore, alternative obesity anthropometric indicies have been developed to modulate the limitations of BMI.

Anthropometry is a simple, reliable, and low-cost method and provides useful information regarding abdominal and genitofemoral adiposity[9]. Identification of the effect of those

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anthropometric parameters on maternal and neonatal outcomes is important in order to reduce or prevent adverse obstetric and neonatal outcomes, which has many implications for the development of maternal and newborn health. Accumulating evidence implies that the distribution of body fat might be a more precise indicator of individual risk, yet there is a dearth of reliable evidence during pregnancy. With this study, we aimed to examine the relationship of anthro-metabolic indices with maternal and neonatal outcomes.

METHODS

This prospective observational study was carried out at a tertiary hospital from January 1, 2023 to July 1, 2023. Ethical approval was obtained from the Research Ethics Committee (Approval number: 197/22.12.2021) and adheres to the principles of Declaration of Helsinki. All participants gave written informed consent prior to entering the study.

The study recruited a total of 320 healthy pregnant women who gave birth to normal healthy, full-term, singleton baby, with minimum reading and writing literacy, and were willing to participate in the study after being informed of its purpose and methodology. Pregnant women with multiple pregnancies, fetal chromosomal aneuploidy and/or congenital deformities, stillbirth, birth before 37 weeks, previous cesarean section, pregnant women who were using any drugs that affect blood glucose, history of taking alcohol, smoking, those with a history of psychological or physical illnesses, history of complicated pregnancy, who had an addiction, those with chronic diseases (e.g., diabetes, chronic hypertension, liver or kidney disease, cardiovascular disease, and thyroid dysfunction), pregnant women with maternal and fetal complications (e.g., preterm birth, prelabor rupture of membranes, preeclampsia, gestational hypertension, gestational diabetes mellitus, oligohydramnios, polyhydramnios, intrahepatic cholelithiasis, placenta previa, and intrauterine growth restriction) were excluded from the study.

Body weight and height of the expectant mother were measured with a digital weight and height scale. Each participant was required to remove shoes, stand upright with arms loosely to the side, and be positioned in the Frankfurt plane, with equal weight distribution. Body weight and height were measured to the nearest 0.1 kg and 0.001 m, respectively.

Hip circumstance (HC), waist circumstance (WC), mid-upper arm circumstance (MUAC), and neonatal head circumference were measured with a non-stretch tape. A standard technique was followed for accurate anthropometric measurements. After emptying the bladder, the participants removed their clothing and footwear, then stood upright with arms hanging loosely at the sides. WC measurement was made at midpoint between the lowest rib and iliac crest during expiration. HC measurement was made at the widest part of the gluteus region over the greater trochanters. Mid-upper arm circumference (MUAC) was measured halfway between the acromion and the olecranon fossa on the non-dominant arm.

Body mass index was categorized according to WHO[10]. GWG is categorized according to the 2009 Institute of Medicine (IOM) recommendations[11].

Body mass index formula[12]: weight (kg)/[height (m)]^2.

A body shape index (ABSI) formula[13]: ABSI=WC(m)/[BMI $^{2/3}$ × Height (m)$^{1/2}$].

The body round index (BRI) formula[13]: BRI=365.2 - 365.5 × $\sqrt{}$(1 - (((WC/2π)^2)/[(0.5 × height)]^2))

Waist-to-hip ratio (WHR) formula: WHR=WC/HC

Birth weight and lengths of infants were measured within 1 h of birth using standardized procedures. We weighed the neonates naked using a digital weighing scale, in a supine position, to the nearest 0.001 kg. An infant meter was used to measure a baby's length. On the board, the body was placed with the legs fully extended, and moderate pressure was applied to the knees. After positioning the head, measurements were taken to 0.001 m.

The date of the last menstrual period was taken to determine gestational age, and confirmation was made with first trimester sonographic crown-rump length measurement. Systolic and diastolic blood pressures were taken during delivery time. The blood pressure values were recorded as millimeters of mercury (mmHg). Detailed sociodemographic information was collected through an interview with the mother. Clinical, biochemical, obstetric, fetal, and neonatal outcomes were abstracted for each patient from patients' hospital medical records. Anthropometric measurements were obtained from the examination of mother–baby pairs. According to The American College of Obstetricians and Gynaecologists, fetal macrosomia was defined as fetal birth weight greater than 4000 g or 4500 g[14]. In the present study, an infant's birth weight above >4000 g was defined as fetal macrosomia. APGAR score of 7 points or less was classified as abnormal, and an APGAR score above 7 was classified as normal[15].

Statistical analysis

The data collected in the study were transferred to the Epi info 7.2 program and analyzed. Descriptive data are presented in the form of mean, standard deviation, minimum, maximum, number, and percentage values. Whether the distributions of continuous variables were normal or not was controlled with the Kolmogorov-Smirnov test. For comparison between two

variables which do not conform to the normal distribution, the Mann-Whitney U-test and Kruskal-Wallis test were employed. A p-value of less than 0.05 was deemed to be indicative of statistical significance.

RESULTS

A total of 336 mothers–children pairs were included in our study. The mean of the maternal age was 27.53±5.07 years, pre-pregnancy BMI 24.13±4.15 kg/m^2, and GWG 14.92±6.83 kg. There were 16 women (4.76%) who gave birth to newborns with macrosomia >4000 g, and 8 women (2.38%) had newborns <2500 g. There were 126 (29.7%) women with pre-pregnancy BMI≥25 kg/m^2, of whom 35 (10.8%) were obese (BMI≥30 kg/m^2). The percentage of women with excessive GWG was 50.2% (169/336).

In the comparison of maternal characteristics and neonatal birth weight, mothers of newborn ≥4000 g had higher gestational age (p=0.003), BMI (p=0.003), GWG (p=0.016), WC (p=0.002), and HC (p=0.001). Birth weight did not significantly differ according to WHR, MUAC, ABSI, and BRI. Neonatal birth weight was associated with maternal fasting blood glucose (p=0.004), but not with systolic and diastolic blood pressure or hemoglobin value (p>0.05) (Table 1).

Gestational weight gain was associated with the mode of delivery (p=0.023). The mean diastolic blood pressure was 66.49±7.29 in the spontaneous vaginal delivery (SVD) group, whereas it was 68.92±7.09 in the cesarian section (CS) group (p=0.017) (Table 2).

It was found that maternal anthropometric measurements were not associated with 1-min APGAR score (p>0.05). GWG (p=0.013), WHR (p=0.005), and ABSI (p<0.001) were associated with longer length of hospital stay. APGAR score ≤7 at 1 min was significantly higher in younger mothers (p=0.036) (Table 3).

Age (p<0.001) and inter-pregnancy interval (p=0.004) were higher in pre-pregnancy underweight/obese mothers. Neonatal birth height was found to be shorter in pre-pregnancy underweight mothers (p=0.046).

Receiver operating characteristic (ROC) analysis revealed that maternal WC (AUC: 0.708, p=0.005), maternal weight (AUC: 0.690, p=0.010), and HC (AUC: 0.680, p=0.015) were sufficient to predict macrosomia (p<0.05).

DISCUSSION

The study demonstrated a significant association between GWG and cesarean delivery, prolonged hospital stay, and macrosomia. It was also found that maternal BMI, WC, and HC measurements during pregnancy were associated with macrosomia. On the contrary, no significant relationship was found between maternal pre-pregnancy weight, BRI, ABSI, MUAC, WHR, and maternal–fetal and birth outcomes.

It is well established that excessive GWG is a risk factor for macrosomia, regardless of pre-pregnancy BMI[16]. The results of our study corroborate the findings of other studies, including a recent meta-analysis involving 1,309,136 women[17]. The researchers reported that high GWG was associated with macrosomia and cesarean delivery. Similarly, a multicenter study also found associations between GWG and adverse pregnancy outcomes, including macrosomia, shoulder dystocia, cesarean birth, and neonatal hypoglycemia[18]. Another prospective cohort study reported that excessive GWG played a crucial role

Table 1. Comparison of neonatal birth weight categories with maternal anthropometric features.

Variables	Birth weights (g)			p-value
	Low birth weight (<2500 g)	Normal birth weight (>2500 g)	Macrosomia (>4000 g)	
Maternal age (years)	30.00 (21.00–37.00)	27.00 (18.00–43.00)	27.00 (22.00–38.00)	0.571¥
Gestational age (weeks)	37.20 (37.00–41.00)	39.60 (37.00–42.00)	40.65 (38.00–42.00)	**0.003**¥
Maternal BMI (kg/m^2)	25.87 (18.37–37.46)	29.39 (21.10–42.76)	31.24 (23.57–46.06)	**0.039**¥
Maternal weight gain during pregnancy (kg)	10.50 (2.00–20.00)	15.00 (-9.00–40.00)	18.50 (10.00–31.00)	**0.016**¥
Maternal waist circumference (cm)	101.00 (90.00–118.00)	110.00 (90.00–129.00)	116.00 (106.00–130.00)	**0.002**¥
Maternal hip circumference (cm)	100.00 (90.00–116.00)	112.00 (90.00–132.00)	116.00 (100.0–128.00)	**0.001**¥
A Body shape index (ABSI) (m11/6 kg2/3)	0.92 (0.82–1.01)	0.91 (0.73–1.07)	0.89 (0.73–1.03)	0.867¥
Body roundness index (BRI)	6.55 (4.26–9.23)	7.45 (4.33–12.46)	7.76 (6.28–9.55)	0.175¥
Maternal glucose level	72.10 (67.30–78.90)	83.80 (51.00–185.00)	79.60 (66.40–116.70)	**0.004**¥

Body mass index, BMI. Categorical variables are shown as n (column %), and continous variables are shown as median (min–max). ¥Kruskal-Wallis test. Statistically significant values are denoted in bold.

Table 2. Comparison of mode of delivery with maternal anthropometric.

Variables	Mode of delivery		p-value
	Spontaneous vaginal delivery	Cesarian section	
Maternal Age (years)	27.00 (18.00–43.00)	27.00 (18.00–40.00)	0.670¥
Gestational age (weeks)	39.60 (37.00–42.00)	40.00 (37.00–42.00)	0.060¥
Maternal weight gain during pregnancy (kg)	15.00 (-9.00–40.00)	17.00 (-3.00–40.00)	**0.023**¥
Maternal waist circumference (cm)	110.00 (90.00–128.00)	110.00 (94.00–130.00)	0.478¥
Maternal hip circumference (cm)	112.00 (90.00–132.00)	110.00 (96.00–128.00)	0.131¥
Waist/hip ratio (WHR)	0.98 (0.86–1.16)	1.00 (0.89–1.19)	0.057¥
Mid-upper arm circumference (MUAC) (cm)	28.00 (21.00–36.00)	27.00 (24.00–34.00)	0.096¥
A body shape index (ABSI) (m11/6 kg2/3)	0.91 (0.73–1.07)	0.90 (0.73–1.00)	0.086¥
Body roundness index (BRI)	7.44 (4.26–12.46)	7.35 (5.05–9.98)	0.881¥
Systolic blood pressure (mmHg)	110.00 (90.00–130.00)	110.00 (100.00–130.00)	0.580¥
Diastolic blood pressure (mmHg)	70.00 (50.00–80.00)	70.00 (50.00–80.00)	**0.017**¥

Body mass index, BMI. Categorical variables are shown as n (column %), continous variables are shown as median (min–max). ¥Mann-Whitney U-test. Statistically significant values are denoted in bold.

Table 3. Comparison of APGAR score at 1-min and length of hospital stay with maternal anthropometric features.

Variables	APGAR score at 1 min		p-value	Length of hospital stay		p-value
	Normal (≥7)	Abnormal (<7)		Normal (≤2)	Abnormal (<2)	
Maternal age (years)	27 (18–43)	24 (19–30)	**0.036**¥	27 (18–43)	27 (18–39)	0.732¥
Gestational age (weeks)	39.60 (37.00–42.00)	40.30 (37.00–41.50)	0.150¥	39.60 (37.00–42.00)	39.40 (37.00–42.00)	0.479¥
Maternal weight gain during pregnancy (kg)	15.00 (-9.00–40.00)	17.00 (1.00–30.00)	0.733¥	14.00 (-5.00–38.00)	16.00 (-9.00–40.00)	**0.013**¥
Maternal waist circumference (cm)	110 (90–130)	110 (96–129)	0.976¥	110 (90–129)	110 (90–130)	0.155¥
Maternal hip circumference (cm)	112 (90–132)	110 (98–120)	0.414¥	112 (90–132)	112 (96–128)	0.448¥
Waist/hip ratio (WHR)	0.98 (0.86–1.19)	1.00 (0.92–1.13)	0.520¥	1.00 (0.87–1.19)	0.98 (0.86–1.13)	**0.005**¥
A body shape index (ABSI) (m11/6 kg2/3)	0.91 (0.73–1.07)	0.92 (0.84–1.00)	0.586¥	0.91 (0.73–1.07)	0.89 (0.73–1.00)	**<0.001**¥
Body roundness index (BRI)	7.43 (4.26–12.46)	7.57 (5.63–9.53)	0.825¥	7.56 (4.26–12.46)	7.34 (4.33–9.98)	0.195¥

Body mass index, BMI. Categorical variables are shown as n (column %), continous variables are shown as median (min–max). ¥Mann-Whitney U-Test. Statistically significant values are denoted in bold.

in macrosomia prediction[19]. These findings suggest that GWG is critical in maternal and neonatal outcomes. Thus, effective public health interventions are necessary in order to prevent excess gestational weight gain[20].

Nguyen et al., found that women delivering macrosomic babies had higher WC compared with controls[21]. Likewise, a large follow-up study by Li et al., suggested that GWG and high WC but not WHR were risk factors for macrosomia[22]. In contrast, a Mendelian randomization analysis of Geng et al., did not find any causal relationship between maternal WC, WHR, and birth weight. However, they noted that genetically predisposed to higher HC was linked to increased birth weight[23]. In our data, WC and HC were associated with macrosomia. According to these data, we can infer that WC and HC measured in the third trimester may be the useful predictors for macrosomia.

Alternative anthropometric measures that standardize BMI, such as ABSI and BRI, have been developed to reflect the health status. In the study of Özler et al., examining the anthropometric indices in the first trimester pregnant women demonstrated that BRI, but not ABSI, may be a reliable predictor for fetal macrosomia in obese pregnant women[24]. Conversely, the present study found no association

between these two indices and neonatal and birth outcomes. A possible explanation for this might be that our study was conducted on healthy women in their third trimester of pregnancy.

This study has several limitations. The present study did not incorporate pre-pregnancy anthropometric characteristics. To address this shortcoming, future studies would benefit from longitudinal data extending from pre-pregnancy period to the postpartum period. It is important to note that, in contrast with previous studies, this study was unique in that participants were measured by a trained health professional, and data were collected prospectively, allowing for accurate data. Unlike studies that focus primarily on the association between anthropometric characteristics and cardiometabolic diseases in a non-pregnant population, this study provides a comprehensive assessment of a variety of anthro-metabolic indices within a specific population. Considering increasing maternal obesity rates as well as the lack of evidence relating to health outcomes in mother–infant dyads, research is of paramount importance. The findings established in this study will guide health care providers and policy makers in developing early intervention strategies.

CONCLUSION

This large, diverse cohort with prospectively collected data showed that maternal BMI, GWG, WC, and HC during pregnancy are important factors in determining clinical and fetal outcomes. Promoting optimal weight gain during pregnancy may reduce adverse maternal and neonatal complications.

AVAILABILITY OF DATA AND MATERIALS

The dataset used and analyzed in the study is available from the corresponding author on reasonable request.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of University of Health Sciences Turkey, Zeynep Kamil Women and Children's Diseases Training and Research Hospital (Date: 22.12.2021, No: 197). Informed consent was obtained from all individual participants included in the study.

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AUTHORS' CONTRIBUTIONS

EK: Conceptualization, Writing – original draft, Writing – review & editing. **LK:** Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing. **NY:** Formal Analysis, Writing – original draft, Writing – review & editing. **ZK:** Conceptualization, Data curation, Supervision, Writing – original draft, Writing – review & editing. **ÖT:** Writing – original draft, Writing – review & editing.

REFERENCES

1. Feng X, Zhu J, Hua Z, Shi Q, Zhou J, Luo P. The prevalence and determinant of overweight and obesity among residents aged 40-69 years in high-risk regions for upper gastrointestinal cancer in southeast China. Sci Rep. 2023;13(1):8172. https://doi.org/10.1038/s41598-023-35477-x
2. World Health Organization. Obesity and overweight. Geneva: World Health Organization; 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
3. Stüber TN, Künzel EC, Zollner U, Rehn M, Wöckel A, Hönig A. Prevalence and associated risk factors for obesity during pregnancy over time. Geburtshilfe Frauenheilkd. 2015;75(9):923-8. https://doi.org/10.1055/s-0035-1557868
4. Denison FC, Aedla NR, Keag O, Hor K, Reynolds RM, Milne A, et al. Care of women with obesity in pregnancy: green-top guideline no. 72. BJOG. 2019;126(3):e62-106. https://doi.org/10.1111/1471-0528.15386
5. Pereda J, Bove I, Pineyro MM. Excessive maternal weight and diabetes are risk factors for macrosomia: a cross-sectional study of 42,663 pregnancies in Uruguay. Front Endocrinol (Lausanne). 2020;11:588443. https://doi.org/10.3389/fendo.2020.588443
6. Chen CN, Chen HS, Hsu HC. Maternal prepregnancy body mass index, gestational weight gain, and risk of adverse perinatal outcomes in Taiwan: a population-based birth cohort study. Int J Environ Res Public Health. 2020;17(4):1221. https://doi.org/10.3390/ijerph17041221
7. Dang AK, Truong MT, Le HT, Nguyen KC, Le MB, Nguyen LT, et al. Anthropometric cut-off values for detecting the presence of metabolic syndrome and its multiple components among adults in Vietnam: the role of novel indices. Nutrients. 2022;14(19):4024. https://doi.org/10.3390/nu14194024
8. Chappell LC, Seed PT, Myers J, Taylor RS, Kenny LC, Dekker GA, et al. Exploration and confirmation of factors associated with uncomplicated pregnancy in nulliparous women: prospective cohort study. BMJ. 2013;347:f6398. https://doi.org/10.1136/bmj.f6398
9. Górnicka M, Szewczyk K, Białkowska A, Jancichova K, Habanova M, Górnicki K, et al. Anthropometric indices as predictive screening tools for obesity in adults; the need to define sex-specific cut-off points for anthropometric indices. Appl Sci. 2022;12(12):6165. https://doi.org/10.3390/app12126165
10. World Health Organization. A healthy lifestyle - WHO recommendations. Geneva: World Health Organization; 2010.

Available from: https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations

11. Institute of Medicine (IOM). Subcommittee on nutritional status and weight gain during pregnancy. In: Nutrition during pregnancy. Washington (DC): National Academies Press; 2009.
12. Thomas DM, Bredlau C, Bosy-Westphal A, Mueller M, Shen W, Gallagher D, et al. Relationships between body roundness with body fat and visceral adipose tissue emerging from a new geometrical model. Obesity (Silver Spring). 2013;21(11):2264-71. https://doi.org/10.1002/oby.20408
13. Krakauer NY, Krakauer JC. A new body shape index predicts mortality hazard independently of body mass index. PLoS One. 2012;7(7):e39504. https://doi.org/10.1371/journal.pone.0039504
14. Obstetrics Gynecology. ACOG practice bulletin no 156: obesity in pregnancy. Obstet Gynecol. 2015 Dec;126(6):e112-e126. doi: 10.1097/AOG.0000000000001211. Erratum in: Obstet Gynecol. 2016;128(6):1450. PMID: 26595582
15. Obstetrics Gynecology. Committee opinion no. 644: the Apgar Score. Obstet Gynecol. 2015;126(4):e52-5. doi: 10.1097/AOG.0000000000001108
16. Sun Y, Shen Z, Zhan Y, Wang Y, Ma S, Zhang S, et al. Effects of pre-pregnancy body mass index and gestational weight gain on maternal and infant complications. BMC Pregnancy Childbirth. 2020;20(1):390. https://doi.org/10.1186/s12884-020-03071-y
17. Goldstein RF, Abell SK, Ranasinha S, Misso M, Boyle JA, Black MH, et al. Association of gestational weight gain with maternal and infant outcomes: a systematic review and meta-analysis. JAMA. 2017;317(21):2207-25. https://doi.org/10.1001/jama.2017.3635
18. Kominiarek MA, Saade G, Mele L, Bailit J, Reddy UM, Wapner RJ, et al. Association between gestational weight gain and perinatal outcomes. Obstet Gynecol. 2018;132(4):875-81. https://doi.org/10.1097/AOG.0000000000002854
19. Lewandowska M. The role of maternal weight in the hierarchy of macrosomia predictors; overall effect of analysis of three prediction ındicators. Nutrients. 2021;13(3):801. https://doi.org/10.3390/nu13030801
20. Hassan M, Alghanimi S, Abdullah E, Rund N, Yehya A. Impact of gestational weight gain on maternal and neonatal clinical outcomes: a retrospective cohort study. Evid Based Women's Health J. 2022;12(4):305-10. https://doi.org/10.21608/EBWHJ.2022.158508.1193
21. Nguyen G, Hayes L, Ngongalah L, Bigirumurame T, Gaudet L, Odeniyi A, et al. Association between maternal adiposity measures and infant health outcomes: a systematic review and meta-analysis. Obes Rev. 2022;23(10):e13491. https://doi.org/10.1111/obr.13491
22. Li S, Rosenberg L, Palmer JR, Phillips GS, Heffner LJ, Wise LA. Central adiposity and other anthropometric factors in relation to risk of macrosomia in an African American population. Obesity (Silver Spring). 2013;21(1):178-84. https://doi.org/10.1002/oby.20238
23. Geng TT, Huang T. Maternal central obesity and birth size: a Mendelian randomization analysis. Lipids Health Dis. 2018;17(1):181. https://doi.org/10.1186/s12944-018-0831-4
24. Ozler S, Kozanhan B, Bardak O, Arıkan MN. Are body roundness index and a body shape index in the first trimester related to foetal macrosomia? J Obstet Gynaecol. 2022;42(3):396-402. https://doi.org/10.1080/01443615.2021.1907565

ORIGINAL ARTICLE

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Did diet compliance and remission reduce oxidative stress in celiac patients?

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SUMMARY

OBJECTIVE: We aimed to examine the effect of remission status on thiol–disulfide homeostasis in celiac patients and thus to indirectly determine the effect of oxidative stress and inflammation caused by non-compliance with the diet.

METHODS: Between February 2019 and December 2021, 117 patients diagnosed with celiac disease were included in this prospective randomized and controlled study. In addition to routine tests of celiac patients, thiol and disulfide measurements were made from the blood both at the beginning of the study and at the end of the first year.

RESULTS: While 52 of the patients (44.4%) were in remission, 65 patients (55.6%) were not. There was an evident increase in native thiol levels of the patients who were initially not in remission but went into at the end of the first year (347.4±46.7 μmol/L vs. 365.3±44.0 μmol/L; p=0.001). Mean plasma disulfide levels of patients with celiac going into remission became reduced in the first year from the level of 14.5±5.1 μmol/L down to 8.9±4.2 μmol/L (p<0.001). In celiac patients who entered remission, disulfide and anti-tissue transglutaminase immunoglobulin A levels decreased in a correlation (r=0.526; p<0.001).

CONCLUSION: Not being in remission in celiac disease leads to increased oxidative stress, and thiol–disulfide homeostasis is an indirect indicator of this. Additionally, providing remission in celiac patients reduces oxidative stress.

KEYWORDS: Celiac disease. Oxidative stress. Thiol-disulfide.

INTRODUCTION

Celiac disease is an immune-mediated systemic disease triggered by the ingestion of gluten and related prolamins found in wheat, barley, rye, and oats in genetically susceptible individuals. People with HLA-DQ2 and HLA-DQ8 genotypes develop autoantibodies against tissue transglutaminase (tTG) enzyme, which plays an important role in pathogenesis, after gluten intake[1]. The prevalence of celiac disease worldwide is estimated to be 1%[2].

In CD patients, there is a proinflammatory response accompanied by intraepithelial lymphocytosis, crypt hyperplasia, and villus atrophy, caused by the activation of antigen-specific T lymphocytes induced by HLADQ2 and DQ8. As celiac patients are exposed to gluten, the production of interleukin (IL)-15, IL-18, and IL-21 increases. This causes an ongoing inflammatory response[3].

Thiol–disulfide hemostasis (TDH) is one of the antioxidant systems of the body. When oxidative damage occurs due to reactive oxygen species (ROS), sulfide-containing thiol groups are oxidized, and disulfide bonds are formed. Disulfide bonds are dynamic covalent bonds that are formed between two thiol groups. These are two-way reactions and the thiol groups formed are re-reduced to thiol groups by redox reaction, and dynamic TDH is maintained[4,5].

In this study, we investigated whether there is a difference in terms of TDH between celiac patients who are in remission and those who are not. We also examined the changes in thiol and disulfide levels in both remitted and non-remitted celiac patients. Thus, we aimed to indirectly evaluate the effect of chronic inflammation caused by noncompliance with the diet on oxidant and antioxidant balance and ROS-related oxidative stress through TDH.

METHODS

Study design and participants

Our study was designed as a prospective randomized controlled study. A total of 117 patients diagnosed with celiac disease

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presenting to the gastroenterology polyclinic between February 2019 and December 2021 were included in our study.

Ethical considerations

The patients participating in the study were informed about the aim of the study. Written informed consent was requested from the patients to participate in the study. Patients who signed the informed consent form were included in the study. This study was approved by the decision of the University of Health Sciences, Diyarbakır Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee dated 26.03.2021 and numbered 719.

Inclusion criteria

Having a diagnosis of celiac disease (patients who were previously positive for celiac autoantibodies (anti-transglutaminase immunoglobulin A (IgA) or anti-endomysium IgA) and were diagnosed with celiac disease according to the MARSH classification by endoscopic biopsy[6]), being over 16 years of age, patients who agree to give a blood sample for thiol–disulfide level in addition to routine laboratory tests at the beginning and end of the study, having normal WBC and CRP levels, and normal albumin level (as it may affect the thiol–disulfide level[7]).

Exclusion criteria

Celiac patients younger than 16 years of age, those with chronic systemic diseases (such as hypertension, diabetes mellitus, heart failure, chronic kidney disease, chronic lung disease, and chronic liver disease), patients with active infection, those with a disease that may cause low albumin in the blood[5], smoking or alcohol[6] users, pregnant celiac patients, patients using antioxidant vitamins and/or herbal products, and patients who did not sign the informed consent form were excluded from the study.

Thiol–disulfide measurement

In our study, native thiol and total thiol concentrations were measured synchronously as a paired test. In the first container, the amount of native thiol groups was measured by using a modified Ellman reagent. In a parallel study, first of all, dynamic disulfide bonds were reduced to free thiol groups by sodium borohydride. It was removed by NaBH4 formaldehyde in order to prevent the unused reduced sodium borohydride from reduction into 5,5´-dithio-bis-2-nitrobenzoic acid (DTNB). After reaction with DTNB, native thiol (NT) and total thiol (TT) levels were determined, and eventually the levels were measured. The result obtained by subtracting the amount of native thiol from the total thiol content and thereafter dividing it by half indicated the disulfide (DS) level[8].

Reference range determined for native thiol (-SH) is 278–826 µmol/L, for total thiol ((S-S)+(-SH)), it is 441–740 µmol/L, and for disulfide (S-S), it is 2–52 µmol/L[9].

When disulfide, native thiol, and total thiol levels were divided by each other, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol rates were obtained. Disulfide/native thiol (sD/sNT), disulfide/total thiol (sD/sTT), and native thiol/total thiol (sNT/sTT) rates were calculated in percentages (%).

Other laboratory tests

When celiac patients come for control, as recommended by the guidelines[6], complete blood count, ferritin, iron, iron-binding capacity, folic acid, vitamin B12, vitamin D, calcium, phosphorus, magnesium, albumin, glucose, urea, creatinine, sodium, potassium, liver function tests, parathormone and DEXA for measuring bone mineralization, thyroid hormones and autoantibodies, and celiac autoantibody levels were checked.

Evaluation of remission

Patients who state that they are on a gluten-free diet (for at least 6 months) were asymptomatic, and those who had the tTG IgA level below the cutoff value of 12 U/mL were considered to be in clinical remission and asymptomatic celiac disease. Patients who were noncompliant with the diet, had malabsorption symptoms, had anemia, vitamin, and mineral deficiency in laboratory parameters, and had tTG IgA level>12 U/mL were clinically evaluated as symptomatic CD. Due to its high specificity, IgA-EMA was used as a confirmation test, especially in cases where tTG IgA had a low titer. Autoantibodies were used to assess compliance with the gluten-free diet[10].

Laboratory parameters of remission and non-remission patients at the baseline and native and total thiol levels as well as disulfide level and rates thereof were statistically compared. Patients who were non-remission at the baseline and patients coming for control a year later were classified based on their state of remission. Native and total thiol levels among these groups as well as disulfide levels and rates thereof were compared with the values of these patients measured at the baseline.

The aim of using this method was to uncover the effect of dietary adherence and remission on thiol–disulfide hemostasis in patients with CD.

Statistical analysis

Kolmogorov-Smirnov, Shapiro-Wilk test, coefficient of variation, and skewness and kurtosis methods were used to control the normal distribution of patient data. While mean and standard deviation values were stated for continuous variables, categorical variables were expressed as %. Independent t-test

or Mann-Whitney U test was used to determine the difference between age, body mass index, dietary adherence, and laboratory parameters between remission and non-remission patients with celiac disease. Paired samples t-test was used for parameters that had normal distribution, and Wilcoxon test was used for parameters that did not have normal distribution to determine the differences between the native and total thiol and disulfide levels and their ratios to each other at the baseline and 1 year later in non-remission celiac patients. Pearson correlation analysis was performed to show the relationship between disulfide and anti-Ttg IgA levels at baseline and in the first year of remission patients with celiac disease. All tests were bilateral, and $p<0.05$ was considered statistically significant. Statistical analyses were performed by using the package program SPSS24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and laboratory data of celiac patients in remission and non-remission are shown in Table 1. While 52 (44.4%) of 117 patients with celiac included in the study were in remission, 65 patients (55.6%) were not. The mean age of the patients was 30.7±10.8. Of the patients, 60 (51.3%) were female and 57 (48.7%) were male. Although 71.8% (n=84) of the patients stated that they were on a diet, 38.1% (n=32) of these patients were not in remission. The mean anti-Ttg IgA value was 7.5±5.8 U/mL in remission patients with CD, but the value was 205.3±124.3 U/mL in non-remission patients ($p<0.001$).

The mean plasma total thiol level was 374.4±46.5 µmol/L at the baseline in celiac patients in remission, while it was 392.7±43.6 µmol/L at the end of the first year ($p<0.001$). In patients who did not go into remission, mean plasma total thiol levels were similar at the baseline and at the first year (382.1±34.5 µmol/L vs. 383.1±36.0 µmol/L; $p=0.392$). While the mean plasma disulfide level of celiac patients in remission decreased from 14.5±5.1 µmol/L to 8.9±4.2 µmol/L in the first year ($p<0.001$), no change was observed in celiac patients who did not go into remission (17.6±3.1 µmol/L vs. 17.7±3.2; $p=0.784$). In addition, serum disulfide/native thiol and disulfide/total thiol ratios reached lower levels in celiac patients who went into remission (Table 2).

Among the celiac patients who were not in remission at the baseline, there were significant decreases in both plasma disulfide and anti-transglutaminase IgA levels at the end of the first year in patients who adhered to the diet and went into remission ($p<0.001$). Furthermore, disulfide and anti-tTG IgA levels decreased in a correlational manner while the patients were in remission ($r=0.526$; $p<0.001$). It was observed that in patients with similar total thiol levels at the beginning, a significant increase occurred in patients who were on diet compared with patients who were not on diet, due to the effect of long-term remission (Figure 1).

Table 1. Demographic and laboratory data of celiac patients in remission and non-remission.

	In remission (n=52)	Not in remission (n=65)	p
Age (years)	35.1±13.7	27.3±8.5	0.001
Gender F/M	24 (46.2%)/28 (53.8%)	36 (55.4%)/29 (44.6%)	0.433
Diet compliance Yes/No	52 (100%)	32 (49.3%)/33 (50.7%)	<0.001
Anti Ttg Ig A (U/mL)	7.5±5.8	205.3±124.3	<0.001
BMI (kg/m^2)	22.2±1.8	20.1±3.8	<0.001
Albumin (g/L)	4.5±0.4	4.4±0.3	0.193
Hgb (g/dL)	14.7±1.9	13.3±2.4	0.005
Iron (µg/dL)	21.4±18.6	8.1±6.1	<0.001
Ferritin (µg/L)	60.6±40.9	20.3±16.5	<0.001
Folate (ng/mL)	10.7±10.2	6.2±4.3	0.026
Vitamin D (ng/mL)	19.1±9.2	18.7±8.4	0.745
Calcium (mg/dL)	9.6±0.6	9.0±0.4	0.294
Phosphorus (mg/dL)	3.4±0.7	3.2±0.5	0.149
DEXA (Z score)	-0.5±1.2	-1.5±1.2	<0.001
WBC (10^3 cell/•1)	7100±1500	7400±1100	0.315
CRP (mg/L)	0.6±0.2	0.5±0.3	0.254

BMI: body mass index; Anti Ttg IgA: anti-transglutaminase antibodies A; Hgb: hemoglobin; DEXA: dual-energy X-ray absorptiometry; WBC: white blood cell; CRP: C-reactive protein.

Table 2. The difference between the thiol–disulfide values 1 year later in celiac patients who were not in remission at the beginning but achieved remission after adhering to the recommended diet, and those who did not comply with the diet and could not achieve remission.

	In remission (n=38)			Not in remission (n=27)		
	Initially	1 year	p	Initially	1 year	p
Native thiol (µmol/L)	347.4±46.7	365.3±44.0	0.001	352.9±30.2	353.5±30.6	0.654
Total thiol (µmol/L)	374.4±46.5	392.7±43.6	<0.001	382.1±34.5	383.1±36.0	0.392
Disulfide (µmol/L)	14.5±5.1	8.9±4.2	<0.001	17.6±3.1	17.7±3.2	0.784
(Disulfide/native thiol)x100 (%)	3.9±1.6	3.6±1.5	0.022	5.5±0.5	5.6±0.6	0.688
(Disulfide/total thiol)x100 (%)	3.8±1.4	3.3±1.4	0.015	4.5±0.4	4.4±0.5	0.559
(Native thiol/total thiol)x100 (%)	92.7±1.8	93.0±2.1	0.772	92.0±1.9	92.2±1.7	0.450

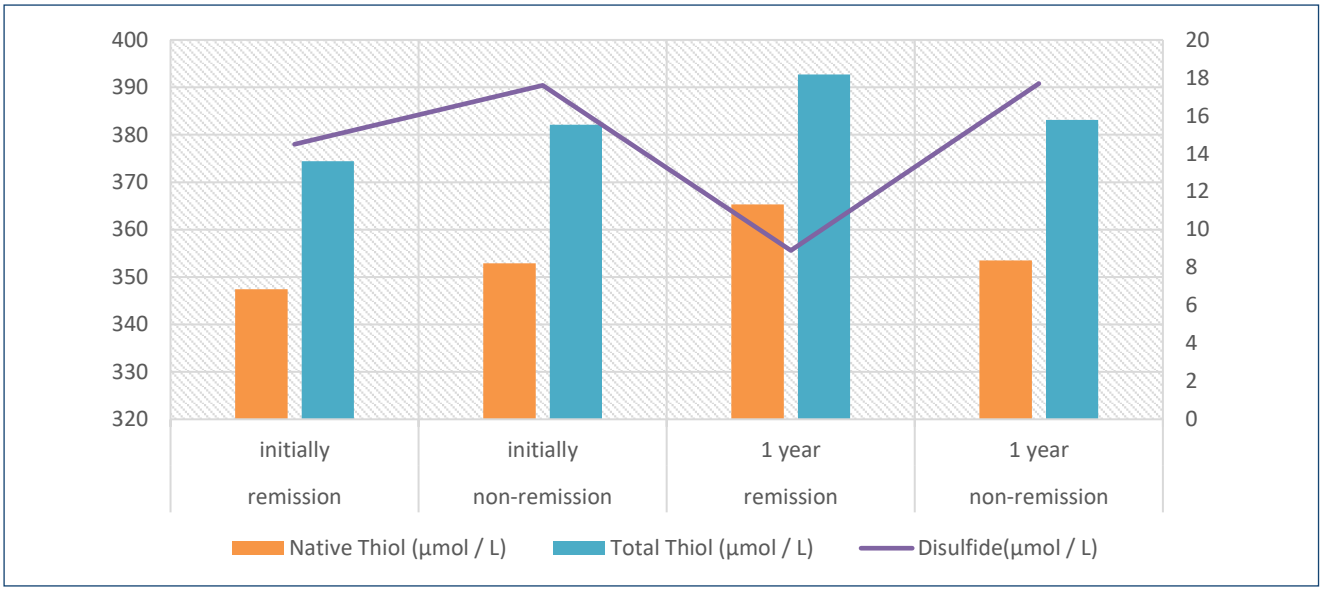

Figure 1. Cumulative change of thiol and disulfide levels in celiac patients in remission compared to non-remission patients.

DISCUSSION

Extracellular redox reactions can regulate tissue homeostasis via its effects on cell proliferation, differentiation, apoptosis, and immune system. Therefore extracellular redox and thiol/disulfide balance, an important component thereof, has a significant impact on, in particular, diseases with an inflammatory progression[11].

In our study, we did not notice any differences with regard to total and native thiol levels between remission and non-remission patients with celiac disease. The underlying reason is that thiol level is affected by many factors including body mass index and that total thiol capacity of each individual is different[12]. However there were significant differences between both groups in terms of disulfide level. Moreover, thiol:disulfide ratios were different. This case may be rather an indicator of an ongoing inflammation constantly triggered by gluten exposure in non-remission celiac patients than total thiol content.

The most important result we reached in this study is the increase in the body's total thiol pool as well as the decrease in disulfide levels in compliance with the gluten-free diet in patients who were initially non-compliant with the diet and were not in clinical remission. This result shows that the gluten-free diet reduces inflammation in the body and increases antioxidant capacity. It was not possible to say the same for patients who were non-compliant with the diet. Even after a year, no significant changes were observed in total thiol and disulfide levels in the body.

A study conducted thereon states that in these patients thiol absorption is not impaired, and TDH is dependent on gliadin toxicity and auto-inflammation[13]. We have the same opinion in regard to this matter. Should thiol absorption be impaired, there would be significant differences between remission and non-remission celiac patients. There could also be a difference between both groups with regard to albumin levels.

However, the fact that the total thiol pool is different for everyone and that these biomarkers have not been compared with methods that directly measure ROS prevents us from reaching a clear conclusion on this subject. A meta-analysis comparing the tests related to oxidative stress may provide us with further opinions.

Nevertheless, we can say that disulfide level and anti-transglutaminase antibody decreased in a correlated manner in patients going into remission at the end of the first year. Anti-transglutaminase antibodies are essentially produced against the inflammation due to gliadin exposure, and against the tissue transglutaminase at the end of deamidation reactions, and in parallel, a large amount of cytokines are released from CD4 T lymphocytes[14,15]. In fact, anti-transglutaminase and disulfide levels reduced with the diet may be an indirect indicator of a reduction in cytokine levels.

Study limitations

The most important limitation of our study was that we could not measure the levels of cytokines such as interleukin and interferon, which are inflammatory parameters. Unfortunately, there are no any such studies in literature either. If we were able to measure these parameters and had a chance to compare them with thiol and sulfide levels, we could obtain more and detailed data about ROS with both the chronic inflammatory situation in CD and the oxidative stress caused by remission. In addition, since the majority of our patients were reluctant to undergo endoscopy, histopathological examination of the duodenal mucosa, which is recommended as the gold standard[16] in determining the remission status of patients, could not be performed.

CONCLUSION

In CD, not being in remission, especially non-compliance with the diet, causes increased oxidative stress. TDH is an indirect indicator of this. Additionally, achieving remission in celiac patients by complying with a gluten-free diet reduces oxidative stress in the body. Maintaining remission in CD by complying with a gluten-free diet may be protective against the risk of developing other chronic and autoimmune diseases.

ETHICS COMMITTEE APPROVAL

Ethics committee approval was received for this study from the Ethics Committee of University of Health Sciences, Diyabakır Gazi Yasargil Education and Research Hospital, who approved this study protocol (Ethics committee approval; Date: 26.03.2021 issue: 719).

INFORMED CONSENT

The patients participating in the study were informed about the aim of the study. Patients who agreed to participate in the study and signed the informed consent form were accepted into the study. We thank all our patients who participated in the study.

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AUTHORS' CONTRIBUTIONS

BE: Conceptualization, Writing – original draft. **FB**: Methodology. **AU**: Project administration, Investigation. **MZA**: Validation. **AY**: Resources. **HK**: Funding acquisition. **NA**: Data curation. **MA**: Software. **SN**: Visualization, Formal Analysis. **OE**: Writing – review & editing.

REFERENCES

1. Sahin Y. Celiac disease in children: a review of the literature. World J Clin Pediatr. 2021;10(4):53-71. https://doi.org/10.5409/wjcp.v10.i4.53
2. Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, et al. Coeliac disease. Nat Rev Dis Primers. 2019;5(1):3. https://doi.org/10.1038/s41572-018-0054-z
3. Garrote JA, Gómez-González E, Bernardo D, Arranz E, Chirdo F. Celiac disease pathogenesis: the proinflammatory cytokine network. J Pediatr Gastroenterol Nutr. 2008;47(Suppl 1):S27-32. https://doi.org/10.1097/MPG.0b013e3181818fb9
4. Nakai K, Tsuruta D. What are reactive oxygen species, free radicals, and oxidative stress in skin diseases? Int J Mol Sci. 2021;22(19):10799. https://doi.org/10.3390/ijms221910799
5. Yi MC, Khosla C. Thiol-disulfide exchange reactions in the mammalian extracellular environment. Annu Rev Chem Biomol Eng. 2016;7:197-222. https://doi.org/10.1146/annurev-chembioeng-080615-033553
6. Rubio-Tapia A, Hill ID, Semrad C, Kelly CP, Greer KB, Limketkai BN, et al. American College of Gastroenterology guidelines update: diagnosis and management of celiac disease. Am J Gastroenterol. 2023;118(1):59-76. https://doi.org/10.14309/ajg.0000000000002075
7. Go YM, Jones DP. Thiol/disulfide redox states in signaling and sensing. Crit Rev Biochem Mol Biol. 2013;48(2):173-81. https://doi.org/10.3109/10409238.2013.764840
8. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014;47(18):326-32. https://doi.org/10.1016/j.clinbiochem.2014.09.026

9. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. Free Radic Biol Med. 2009;47(10):1329-38. https://doi.org/10.1016/j.freeradbiomed.2009.08.021
10. Farina E, Roncoroni L, Lombardo V, Scricciolo A, Vecchi M, Doneda L, et al. Clinical value of tissue transglutaminase antibodies in celiac patients over a long term follow-up. Nutrients. 2021;13(9):3057. https://doi.org/10.3390/nu13093057
11. Moriarty-Craige SE, Jones DP. Extracellular thiols and thiol/disulfide redox in metabolism. Annu Rev Nutr. 2004;24:481-509. https://doi.org/10.1146/annurev.nutr.24.012003.132208
12. Balcerczyk A, Bartosz G. Thiols are main determinants of total antioxidant capacity of cellular homogenates. Free Radic Res. 2003;37(5):537-41. https://doi.org/10.1080/1071576031000083189
13. Kaplan M, Ates I, Yuksel M, Ozderin Ozin Y, Alisik M, Erel O, et al. Thiol/disulphide homeostasis in celiac disease. World J Gastrointest Pharmacol Ther. 2017;8(2):120-6. https://doi.org/10.4292/wjgpt.v8.i2.120
14. Lexhaller B, Ludwig C, Scherf KA. Comprehensive detection of isopeptides between human tissue transglutaminase and gluten peptides. Nutrients. 2019;11(10):2263. https://doi.org/10.3390/nu11102263
15. Valitutti F, Fasano A. Breaking down barriers: how understanding celiac disease pathogenesis informed the development of novel treatments. Dig Dis Sci. 2019;64(7):1748-58. https://doi.org/10.1007/s10620-019-05646-y
16. Wahab PJ, Meijer JW, Mulder CJ. Histologic follow-up of people with celiac disease on a gluten-free diet: slow and incomplete recovery. Am J Clin Pathol. 2002;118(3):459-63. https://doi.org/10.1309/EVXT-851X-WHLC-RLX9

ORIGINAL ARTICLE

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Implementation and use of electronic patient records in the Brazilian Air Force: a cross-sectional study[1]

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SUMMARY

OBJECTIVE: The objective was to analyze the implementation and use of the electronic patient record in the health services of the Brazilian Air Force.

METHODS: This is a cross-sectional study carried out with 234 physicians, between March and May 2021. The data collection instrument was sent by email. The electronic patient record was implemented in the Air Force approximately 3 years ago (64.5%), and about 81% of the physicians received training to operate it.

RESULTS: The most common records involve data related to consultations (90.1%) and interviews with physical examination (67.1%). Physicians cited that information storage (75.6%), agility, and feasibility of recording (55.1%) were the main advantages of the electronic patient record. As disadvantages, problems in electronic equipment (69.7%) and system errors (65%) were reported. Most participants considered that the implementation had a positive impact on work dynamics (75.6%) and productivity (66.7%), mainly regarding the components "Work processes" (57.3%) and "Amount of carried out activities" (21.4%). Keeping records was significantly associated with the job position ($p<0.001$), type of unit ($p=0.008$), time of implementation ($p<0.001$), and participation in training ($p=0.028$).

CONCLUSION: The implementation of the electronic patient record in the Air Force was recently done, and just over half of the physicians were trained prior to the implementation. The tool is considered compatible with work processes and has a positive effect on productivity.

KEYWORDS: Electronic health records. Health information systems. Health planning.

INTRODUCTION

The electronic patient record (EPR) is defined by the Federal Council of Medicine as a document consisting of information generated from facts, events, and situations related to the patient's health and the care provided, which has a legal, confidential, and scientific characteristic, in addition to allowing communication between members of the multidisciplinary team[1].

The implementation of the EPR in Brazilian territory is regulated by Laws 13,709 and 13,787 of 2018; however, it is not widely disseminated in public and private health systems. These laws regulate personal data protection and patient records handling. The former is the base for all the sectors of our society regarding personal data, including health data and data from patients. The latter is the base for digitization and use of computerized systems for the custody, storage, and handling of patient records and are available at https://www.planalto.gov.br/ccivil_03/_ato2015-2018/2018/lei/l13709.htm and https://www.gov.br/conarq/pt-br/legislacao-arquivistica/leis-e-decretos-leis/lei-no13-787-de-27-de-dezembro-de-2018#:~:text=Disp%C3%B5e%20sobre%20a%20digitaliza%C3%A7%C3%A3o%20e,manuseio%20de%20prontu%C3%A1rio%20de%20paciente. From the creation of the e-SUS Primary Care system, which seeks to computerize Primary Health Care, and the publication of the User Manual of the Citizen Electronic Record System, Brazil has strengthened implementation actions in all regions[2]. It is noteworthy that the implementation and the adequate use of the EPR increase the quality and organization of health care, integrating the professionals involved in patient care and subsidizing a shared and more assertive health management[3].

The Armed Forces are permanent and regular national institutions, organized hierarchically, consisting of the Navy, the Army, and the Air Force, which are intended to defend the Homeland and guarantee constitutional powers. The Air Force Health System (SISAU, Sistema de Saúde da Aeronáutica),

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implemented in 1976 and reformulated in 1983, carries out activities related to the diagnosis and prevention of diseases, conservation or health recovery, and rehabilitation, covering all services provided in hospitals, offices, specialized clinics, and laboratories, or in home care.

Considering the advantages related to the use of EPR in health institutions and the importance of continuous improvement processes in care, the aim of this study was to analyze the implementation and use of EPR in the health services of the Brazilian Air Force. The main hypothesis of this study is that EPR, as a beneficial tool in the process of care, improves physicians' work as well as improves information registration and increases security for patients.

METHODS

Study design and location

This is a cross-sectional study carried out at the Air Force Health System (SISAU).

Study population and sample

The population consisted of individuals who are currently commissioned Air Force physicians.

The Brazilian Air Force currently has 1,767 doctors as its staff. Of these, 598 work in health care services where EPR has already been implemented. This information was considered to calculate the sample size of the study, according to the formula for cross-sectional studies with cross-sectional outcomes and finite population, as presented below:

$$n = \frac{Z^2_{a/2} P.(1-P).N}{Z^2_{a/2} P.(1-P) + (N-1).e^2}$$

Confidence level=99%
n=598
p=0.5
e=5%

The final sample size, defined by sample calculation, included 234 participants.

Data collection

The study invitation was sent by email, between March and May 2021. The EPR was implemented in 2019, but there were no records of evaluations of the implementation stages and the impact of PEP on the work process.

The data collection instrument was developed using the Google Forms® platform, based on the critical components of the EPR implementation pointed out in the literature[4], and consisted of four blocks: sociodemographic characteristics, training and professional experience, conditions for EPR operability and impacts on work dynamics and potentialities, and challenges in the implementation.

The Google Forms® platform has general data protection regulation (GDPR) in accordance with resolution 466/2012, which regulates research with human beings in Brazil (https://conselho.saude.gov.br/resolucoes/2012/Reso466.pdf). In this policy, Google commits to encrypting data collected by the Forms® feature at multiple levels, by forcing the adoption of hypertext transfer protocol secure (HTTPS) in all transmissions, and the use of perfect forward secrecy (PFS) in all services. It is also committed to encrypting message transmissions with other servers using Transport Layer Security (TLS) and encryption keys relevant to the validation and key exchange phases. These procedures protect messaging communications when respondents fill out and submit their information. With this, the company Google assures users of its Forms® resource regarding the security of the data entered (https://policies.google.com/privacy?hl=en-US). The platform also has terms of use and privacy policies that guarantee the maintenance of data anonymity and non-sharing of collected information. The platform's GDPR, terms of use, and privacy policies can be accessed at https://www.termsfeed.com/blog/terms-conditions-google-forms/.

The first screen of the instrument contained the free and informed consent form (TCLE). In case of agreement, the subsequent screen was displayed. Otherwise, the participants viewed the screen that ended the contract. There were four blocks of questions, divided into the following sections:

Block I: sex, age, marital status, children, state/municipality where you live and work, personal/family income (7 questions);

Block II: time since graduation, institution completing course, postgraduate degree, area of specialization, working time/weekly workload in aeronautics, time using EPR, use of other forms of records, abandonment of registration in EPR, work in another location… (17 questions);

Block III: EPR implementation time, training, duration, content, confidence in the EPR, time spent on records, satisfaction… (21 questions);

Block IV: potentials and challenges in implementing the patient's electronic medical record: speed, security, reliability, professionals responsible for adjustments to the EPR, interest, strengths and weaknesses (7 questions).

Data collection took place after approval by the Research Ethics Committee of the University of Fortaleza, under number 4,674,238.

Data analysis

The data were exported to a Microsoft Excel® spreadsheet (Microsoft, Albuquerque, New Mexico, USA, 1985–2003) and subsequently transferred to the Stata v.15 software (StataCorp LLC, College Station, Texas, USA, 1985–2017). Descriptive analysis was performed by calculating the position and dispersion measures for quantitative variables, and the frequencies for categorical variables. The inferential analysis began by checking the normality of quantitative variables using Shapiro-Wilk test. Mean difference tests were used to verify the existence of statistically significant differences between the means of numerical responses, depending on the distribution of categorical variables belonging to the blocks of the data collection instrument. For dichotomous variables, Student's t-test or Mann-Whitney test was applied. For polychotomous variables, a one-way ANOVA or Kruskal-Wallis test was applied, depending on the normality and variance of the data. For categorical variables, Pearson's chi-square test or Fisher's exact test was applied. The significance level was set at 5%.

RESULTS

The study included 234 physicians, with a mean age of 39.25 (±7.53) years, predominantly female (56.8%), and who had graduated 14.17 (±7.58) years ago (Table 1). As for postgraduate studies, residency predominated (61.2%), work and reside in the same municipality (93.6%), average work experience of 9.82 (±7.82) years in hospitals (55. 5%) and in the Health Squad (34.1%), and an average workload of 33.70 (±10.86) h per week.

According to the participants, the EPR was implemented in the Air Force 3 years ago or less (64.5%). About 81% received training to operate it and the most present contents were access to the system (70.9%), filling out (70.0%), and medical record items (65.8%).

The EPR records were carried out by approximately 90% of the physicians. However, a significant number stopped using it at some point, mainly due to structural reasons (Table 2).

The purposes of using the EPR were consultations (90.1%), interviews and physical examinations (67%), evolution (62.8%), prescriptions (38%), discharge (28.2%), admission (18.8%), and requesting tests (1.3%). Information storage (75.6%), agility, and feasibility of recording (55.1%) were the main advantages of the EPR. As disadvantages, the following were reported: problems in electronic equipment (69.7%) and system errors (65%).

As for satisfaction with EPR use, 56.8% considered themselves satisfied with the implementation, 56.4% with quality, 51.7% with security, and 58.1% with usability. Most participants considered that the implementation had a positive impact on work dynamics (75.6%) and productivity (66.7%), mainly regarding the components "Work processes" (57.3%) and "Amount of carried out activities" (21.4%).

As for the advantages of using the EPR, agility and feasibility were mentioned by 55.1% of the participants and storage of information by 75.6%. However, for 66.7% there was no increase in the efficiency of care, 91% did not observe team satisfaction, and 90.6% of physicians did not relate the EPR to a better relationship with patients. The main strengths were the EPR as a care tool (99.4%), the health professionals' interest (71.4%), and the quality of the EPR (70.1%). As weaknesses, the time spent (55.1%) and the level of training (46.2%) were mentioned.

Data recording was significantly associated with the participants' rank ($p<0.001$), type of unit where they are stationed ($p=0.008$), time of implementation ($p<0.001$), and participation in training ($p=0.028$) (Table 3).

Table 1. Sociodemographic and occupational characteristics of the physicians in the Brazilian Air Force.

Variables	n	%
Sex		
Female	133	56.8
Male	101	43.2
Age range (years)		
≤35	85	36.3
≥35	149	63.7
Rank in the Air Force		
Lieutenant	120	51.2
Captain	60	25.6
Major	36	15.4
Colonel	10	4.3
Lieutenant colonel	8	3.5
Time since graduation (years)		
<5	31	13.2
6–20	162	69.2
>20	41	17.6
Work experience as a physician in the Air Force (years)		
Up to 5 years	99	42.3
6–15 years	86	36.8
16 years or more	49	20.9
Type of unit where they work		
Health Squad	85	36.3
Hospital	130	55.6
Other	19	8.1

Fortaleza, CE, Brazil, 2021 (n=234). Source: study data.

Table 2. Characterization of the implementation and use of the electronic patient record in the health services of the Brazilian Air Force.

Implementation			Use		
Variables	**n**	**%**	**Variables**	**n**	**%**
Time			Time		
≤3 years	151	64.5	≤2 years	172	73.5
>3 years	66	28.2	>2 years	62	26.5
Does not know	10	4.3	EPR records		
Under implementation	7	3.0	Yes	211	90.2
Participation in training			No	9	3.9
Yes	190	81.2	Sometimes	14	5.9
No	44	18.8	Did you stop using it at some point?		
Duration of training			Yes	171	73.1
Up to 1 month	169	88.9	No	63	26.9
Up to 6 months	6	3.2	Reasons for not using EPR		
EPR records	3	1.6	Structural	151	88.4
Does not know	12	6.3	Work dynamics	10	5.8
Training before the implementation			Other	10	5.8
Yes	131	56.0			
No	103	44.0			

Fortaleza, CE, Brazil, 2021 (n=234). Source: study data.

DISCUSSION

The implementation of the EPR in the Brazilian Air Force has been a recent event. It was found that 44% of physicians did not receive any training before the implementation, and 19% did not participate in any training at any time. It is known that the lack of training is a threat to the implementation of the EPR and can increase the chances of errors, recording delays, reduced satisfaction, and acceptability[4,5]. This was confirmed by the association between participation in training and electronic records in clinical practice (p=0.028). The participants considered the level of training a weakness related to EPR use, which can interfere with the incorporation of the tool in daily activities[3]. This study advances the understanding of the use of EPR by the physicians in the Brazilian Air Force, which is still less explored in the national scientific research. Given the positive perception about the aspects of EPR in the institution, there has been a successful implementation of electronic health records. It is noteworthy that this study represents, until November 2021, the first investigation to evaluate the implementation and the use of EPR in the Brazilian Armed Forces.

This study showed that consultations, interviews, physical examinations, and evolution were the main EPR purposes used by the physicians. The central role that the use of these characteristics plays in optimizing work processes, increasing the quality of records, fostering interprofessional discussion, and encouraging the achievement of best health practices is highlighted[6]. However, there was a need to offer training aimed at using it in admissions, discharge, and drug prescriptions.

Although approximately 55% of the physicians reported that the EPR implementation resulted in agility and feasibility in data recording, 66.7% did not observe an increase in the efficiency of care. Therefore, although the EPR contributed to the work dynamics, the increase in efficiency, which is one of the objectives of its implementation, is not being fully achieved. The use of artificial intelligence mechanisms is a strategy that can encourage this scenario, as it expands the possibilities of effective decision-making[7,8]. While physicians may perceive the EPR as a tool that requires more time to make records, patients express satisfaction with the records in information systems with interoperability.

The use of EPR was considered relevant to increase the productivity by most physicians. However, in the study by Kaneko et al., no significant differences or decreases in productivity were observed when compared with the use of traditional paper recording[9]. Hence, the perception of the impact of the EPR on work processes must be periodically reassessed to support continuous improvement strategies.

Table 3. Association between sociodemographic and labor variables and data recording in the electronic patient record in the Brazilian Air Force.

Variables	Data recording in EPR			p-value
	Yes n (%)	No n (%)	Sometimes n (%)	
Rank in Air Force				
Lieutenant	112 (93.4)	1 (0.8)	7 (5.8)	<0.001†
Captain	55 (91.7)	1 (1.7)	4 (6.6)	
Major	27 (75.0)	7 (19.4)	2 (5.6)	
Colonel	10 (100.0)	0	0	
Lieutenant colonel	7 (87.5)	0	1 (12.5)	
Type of unit where they work				
Health Squad	73 (85.9)	3 (3.5)	9 (10.6)	0.008†
Hospital	124 (95.4)	4 (3.1)	2 (1.5)	
Other	14 (73.7)	2 (10.5)	3 (15.8)	
Time of EPR implementation at the unit				
Up to 6 months	37 (90.2)	1 (2.5)	3 (7.3)	<0.001†
6 months to 1 year	30 (85.7)	1 (2.9)	4 (11.4)	
1–2 years	71 (94.6)	2 (2.7)	2 (2.7)	
3–5 years	58 (95.1)	1 (1.6)	2 (3.3)	
5 years or longer	0	4 (80.0)	1 (20.0)	
Under implementation	1 (14.3)	4 (57.1)	2 (28.6)	
Does not know	10 (100.0)	0	0	
Participation in training to operate EPR				
Yes	176 (92.6)	5 (2.6)	9 (4.8)	0.028‡
No	35 (79.5)	4 (9.1)	5 (11.4)	
Prior training to EPR implementation				
Yes	118 (90.1)	6 (4.6)	7 (5.3)	0.733‡
No	93 (90.3)	3 (2.9)	7 (6.8)	

Fortaleza, CE, Brazil, 2021 (n=234). ‡Chi-square test. †Fisher's exact test. Source: study data.

The participants' rank in the Air Force and the time since the EPR was implemented in the work unit were associated with record performing ($p<0.001$ and $p<0.001$, respectively). It was verified that the frequency of recording in the EPR increased according to the time of implementation in the unit ($p<0.001$). In this process, the expansion of its use is relevant as it generates new technical, professional, and ethical challenges[10] for the improvement of the EPR and, consequently, greater inclusion into the service routine.

The Brazilian Air Force has the potential to improve and expand the implementation of the EPR, since the health professionals' interest and the system quality were considered strengths by more than 70% of the participants in this study. Both aspects are considered crucial for the usability and operability success of the EPR[11].

The results allowed mapping the usability characteristics of the EPR and existing strengths, which should be promoted. Additionally, aspects for continuous improvement were identified, such as corrections of system errors and problems in electronic equipment, aiming to increase operational efficiency, user satisfaction, and the quality of care.

One limitation of this study is the gathering of information through one professional category. However, it is noteworthy that the study promotes substantial progress in understanding the use of EPR in the Air Force, whose health care is still less explored in scientific research. Given the positive perception, the institution can be considered a national reference regarding the implementation of the EPR. It is noteworthy that this is the first study, up to the moment of its conclusion, to assess the implementation and use of the EPR in the Brazilian Armed Forces. Given that software and operability updates are dynamic and constant, it is possible that perceptions have changed over time. Each professional uses specific EPR functionalities, and perceptions about EPR may differ between the members of the healthcare team.

It is suggested that, based on the knowledge provided in this research, longitudinal and prospective studies should be carried out to compare the efficiency, satisfaction, patient safety, and quality of care in Brazilian health services that adopt manual and electronic records. This comparison is important for both public and private spheres, including, but not limited to, the Brazilian Armed Forces. Expanding the use of artificial intelligence to improve clinical decision-making in healthcare and optimize the healthcare services offered the relationship between EPR and big data complex sets of data through various techniques, which include machine learning, enabling the manipulation and application of data stored in EPR.

CONCLUSION

The implementation of the EPR in the Brazilian Air Force is recent, and just over half of the physicians were previously trained to use it. The most frequent recordings are those related to consultations, interviews, physical examinations, and evolution, with underutilization of the system for admissions, discharge, and drug prescriptions.

According to the physicians' assessment, the main advantages of the EPR are data storage, agility, and feasibility of the recording, whereas the disadvantages are equipment problems and system errors. The EPR is considered compatible with work processes and has a positive effect on productivity.

Future studies should investigate the efficiency, satisfaction, patient safety, and quality of care in health services that adopt manual and electronic data recording. In the Brazilian Air Force, it is suggested that EPR user satisfaction surveys should be carried out periodically, with a view to improvement.

Multiple possibilities for expanding the use of PEP in the Brazilian Armed Forces exist, mainly in the application of approaches that allow processing, understanding, and learning from health information recording systems. This scenario will be feasible with techniques, computational methods that employ methods of organization, interpretation, and recognition of patterns in health information registered in the Brazilian Armed Forces, which will contribute to promoting practices developed in its health system on the effectiveness of treatments and clinical decisions aligned with the Health Surveillance model in the Brazilian Unified Health System.

AUTHORS' CONTRIBUTIONS

PMVB: Conceptualization, Investigation, Methodology, Project administration, Validation, Writing – original draft. **LJESV:** Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Supervision, Writing – review & editing. **GBSJ:** Formal Analysis, Supervision, Writing – review & editing. **GVL:** Investigation, Validation, Writing – original draft. **JGRO:** Data curation, Formal Analysis, Supervision, Writing – review & editing.

REFERENCES

1. Conselho Federal de Medicina. Resolução nº 1.638/02, de 9 de agosto de 2002. Brasília (DF): Conselho Federal de Medicina. 2002. [cited on 2021 Jan 10]. Available from: https://sistemas.cfm.org.br/normas/visualizar/resolucoes/BR/2002/1638
2. Ministério da Saúde (BR). E-SUS Atenção Básica: manual de uso do sistema com prontuário eletrônico do cidadão PEC – Versão 3.2. Brasília (DF): Ministério da Saúde; 2019.
3. Toledo PPDS, Santos EMD, Cardoso GCP, Abreu DMF, Oliveira AB. Electronic health record: a systematic review of the implementation under the national humanization policy guidelines. Cien Saude Colet. 2021;26(6):2131-40. https://doi.org/10.1590/1413-81232021266.39872020
4. Aguirre RR, Suarez O, Fuentes M, Sanchez-Gonzalez MA. Electronic health record implementation: a review of resources and tools. Cureus. 2019;11(9):e5649. https://doi.org/10.7759/cureus.5649
5. Ting J, Garnett A, Donelle L. Nursing education and training on electronic health record systems: an integrative review. Nurse Educ Pract. 2021;55:103168. https://doi.org/10.1016/j.nepr.2021.103168
6. Hedian HF, Greene JA, Niessen TM. The electronic health record and the clinical examination. Med Clin North Am. 2018;102(3):475-83. https://doi.org/10.1016/j.mcna.2017.12.009
7. Chi EA, Chi G, Tsui CT, Jiang Y, Jarr K, Kulkarni CV, et al. Development and validation of an artificial intelligence system to optimize clinician review of patient records. JAMA Netw Open. 2021;4(7):e2117391. https://doi.org/10.1001/jamanetworkopen.2021.17391
8. Yang X, Mu D, Peng H, Li H, Wang Y, Wang P, et al. Research and application of artificial intelligence based on electronic health records of patients with cancer: systematic review. JMIR Med Inform. 2022;10(4):e33799. https://doi.org/10.2196/33799
9. Kaneko K, Onozuka D, Shibuta H, Hagihara A. Impact of electronic medical records (EMRs) on hospital productivity in Japan. Int J Med Inform. 2018;118:36-43. https://doi.org/10.1016/j.ijmedinf.2018.07.008
10. Evans RS. Electronic health records: then, now, and in the future. Yearb Med Inform. 2016;Suppl 1(Suppl 1):S48-61. https://doi.org/10.15265/IYS-2016-s006
11. Wolfe L, Chisolm MS, Bohsali F. Clinically excellent use of the electronic health record: review. JMIR Hum Factors. 2018;5(4):e10426. https://doi.org/10.2196/10426

ORIGINAL ARTICLE

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Evolution of the quality of life of total laryngectomy patients using electrolarynx

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SUMMARY

OBJECTIVE: Therapy and vocal rehabilitation in laryngeal cancer impact patients' quality of life. The objective of this study was to evaluate the evolution of the quality of life of patients with laryngeal cancer submitted to total laryngectomy and using electrolarynx.

METHODS: This is an observational study with a cross-sectional design and a quantitative approach. It was conducted between April 2022 and January 2023 in a Brazilian cancer hospital. For data collection, a quality of life questionnaire, validated for patients with head and neck cancer at the University of Washington, was applied in two phases: from 7 days after total laryngectomy and, subsequently, from 70 days after surgery using electronic larynx for at least 60 days. The inclusion criteria were patients undergoing total laryngectomy included on the Aldenora Bello Cancer Hospital's election list to receive the electronic larynx. Patients who did not sign the informed consent form were not included.

RESULTS: The sample consisted of 31 patients, of which approximately 84% were men and approximately 93% at the age of 50 years or older. When comparing the phases, it is possible to observe that the item speech had the greatest progress, while chewing had the least. Only the item recreation, swallowing, taste, and saliva did not show any statistical significance. The score for the general quality of life questions increased.

CONCLUSION: Electronic larynx is a viable and useful method of voice rehabilitation. Our data suggest that the use of the electrolarynx as a postlaryngectomy method of verbal communication is responsible for positive effects on patients' quality of life.

KEYWORDS: Laryngeal neoplasms. Laryngectomy. Larynx artificial. Quality of life.

INTRODUCTION

Head and neck cancers include neoplasms of the upper aerodigestive tract, including the larynx[1]. The main risk factors are smoking and alcoholism[2]. In the larynx, most cancers develop in the glottis and supraglottic region, and their histological predominance is squamous cells[3].

The global estimate for 2018 was 1,454,892 cases of head and neck cancers, with laryngeal cancer (LC) corresponding to 12.2%[4]. In Brazil, there is an estimate of 39,610 new diagnoses per year in the 3-year period from 2023 to 2025, with LC accounting for 20%[5]. Furthermore, the prevalence of LC is higher in men over 40 years old[3], with low education and income and a history of smoking and drinking alcohol[1,6].

Laryngeal cancer presents high morbidity due to the role of the larynx in voice, swallowing, and quality of life (QoL)[7]. According to the Brazilian Department of Informatics of the Unified Health System (DATASUS), diagnoses occur in advanced stages[8]. This scenario is similar to the international context and corroborates aggressive treatments associated with sequelae[9].

Currently, conventional treatments are effective in advanced stages and include total laryngectomy (TL) with or without other modalities[7]. However, changes arising from therapy affect communication, swallowing, respiratory physiology, and psychosocial aspects[10].

To interfere with communication and psychosocial aspects after surgery, vocal rehabilitation can be initiated with the use of devices, such as the electronic larynx (EL)[11]. This device acts by vibrating the pharyngomucosal segment, reducing patients' anxiety due to lack of oral communication[9].

Given the incidence of cancer and its impact on health, therapy, and vocal rehabilitation, this research study is justified by the relevance of evaluating the QoL of LC patients. In this context, the objective was to evaluate the evolution of the QoL of total laryngectomized patients using EL. Thus, the hypothesis is that there might be an improvement in those patients' QoL.

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METHODS

Ethical considerations

This study was approved by the Research Ethics Committee of the University Hospital of the Federal University of Maranhão in February 2022 under Opinion Number 5261052. All the participants voluntarily agreed to participate and signed the informed consent form.

Study design

This is a quantitative cross-sectional observational study, which was held between April 2022 and January 2023 at the Aldenora Bello Cancer Hospital located in São Luís, Maranhão.

Settings and participants

The sample consisted of 31 patients who underwent TL between April 2022 and January 2023. The inclusion criteria were patients included in the Aldenora Bello Hospital system who have the diagnosis of LC, treatment with TL, use of EL, and voluntary participation through a properly signed free and informed consent form. Patients who did not sign the informed consent form were not included. It is important to explain that the EL is the only method of vocal rehabilitation available at the mentioned hospital, which receives these devices from the local city hall.

Data collection took place in two phases: the first one from 7 days after TL and, subsequently, the second phase from 70 days after surgery using EL for at least 60 days. Patients were numbered in the order of inclusion to ensure confidentiality.

Variables

To evaluate the progress of the intrinsic aspects of their physical and mental health, the participants were asked to answer twice, in the aforementioned interval, the QoL questionnaire, validated for patients with head and neck cancer at the University of Washington[12]. This tool addresses questions about patients' health and QoL during the past 7 days, through 12 items: pain, appearance, activity, recreation, swallowing, chewing, speech, shoulder, taste, saliva, mood, and anxiety. Each item scores between 0 and 100; values close to the minimum indicate worse QoL, while values close to the maximum points indicate better QoL. Thus, the total score varies between 0 and 1,200. Furthermore, patients were supposed to choose at most three items considered to have the greatest impact on their life in the 7 days preceding the questionnaire. They should answer three general questions as well about QoL in the month before developing cancer, during the past 7 days, and aspects that contributed to their well-being in the last 7 days. In the end, they could also mention the problems considered to be relevant in terms of their QoL, which were not mentioned in the questionnaire.

Statistical analysis

Ages were analyzed by mean, standard deviation, and percentage. The representation of men and women was expressed as a percentage. As for the 12 domains, the paired Student's t-test assessed the significance, by generating the p-value ($p<0.05$) between the mean values per question of the phases. In addition, to assess QOL, the Composite Score (the ratio between the total score of the domains and the number of domains) was calculated between the phases, being analyzed as positive the one closer to the value 100 and negative to the value 0.

The question about the most relevant problems in the past 7 days with the possibility of up to three choices and the general questions about QoL were evaluated using Fisher's exact test, which offered the p-value ($p<0.05$), in order to verify the significance between the phases.

RESULTS

In the first phase, 32 participants were included. However, the final sample consisted of 31 analyses, due to the exclusion of a patient who died before the second data collection. Table 1 shows demographic details.

Table 2 shows the analysis of the domains when comparing the significance between the phases. Only the item recreation, swallowing, taste, and saliva were not statistically significant. Furthermore, Table 2 shows the most relevant issues during the past 7 days.

In Table 3, the general questions about QoL have an increase in the percentage of assertions that were evaluated as positive.

Table 1. Demographic details.

Sociodemographic characteristics (n=31)		
Age (years)	Median of age	Standard deviation
	64.6	11.6
Sex	Male	Female
	87.0%	12.9%
Occupation	Farmer	Mason
	6.4%	6.4%

n: number of patients with laryngectomies.

Table 2. Analysis of the 12 domains and the most relevant issues during the past 7 days.

Analysis of the 12 domains (n=31)			
First phase		**Second phase**	
Domains	**Average score per question**	**Average score per question**	**p-value**
Pain	58	87.9	**<0.001**
Appearance	65.3	85.4	**<0.001**
Activity	64.5	87.0	**<0.001**
Recreation	58	87	**<0.001**
Swallowing	63.4	77.5	0.067
Chewing	70.9	82.2	0.182
Speech	30	65.8	**<0.001**
Shoulder	79.5	94.6	**0.017**
Taste	65.6	76.3	0.182
Saliva	83.9	80.6	0.572
Humor	50.8	83.8	**<0.001**
Anxiety	52.7	93.6	**<0.001**

Most relevant issues during the past 7 days (n=31)					
First phase			**Second phase**		
Domains	**Sample n=31**	**Percentage (%)**	**Sample n=31**	**Percentage (%)**	**p-value**
Pain	8	28.8%	4	12.9%	0.335
Appearance	5	13.1%	1	3.2%	0.195
Activity	2	6.4%	2	6.4%	1
Recreation	1	3.2%	0	0%	0.990
Swallowing	8	28.8%	12	38.7%	0.415
Chewing	2	6.4%	3	9.6%	1
Speech	24	77.4%	9	29%	**<0.001**
Shoulder	3	9.6%	2	6.4%	1
Taste	1	3.2%	2	6.4%	1
Saliva	2	6.4%	7	22.5%	0.146
Humor	8	25.8%	3	9.6%	0.182
Anxiety	6	19.3%	3	9.6%	0.472

n: number of patients with laryngectomies. Statistically significant values are indicated in bold.

DISCUSSION

The data inherent to age and sex obtained are consistent with the DATASUS information that found a higher prevalence in men and the elderly[8]. It is important to emphasize the follow-up by medical specialties jointly with other healthcare areas after TL. The speech therapy sector carried out interventions related to voice, swallowing, and breathing which are important for communication, QoL, and social and professional reintegration[13]. In addition, there is the possibility of adding other healthcare providers to the therapy, such as physical therapists, occupational therapists, and psychologists. In short, QoL is impacted by TL and requires multidisciplinary actions starting in the postoperative period[14].

Total laryngectomy is an important resource in advanced LC, despite the physical and psychological morbidity related to respiratory and communicative changes. Furthermore, pharyngocutaneous fistula and surgical wound infection are frequent complications associated with increased length of hospital stay and the need for a new surgical intervention[15]. In this context, functional and structural changes and possible treatment complications impact patients' QoL, mainly in the elderly[16].

Table 3. General questions about quality of life.

	First phase		Second phase		
Comparison of QoL with the last month before developing cancer					
Much better	1	3.2%	17	54.8%	**<0.001**
Somewhat better	2	6.4%	9	29%	**0.042**
About the same	2	6.4%	4	12.9%	0.671
Somewhat worse	20	64.5%	0	0%	**<0.001**
Much worse	6	19.3%	1	3.2%	0.103
QoL related to health in the last 7 days					
Great	0	0%	2	6.4%	0.491
Very good	0	0%	14	45.1%	**<0.001**
Good	9	29%	13	41.9%	0.426
Average	9	29 %	0	0%	**0.002**
Bad	11	35.4%	2	6.4%	**0.010**
Very bad	2	6.4%	0	0%	0.491
QoL related to factors relevant to well-being in the past 7 days					
Great	0	0%	2	6.4%	0.419
Very good	0	0%	12	38.7%	**<0.001**
Good	12	38.7%	14	45.1%	0.797
Average	10	32.2%	2	6.4%	**0.021**
Bad	9	29.0%	1	3.2%	**0.012**
Very bad	0	0%	0	0%	–

Statistically significant values are indicated in bold.

Moreover, chemotherapy and radiation therapy can be integrated into treatment and cause sequelae. The impact of radiotherapy is proportional to the number of sessions,and may cause complications, such as mucositis and tissue necrosis[17]. Therefore, the emotional and functional consequences inherent to the therapies reduce psychological and physical comfort, as chemoradiotherapy is associated with sequelae that affect the QoL[17].

In our research study, speech presents the best comparative result between the phases. In addition to being the only item with statistical significance in the most relevant problems during the past 7 days, it is possible to infer the reduction of the negative impact of the absence of oral communication on the QoL due to the vocal rehabilitation with electrolarynx.

The possibility of adapting to aesthetic and functional changes is ratified in the evolution of appearance, activity, and recreation domains. For the latter two, it is inferred the return to a satisfactory routine without limitations in carrying out activities relevant to daily life and well-being.

Mood and anxiety, after treatment and process of adapting to changes, improve and are relevant given the impact on mental health justified by the association between vocal changes and poor communication that contribute to social isolation[17]. Therefore, EL enables dialogue in the absence of oral communication.

In line with the evolution of the activity item, a Spanish study found that most patients remained active after TL, with a relationship with vocal rehabilitation. To the new vocal condition, the emotional aspects advance together with the social function[18]. In this study, the statistical approval of the item's mood, anxiety, activity, and recreation is in agreement. The progress of the issue on QoL related to activities inherent to well-being is considered a means of admitting the relevance of vocal rehabilitation.

In a research study by the National Cancer Institute with patients undergoing treatment for LC, despite different stages and therapies, responses in the domains of swallowing, chewing, taste, and saliva were not considered significant[9], as well as in our study. It is important to mention the early intervention in adapting to changes inherent to swallowing and chewing.

Another study at the University of North Carolina found that survivors of head and neck squamous cell carcinoma are affected by mental disorders associated with greater pain and negative QoL results[19]. Anxiety, in addition to general questions about QoL, is concordant, since in the first phase, which presents a short interval with TL, these questions are affected concomitantly.

In patients with head and neck cancers, pain is common after curative treatment. Among the most affected areas, the shoulder is mentioned, and arm disability may coexist. Widespread distribution of pain is frequent and there may not be limitations to areas irradiated in radiotherapy treatments. However, multimodal interventions and pain treatment reduce awareness[20]. It is possible to relate to the retrogression in the intensity and presence of pain and alterations in the shoulder between the phases.

In the evaluation of the composite score, there is an improvement between the phases, and this scenario is confirmed by the evolution of positive answers in the general questions and in the pain, mood, and anxiety domains. The progress with longer intervals after surgery and rehabilitation is confirmed.

Laryngeal cancer and treatment change how patients see themselves, interact, and play their social role[19]. Despite the favorable results, adjuvant treatments and relapses have an impact on the evolution. In this sample, in addition to the exclusion of the deceased patient, there was the death of four individuals who completed the two phases. On the contrary, it is important to consider the improvement after discharge from clinical treatment.

Despite the methodological limitations inherent to the design, the results were able to describe important aspects of the QoL of this population.

CONCLUSION

Electrolarynx is a viable and useful method of voice rehabilitation for patients who have had laryngectomies, and according to these data, it is responsible for positive effects on patients' QoL. Significant results were observed for the pain, appearance, activity, recreation, speech, shoulder, mood, and anxiety domains. In addition, general questions about QoL progressed. However, the swallowing, chewing, taste, and saliva domains were rejected in the statistics. Furthermore, only speech showed statistical significance in the comparison between the phases of the most relevant problems during the past 7 days.

AUTHORS' CONTRIBUTIONS

LEFM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **ICS:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. **JRRJ:** Formal Analysis, Software. **PCL:** Supervision. **AVDF:** Supervision. **CMBO:** Supervision. **ECRM:** Supervision.

REFERENCES

1. Silva FA, Rousseng SC, Tavares MG, Souza CP, Mozzini CB, Benetti M. Perfil epidemiológico dos pacientes com câncer de cabeça e pescoço em um centro oncológico no sul do Brasil. Rev Bras Cancerol. 2020;66(1):1-8.
2. American Cancer Society. Risk factors for laryngeal and hypopharyngeal cancers. 2021. Available from: https://www.cancer.org/cancer/laryngeal-and-hypopharyngeal-cancer/causes-risks-prevention/risk-factors.html
3. Instituto Nacional de Câncer. Câncer de laringe. 2022. Available from: https://www.gov.br/inca/pt-br/assuntos/cancer/tipos/laringe
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492
5. Sociedade Brasileira de Cirurgia de Cabeça e Pescoço (SBCCP). Previsão de 2,1 milhões de novos casos de câncer no Brasil em três anos. 2022. Available from: https://sbccp.org.br/Noticias/-previsao-de-21-milhoes-de-novos-casos-de-cancer-no-brasil-em-trs-anos/
6. Figueiredo IC, Vendramini SHF, Lourenção LG, Sasaki NSGMDS, Maniglia JV, Padovani Junior JA, et al. Profile and speech-language rehabilitation of patients with laryngeal cancer. Codas. 2019;31(1):e20180060. https://doi.org/10.1590/2317-1782/20182018060
7. Forastiere AA, Ismaila N, Lewin JS, Nathan CA, David J, Eisbruch A, et al. Use of larynx-preservation strategies in the treatment of laryngeal cancer: American society of clinical oncology clinical practice guideline update. J Clin Oncol. 2018;36(11):1143-69. https://doi.org/10.1200/JCO.2017.75.7385
8. Conceição MVM, Virginio VRR. Análise espacial e série temporal do câncer de laringe no brasil entre os anos de 2010-2019 [dissertação]. São Cristovão: Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe; 2022.
9. SOUZA FGR. Avaliação da qualidade de vida em pacientes com câncer de laringe [tese]. Rio de Janeiro (RJ): Doutorado em Oncologia, Instituto Nacional de Câncer; 2021.
10. Scott AJ, McGuire JK, Manning K, Leach L, Fagan JJ. Quality of life after total laryngectomy: evaluating the effect of socioeconomic status. J Laryngol Otol. 2019;133(2):129-34. https://doi.org/10.1017/S0022215119000215
11. Souza FGR, Santos IC, Bergmann A, Thuler LCS, Freitas AS, Freitas EQ, et al. Quality of life after total laryngectomy: impact of different vocal rehabilitation methods in a middle income country. Health Qual Life Outcomes. 2020;18(1):92. https://doi.org/10.1186/s12955-020-1281-z
12. Vartanian JG, Carvalho AL, Yueh B, Furia CL, Toyota J, McDowell JA, et al. Brazilian-Portuguese validation of the University of Washington Quality of life questionnaire for patients with head and neck cancer. Head Neck. 2006;28(12):1115-21. https://doi.org/10.1002/hed.20464
13. Carlos MC, Santos NMS, Fortaleza MJC, Silva CM, Porto VFA. Instrumentos e reabilitação fonoaudiológica: as contribuições em pacientes laringectomizados. Gep News. 2021;5(1):205-17.
14. Lopes LIL. Os impactos socias em pacientes que realizam a laringectomia total. Rev Multidisciplin Saúde. 2021;2(4):316. https://doi.org/10.51161/rems/3263
15. Martins S, Silveira H, Sousa M, Santos M, Vaz RP. Complicações pós laringectomia total – fatores de risco e abordagem do doente no perioperatório. Revista Portuguesa de Otorrinolaringologia e Cirurgia de Cabeça e Pescoço. 2020;58(4):173-9.
16. Güneş S, Orhan KS, Başaran B, Çelik M, Kıyak E. Comparison of long-term functional results between standard supracricoid laryngectomy and modified technique with sternohyoid muscle. Braz J Otorhinolaryngol. 2019;85(3):344-50. https://doi.org/10.1016/j.bjorl.2018.02.007
17. Rosa MED, Mituuti CT, Ghirardi ACAM. Correlation between the voice handicap and swallowing quality of life in patients with laryngeal cancer submitted to chemoradiotherapy. Codas. 2018;30(2):e20170060. https://doi.org/10.1590/2317-1782/20182017060
18. Costa JM, López M, García J, León X, Quer M. Impact of total laryngectomy on return to work. Acta Otorrinolaringol Esp (Engl Ed). 2018;69(2):74-9. https://doi.org/10.1016/j.otorri.2017.02.006
19. Lenze NR, Bensen JT, Yarbrough WG, Shuman AG. Characteristics and outcomes associated with anxiety and depression in a head and neck cancer survivorship cohort. Am J Otolaryngol. 2022;43(3):103442. https://doi.org/10.1016/j.amjoto.2022.103442
20. Hernández SH, Guía VGJ, Núñez JM, Ciuró AH, Otero AN, Mohedo ED, et al. Widespread distribution and altered pain processing in head and neck cancer survivors at long-term after treatment. Support Care Cancer. 2023;31(7):394. https://doi.org/10.1007/s00520-023-07846-9

ORIGINAL ARTICLE

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Epidemiological analysis of congenital syphilis in the State of Paraná, Brazil

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SUMMARY

INTRODUCTION: Congenital syphilis is a complex public health issue caused by the transmission of *Treponema pallidum*. Brazil has high incidence rates, with a distinct transmission pattern surpassing other notifiable diseases.

OBJECTIVE: The objective of this study was to examine epidemiological trends, incidence rate, mortality, geographical distribution, prenatal care, and diagnostic determination timing of congenital syphilis in Paraná State.

METHODS: Data from Department of Informatics of the Single Health System were used to analyze the period from 2015 to 2021 in Paraná. Linear regression and t-tests were employed to assess significance. Statistical significance was determined by $p<0.05$.

RESULTS: A total of 5,096 notifications of congenital syphilis were recorded in Paraná over the examined period. The metropolitan region is a notable clustering of cases, following Londrina, Maringá, and Foz do Iguaçu. The age group with the highest cases is found between 20 and 24 years (34.93%). Regarding maternal education, a higher occurrence was noticed in incomplete lower secondary education mothers (22.12%). Regarding ethnic background, 3,792 women were identified as white, which was the majority of this analysis (74.41%). Diagnosed maternal syphilis throughout the prenatal phase during 2015–2018 exhibited a noteworthy increase ($p<0.05$). Most women received prenatal care ($p<0.05$), even though a significant number received the diagnosis at the delivery or after it. The average infant mortality rate associated with congenital syphilis in Paraná was 0.03.

CONCLUSION: Paraná State serves as a representative sample of this epidemiological situation, providing significant insights into the intricacies of congenital syphilis incidence. Further comparative investigations including diverse regions within Brazil are necessary.

KEYWORDS: Epidemiology. Syphilis. Congenital. Maternal-child health services.

INTRODUCTION

Congenital syphilis remains a difficult and challenging issue in the field of global public health, as it is caused by the vertical transmission of the etiological agent, *Treponema pallidum*. The illness in question is characterized by a complex interplay of biological, social, and health-related elements, resulting in notable resilience despite advancements in medical and technology interventions[1,2].

In the specific context of Brazil, this disorder assumes a very concerning magnitude, considering the documented high incidence. Congenital syphilis exhibits a distinctive pattern of transmission, characterized by infection rates that frequently surpass those of other illnesses that are subject to mandatory reporting. The reports have been conducted by thoroughly examining and analyzing data by the Department of Informatics of the Single Health System (DATASUS), which has been prepared by the Notification Accidents Information System (SINAN). The aforementioned departments facilitate research efforts aimed at delineating the epidemiological panorama of congenital syphilis, hence enabling the detection of patterns and the development of focused treatments[2].

At the national level, a more comprehensive understanding of the intricacies of congenital syphilis emerges when we examine the broader context. The intricate nature of disease transmission is inherently intertwined with systemic factors and the vulnerability of healthcare systems. The dearth of high-quality prenatal care and inadequate allocation of resources for the advancement of sexual and reproductive health education are critical determinants[3].

The issue of congenital syphilis extends beyond the borders of Brazil and has global implications, irrespective of a country's level of development. The persistence of this illness in both developed and developing nations underscores the existing disparities in health systems and the obstacles that hinder access to reproductive health treatments. Insufficient access to education and less knowledge on the prevention of vertical transmission exacerbate the current circumstances[4,5]. According to the World Health Organization, the establishment of diagnostic,

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therapeutic, and preventive guidelines for congenital syphilis aims to foster a worldwide alliance in combating this ailment[6].

The thorough analysis of the incidence of congenital syphilis in the state of Paraná yields a significant and insightful viewpoint. The comprehensive examination of the data accessible in SINAN and Information System in Live Births (SINASC), facilitated by DATASUS, has the potential to unveil distinct geographical, demographic, and temporal trends pertaining to this location. This information might provide valuable insights for the development of preventative and control methods tailored to the specific local context. By comprehending congenital syphilis in this framework, it becomes feasible to tackle not only the clinical facets but also the social and behavioral factors that impact the transmission of the ailment[3].

The main aim of this study was to conduct a comprehensive and evidence-based examination of the prevalence of congenital syphilis in the state of Paraná, focusing on the period from 2015 to 2021. The study was grounded in the recognition that Paraná serves as a representative sample of this epidemiological situation, providing significant insights into the intricacies of congenital syphilis incidence and the potential consequences of its determinants in specific places.

METHODS

This study was conducted as a transversal quantitative research. The collection of data on recorded cases of congenital syphilis was conducted by leveraging the information maintained within SINAN and SINASC, which covers Paraná's geographical region. The dataset covers the period from January 2015 to December 2021. The factors that were examined in this study include the rate of incidence, infant age at the time of diagnosis, the technique utilized for a definite diagnosis, death instances, provision of prenatal care, and the diagnostic determination timing.

The data were systematically arranged in tabular format, by using the Microsoft Excel software, encompassing both absolute numerical values and corresponding relative percentages. The analysis involved an investigation of the variables and their distribution throughout the years under consideration. There was no need to provide the assessment report to the Research Ethics Committee due to the utilization of publicly available data.

The statistical analysis was conducted using the GraphPad Prism 10 software (GraphPad Software Inc., San Diego, CA, USA). The incidence data were analyzed using simple linear regression to determine the slopes, standard error, R-squared, and p-value. The t-test was employed to analyze variance. The 95% confidence interval was computed for each trend and determined to have a significant level (p-value) of 0.05. A $p<0.05$ was the threshold for statistical significance. The results obtained were subsequently presented.

RESULTS

A total of 5,096 notifications of congenital syphilis were recorded in the state of Paraná over the examined period, utilizing the secondary data provided by the DATASUS platform. In terms of the yearly distribution, there was a discernible decline in the incidence of the disease, as evidenced by 867 diagnoses in 2019 and 351 cases in 2021, which, respectively, marked the greatest and lowest recorded values over the past decade.

When examining the partitioning of the Paraná State into Health Regions, it becomes apparent that there is a notable clustering of cases in Curitiba and the surrounding metropolitan region, which constitutes the second health region. These areas collectively accounted for a total of 2,282 cases over the specified time of inquiry. The Health areas of Londrina, Maringá, and Ponta Grossa, which have dense populations, have elevated notification rates, with corresponding case counts of 509, 445, and 214. It is noteworthy to mention the significant occurrence of cases in the Paranaguá region (239 cases) and the Foz do Iguaçu region (341 cases), with the former being a port area and the latter serving as a border zone between Argentina and Paraguay. The high prevalence of syphilis in some regions can be attributed to the substantial movement of individuals in these locations, as syphilis is mostly spread through sexual contact[5,7].

Furthermore, it has been observed that health areas located at a considerable distance from major metropolitan centers demonstrate the lowest rates of congenital syphilis. Notable examples include the areas of Irati (with 12 reported cases), Jacarezinho (with 22 reported cases), and Ivaiporã (with 23 reported cases). The observed gap may be ascribed to a diminished population contingent in these places or the potential underreporting of the analyzed cases[7,8]. Figure 1 graphically depicts the spatial distribution of cases within the state of Paraná through the utilization of a map of circles.

The analysis regarding the maternal age group has demonstrated its substantial influence on the incidence of congenital syphilis, as shown in Table 1. The age group between 20 and 24 years has the highest concentration of people (34.93%) followed by the age group between 25 and 29 years (22.12%). On the contrary, the obtained data showed that the maternal age groups at the extremes had the lowest proportions of confirmed cases in neonates. The degree of maternal education is an additional factor that contributes to the risk of *T. pallidum* bacterial transmission[7].

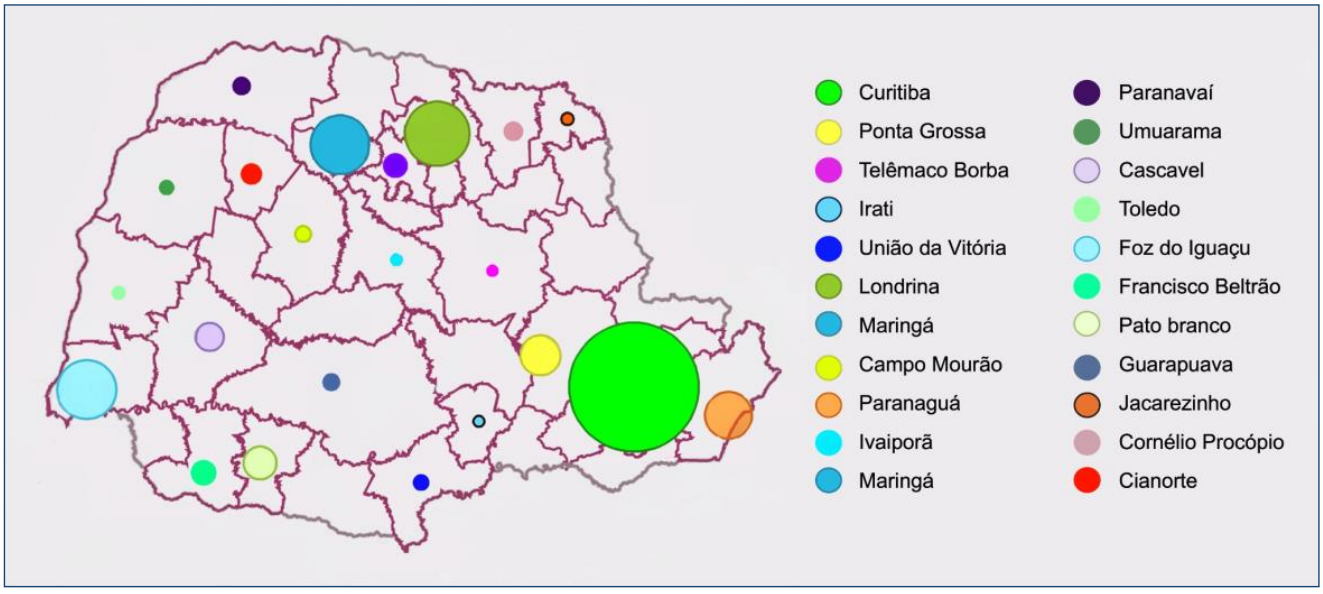

Figure 1. Map displaying circles distributed proportionally to the notifications of congenital syphilis cases in each health region in the state of Paraná. Source: the authors.

The findings of the analysis suggest that there was a higher occurrence of the mentioned incidence in infants born from mothers with incomplete lower secondary education (21.27%). This was followed by mothers who completed higher secondary education (18.82%), those who did not finish higher secondary education (14.50%), and those who completed lower secondary education (12.85%). On the contrary, it is noteworthy that the proportion of women who have attained a higher level of education, such as university level, for example, either completed or partial, is just 3.30% of the total instances, as depicted in Table 1. The correlation between younger age groups and lower educational level is associated with risk behavior, hence facilitating the transmission of the disease[7].

The ethnic background of the mother might offer significant insights into identifying the factors that contribute to the risk linked with the condition. This investigation identified 3,792 women as belonging to the white ethnic group. Additionally, the analysis identified 743 women as mixed, 104 as black, 16 as yellow, and 12 as indigenous. However, information for 429 cases was unavailable.

Regarding the monitoring of pregnant individuals and the timing of congenital syphilis notification, Table 2 provides a comprehensive overview of the trends observed between 2015 and 2021. This table illustrates the various stages of diagnosis, such as prenatal care, delivery/curettage, and the postpartum periods, shedding light on the intricacies associated with these instances. It was comprehended by a thorough individual analysis of each instance that the occurrence of maternal syphilis throughout the prenatal phase exhibited a noteworthy increase ($p<0.05$) of occurrences from 2015 to 2018. Upon analyzing the occurrence of maternal syphilis after labor or curettage, no statistically significant increases or decreases in cases were observed ($p>0.05$). With respect to the occurrence of maternal syphilis following delivery, the data presented in Table 2 do not indicate any statistically significant changes in the number of cases, as demonstrated by a $p>0.05$.

Moreover, when considering the frequency of diagnosed women who received prenatal care, as depicted in Table 2, it becomes apparent that a significant majority of diagnoses in pregnant women occurred during the prenatal period (4,560 cases). This finding demonstrates a statistically significant difference ($p<0.05$) between the group of women who underwent prenatal treatment and those who did not. Therefore, the results show that a lot of the pregnant women who were diagnosed with congenital syphilis had received prenatal care. This is different from cases that were found during childbirth or curettage procedures, as well as in the time after giving birth. Consequently, additional investigations are warranted to elucidate the factors contributing to the delayed diagnosis of these patients during the prenatal stage. Moreover, there is a required critique regarding the significance of systematic monitoring of pregnant women and the administration of syphilis detection tests throughout the gestational period and during delivery or curettage procedures in cases of abortions[9].

According to the guidelines established by the Ministry of Health of Brazil, it is suggested to perform serological testing for syphilis during prenatal visits, irrespective of the maternal illness history[9-11]. In Paraná, the data reveal that prenatal care was not received by nearly 10% of pregnant mothers (482 out

Table 1. Analysis of the maternal epidemiological profile of reported cases of congenital syphilis.

Variants	n	%	95%CI	p-value
Mother's level of education				
Ignored/null	1,058	20.76	76.00–215.00	<0.0001
No schooling	45	0.88	2.00–17.00	<0.0001
Incomplete primary	207	4.06	16.00–36.00	<0.0001
Complete primary	151	2.96	8.00–30.00	<0.0001
Incomplete lower secondary	1,084	21.27	69.00–214.00	<0.0001
Complete lower secondary	655	12.85	41.00–135.00	<0.0001
Incomplete higher secondary	739	14.50	54.00–156.00	<0.0001
Complete higher secondary	959	18.82	74.00–189.00	<0.0001
Incomplete university	91	1.79	7.00–23.00	<0.0001
Complete university	77	1.51	2.00–17.00	<0.0001
Not applied	30	0.59	2.00–7.00	<0.0001
Total	5,096			
Mother's age group (years)				
Ignored/null	51	1.00	1.00–17.00	<0.0001
10–14	33	0.65	1.00–9.000	<0.0001
15–19	1,033	20.27	57.00–196.00	<0.0001
20–24	1,780	34.93	129.00–316.00	<0.0001
25–29	1,127	22.12	87.00–204.00	<0.0001
30–34	588	11.54	37.00–105.00	<0.0001
35–39	366	7.18	24.00–65.00	<0.0001
40–44	109	2.14	10.00–21.00	<0.0001
45–49	9	0.18	0.00–3.00	<0.0001
Total	5,096			
Mother's ethnicity				
White	3,792	74.41	408.70–674.80	<0.0001
Black	104	2.04	11.30–18.42	<0.0001
Yellow	16	0.31	1.41–3.16	<0.0001
Mixed	743	14.58	71.41–140.90	<0.0001
Indigenous	12	0.24	3.09	<0.0001
Ignored	429	8.42	42.81–74.33	<0.0001
Total	5,096			

Source: the authors.

of 4,543) whose infants were diagnosed with congenital syphilis, excluding instances with missing information. The assessment of the fetus before birth is of utmost importance in facilitating appropriate fetal growth, identifying disorders in the gestational period, and implementing timely interventions to impede the advancement of these ailments[3,10,11].

The assessment of disease incidence can be informed by analyzing death rates associated with congenital syphilis. During the investigation period in Paraná, a total of 37 deaths officially related to this particular illness were registered. The distribution of fatalities with respect to age groups, as shown in Table 2, is as follows: there were 25 fatalities recorded within the initial 6 days of life, followed by four deaths between 7 and 27th days, with an additional eight deaths occurring from 28 to 364th days of life. Moreover, based on the information that was available, it was feasible to compute the yearly mortality rate utilizing the

Table 2. Epidemiological linear regression analysis of congenital syphilis cases in Paraná from 2015 to 2021, including maternal notification, incidence during prenatal care, at delivery, after delivery, and infant's mortality incidence.

Variants					
Incidence of diagnosed women who underwent prenatal care during 2015–2021					
Realized prenatal diagnosis	Confirmed cases (n)	%	p-value		
No	54	1.06	0.003216		
Yes	4,560	89.48			
Ignored	482	9.46			
Total	5,096				
Diagnosis during prenatal care					
Period	Y-intercept	X-intercept	95%CI	p-value	R^2
2015–2021	50,198	2,040	−95.54 to 46.33	0.4134	0.284
2015–2018	-117787	2,007	31.21–86.19	0.0116	0.9768
2018–2021	271,345	2,023	-340.60 to 72.38	0.1078	0.7961
At the time of delivery/curettage					
2015–2021	7,775	2,054	-22.36 to 14.78	0.6226	0.05206
2015–2018	-37565	2,009	-16.10 to 53.50	0.147	0.7277
2018–2021	57,690	2,024	-81.96 to 24.96	0.1488	0.7246
After delivery					
2015–2021	7,243	2,028	-7.97 to 0.8292	0.0913	0.4654
2015–2017	-12051	2,009	-1.33 to 13.34	0.0611	0.9908
2017–2021	12,551	2,024	-15.87 to 3.467	0.1339	0.5814

Source: the authors.

SINASC data of the state of Paraná, encompassing the period from 2015 to 2021. The findings of the study indicate that the average infant mortality rate associated with congenital syphilis in the state of Paraná for the period 2015–2021 was 0.03 deaths per 1,000 live births. With regard to the yearly rate, it is worth noting that in the year 2015, there was a rate of 0.05; however, in the year 2021, the rate was recorded at 0.01.

The incidence of congenital syphilis cases in Paraná, totaling 5,096 records, appears to be significantly high when contrasted with epidemiological data from the surrounding states in the southern region of Brazil. In comparison, Santa Catarina had a lower number of cases, totaling 3,866, while Rio Grande do Sul recorded the highest incidence with 11,953 cases[2]. Such disparities indicate epidemiological variations within the region, suggesting the need for more in-depth analyses to understand the factors determining these discrepancies and implement effective strategies for preventing and controlling congenital syphilis[12,13].

CONCLUSION

The results pertaining to the epidemiological characteristics of congenital syphilis in the state of Paraná align with the guidelines established by the Ministry of Health for the prevention, diagnosis, and treatment of this ailment. The analysis of the data indicates that among the main factors contributing to the progression of more severe manifestations of the disease and mortality are delayed diagnosis and insufficient treatment. This underscores the critical need for prenatal monitoring and administering non-treponemic testing to pregnant mothers and babies as a preventative intervention. The imperative of promoting sexual education arises as crucial in the effort to mitigate the incidence of sexually transmitted illnesses.

In the context of a prospective study on congenital syphilis, it is recommended to do further comparative investigations including diverse regions within Brazil. These studies would offer a complete perspective on the condition at a national scale, taking into account the public health network's ability to provide care to the susceptible population affected by this disease.

AUTHORS' CONTRIBUTIONS

FVB: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **JPGP:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **CO:** Writing – review & editing.

REFERENCES

1. Soares LG, Zarpellon B, Soares LG, Baratieri T, Lentsck MH, Mazza VD. Sífilis gestacional e congênita: características maternas, neonatais e desfecho dos casos. Rev Bras Saúde Matern Infant. 2017;17:781-9.
2. Brasil, Ministério da Saúde. DATASUS – Tabnet. 2023. Available from: http://datasus.saude.gov.br/
3. Barbosa DR, Almeida MG, Silva AO, Araújo AA, Santos AG. Perfil epidemiológico dos casos de sífilis gestacional. Rev Enferm UFPE On Line. 2017;11(5):1867-74.
4. Magalhães DM, Kawaguchi IA, Dias A, Calderon ID. Sífilis materna e congênita: ainda um desafio. Cad Saúde Pública. 2013;29(6):1109-20. PMID: 23778543
5. Ramos MG, Boni SM. Prevalência da sífilis gestacional e congênita na população do Município de Maringá–PR. Saúde Pesquisa. 2018;11(3):517-26.
6. World Health Organization. WHO guidelines for the treatment of *Treponema pallidum* (syphilis). Geneva: WHO; 2023.
7. Silva GA, Oliveira CM. O registro das doenças de notificação compulsória: a participação dos profissionais da saúde e da comunidade. Rev Epidemiol Controle Infecç. 2014;4(3):215-20.
8. Padovani C, Oliveira RR, Pelloso SM. Sífilis na gestação: associação das características maternas e perinatais em região do sul do Brasil. Rev Lat Am Enfermagem. 2018;26:e3019. https://doi.org/10.1590/1518-8345.2305.3019
9. Ministério da Saúde e Secretaria de Vigilância em Saúde, Brasil. Protocolo clínico e diretrizes terapêuticas para prevenção da transmissão vertical de HIV, sífilis e hepatites virais. Ministério da Saúde e Secretaria de Vigilância em Saúde; 2020.
10. Figueiredo DCMM, Figueiredo AM, Souza TKB, Tavares G, Vianna RPT. Relação entre oferta de diagnóstico e tratamento da sífilis na atenção básica sobre a incidência de sífilis gestacional e congênita. Cad Saúde Pública. 2020;36(3):e00074519. https://doi.org/10.1590/0102-311X00074519
11. Oliveira VDS, Rodrigues RL, Chaves VB, Santos TS, Assis FM, Ternes YMF, et al. Aglomerados de alto risco e tendência temporal da sífilis congênita no Brasil. [High-risk clusters and temporal trends in congenital syphilis infection in Brazil]. Rev Panam Salud Publica. 2020;44:e75. https://doi.org/10.26633/RPSP.2020.75
12. Domingues RM, Szwarcwald CL, Souza Junior PR, Leal MC. Prevalence of syphilis in pregnancy and prenatal syphilis testing in Brazil: birth in Brazil study. Rev Saude Publica. 2014;48(5):766-74. https://doi.org/10.1590/s0034-8910.2014048005114
13. Cooper JM, Michelow IC, Wozniak PS, Sánchez PJ. In time: the persistence of congenital syphilis in Brazil – more progress needed! Rev Paul Pediatr. 2016;34(3):251-3. https://doi.org/10.1016/j.rppede.2016.06.004

ORIGINAL ARTICLE

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Epidemiological analysis of congenital glaucoma: a national scenario

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SUMMARY

INTRODUCTION: Congenital glaucoma is a disease that involves increased intraocular pressure and can result in irreversible visual deterioration. The study of epidemiology allows the delineation of the characteristics associated with patients and specific risk factors.

OBJECTIVE: The objective of this study was to examine epidemiological trends, place of residence, duration of gestation, sex, and race of the newborn diagnosed with congenital glaucoma in Brazil.

METHODS: Data from SINASC (National Live Birth System) were used to analyze the period from 2017 to 2021 in Brazil. Linear regression and analysis of variance tests were employed to assess significance. The statistical significance was determined by $p<0.05$.

RESULTS: A total of 47 cases of congenital glaucoma were identified in Brazil during the study period, with the highest incidence between the years of 2018 and 2021. The analysis of the distribution indicated that the states with the highest incidence were São Paulo, followed by Rio Grande do Sul and Pernambuco. Approximately 60% of cases occurred in male individuals, compared with 19 female cases. The ethnic analysis showed the highest incidence among whites and mixed. Regarding the length of pregnancy, statistical differences were observed between newborns of different periods of gestation. Infants born from pregnancies lasting between 28 and 31 weeks and 32 and 36 weeks were significant when analyzed with the group between 37 and 41 weeks.

CONCLUSION: Studies on the mechanisms of congenital glaucoma seek to improve knowledge about the disease. Epidemiological evaluation is essential for identifying demographic and clinical patterns of the disease.

KEYWORDS: Glaucoma. Epidemiology. Infant, newborn, diseases.

INTRODUCTION

Glaucoma is a group of conditions that are defined by the presence of optical neuropathy, which has the potential to progressively deteriorate over time. This condition is closely associated with an elevation in intraocular pressure (IOP), which can initiate an irreversible process leading to visual degeneration[1]. As a result of the prevalence of hereditary disorders within the category of pathologies that contribute to juvenile vision loss, congenital glaucoma is identified as a relatively uncommon condition, accounting for around 20% of childhood blindness diagnoses[2,3].

In the field of epidemiology, it has been discovered that congenital glaucoma occurs in around 1 out of every 10,000 persons, with a higher prevalence among males[4-6]. The pathology's underlying etiology is connected with an aberration in the drainage mechanism of the aqueous humor. This defect is known as primary congenital glaucoma, and it can also be secondary to other clinical or iatrogenic disorders. As a consequence, it leads to increased IOP[7].

The majority of cases of congenital glaucoma are considered random, while around 10% of cases have a recessive genetic pattern. The physiopathology of this condition involves a disruption in the development of the trabecular meshwork architecture and the angular shape of the anterior chamber[7]. However, there is no apparent association with other significant ocular abnormalities. The condition known as isolated trabecular dysgenesis is distinguished by the absence of a specific portion of the ciliary body, resulting from the occlusion of the trabecule by a transparent amorphous material[4,7].

The patient commonly exhibits symptoms such as excessive tearing, involuntary eyelid spasms, and sensitivity to light. Furthermore, manifestations such as corneal edema and opacity, excavation of the optical disk, and abnormal expansion in the size of the eyeball can also be observed. The clinical assessment necessitates the performance of many tests, including ophthalmoscopy, tonometry, gonioscopy, and pachymetry. These procedures are crucial for both the diagnosis and monitoring of this particular condition[4,8-10].

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The diagnostic criteria encompass a positive IOP measurement over 21 mmHg, accompanied by certain structural alterations, such as a corneal diameter increase of more than 1.5 mm or an asymmetry of more than 1.5 mm. Furthermore, these characteristics encompass the concomitant occurrence of progressive myopia, accompanied by an augmentation in the corneal diameter and/or axial length of the ocular organ, an escalated excavation of the optical disk exceeding 20%, and the necessity for surgical intervention to regulate IOP[11,12].

Surgical operations are frequently employed as the primary therapeutic strategy, which has the potential to maintain visual function for an extended period of time[13]. The initial procedure often involves the performance of goniotomy, although, in certain circumstances, further interventions such as trabeculectomy and trabeculotomy may be necessary[2,4,14].

The formation of a diagnosis for congenital glaucoma is heavily reliant on the identification of the observed pattern in afflicted people, given the symptoms associated with this condition are non-specific[15]. In this particular context, it is imperative to undertake a comprehensive national epidemiological study on this particular pathology. This study acts as a fundamental approach to delineate the characteristics linked to patients, with the ultimate goal of estimating specific risk factors and enhancing diagnostic considerations within the field of pediatric ophthalmology.

METHODS

This study encompasses a quantitative research methodology, which is distinguished by its descriptive approach and utilization of a cross-design. The data collection process for recording cases of congenital glaucoma (CID 10 Q150) involved utilizing the information provided in the "Anomalia ou defeitos congênitos em Nascidos Vivos" section inside the SINASC database, which is accessible through the DATASUS portal[16]. This platform covers the entirety of the Brazilian area. The data set encompasses a period between 2017 and 2021. The variables examined in this study comprise the patient's location of residence, duration of gestation, as well as the sex and race of the infant.

For the collection of data, it was subjected to analysis using the Microsoft Excel software to facilitate the organization of the data into suitable tables for further statistical analysis. The data set utilized encompassed both absolute numerical values and their associated relative percentages. The analysis involved an investigation of the variables and their distribution throughout the years under consideration. The absence of a necessity to submit an assessment report to the Research Ethics Committee can be attributed to the utilization of publicly available data in the study.

The statistical analysis was conducted using the GraphPad Prism 10 software, developed by GraphPad Software Inc., San Diego, CA, USA. The incidence data were subjected to analysis using simple linear regression to determine the inclinations, standard error, R^2, and p-value. The analysis of variance (ANOVA) test was utilized to examine variance. A significance level (p) of 0.05 was used to define the 95% confidence interval for each trend. The determination of statistical significance was based on a p below the threshold of 0.05.

The research was conducted systematically, encompassing many discrete stages. The phases encompassed a comprehensive examination of pertinent scholarly literature, the execution of research activities, and the compilation of data specifically pertaining to the prevalence of congenital glaucoma in Brazil between 2017 and 2021. The findings derived from the research were later displayed and discussed.

RESULTS

Congenital glaucoma is a multifaceted condition that is defined by an increase in IOP and is linked to a specific form of trabecular dysgenesis. Irreversible blindness represents the most severe prognosis, underscoring the importance of understanding the risk and epidemiological factors associated with this condition. Such knowledge is crucial for enhancing the understanding of this disease within the context of differential diagnoses of eye symptoms, facilitating timely treatment, and minimizing morbidity[2,3].

The analysis of data sets obtained from the DATASUS-TABNET system enabled a thorough examination of the prevalence and characteristics of congenital glaucoma in Brazil from 2017 to 2021. The acquisition of pertinent data facilitated the development of an epidemiological profile in relation to the specified theoretical framework.

During the study period, a total of 47 incidences of congenital glaucoma were found in Brazil, as shown in Figure 1. The distribution of occurrences throughout different years is as follows: in 2017, there were six cases, accounting for 12.77% of the total; in 2018, there were 17 cases, representing 36.17%; in 2019, there were eight cases, accounting for 17.02%; in 2020, there were five cases, representing 10.64%; and in 2021, there were 11 cases, accounting for 23.40%.

The analysis of the distribution of cases by Federation Unit (state), as demonstrated in Table 1, indicated that the states with the highest incidence of congenital glaucoma in the period evaluated were São Paulo (n=23, 48.94%), followed by Rio Grande do Sul (n=5, 10.64%) and Pernambuco (n=5, 10.64%). There were two notifications in each of the following states: Amazonas, Ceará, Minas Gerais, and Goiás. The states of Maranhão, Piauí, Alagoas, Sergipe, Rio de Janeiro, and Santa Catarina appointed one notification each.

The rate of consanguinity is a contributing element that might lead to a rise in the number of instances within a specific geographical area. In populations characterized by a high prevalence index, the occurrence of primary congenital glaucoma may be significantly elevated, up to 10-fold, and the onset of the illness may occur at an earlier age and exhibit more severe manifestations[17].

In relation to the circumstances surrounding pregnancy, it has been reported that all pregnant individuals had prenatal care during their pregnancies. However, it should be noted that prenatal visits do not encompass any targeted preventative measures for the occurrence of congenital glaucoma. Nevertheless, it is recommended to conduct genetic surveillance after the birth of a child with the aforementioned condition to evaluate the risk associated with future pregnancies[2,4,18].

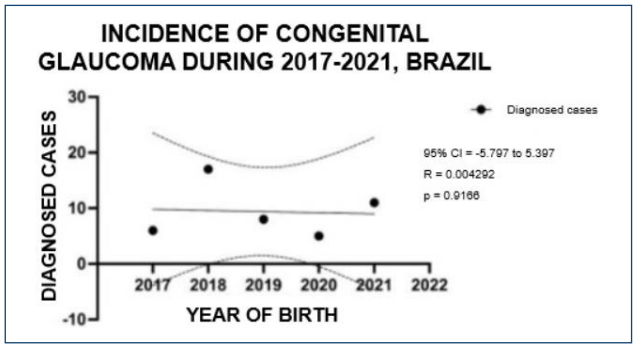

Figure 1. Analysis of the distribution of cases diagnosed with congenital glaucoma during the period 2017–2021, Brazil. Source: The authors.

Table 1. Demographic analysis of the infant diagnosed with congenital glaucoma, 2017–2021, Brazil.

Variants	n	%	95%CI	p-value
Infant's state of birth				
Amazonas	2	4.26%	-0.2801 to 1.080	0.1942
Maranhão	1	2.13%	-0.3553 to 0.7553	0.1604
Piauí	1	2.13%	-0.3553 to 0.7553	0.1178
Ceará	2	4.26%	-0.2801 to 1.080	0.1192
Pernambuco	5	10.64%	-1.776 to 3.776	0.3505
Alagoas	1	2.13%	-0.3553 to 0.7553	0.1178
Sergipe	1	2.13%	-0.3553 to 0.7553	0.1178
Minas Gerais	2	4.26%	-0.2801 to 1.080	0.1487
Rio de Janeiro	1	2.13%	-0.3553 to 0.7553	0.1178
São Paulo	23	48.94%	0.8134 to 8.387	0.0959
Santa Catarina	1	2.13%	-0.3553 to 0.7553	0.1460
Rio Grande do Sul	5	10.64%	-0.2417 to 2.242	0.1618
Goiás	2	4.26%	-0.2801 to 1.080	0.1192
Total	47	100.00%		

Source: the authors.

Significant disparities are observed between the genders of patients diagnosed with congenital glaucoma, as indicated in Table 2, in terms of underlying patient features. Around 60% of the cases (n=28) were found in male individuals, whereas the remaining 19 cases were found in females. The provided evidence supports the theory that the condition has a higher prevalence in males compared with females, with the underlying mechanism remaining unknown[9,17].

The analysis of the ethnic background of individuals diagnosed with congenital glaucoma revealed a notably greater prevalence among whites (n=23, 48.94%), which was statistically significant. Additionally, mixed individuals (n=22, 46.81%) also exhibited an elevated incidence of this condition. The number of notifications submitted by blacks and indigenous infants was limited to one each. The racial composition of the patients aligns with previous research on the epidemiological profile of this particular condition, where 83.3% of the patients were identified as individuals of mixed racial background[6]. In a study conducted in the United States, evaluating the Dallas Glaucoma Registry (DGR), assessing the incidence of congenital primary glaucoma across different ethnic groups revealed that Hispanics had a prevalence rate of 39%, Caucasians had a prevalence rate of 30%, and African Americans had a prevalence rate of 10%. The aforementioned indicators indicate that the breed of a subject has a significant impact on the probability of illness development[18,19].

The duration of gestation is a crucial factor to consider when examining the epidemiological profile of congenital glaucoma. This pathological condition has been observed to potentially exhibit an association with prematurity, particularly due to the early development of internal angle glaucoma paralysis during a

Table 2. Epidemiological profile analysis of newborns diagnosed with congenital glaucoma, 2017–2021, Brazil.

Variants	n	%	95%CI	p-value
Infant's gender				
Male	28	59.57	5.181–8.419	0.0397
Female	19	40.43	0.7169–4.483	0.0007
Male vs. female	47	100.00	3.487–4.913	<0.0001
Infant's ethnicity				
White	23	48.94	0.4261–8.774	0.0482
Black	1	2.13	-0.3553 to 0.7553	0.0550
Mixed	22	46.81	1.162–7.638	0.1403
Indigenous	1	2.13	-0.3553 to 0.7553	0.0609
Infant's birth period of gestation				
28–31 weeks	3	6.38	-0.5106 to 1.711	0.0332
32–36 weeks	8	17.02	0.1843–3.016	0.045
37–41 weeks	36	76.60	2.604–11.80	0.1882

Source: the authors.

certain period in the third trimester of pregnancy[20]. In contrast to these findings, notable statistical disparities were detected across neonates of varying gestational durations. The study observed a subset of infants born from pregnancies lasting between 28 and 31 weeks (n=3, 6.38%) and 32 and 36 weeks (n=8, 17.02%) with a statistically significant association between these gestational age groups and the occurrence of congenital glaucoma. However, the group of infants born between 37 and 41 weeks (n=36, 76.60%) also exhibited an important correlation with the development of congenital glaucoma. This finding is supported by the data presented in Table 2. It is noteworthy to mention that there were no cases of congenital glaucoma observed in pregnancies occurring before 28 weeks gestation throughout the timeframe under investigation.

CONCLUSION

Understanding the physiopathological basis causing congenital glaucoma remains an emerging topic, with research ongoing to unravel the processes of the condition. Therefore, in addition to the foregoing research, the epidemiological examination plays a crucial role in identifying the patient profile, addressing related risk factors, and allowing the identification of demographic and clinical trends.

Consequently, congenital glaucoma represents an exciting area of medical and scientific study, which demands interdisciplinary collaboration and continued research to improve our understanding, diagnosis, and treatment of this complicated disorder.

AUTHORS' CONTRIBUTIONS

JPGP: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **FVB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **RNB:** Conceptualization, Data curation, Formal Analysis, Writing – original draft. **CO:** Writing – review & editing.

REFERENCES

1. Barkan O. Pathogenesis of congenital glaucoma: gonioscopic and anatomic observation of the angle of the anterior chamber in the normal eye and in congenital glaucoma. Am J Ophthalmol. 1955;40(1):1-11. PMID: 14388087
2. Silva IM. Diagnóstico do glaucoma congênito - revisão sistemática. Rev Med Minas Gerais. 2018;28(Suppl 7):6-9.
3. Stein JD, Khawaja AP, Weizer JS. Glaucoma in adults-screening, diagnosis, and management: a review. JAMA. 2021;325(2):164-74. https://doi.org/10.1001/jama.2020.21899
4. Brink DBC, Brasil MBQ, Brink GB. Perfil epidemiológico dos pacientes com glaucoma congênito atendidos no Hospital Regional de São José. Rev Bras Oftalmol. 2015;74(3):133-7. https://doi.org/10.5935/0034-7280.20150029
5. Lopes AB, Viana LA, Faria IS, Campos VAA, Paiva MGS, Araújo FN, et al. Hipertensão ocular: uma revisão narrativa sobre o glaucoma. Rev Eletrônica Acervo Cient. 2022;41:e9987. https://doi.org/10.25248/reac.e9987.2022
6. Xavier TC, Castro Leão RF, Besteiro AVM, Trindade Junior CJS, Fernandes ACM. Perfil epidemiológico dos pacientes com glaucoma congênito no Hospital Universitário Bettina Ferro de Souza. Braz J Health Rev. 2023;6(2):5825-37. https://doi.org/10.34119/bjhrv6n2-110
7. Kanski JJ, Bowling B. Oftalmologia clínica. 7th ed. Amsterdã: Elsevier; 2012.
8. Silva GD, Orrêa JPN, Almeida JAE, Moribe JH, Flores KS, Biberg-salum TG, et al. Procedimentos cirúrgicos eleitos nas condutas de uma série de casos de glaucoma congênito na rede pública de campo grande - MS. Ensaios Ciência Biol Agrárias Saúde. 2019;23(3):185-8. https://doi.org/10.17921/1415-6938.2019v23n3p185-188
9. González VC, Mendoza CV. Clinical characteristics in congenital glaucoma: asociación para evitar la ceguera en México: 5 years' review. Rev Mex Oftalmol. 2015;89(3):150-3.
10. Rodríguez MM, Méndez STJ, Naranjo FR. Glaucoma congénito primario: ¿cuándo es preciso sospechar? Rev Cub Oftal. 2019;32(3):1-12.
11. Drechsler J, Lee A, Maripudi S, Kueny L, Levin MR, Saeedi OJ, et al. Corneal structural changes in congenital glaucoma. Eye Contact Lens. 2022;48(1):27-32. https://doi.org/10.1097/ICL.0000000000000844
12. Schuster AK, Erb C, Hoffmann EM, Dietlein T, Pfeiffer N. The diagnosis and treatment of glaucoma. Dtsch Arztebl Int. 2020;117(13):225-34. https://doi.org/10.3238/arztebl.2020.0225
13. Grehn F. New horizons in congenital glaucoma surgery. Acta Ophthalmol. 2018;96(1):7-8. https://doi.org/10.1111/aos.13589
14. Ibrahim LF, Silva SAR, Prata TS, Kanadani FN. Short-term results of ab-interno trabeculotomy using Kahook dual blade in patients with primary congenital glaucoma. Arq Bras Oftalmol. 2021;84(4):380-2. https://doi.org/10.5935/0004-2749.20210048
15. Miranda BG, Alvarenga PHA, Bertuccio CO, Almeida LV, Carvalho PV, Caldas RG, et al. Desafios do diagnóstico precoce do glaucoma congênito. Braz J Health Rev. 2022;5(2):4660-70. https://doi.org/10.34119/bjhrv5n2-054
16. Ministério da Saúde Brasil. DATASUS (Departamento de Informática do SUS) - SINASC. Ministério da Saúde. 2023.
17. Papadopoulos M, Cable N, Rahi J, Khaw PT. The British infantile and childhood glaucoma (BIG) eye study. Invest Ophthalmol Vis Sci. 2007;48(9):4100-6. https://doi.org/10.1167/iovs.06-1350
18. Dietze J, Blair K, Havens SJ. Glaucoma. Tampa (FL): StatPearls; 2022.
19. Fung DS, Roensch MA, Kooner KS, Cavanagh HD, Whitson JT. Epidemiology and characteristics of childhood glaucoma: results from the Dallas glaucoma registry. Clin Ophthalmol. 2013;7:1739-46. https://doi.org/10.2147/OPTH.S45480
20. Ko F, Papadopoulos M, Khaw PT. Primary congenital glaucoma. Prog Brain Res. 2015;221:177-89. https://doi.org/10.1016/bs.pbr.2015.06.005

ORIGINAL ARTICLE

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Evaluation of mucin-1, nuclear factor κB, and hemoglobin A1c levels in obese and non-obese individuals

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SUMMARY

OBJECTIVE: Obesity is a chronic multisystem disease associated with increased morbidity and mortality. Obesity, which is a complex, multifactorial, and heterogeneous condition, is thought to result from the interaction of environmental, physiological, and genetic factors. In this study, the relationship between serum levels of hemoglobin A1c, mucin-1, and nuclear factor κB in obese and healthy cohorts was evaluated along with biochemical and gene expressions and with demographic and clinical covariates, and their effects on obesity were evaluated.

METHODS: This case–control study included a total of 80 individuals, 40 healthy controls and 40 obesity patients, consisting of female and male aged between 18 and 63 years. Hemoglobin A1c, mucin-1, and nuclear factor κB levels were determined by ELISA in serum samples obtained from patients. In addition, aspartate aminotransferase, alanine transaminase, low density lipoprotein, and glucose values were measured. The gene expressions of the same markers were analyzed by quantitative real-time polymerase chain reaction, and their regulation status was defined.

RESULTS: Serum levels of hemoglobin A1c, mucin-1, and nuclear factor κB were found to be high in obese individuals ($p<0.05$). The gene expression of these serum markers was found to be upregulated. Of the anthropometric measurements, waist circumference and body mass index were correlated with both serum markers and gene expressions ($p<0.05$).

CONCLUSION: In addition to the known association of hemoglobin A1c and nuclear factor κB with obesity, serum levels of mucin-1 as well as upregulation of genes point to its modifier effect on obesity. These parameters can be the powerful markers in the diagnosis of obesity.

KEYWORDS: Obesity. MUC1. NF-κB. HbA1c.

INTRODUCTION

Obesity is a serious health problem all over the world. Obesity, which is caused by a decrease in physical activity and the effects of various genetic factors, causes hypertension, dyslipidemia, insulin resistance, and severe psychological stress, and it is seen with an increasing frequency in childhood[1].

Obesity increases *NF-kβ* active in the liver and skeletal muscle and the transcription of *NF-kβ* target genes[2]. The *NF-κB* pathway can be activated by cellular stress, inflammatory cytokines, growth factors, or ultraviolet rays[3]. Overfeeding activates the hypothalamic NF-κβ pathway through elevated endoplasmic reticulum stress in the hypothalamus. According to the results obtained here, the hypothalamic *IKKb/NF-κβ* pathway is a general neural mechanism for the energy imbalance underlying obesity[4]. The *MUC1* protein encodes a membrane-bound protein that is a member of the MUC1 family and is also involved in intracellular signaling. This protein is expressed on the apical surface of epithelial cells lining the mucosal surfaces of many different tissues, including lung, breast, stomach, and pancreas[5]. The *MUC1* gene promoter contains potential binding sites for *NF-κB* and similar transcriptional regulatory factors[6].

Within this transcriptionally important region of the *MUC1* promoter, the putative region for *NF-κB* overlaps with an AP-3 (activator protein 3) element and a STAT (signal converter and activator of transcription)[7]. In many people, increased glucose needs can result in sustained insulin release, constant cravings for food, and eventually obesity[8].

The data obtained from this study will provide information about the role and diagnostic power of two known and one newly proposed serum markers as well as gene expressions in the formation of obesity in the metabolism of the disease.

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METHODS

Clinical characteristics of subjects

The study included a total of 80 participants: 40 healthy control individuals (18–62 years old, female=26, male=14) and 40 individuals with obesity (18–63 years old, female=27, male=13). The study group was composed of individuals who applied to Gaziantep University Faculty of Medicine Endocrinology outpatient clinic. Research permission was obtained from Gaziantep University Clinical Research Ethics Committee (Ethical number: 2022/51), and a voluntary consent form was obtained from the participants. The study was supported by Gaziantep University Scientific Research Projects Management Unit (Project number: TF.YLT.22.38). In addition to the family history of all individuals, age, gender, height, weight, body mass index, and waist circumference measurements were taken (exercise status and duration, meal content and amount of consumption, water consumption, fat consumption, etc.)[9].

Inclusion and exclusion criteria

The inclusion criterion was to be over 18 years old. Individuals who were considered obese were classified according to the international standards as a result of body mass index (BMI), waist circumference, and skinfold thickness measurements of both patients and controls (https://www.cdc.gov/obesity/basics/adult-defining.html). The diagnosis of concomitant insulin resistance, hypertension, and atherosclerosis was questioned in the patient group and was not included. Among healthy control subjects, individuals with a BMI of <80 for women and <94 for men with a waist circumference were included in the study. Patient exclusion criteria were also valid for control subjects.

Measurement of protein activity

Some of the sera obtained from all blood samples were used for the measurement of the protein levels, and the other part was used for the determination of aspartate aminotransferase (AST), alanine transaminase (ALT), glucose, and low density lipoprotein (LDL) levels. HbA1c (CEA190Hu, SCN Life Science & Technology Company, Missouri, TX, USA), NF-κB (SEB824Hu, SCN Life Science & Technology Company, Missouri, TX, USA), and MUC1 (SEA413Hu, SCN Life Science & Technology Company, Missouri, TX, USA) serum levels were determined by ELISA reader from the serum samples according to the commercial protocol.

Quantitative analysis of gene expression

A volume of 3 mL of peripheral blood sample was obtained from all individuals, and RNA isolation was performed by modifying the Trizol method[10]. Quantification of the analyzed total RNA samples was performed with a spectrophotometer. Totally, 20 µl cDNA reaction was prepared, which contained5x RT buffer, dNTP, OneScript®Plus RTase, and 2 ng RNA. The samples were incubated at 55°C for 15 min and at 85°C for 5 min in a thermal cycler. Analysis of the expression changes of *HbA1c* [Chromosome 16, NC_000016.10 (176680.177522)], *NF-κB* [Chromosome 4, NC_000004.12 (102501359.102617302)], and *MUC1* [Chromosome 1, NC_000001.11 (155185824.155192915)] genes was carried out by quantitative real-time polymerase chain reaction (qRT-PCR). Normalization of the expressional changes of genes was performed relative to β-actin (*ACTB*) endogenous control. A volume of 25 µl reaction mixture was prepared containing 2X SYBR Green Master Mix, 10X Quanti Tect primer assay, 2 µg cDNA, and ddH_2O. Samples were incubated in a real-time PCR device for 5 min, 1 cycle at 95°C, 5 s at 95°C, and 40 cycles at 60°C for 10 s. Each sample was replicated three times, and the mean DDCt values were calculated[11].

Statistical analysis

Mann-Whitney U test (comparison between two groups) was used to compare the gene expression levels and clinicopathological data. Descriptive statistics of the data obtained from the study are given with mean, standard deviation for numerical variables, and frequency and percentage analysis for categorical variables. The normal distribution test of *HbA1c*, *NF-κB*, and *MUC1* variables was analyzed using the Shapiro-Wilk test. It was determined that the variables other than the *NF-κB* variable did not conform to the normal distribution ($p<0.05$). Independent samples t-test and Mann-Whitney U test were used to compare these variables according to the study groups. ROC analysis was used to determine the cutoff point for *HbA1c*, *NF-κB*, and *MUC1* variants. All data obtained from patients and laboratory studies were analyzed using the SPSS 22.0 program. $p<0.05$ was considered significant. The diagnostic power of gene expressions was evaluated by receiver operating characteristic (ROC) curve analysis. The diagnostic power was determined by the area under the curve (AUC) values classification[12]. To detect a difference of 23 units (effect size 23) between the biochemical and expressional measurements of the obesity and control groups, the required minimum sample size was calculated as 40 individuals in each group, under the conditions of 5% Type I error and 80% power (Type II error 0.20). Power analysis was performed with the SPSS 22.0 package program.

RESULTS

Clinical and demographic factors

This is a case–control study in randomly selected individuals who were not related but age-sex matched. The cohort consisted of a total of 80 individuals between the ages of 18 and 63 years, with 40 healthy controls and 40 obesity patients.

When the average age of the groups was examined, it was determined that the highest percentile was between the ages of 36 and 50 years. It shows that the risk of developing obesity increases with the contribution of covariates in the transition from young to middle age and old age (Figure 1 and Table 1). Descriptive statistics differed significantly between groups for weight, BMI, waist circumference, *HbA1c*, *MUC1*, and *NF-κB*. Weight, waist circumference, and BMI were high in patients as expected. HbA1c, MUC1, and NF-κB serum levels were high, and the significant difference was detected ($p<0.05$). Glucose levels were not significant. The parameter indicating the absence of diabetes and diabetes onset in individuals indicated the trend with *HbA1c*. ALT and LDL levels were not significant ($p>0.05$). Elevated AST level may be a sign of many diseases. However, due to the absence of these diseases in the clinical data of our patient group, it is thought to be associated with obesity. Although an increase was observed in LDL levels in the patient group, there was no significant difference ($p>0.05$). The fact that patients are taking cholesterol medication (n=26) may affect the significance. This suggests the association of obesity with cholesterol in patients. The positive correlation between *HbA1c*, BMI, and waist circumference, which is used in the diagnosis of diabetes and pre-diabetes, indicates that individuals are at risk of developing diabetes. Therefore, in accordance with the literature, it is a marker that can be investigated for diabetes risk in obese individuals. There is also a correlation between *HbA1c*, *MUC1*, and *NF-κB*. In obese individuals, these three parameters with high serum levels seem to be associated with obesity risk in the absence of diabetes.

Gene expression analysis of groups

To clarify the question of whether the direction of the relationship for *HbA1c* and *NF-κB* is due to obesity or obesity caused

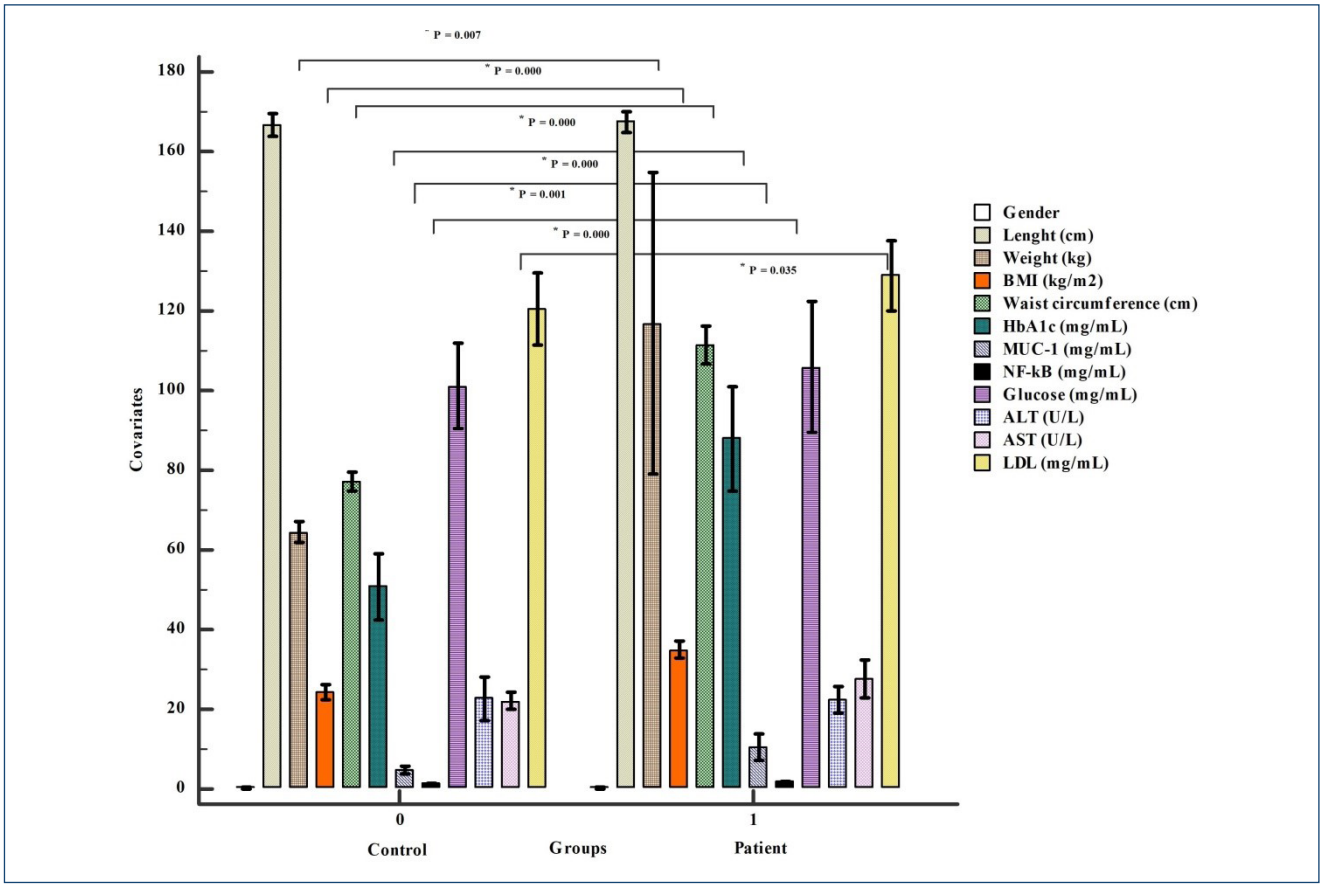

Figure 1. Clustered multiple variable graph. The bar graph represents the 95%CI values for the mean of the covariates in the groups. Weight, BMI, waist circumference, *HbA1c*, *MUC1*, *NF-κB*, and AST were statistically significant ($p<0.005$).

by these factors, and to show whether *MUC1* has a role in these links, it was determined that all three genes were upregulated in obesity and were statistically significant between groups ($p<0.05$). *HbA1c*, *NF-κB,* and *MUC1* genes were found to be upregulated and statistically significant in obese individuals ($p<0.05$) (Table 2).

In the correlation analyses for the three genes, the expression changes were statistically significant for *HbA1c–MUC1* (Pearson correlation=0.984) and *HbA1c–NF-κB* (Pearson correlation=0.938) (correlation is significant at the 0.01 level) (2-tailed) ($p=0.000$). In the analysis of gene expressions, the correlation of which was investigated for clinical and demographic data, it was found that they were correlated with all parameters except ALT and were statistically significant. Obtained data show that there is a correlation between gene expressions

Table 1. Descriptive statistics of cohorts.

Covariates	Groups	Mean	df	SD	SE	95%CI	p-value
Gender	Control	0.35	34	0.483	0.076	0.20–0.50	0.816
	Patient	0.33	33	0.474	0.075	0.17–0.48	
Lenght (cm)	Control	166.65	34	9.404	1.487	163.64–169.66	0.674
	Patient	167.48	33	7.994	1.264	164.92–170.03	
Weight (cm)	Control	64.48	34	7.776	1.229	61.99–66.96	**0.007***
	Patient	116.88	33	18.669	18.763	78.92–154.83	
BMI (kg/m^2)	Control	24.09	34	5.94	0.93922	22.190–25.990	**0.000***
	Patient	34.992	33	6.057	0.9577	33.055–36.930	
Waist circumference (cm)	Control	77.18	34	8.041	1.271	74.60–79.75	**0.000***
	Patient	111.38	33	14.565	2.303	106.72–116.03	
HbA1c (mg/mL)	Control	50.745	34	50.745	4.13301	42.385–59.104	**0.000***
	Patient	87.869	33	87.869	6.57524	74.570–101.168	
MUC1 (mg/mL)	Control	4.693	34	4.693	0.50313	3.675–5.710	**0.001***
	Patient	10.5	33	10.5	1.74677	6.954–14.046	
NF-κB (mg/mL)	Control	1.306	34	1.306	0.5006	1.145–1.466	**0.000***
	Patient	1.904	33	1.94	0.41727	1.807–2.074	
Glucose (mg/mL)	Control	101.14	34	101.14	31.956	90.48–111.79	0.622
	Patient	105.89	33	105.89	48.853	89.60–122.18	
ALT (U/L)	Control	22.65	34	22.65	16.794	17.05–28.25	0.952
	Patient	22.46	33	22.46	9.714	19.22–25.70	
AST (U/L)	Control	22.11	34	22.11	6.729	19.84–24.39	**0.035***
	Patient	27.59	33	27.59	13.783	23.00–32.19	
LDL (mg/mL)	Control	120.53	34	120.53	25.773	111.54–129.52	0.181
	Patient	128.84	33	128.84	25.973	120.18–137.50	

*p<0.05 is significant, SD: standard deviation; SE: standard error; CI: confidence interval.

Table 2. Gene expression analysis of groups (according to DDCt values).

Covariates	Groups	Mean	df	SD	SE	95%CI	p-value
HbA1c	Control	7.246	1	0.477	0.075	7.093–7.399	**0.001***
	Patient	13.438	1	0.303	0.048	13.341–13.534	
MUC1	Control	6.075	1	0.534	0.084	5.905–6.246	**0.001***
	Patient	12.752	1	0.495	0.078	12.594–12.911	
NF-κB	Control	8.158	1	0.59	0.093	7.964–8.347	**0.001***
	Patient	12.699	1	1.039	0.164	12.367–13.032	

*p<0.05 is significant, SD: standard deviation; SE: standard error; CI: confidence interval.

and BMI, waist circumference, serum *HbA1c*, *MUC1*, *NF-κB* values, and liver enzyme levels in obese individuals.

Upregulations of *HbA1c* and *NF-κB* and high serum levels have been shown to be associated with obesity.

Diagnostic power of gene expressions and covariates with receiver operating characteristic analysis

In the ROC curve analyses performed to determine the diagnostic power of the datasets, BMI and waist circumference measurements were found to be excellent. *NF-κB*, *HbA1c*, *MUC1*, and AST levels were determined as very good, good, satisfactory, and unsatisfactory, respectively, for serum levels. In multivariate analyses based on the ROC values of the datasets, covariates were modeled together, and their diagnostic power was evaluated. It was determined that there was diagnostic power in binary combinations (except *MUC1* and AST) with *HbA1c*. For *MUC1*, the diagnostic power was statistically significant when evaluated together with *NF-κB*, BMI, waist circumference, and AST.

The diagnostic power of the upregulation of genes in obese individuals was evaluated with the ROC curve. Genes were found to have "excellent" diagnostic power in differentiating obese from healthy individuals.

DISCUSSION

Obesity is a complex, multifactorial disease involving genetic and environmental factors. There is an increase in the prevalence of obesity worldwide in both developed and developing countries. There are no studies on the reflections of the relationship between *MUC1* and the other two genes (*HbA1c* and *NF-κB*) in obesity physiology. Obesity can affect the level of *HbA1c*. There was a significant correlation between anthropometric measurements and *HbA1c*, with *HbA1c* levels being higher in the obese group, and significant patient scores with HbA1c and high body fat indicate the relationship of the molecule with obesity[13]. Our data also support these findings.

During this process, *MUC1* is thought to be involved in cell growth and division (proliferation), helping cells stick together (cell adhesion), cell movement (motility), and cell survival. Some researchers suggest that in the nucleus, *MUC1-CT* helps control the activity of other genes[14-16]. *MUC1* can also provide information on the direction of the relationship between obesity and cancer. Several possible mechanisms have been proposed to explain how obesity may increase the risks of some cancers[17,18]. Current results have shown that the *MUC1* gene may play a critical role in determining obesity[19]. The data in this study indicate that upregulation of *MUC1* is associated with an increased risk of obesity. *MUC1* is known to regulate the expression of metabolic genes as a transcriptional activator[20]. The role of this mission is great in its relationship with obesity. *MUC1* receptor tyrosine kinase affects the activity and stability of transcription factors by regulating metabolic activities, due to its modulatory role in the signaling of the receptor. The correlation analyses suggest that *NF-κB* may have a role in this task.

Nuclear factor κB pathway, cellular stress, inflammatory cytokines, growth factors, ultraviolet rays, etc. can be activated. Activated *NF-kB* is transported from the cytoplasm to the nucleus and then binds to a specific DNA sequence, forming a DNA–NF-κB complex[3]. Inappropriate activation of *NF-κB* is associated with a number of inflammatory diseases, while persistent inhibition of *NF-κB* leads to inappropriate immune cell development or delayed cell growth[21].

Receiver operating characteristic analyses are helpful in eliminating the confounding effects of clinical risk factors and environmental exposures. The analyses were carried out to predict the variables and their effects indicate that genes have biomarker potential. These genes are possible targets for detecting disease as they move away from a healthy state. These genes can be used to determine the appropriate option in various clinical conditions with a threshold value, to establish the balance of sensitivity and susceptibility, and to ensure the natural balance that exists between sensitivity and susceptibility.

In conclusion, *MUC1* appears to be capable of modulation on its own, without being overshadowed by *HbA1c* and *NF-κB* in obesity. However, diagnostic tests indicate that there is no strong marker to distinguish patients from healthy ones. For a better understanding of *MUC1* pathology and physiology, studying in large-scale groups will make it possible to use it as a treatment target.

DATA AVAILABILITY STATEMENT

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

ETHICS APPROVAL

The work described in this article has been carried out by the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Approval was obtained from the Gaziantep University Faculty of Medicine Experimental Animal Research Ethics Committee for our study

(protocol number: 2022/51). Gaziantep University Clinical Research Ethics Committee approval was obtained (date: March 09, 2022; Number: 2022/51).

AUTHORS' CONTRIBUTIONS

ASB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ŞGY**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **MF**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **HU**: Data curation, Formal Analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **EA**: Formal Analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **ZAS**: Formal Analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing.

REFERENCES

1. Hill JO, Wyatt HR, Peters JC. Energy balance and obesity. Circulation. 2012;126(1):126-32. https://doi.org/10.1161/CIRCULATIONAHA.111.087213
2. Ferrante AW. Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. J Intern Med. 2007;262(4):408-14. https://doi.org/10.1111/j.1365-2796.2007.01852.x
3. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:17023. https://doi.org/10.1038/sigtrans.2017.23
4. Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D. Hypothalamic IKKbeta/NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. Cell. 2008;135(1):61-73. https://doi.org/10.1016/j.cell.2008.07.043
5. Junker WM, Moniaux N, Batra SK. MUC17 (mucin 17, cell surface associated). 2008.
6. Gendler SJ. MUC1, the renaissance molecule. J Mammary Gland Biol Neoplasia. 2001;6(3):339-53. https://doi.org/10.1023/a:1011379725811
7. Lagow EL, Carson DD. Synergistic stimulation of MUC1 expression in normal breast epithelia and breast cancer cells by interferon-gamma and tumor necrosis factor-alpha. J Cell Biochem. 2002;86(4):759-72. https://doi.org/10.1002/jcb.10261
8. Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. Trends Mol Med. 2014;20(6):332-42. https://doi.org/10.1016/j.molmed.2014.02.007
9. Reethesh SR, Ranjan P, Arora C, Kaloiya GS, Vikram NK, Dwivedi SN, et al. Development and validation of a questionnaire assessing knowledge, attitude, and practices about obesity among obese individuals. Indian J Endocrinol Metab. 2019;23(1):102-10. https://doi.org/10.4103/ijem.IJEM_487_18
10. Avison M. Chapter 2: Isolation and analysis of RNA. In: Measuring gene expression. 2nd ed. Milton Park: Taylor & Francis; 2008, p. 41-90.
11. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402-8. https://doi.org/10.1006/meth.2001.1262
12. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5(9):1315-6. https://doi.org/10.1097/JTO.0b013e3181ec173d
13. Sarnings W, Aman AM, Rasyid H, Bakri S, Sanusi H, Zainuddin AA. Obesity measurement index is associated with hemoglobin a1c level in young adult without diabetes: a single-center cross-sectional study. J Endocrinol Metabol. 2022;12(4-5):140-5. https://doi.org/10.14740/jem823
14. Fanni D, Fanos V, Monga G, Gerosa C, Nemolato S, Locci A, et al. MUC1 in mesenchymal-to-epithelial transition during human nephrogenesis: changing the fate of renal progenitor/stem cells?. J Matern Fetal Neonatal Med. 2011;24(Suppl 2):63-6. https://doi.org/10.3109/14767058.2011.613159
15. Hattrup CL, Gendler SJ. Structure and function of the cell surface (tethered) mucins. Annu Rev Physiol. 2008;70:431-57. https://doi.org/10.1146/annurev.physiol.70.113006.100659
16. Singh PK, Hollingsworth MA. Cell surface-associated mucins in signal transduction. Trends Cell Biol. 2006;16(9):467-76. https://doi.org/10.1016/j.tcb.2006.07.006
17. Friedenreich CM, Ryder-Burbidge C, McNeil J. Physical activity, obesity and sedentary behavior in cancer etiology: epidemiologic evidence and biologic mechanisms. Mol Oncol. 2021;15(3):790-800. https://doi.org/10.1002/1878-0261.12772
18. Liu XZ, Pedersen L, Halberg N. Cellular mechanisms linking cancers to obesity. Cell Stress. 2021;5(5):55-72. https://doi.org/10.15698/cst2021.05.248
19. Teng MS, Wu S, Hsu LA, Chou HH, Ko YL. Pleiotropic effects of functional MUC1 variants on cardiometabolic, renal, and hematological traits in the taiwanese population. Int J Mol Sci. 2021;22(19):10641. https://doi.org/10.3390/ijms221910641
20. Mehla K, Singh PK. MUC1: a novel metabolic master regulator. Biochim Biophys Acta. 2014;1845(2):126-35. https://doi.org/10.1016/j.bbcan.2014.01.001
21. Tuovinen EA, Kuismin O, Soikkonen L, Martelius T, Kaustio M, Hämäläinen S, et al. Long-term follow up of families with pathogenic NFKB1 variants reveals incomplete penetrance and frequent inflammatory sequelae. Clin Immunol. 2023;246:109181. https://doi.org/10.1016/j.clim.2022.109181

ORIGINAL ARTICLE

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Quality of sleep in individuals with systemic sclerosis and its correlation with functional disability and quality of life: a cross-sectional study

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SUMMARY

OBJECTIVE: This study aimed to evaluate the quality of sleep in individuals with systemic sclerosis and its correlation with the quality of life and disability.

METHODS: This is a cross-sectional study, carried out in a tertiary service of a university hospital. Inclusion criteria were diagnosis of systemic sclerosis according to the criteria of the American College of Rheumatology/European League Against Rheumatism 2013 or the preliminary criteria of the American College of Rheumatology 1980, age ≥ 18 years; regularly monitored at the outpatient clinic of rheumatology. Clinical and demographic data of the patients were obtained through a structured interview and evaluation of the medical records. Sleep quality was assessed using the Pittsburgh Sleep Quality Index questionnaire, daytime sleepiness using the Epworth Sleepiness Scale, quality of life using 12-item short-form health survey, and disability using the scleroderma health assessment questionnaire.

RESULTS: A total of 50 patients with systemic sclerosis were included, with 92% female, mean age 48.9 years, mean disease duration 8.9 years, and 60% limited cutaneous form. Most systemic sclerosis patients (84%) have poor sleep quality and 20% have excessive daytime sleepiness. There was a significant negative correlation between Pittsburgh Sleep Quality Index and the physical and mental components of the 12-item short-form health survey (r=-0.42, p=0.003 and r=-0.43, p=0.002, respectively) and a positive correlation with the scleroderma health assessment questionnaire (r=0.52, p=<0.001).

CONCLUSION: This study showed that poor sleep quality is a very common finding among systemic sclerosis patients, and it negatively affects both the quality of life and the degree of disability.

KEYWORDS: Scleroderma, systemic. Sleep quality. Daytime sleepiness. Quality of life.

KEYPOINTS:

- Sleep quality is an unmet need in patients with systemic sclerosis
- Poor sleep quality is very common in patients with systemic sclerosis
- Poor sleep quality correlated with worse quality of life and greater disability

INTRODUCTION

Systemic sclerosis (SSc) is a rare autoimmune disease characterized by vascular involvement, autoimmunity, and progressive fibrosis of the skin and internal organs. Given its heterogeneous clinical manifestations and its chronic and progressive character, patients (SSc) have significant functional impairment and quality of life. While the traditional medical approach has generally focused on treating target organ manifestations such as in the skin, lungs, and heart, patients may perceive other manifestations as more important or debilitating. Factors such as fatigue, depression, dissatisfaction with their body image, joint problems, sexual dysfunction, sleep disturbances, and pruritus are some obstacles that these patients face to fully achieve well-being[1,2]. Thus, the purpose of treatment should be to maintain control of the disease and to ensure improvement in the quality of life through a comprehensive analysis of the patient and multidisciplinary support[3].

Sleep quality is an important component of quality of life, but the impact of SSc on the sleep of affected individuals is still a poorly studied issue[4-6]. In a large Canadian cohort, difficulty sleeping was reported as one of the five highest-rated symptoms in terms of frequency and moderate-to-severe impact on daily activities[2]. A study with polysomnography showed changes such as reduced sleep efficiency and increased amount

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of periodic limb movements[7], while another study showed a high frequency of obstructive sleep apnea in patients with SSc[8]. Possible causes of sleep disorders in SSc include fatigue, functional limitations, skin deformities, pain, restless legs syndrome, dyspnea, gastroesophageal reflux, and psychological disorders such as depression[6]. Therefore, the objective of this study was to evaluate the quality of sleep in individuals with SSc and its correlation with the quality of life and disability.

METHODS

Patients

This is a cross-sectional study which was approved by the local ethics committee. Data were collected from 50 patients with SSc treated at the Rheumatology Service of the Hospital das Clínicas of the Federal University of Pernambuco (HC-UFPE), who were selected according to outpatient care using the eligibility criteria.

Inclusion criteria were as follows: over 18 years of age; diagnosis of SSc according to the preliminary criteria of the American College of Rheumatology[9] or the criteria of the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2013[10]; and regularly monitored at the outpatient clinic of rheumatology at HC-UFPE. Exclusion criterion was diagnosis of localized scleroderma or known diagnosis of mental retardation or dementia.

Demographic and clinical information were obtained from an interview of the patients by a trained evaluator, and then the questionnaires were applied. Complementary information regarding the diagnosis, complementary exams, and treatment employed was obtained from medical records.

The study was approved by the Committee on Ethics in Research of Human Beings UFPE (CAAE- 77235517.8.1001.5208) in accordance with the precepts of the Brazilian Health Council. Informed consent was obtained from all individual participants included in the study.

Assessment instruments

Sleep quality was evaluated using the Pittsburgh Sleep Quality Index (PSQI) questionnaire, which was developed in 1989[11] and assesses sleep quality in a standardized questionnaire, which can be easily answered and is validated for Brazilian Portuguese[12]. It consists of 19 questions answered by self-report and 5 answered by a roommate if they have one. The instrument assesses 7 sleep components: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep changes, use of sleep medication, and daytime sleep dysfunction. The score ranges from 0 to 3 for each component, with a maximum score of 21 points. Scores ≥ 5 indicate poor sleep quality[13]. The Epworth Sleepiness Scale (ESS-BR) was used to assess daytime sleepiness. The questions are answered through self-report involving six daily situations and the chance for the patient to fall asleep when performing them. The score ranges from 0 to 18, and a score greater than 10 indicates excessive daytime sleepiness[13].

Quality of life assessment was performed using the 12-Item Short-Form Health Survey (SF-12) instrument, a questionnaire consisting of 12 questions and has two domains: physical and mental[14]. The values for each domain are transformed into a scale ranging from 0 to 100, where 0 is equivalent to a worse quality of life and 100 to a better quality of life[15]. Functional disability was assessed using scleroderma health assessment questionnaire (SHAQ)[16]. It consists of 20 items spread over eight domains together with five additional domains that assess the dysfunctions caused by the symptoms of the disease. A visual analog scale is used to evaluate the additional domains. The values of each domain vary from 0 to 3, and the total score is obtained after adding all the values and dividing the total by 13[16].

Statistical analyses

The data collection took place from August 2019 to March 2020. The collected data were organized in the Excel XP 2016 Microsoft® spreadsheets and analyzed using the GraphPad Prism 6.0 software program. Descriptive statistics were performed using mean and standard deviation for variables with normal distribution, a median and interquartile range for those with non-normal distribution, and frequency (percentage) for qualitative variables. Possible differences between means in the intergroup analysis were verified using the Student's t-test for independent samples when the sample presented normal distribution and Mann-Whitney test in cases of non-Gaussian distribution. Pearson's (samples with normal distribution) or Spearman's correlation test (samples with non-Gaussian distribution) was used to assess the relationship between two continuous variables. $p<0.05$ were considered significant. For correlation magnitude, it was considered that 0.1 is a small magnitude, 0.5 is a medium magnitude, and 0.8 is a large magnitude[17].

RESULTS

The demographic and clinical characteristics of the assessed patients are described in Table 1. About 42 patients (84%) had a PSQI score ≥ 5, indicating poor sleep quality, and 10 patients (20%) had excessive daytime sleepiness (ESS-BR>10).

Table 2 presents the correlations between sleep quality and the outcomes of disability and quality of life. It can be seen that

Table 1. Demographic and clinical characteristics of patients with systemic sclerosis (n=50).

Sociodemographic and clinical characteristics	
Age (years) median (IQR)	52.0 (37.7–58.2)
Female n (%)	46 (92%)
Disease duration (years) median (IQR)	7.0 (4.0–13.0)
Clinical subset n (%)	
Limited cutaneous	30 (60%)
Diffuse cutaneous	18 (36%)
Sine scleroderma	2 (4%)
Overlap	6 (12%)
Clinical manifestations n (%)	
Raynaud's phenomenon	49 (98%)
Interstitial lung disease	34 (68%)
Esophageal dysmotility	39 (78%)
Digital ulcers	26 (52%)
Myopathy	5 (10%)
Pulmonary arterial hypertension	5 (10%)
Arthritis	34 (68%)
Telangiectasia	15 (30%)
Calcinosis	5 (10%)
PSQI mean (±SD)	9.63 (±4.88)
ESS-BR mean (±SD)	6.62 (±5.23)
SHAQ mean (±SD)	1.42 (±0.73)
SF-12 PCS mean (±SD)	35.36 (±10.28)
SF-12 MCS mean (±SD)	42.60 (±12.38)

ESS-BR: Epworth Sleepiness Scale–Portuguese version; IQR: interquartile range; MCS: mental component summary; PCS: physical component summary; PSQI: Pittsburgh Sleep Quality Index; SD: standard deviation; SF-12: 12-item short-form health survey; SHAQ: scleroderma health assessment questionnaire.

Table 2. Correlation between sleep quality and age, disease duration, disability, and quality of life in patients with systemic sclerosis.

	Sleep quality (PSQI)		
	R	95%CI	p-value
Age	-0.09	-0.37 to 0.20	0.53
Disease duration	-0.03	-0.33 to 0.28	0.86
ESS-BR	-0.09	-0.37 to 0.20	0.55
SHAQ	0.52	0.26 to 0.70	<0.001
SF-12 PCS	-0.42	-0.63 to -0.14	0.003
SF-12 MCS	-0.43	-0.64 to -0.16	0.002

ESS-BR: Epworth Sleepiness Scale–Portuguese version; MCS: mental component summary; PCS: physical component summary; PSQI: Pittsburgh Sleep Quality Index; SF-12: 12-item short-form health survey; SHAQ: scleroderma health assessment questionnaire.

there was a significant negative correlation between the components of the SF-12 and the PSQI and a significant positive correlation between SHAQ and PSQI. No associations were observed between sleep quality and demographic characteristics or clinical manifestations (Table 3).

DISCUSSION

In the present study, we showed that most patients with SSc have poor sleep quality, which in turn was associated with the worse quality of life and greater disability. Although it is an important aspect related to the quality of life and a frequent complaint among patients, few previous studies have evaluated the sleep quality in individuals with SSc[1-9]. Sleep assessment in patients with autoimmune rheumatic diseases has been shown to be extremely important. In addition to the recognized impact on fatigue, mood, productivity, and quality of life, sleep disturbances have been associated with increased systemic inflammation and greater sensitivity to pain. Furthermore, recent studies have suggested an association between sleep disorders and an increased risk of chronic diseases, including autoimmune diseases[18,19].

Considering a PSQI cutoff of 5, we observed that most patients with SSc (84%) had poor sleep quality. The mean PSQI score in our patients was high, similar to that described in a study with

Table 3. Associations of sleep quality Pittsburgh Sleep Quality Index with the clinical characteristics in patients with systemic sclerosis.

Characteristic	Status	Mean PSQI (±SD)	p-value
Sex	Female	9.9 (±4.8)	0.18
	Male	6.5 (±4.8)	
Clinical subset	Limited	9.9 (±4.9)	0.69
	Diffuse	9.4 (±5.1)	
Interstitial lung disease	Yes	10.2 (±5.1)	0.24
	No	8.4 (±4.2)	
Esophageal dysmotility	Yes	10.0 (±5.0)	0.30
	No	8.2 (±4.3)	
Digital ulcers	Yes	10.5 (±4.9)	0.17
	No	8.6 (±4.7)	
Pulmonary arterial hypertension	Yes	11.0 (±6.6)	0.51
	No	9.5 (±4.7)	
Myopathy	Yes	8.4 (±3.9)	0.56
	No	9.8 (±5.0)	
Arthritis	Yes	10.1 (±4.9)	0.35
	No	8.7 (±4.9)	

Italian[20] and Brazilian patients[4], and higher than that described in Turkish patients[6]. Although our study did not evaluate a control group, these previous studies had already shown that SSc patients had higher PSQI scores compared with healthy individuals and patients with rheumatoid arthritis[6,20].

We found a significant correlation between disability and quality of life with sleep quality. These findings are in line with those described by other authors, who also found a correlation between sleep quality and disability and worse quality of life[4,6]. This finding is probably because quality sleep is an important component of quality of life, so poor sleep quality can affect this perception and other symptoms such as pain and fatigue that influence disability.

Although previous studies have shown an association between sleep disturbance and clinical manifestations of the disease, such as dysphagia, gastroesophageal reflux, pulmonary impairment, and pain[4,6], we were unable to establish any association between these variables. In this study, the assessment of clinical manifestations was made according to the medical records, which did not necessarily reflect the activity of these manifestations. Therefore, it is possible that the lack of association between sleep quality and clinical manifestations of SSc is due to the absence of disease activity and good control of symptoms related to esophageal or pulmonary involvement. Furthermore, it is possible that sample size and demographic and clinical differences between patients in different studies may also be implicated.

Excessive daytime sleepiness can have an important impact on quality of life and functional impairment, in addition to being associated with the risk of morbidity and mortality related to cardiovascular, psychiatric, and neurodegenerative pathologies. Among the associated causes, sleep deprivation, obstructive sleep apnea, psychiatric or central nervous system disorders, and side effects of medications were common[21]. Taylor-Gjevre et al., evaluated a sample of patients with different rheumatic diseases and found excessive sleepiness in about 25.7% of patients and demonstrated a correlation with fatigue, quality of life, and disability[22]. In the present study, 20% of patients with SSc had excessive daytime sleepiness, but we did not observe an association with sleep quality or with the other clinical parameters evaluated.

This study has some limitations. The small sample size may have limited the detection of associations between sleep quality and clinical manifestations of the disease. Factors such as pain, anxiety, depression, and fatigue, in addition to more objective sleep assessments such as polysomnography, were not evaluated. Furthermore, the cross-sectional design of our study does not enable establishing causal relationships between the studied variables. Therefore, prospective studies with a larger number of patients are needed to better assess the factors that should be modified to improve sleep quality in patients with SSc.

In summary, this study highlights the high percentage of patients with compromised sleep quality and the high frequency of excessive daytime sleepiness in patients with SSc, reinforcing the importance of assessing sleep health in patients with SSc. Depending on the underlying cause, interventions such as guidance on sleep hygiene, weight loss, adjustment of medications in use, and control of predisposing factors, such as pain and gastroesophageal reflux, may be sufficient to achieve better sleep quality. In more specific situations, evaluation by a sleep specialist may be necessary[21].

CONCLUSION

This study showed that poor sleep quality is a very common finding among SSc patients. It also demonstrated that poor sleep quality negatively affects both the quality of life and the degree of disability of individuals with scleroderma. Therefore, we emphasize this as an important aspect to be evaluated and treated in patients with SSc.

AUTHORS' CONTRIBUTIONS

GSS: Validation, Visualization, Writing – original draft. **MFB:** Investigation, Validation, Visualization, Writing – original draft. **DNM:** Investigation, Validation, Visualization, Writing – original draft. **AST:** Formal Analysis, Validation, Visualization, Writing – original draft. **RSGG:** Investigation, Validation, Visualization, Writing – original draft. **ALBPD:** Validation, Visualization, Writing – review & editing. **ATD:** Conceptualization, Formal Analysis, Validation, Visualization, Writing – review & editing.

REFERENCES

1. Almeida C, Almeida I, Vasconcelos C. Quality of life in systemic sclerosis. Autoimmun Rev. 2015;14(12):1087-96. https://doi.org/10.1016/j.autrev.2015.07.012

2. Bassel M, Hudson M, Taillefer SS, Schieir O, Baron M, Thombs BD. Frequency and impact of symptoms experienced by patients with systemic sclerosis: results from a Canadian National Survey. Rheumatology (Oxford). 2011;50(4):762-7. https://doi.org/10.1093/rheumatology/keq310

3. Shah AA, Wigley FM. My approach to the treatment of scleroderma. Mayo Clin Proc. 2013;88(4):377-93. https://doi.org/10.1016/j.mayocp.2013.01.018
4. Figueiredo FP, Aires GD, Nisihara R, Skare TL. Sleep disturbance in scleroderma. J Clin Rheumatol. 2021;27(6S):S242-5. https://doi.org/10.1097/RHU.0000000000001437
5. Milette K, Razykov I, Pope J, Hudson M, Motivala SJ, Baron M, et al. Clinical correlates of sleep problems in systemic sclerosis: the prominent role of pain. Rheumatology (Oxford). 2011;50(5):921-5. https://doi.org/10.1093/rheumatology/keq411
6. Sariyildiz MA, Batmaz I, Budulgan M, Bozkurt M, Yazmalar L, Inanir A, et al. Sleep quality in patients with systemic sclerosis: relationship between the clinical variables, depressive symptoms, functional status, and the quality of life. Rheumatol Int. 2013;33(8):1973-9. https://doi.org/10.1007/s00296-013-2680-9
7. Prado GF, Allen RP, Trevisani VM, Toscano VG, Earley CJ. Sleep disruption in systemic sclerosis (scleroderma) patients: clinical and polysomnographic findings. Sleep Med. 2002;3(4):341-5. https://doi.org/10.1016/s1389-9457(02)00013-8
8. Çakır Edis E, Mutlucan Eraslan R, Hatipoğlu O. Polysomnography findings and risk factors for sleep-disordered breathing in patients with systemic sclerosis. Arch Rheumatol. 2021;36(3):360-5. https://doi.org/10.46497/ArchRheumatol.2021.8415
9. Masi AT. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American rheumatism association diagnostic and therapeutic criteria committee. Arthritis Rheum. 1980;23(5):581-90. https://doi.org/10.1002/art.1780230510
10. Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2013;72(11):1747-55. https://doi.org/10.1136/annrheumdis-2013-204424
11. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213. https://doi.org/10.1016/0165-1781(89)90047-4
12. Bertolazi AN, Fagondes SC, Hoff LS, Dartora EG, Miozzo IC, Barba ME, et al. Validation of the Brazilian Portuguese version of the Pittsburgh Sleep Quality Index. Sleep Med. 2011;12(1):70-5. https://doi.org/10.1016/j.sleep.2010.04.020
13. Bertolazi AN, Fagondes SC, Hoff LS, Pedro VD, Menna Barreto SS, Johns MW. Portuguese-language version of the Epworth Sleepiness Scale: validation for use in Brazil. J Bras Pneumol. 2009;35(9):877-83. https://doi.org/10.1590/s1806-37132009000900009
14. Andrade TL, Camelier AA, Rosa FW, Santos MP, Jezler S, Pereira e Silva JL. Applicability of the 12-item short-form health survey in patients with progressive systemic sclerosis. J Bras Pneumol. 2007;33(4):414-22. https://doi.org/10.1590/s1806-37132007000400010
15. Ware J, Kosinski M, Keller SD. A 12-item short-form health survey: construction of scales and preliminary tests of reliability and validity. Med Care. 1996;34(3):220-33. https://doi.org/10.1097/00005650-199603000-00003
16. Orlandi AC, Cardoso FP, Santos LM, Cruz VG, Jones A, Kyser C, et al. Translation and cross-cultural adaptation of the scleroderma health assessment questionnaire to Brazilian Portuguese. Sao Paulo Med J. 2014;132(3):163-9. https://doi.org/10.1590/1516-3180.2014.1323621
17. Cohen J. A power primer. Psychol Bull. 1992;112(1):155-9. https://doi.org/10.1037//0033-2909.112.1.155
18. Hsiao YH, Chen YT, Tseng CM, Wu LA, Lin WC, Su VY, et al. Sleep disorders and increased risk of autoimmune diseases in individuals without sleep apnea. Sleep. 2015;38(4):581-6. https://doi.org/10.5665/sleep.4574
19. Garbarino S, Lanteri P, Bragazzi NL, Magnavita N, Scoditti E. Role of sleep deprivation in immune-related disease risk and outcomes. Commun Biol. 2021;4(1):1304. https://doi.org/10.1038/s42003-021-02825-4
20. Bagnato GL, Fiorenza A, Cordova F, Roberts WN, Moore C, Greco D, et al. Clinical, autoimmune, and psychiatric parameters correlate with sleep disturbance in patients with systemic sclerosis and rheumatoid arthritis. Clin Exp Rheumatol. 2016;34 Suppl 100(5):49-55. PMID: 27192425
21. Pérez-Carbonell L, Mignot E, Leschziner G, Dauvilliers Y. Understanding and approaching excessive daytime sleepiness. Lancet. 2022;400(10357):1033-46. https://doi.org/10.1016/S0140-6736(22)01018-2
22. Taylor-Gjevre RM, Gjevre JA, Nair B, Skomro R, Lim HJ. Hypersomnolence and sleep disorders in a rheumatic disease patient population. J Clin Rheumatol. 2010;16(6):255-61. https://doi.org/10.1097/RHU.0b013e3181eeb2df

ORIGINAL ARTICLE

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Molecular monitoring by *CDKN2A/p16*^{INK4A} and *RB1* gene methylation in breast cancer

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SUMMARY

OBJECTIVE: This prospective study aimed to provide a comprehensive analysis of the methylation status of two pivotal genes, *CDKN2A/p16*^{INK4A} (cyclin-dependent kinase inhibitor 2A) and *RB1* (retinoblastoma transcriptional corepressor 1), in breast cancer patients.

METHODS: Samples were obtained from 15 women diagnosed with breast cancer and who underwent a total mastectomy. DNA was extracted from the tumor, non-tumor tissue, and peripheral blood (circulating cell-free DNA). The methylation pattern of cell-free DNA extracted from blood collected on the day of mastectomy was compared with the methylation pattern of cell-free DNA from blood collected 1 year post-surgery. The methylation analysis was carried out by sodium bisulfite conversion and polymerase chain reaction, followed by electrophoresis.

RESULTS: Methylation of *CDKN2A/p16*^{INK4A} was identified in 13 tumor samples and 12 non-tumor tissue samples. Two patients exhibited *CDKN2A/p16*^{INK4A} methylation in the cell-free DNA of the first blood collection, while another showed methylation only in the cell-free DNA of the subsequent blood collection. Regarding *RB1*, 11 tumors and 8 non-tumor tissue samples presented methylation of the gene.

CONCLUSION: This study presents a novel approach for monitoring breast cancer patients through the analysis of cell-free DNA methylation. This analysis can detect changes in methylation patterns before any visible sign of cancer appears in breast tissue and could help predict the recurrence of malignant breast tumors.

KEYWORDS: Retinoblastoma. Breast neoplasms. DNA methylation.

INTRODUCTION

Activation of oncogenes and inactivation of tumor suppressor genes are genetic oscillations related to cancer development[1,2]. DNA methylation, which is an epigenetic mechanism, can influence the expression of tumor suppressors and genes related to uncontrolled cell proliferation. The mechanism can inactivate gene expression and consequently prevent protein synthesis.

The *CDKN2A/p16*^{INK4A} (cyclin-dependent kinase inhibitor 2A), located at chromosome 9 (9p21.3), is a tumor suppressor gene that encodes *p16*^{INK4A}, a protein involved in cell cycle regulation[1,3,4]. The *p16*^{INK4A} participates in the G1-to-S-phase checkpoint and may interrupt the cell cycle in response to various stressors, leading to the inhibition of cell proliferation[1,5]. Additionally, p16INK4A promotes apoptosis of tumor cells and can increase sensitivity to chemotherapy in breast cancer[3].

An investigation found a positive correlation between hypermethylation of *CDKN2A/p16*^{INK4A} and breast cancer progression and also verified that *CDKN2A/p16*^{INK4A} hypermethylation impacts the tumor's grade and stage[3].

The *RB1* (retinoblastoma transcriptional corepressor 1) is also a tumor suppressor gene and is located on chromosome 13 (13q14.2). *RB1* encodes the retinoblastoma protein (Rb), and alterations in its tumor suppressor pathway can be considered a potential risk for the development of breast cancer[6-8]. *RB1* can be inactivated by several mechanisms, including alterations in phosphorylation, viral oncoproteins, and promoter hypermethylation[7].

The analysis of methylation may be performed in circulating cell-free DNA (cfDNA) using liquid biopsy, a promising method for early detection and monitoring of breast cancer[9]. Liquid biopsy is non-invasive and easy to perform, and analysis

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of cfDNA has a hopeful potential for the study of cancer biomarkers, overcoming difficulties in obtaining and repeating biopsies of metastatic tissues[9-12].

This study analyzed the methylation statuses of *CDKN2A*/*p16*^{INK4A} and *RB1* genes in the tumor, non-tumor tissue, and cfDNA of breast cancer patients. The methylation was monitored in cfDNA at two different time points: the day of mastectomy and 1 year post-surgery.

METHODS

Study design

This prospective study included 15 women aged between 44 and 78 years (mean age 56.7±9.6 years) diagnosed with breast carcinoma. The patients were treated at the "Instituto de Ginecologia" of "Universidade Federal do Rio de Janeiro," Brazil, between October 2018 and July 2021. All patients underwent a total mastectomy of one breast. The patients were interviewed and invited to take part in the study. After receiving all the necessary information, those who agreed to participate signed the consent form. The number of patients enrolled is smaller than the ideal due to limitations in the availability of women operated on during the study period.

Data collection and ethical aspects

The recruitment occurred between October 2018 and July 2021. The medical records served as the basis for obtaining clinical and demographic data. The institutional ethics committee approved the study protocol (certificate: CAAE nº 91406118.6.0000.5257 from August 29, 2018).

Material collection

After pre-mastectomy, samples of the tumor, non-tumor tissue, and 5 mL of peripheral blood were collected immediately for DNA analysis. Approximately 1 year after the surgery, patients were invited for a new blood collection, and a second cfDNA analysis was performed.

Extraction of DNA from the tumor, surrounding tissue, and blood serum

The DNA extraction from tumor and non-tumor tissues was performed by the phenol:chloroform method[13], using the Ultra Pure™ Phenol:Chloroform:Isoamyl Alcohol, from Invitrogen™, Cat. No. 15593-031. Quick-gDNA™ MiniPrep Kit (Zymo Research) Cat. No. D3024 was used for cfDNA extraction from blood serum according to the manufacturer's protocol.

Methylation mechanism

Sodium bisulfite conversion and MSP (methylation-specific polymerase chain reaction (PCR)) were adopted to analyze DNA methylation using the EZ DNA Methylation-Gold TM Kit, Cat. No: D5005, Zymo Research, according to the manufacturer's protocol.

Polymerase chain reaction

The DNA integrity was confirmed through amplification of exon 5 of the *p53* gene as previously described[14]. For the *CDKN2*/*p16*^{INK4A} amplification, two pairs of primers were used as follows: *CDKN2A*/*p16*^{INK4A}-U (unmethylated) forward, 5′-TATTAGAGGGTGGGGTGGATTGT-3′ and *CDKN2A*/*p16*^{INK4A}-U reverse, 5′-CAACCCCAAACCACAACCATAA-3′ producing a fragment of 151 base pairs and *CDKN2A*/*p16*^{INK4A}-M (methylated) forward, 5′-TTATTAGAGGGTGGGGCGGATCGC-3′ and *CDKN2A*/*p16*^{INK4A}-M reverse, 5′-ACCCCGAACCGCGACCGTAA-3′ producing a fragment of 150 base pairs[15]. The polymerase used for the MSP was the GoTaq G2 Hot Start Green Master Mix, Cat. No: M7422, Promega. PCR conditions were as follows: initial denaturation at 96°C for 7 min, followed by 35 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The final extension was performed at 72°C for 7 min. *RB1*-U (unmethylated) forward, 5′-GGGAGTTTTGTGGATGTGAT-3′ and *RB1*-U reverse, 5′-ACATCAAAACACACCCCA-3′ producing a fragment of 172 base pairs and *RB1*-M (methylated) forward, 5′-GGGAGTTTCGCGGACGTGAC-3′ and *RB1*-M reverse, 5′-ACGTCGAAACACGCCCCG-3′ producing a fragment of 172 base pairs[16]. The polymerase used for the MSP was the GoTaq G2 Hot Start Green Master Mix, Cat. No: M7422, Promega. PCR conditions were as follows: initial denaturation at 96°C for 7 min, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The final extension was performed at 72°C for 5 min.

Gel electrophoresis and staining

After amplification, PCR products were separated on polyacrylamide gels at a concentration of 10%. Each electrophoretic run had the addition of a negative control and a DNA marker. Gels were stained by the silver nitrate method, allowing visualization of the DNA bands as previously described[17]. In short, DNA fixation with methanol and acetic acid occurred in the first step, followed by impregnation with silver nitrate in the next step and, finally, with sodium hydroxide (NaOH) and formaldehyde to reveal the DNA bands.

RESULTS

According to the data obtained from medical records, all patients had advanced disease (stage III or IV). Figure 1 shows the methylation statuses of *CDKN2A/p16*^{INK4A} and *RB1* in the tumor, non-tumor tissue, and cfDNA (from the first and second blood collections). *CDKN2A/p16*^{INK4A} methylation was detected in 13 tumors and 12 non-tumor tissue samples. Only two patients (2 and 3) presented *CDKN2A/p16*^{INK4A} methylation in the cfDNA from the blood of the first collection, and only patient 14 presented it in the cfDNA from the blood of the second collection. Furthermore, *RB1* methylation was detected in 11 tumors and 8 non-tumor tissues. Patient 8 showed a weak band of *RB1* methylation in the cfDNA from the blood of the first collection and a strong band of methylation in the cfDNA from the blood of the second collection. Moreover, patient 14 showed a weak band of methylation only in the cfDNA from the second collection.

Tables 1 and 2 present the methylation panels of *CDKN2A/p16*^{INK4A} and *RB1*, respectively.

DISCUSSION

This study investigated the methylation statuses of *CDKN2A/p16*^{INK4A} and *RB1* genes in breast cancer patients. DNA from the tumor, non-tumor tissue, and blood serum (cfDNA) was analyzed. The analyses showed that the vast majority of tumor samples had methylation of both *CDKN2A/p16*^{INK4A} (13/15) and *RB1* (11/15). This result was expected as methylation of these genes is related to breast cancer development, as pointed out by Cheng et al.[3] and Yao[7]. Regarding the non-tumor tissue, the majority of samples (although slightly less than in tumors) also showed methylation of *CDKN2A/p16*^{INK4A} (12/15) and *RB1* (8/15). This confirms that non-tumor tissue, although apparently free of malignancy, is part of the tumor microenvironment. These changes were not detected by histopathological examination, but

Table 1. Methylation panel of *CDKN2A/p16*^{INK4A} gene in the tumor, non-tumor tissue, and cell-free DNA of breast cancer patients.

Patient number	Tumor	Non-tumor tissue	cfDNA first collection	cfDNA second collection	NC
1	U	M	U	Death	Yes
2	M	M	M	Death	No
3	M	M	M	U	Yes
4	M	M	U	U	Yes
5	M	M	U	U	Yes
6	M	M	U	U	Yes
7	M	M	U	NR	Yes
8	M	U	U	U	Yes
9	M	M	U	NR	No
10	M	M	U	U	No
11	M	U	U	NR	Yes
12	M	M	U	U	Yes
13	U	U	U	U	Yes
14	M	M	U	M	No
15	M	M	U	U	Yes

CDKN2A/p16^{INK4A}: cylclin-dependent kinase inhibitor 2A; cfDNA: circulating cell-free DNA; M: methylated; U: unmethylated; NR: no return to second blood collection for reason other than death; NC: neochemotherapy.

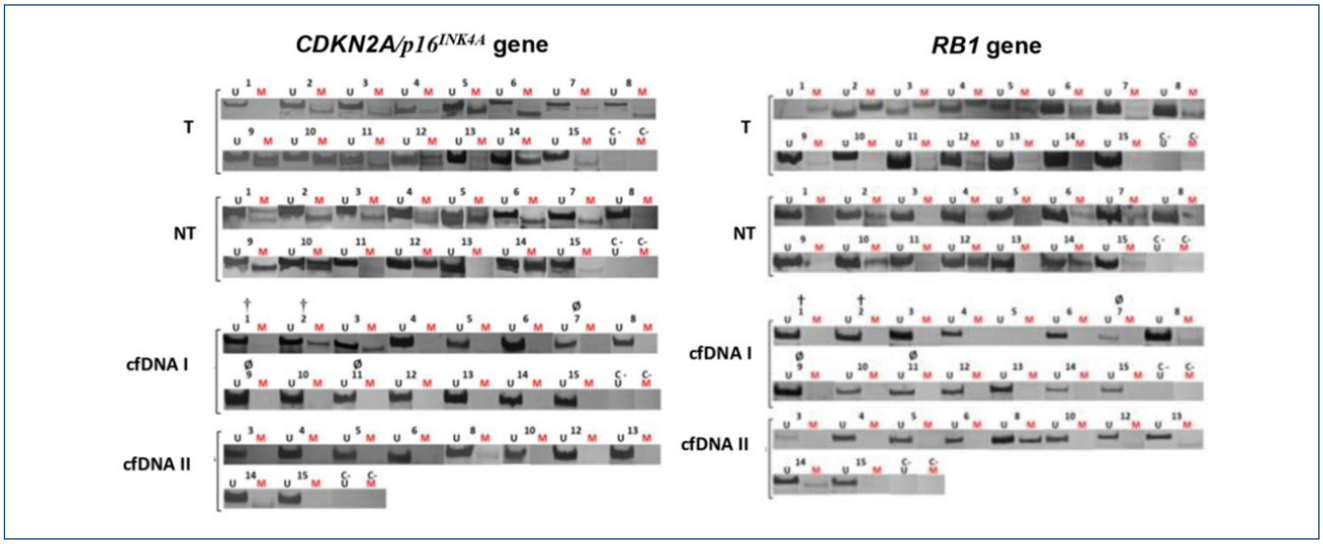

Figure 1. Methylation of *CDKN2A/p16*^{INK4A} and *RB1* genes in breast cancer patients. *CDKN2A/p16*^{INK4A}: cyclin-dependent kinase inhibitor 2A; *RB1*: retinoblastoma transcriptional corepressor 1; T: tumor; NT: non-tumor tissue; cfDNA I: circulating cell-free DNA of the first blood collection; cfDNA II: circulating cell-free DNA of the second blood collection; M: methylated DNA; U: unmethylated DNA; C−: negative control; †: death; Ø: no return to the second blood collection for other reason than death. Numbers correspond to patients.

Table 2. Methylation panel of *RB1* gene in the tumor, non-tumor tissue, and cell-free DNA of breast cancer patients.

Patient number	Tumor	Non-tumor tissue	cfDNA first collection	cfDNA second collection	NC
1	M	U	U	Death	Yes
2	M	M	U	Death	No
3	M	U	U	U	Yes
4	M	U	U	U	Yes
5	M	U	U	U	Yes
6	M	M	U	U	Yes
7	M	M	U	NR	Yes
8	M	M	M	M	Yes
9	M	U	U	NR	No
10	U	M	U	U	No
11	M	U	U	NR	Yes
12	M	M	U	U	Yes
13	U	U	U	U	Yes
14	U	M	U	M	No
15	U	M	U	U	Yes

RB1: retinoblastoma transcriptional corepressor 1; cfDNA: circulating free DNA; M: methylated; U: unmethylated; NR: no return to second blood collection for reason other than death; NC: neochemotherapy.

the molecular analysis of methylation indicated that the tissue considered tumor-free was already in the process of molecular modification with potential for future cancerization.

Only a small number of cfDNA samples showed methylation of *CDKN2A*/*p16*^{INK4A} (2 and 3 in the first collection, and none in the second collection) and *RB1* (8 in the first collection and 8 and 14 in the second collection). This must be related to the chemotherapy treatment given to patients before mastectomy. As highlighted by Kujala et al.[11], chemotherapy reduces the concentration of tumor DNA in the blood.

Patient 14 had no methylation in either *CDKN2A*/*p16*^{INK4A} or *RB1* in the cfDNA of the first blood collection. In contrast, both genes were methylated in the cfDNA of the second blood collection. In addition, patient 8 showed a weak band of *RB1* methylation in the cfDNA from the blood of the first collection and a strong band in the cfDNA of the second collection. The fact that the bands corresponding to methylation are stronger in the cfDNA from the second blood collection suggests a possible recurrence of the disease. Consequently, an increased concentration of tumoral DNA released into the bloodstream is observed, which is visualized as stronger DNA bands by gel electrophoresis.

CONCLUSION

This study presented a novel approach for monitoring breast cancer patients through the assessment of methylation in cfDNA. Once liquid biopsy is non-invasive and easy to perform, the long-term follow-up of patients is facilitated. The cfDNA analysis proposed here can detect changes in methylation patterns before any visible sign of disease appears in breast tissue. This suggests that the study of cancer-related gene methylation in cfDNA could help predict the recurrence of malignant breast tumors.

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AUTHORS' CONTRIBUTIONS

LFQ: Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **MSMS:** Conceptualization, Data curation, Methodology, Writing – original draft, Writing – review & editing. **FCR:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **SLBR:** Methodology, Writing – review & editing. **HSPS:** Funding acquisition, Writing – review & editing. **MGCC:** Conceptualization, Formal Analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

REFERENCES

1. Aftab A, Shahzad S, Hussain HMJ, Khan R, Irum S, Tabassum S. *CDKN2A/P16INK4A* variants association with breast cancer and their in-silico analysis. Breast Cancer. 2019;26(1):11-28. https://doi.org/10.1007/s12282-018-0894-0
2. Kontomanolis EN, Koutras A, Syllaios A, Schizas D, Mastoraki A, Garmpis N, et al. Role of oncogenes and tumor-suppressor genes in carcinogenesis: a review. Anticancer Res. 2020;40(11):6009-15. https://doi.org/10.21873/anticanres.14622
3. Cheng T, Wu Y, Liu Z, Yu Y, Sun S, Guo M, et al. CDKN2A-mediated molecular subtypes characterize the hallmarks of tumor microenvironment and guide precision medicine in triple-negative breast cancer. Front Immunol. 2022;13:970950. https://doi.org/10.3389/fimmu.2022.970950
4. Andersson N, Saba KH, Magnusson L, Nilsson J, Karlsson J, Nord KH, et al. Inactivation of *RB1*, *CDKN2A*, and *TP53* have distinct effects on genomic stability at side-by-side comparison in karyotypically normal cells. Genes Chromosomes Cancer. 2023;62(2):93-100. https://doi.org/10.1002/gcc.23096

5. Inoue K, Fry EA. Aberrant expression of p16INK4a in human cancers – a new biomarker? Cancer Rep Rev. 2018;2(2). https://doi.org/10.15761/CRR.1000145
6. Linn P, Kohno S, Sheng J, Kulathunga N, Yu H, Zhang Z, et al. Targeting RB1 loss in cancers. Cancers (Basel). 2021;13(15):3737. https://doi.org/10.3390/cancers13153737
7. Yao Y, Gu X, Xu X, Ge S, Jia R. Novel insights into RB1 mutation. Cancer Lett. 2022;547:215870. https://doi.org/10.1016/j.canlet.2022.215870
8. Abbasi-Kolli M, Shahbazi S, Geranpayeh L. Down-regulation of RB1 and miR-132 in ductal carcinoma of the breast. Int J Mol Epidemiol Genet. 2023;14(1):1-10. PMID: 37214588.
9. Vietri MT, D'Elia G, Benincasa G, Ferraro G, Caliendo G, Nicoletti GF, et al. DNA methylation and breast cancer: a way forward (review). Int J Oncol. 2021;59(5):98. https://doi.org/10.3892/ijo.2021.5278
10. Sher G, Salman NA, Khan AQ, Prabhu KS, Raza A, Kulinski M, et al. Epigenetic and breast cancer therapy: promising diagnostic and therapeutic applications. Semin Cancer Biol. 2022;83:152-65. https://doi.org/10.1016/j.semcancer.2020.08.009
11. Kujala J, Hartikainen JM, Tengström M, Sironen R, Auvinen P, Kosma VM, et al. Circulating cell-free DNA reflects the clonal evolution of breast cancer tumors. Cancers (Basel). 2022;14(5):1332. https://doi.org/10.3390/cancers14051332
12. Main SC, Cescon DW, Bratman SV. Liquid biopsies to predict CDK4/6 inhibitor efficacy and resistance in breast cancer. Cancer Drug Resist. 2022;5(3):727-48. https://doi.org/10.20517/cdr.2022.37
13. Maccormick TM, Carvalho CES, Bravo Neto GP, Carvalho MDGDC. Comparative analysis of glutathione transferase genetic polymorphism, *Helicobacter pylori* and Epstein-Barr virus between the tumor area and the proximal and distal resection margins of gastric cancer. Rev Col Bras Cir. 2019;46(1):e2068. https://doi.org/10.1590/0100-6991e-20192068
14. Pestaner JP, Bibbo M, Bobroski L, Seshamma T, Bagasra O. Potential of the in situ polymerase chain reaction in diagnostic cytology. Acta Cytol. 1994;38(5):676-80. PMID: 8091896
15. Rosas SL, Koch W, Costa Carvalho MG, Wu L, Califano J, Westra W, et al. Promoter hypermethylation patterns of p16, O6-methylguanine-DNA-methyltransferase, and death-associated protein kinase in tumors and saliva of head and neck cancer patients. Cancer Res. 2001;61(3):939-42. PMID: 11221887.
16. Sippl C, Urbschat S, Kim YJ, Senger S, Oertel J, Ketter R. Promoter methylation of RB1, P15, P16, and MGMT and their impact on the clinical course of pilocytic astrocytomas. Oncol Lett. 2018;15(2):1600-6. https://doi.org/10.3892/ol.2017.7490
17. Silva MM, Fonseca CO, Moura-Neto R, Carvalho JF, Quirico-Santos T, Carvalho MG. Influence of GSTM1 and GSTT1 polymorphisms on the survival rate of patients with malignant glioma under perillyl alcohol-based therapy. Genet Mol Res. 2013;12(2):1621-30. https://doi.org/10.4238/2013.May.14.2

ORIGINAL ARTICLE

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Association between uterine leiomyoma and fragmented QRS waves: a prospective case-control study

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SUMMARY

OBJECTIVE: The aim of this study was to evaluate the relationship between uterine leiomyoma and fragmented QRS, a non-invasive indicator of cardiovascular risk and myocardial ischemia, in women with uterine leiomyoma.

METHODS: In this prospective case-control study, a total of 47 patients diagnosed with uterine leiomyoma (case group) and 47 healthy individuals without uterine leiomyoma (control group) who had undergone bilateral tubal ligation surgery were included. Various demographic, clinical, and laboratory parameters and the presence of fragmented QRS were recorded.

RESULTS: The leiomyoma group showed significantly higher body mass index (27.46±2.18 vs. 25.9±2.87 kg/m^2, p=0.005) and waist circumference (91.34±9.30 vs. 84.97±9.3 cm, p=0.001) compared with the control group. Uterine volumes were also significantly higher in the leiomyoma group (235.75±323.48 vs. 53.24±12.81 mm^3, p<0.001). The presence of fragmented QRS was detected in 18.1% of the patients. Multiple regression analysis identified age, fasting blood glucose value, and the presence of fragmented QRS as independent risk factors for the presence of leiomyoma.

CONCLUSION: This study provides valuable insights into the relationship between uterine leiomyoma and fragmented QRS. The presence of fragmented QRS was identified as an independent risk factor for the presence of leiomyoma. Further research is needed to better understand the underlying mechanisms connecting uterine leiomyoma and cardiovascular health.

KEYWORDS: Cardiovascular risk. Fibroids. Electrocardiography. Leiomyoma.

INTRODUCTION

Uterine leiomyomas, which are also known as fibroids or myomas, are the most common benign monoclonal tumors of the uterus, originating from uterine smooth muscle cells and fibroblasts[1]. They affect up to 80% of women by the age of 50 years and are associated with significant morbidity, including heavy menstrual bleeding, pelvic pain, infertility, and pregnancy complications[2].

The pathophysiology of uterine leiomyomas is not fully understood, but there is evidence that genetic, epigenetic, hormonal, environmental, proinflammatory, angiogenic, and growth factors play a role[3-5]. Recent studies have shown that cardiometabolic risk factors contribute to the pathogenesis of uterine leiomyomas[6-10].

Fragmented QRS (fQRS) is defined as the presence of an extra R wave (R') or notching of the R or S wave or multiple R' waves in two contiguous leads corresponding to a main coronary artery. fQRS is an ECG finding resulting from the heterogeneous activation of the ventricles due to myocardial scar. Studies have shown that fQRS is an indicator of poor prognosis in patients with acute myocardial infarction and a risk marker for arrhythmic events in cardiomyopathies[11].

Investigating the relationship between fQRS and uterine leiomyomas is important[12]. By identifying the presence of fQRS in women with uterine leiomyomas, clinicians could potentially stratify patients according to their risk of cardiovascular events and provide appropriate treatment and follow-up. This could improve patient outcomes and quality of life.

To the best of our knowledge, there is no study investigating the relationship between uterine leiomyoma and fQRS in the literature. Common mechanisms may be shared in the development of uterine leiomyomas and atherosclerosis. Therefore, the aim of this study was to evaluate the relationship between uterine leiomyoma and the non-invasive and easily accessible indicator of cardiovascular risk and myocardial ischemia, fQRS, in women with uterine leiomyoma.

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METHODS

In this prospective case-control study, there were 47 patients diagnosed with uterine leiomyoma in the case group and 47 healthy individuals in the control group who had undergone surgery at a tertiary referral hospital between August 2021 and March 2022 for bilateral tubal ligation, in whom uterine leiomyoma was not detected on ultrasound. The necessary sample size was calculated using the G*Power 3.1.9.2 package program, based on the information obtained from the study by Zimmerman et al.[13] with a power of 95%. Accordingly, the sufficient sample size was found to be 94 (n=47, 2 groups) when the error margin was 0.05 (alpha), the power of the study was 95% (power), and the effect size was 0.69. The study protocol was approved by the local ethics committee of the hospital.

Informed verbal consent was obtained from all patients who participated in the study by providing them with information about the research. Interviews with the patient and control groups were conducted face-to-face. Obstetric and gynecological history, including age, gravidity, parity, vaginal and cesarean delivery numbers, last menstrual period dates, menstrual patterns, contraceptive methods, previous surgeries, medication use, and medical background, was recorded. Patients were subjected to pelvic and physical examinations, and their heights and weights were measured to calculate their body mass index (BMI). Routine venous blood samples were taken from the antecubital area after a 10-h fast from preoperative patients. Fasting blood glucose, hemoglobin A1c (HbA1c), insulin, complete blood count, lipid profile, and biochemical parameters were analyzed from these samples. The "homeostasis model assessment-estimated insulin resistance (HOMA-IR)" formula was used to calculate insulin resistance: HOMA-IR=Fasting insulin Uiu-mL×FPG (mg/dL)/405. Preoperative ultrasound examinations were performed by the responsible researcher. Uterine leiomyomas were identified according to FIGO 2018, and their volumes were calculated using the ellipsoid formula (length×width×depth×0.52) by measuring the width, length, and depth of uterine leiomyomas and the uterus. The recorded data were entered into the patient form.

The ECONET Cardio M Plus ECG recording device was used to obtain a standard 12-lead surface ECG from all patients, and the ECGs were analyzed by an independent cardiologist who was blinded to the study. FQRS was defined as the presence of an RSR pattern and/or notching in the R and S waves in two adjacent leads corresponding to a major coronary artery region.

Statistical analysis

The data were statistically analyzed using the SPSS (Statistical Package for the Social Science) Version 26 (SPSS, Chicago, IL, USA) program. The normal distribution of the data was evaluated by considering histogram and skewness-kurtosis values. Pearson's chi-square test and Fisher's exact test were used to compare the categorical variables between the groups. Categorical data were expressed as n (number) and percentages (%). Independent-samples t-test was used to compare the ordinal data of the groups, and ordinal data were presented as mean±standard deviation. Pearson correlation analysis was used to examine the correlations between variables. The relation between variables was also examined using univariate and multivariate regression analyses. The data were evaluated at a 95% confidence interval, and significance was accepted at $p<0.05$.

RESULTS

The demographic and clinical characteristics of all patients are summarized in Table 1. The leiomyoma group had a significantly higher BMI (27.46±2.18 vs. 25.9±2.87 kg/m^2, p=0.005) and waist circumference (91.34±9.30 vs. 84.97±9.3 cm, p=0.001) compared with the control group. The uterine volumes of the leiomyoma group were statistically higher than the control group (235.75±323.48 vs. 53.24±12.81 mm^3, p<0.001) (Table 1). The comparison of laboratory and ECG parameters of the two groups is shown in Table 1.

When the leiomyoma characteristics of the study group were examined, a single leiomyoma was observed in 51.1% of the group, while the rate of those with more than three leiomyomas was 17%. When leiomyomas were evaluated according to the FIGO classification in our study, type 4 (27.6%) and type 5 (23.4%) myomas were more commonly observed, while type 1 (4.2%), type 2 (2.2%), and type 7 (2.2%) were less detected.

When the leiomyoma group was divided into two groups according to the presence of fQRS, there was no significant difference in terms of age, BMI, waist circumference, endometrial thickness, uterine and leiomyoma volume, and between the fQRS positive (n=14) and fQRS negative (n=33) groups (p>0.05) (Table 2).

When we look at the correlation between the Leiomyoma properties and some variables, a negative correlation was observed between age and leiomyoma volume (r=-0.431, p=0.002). Positive correlations were observed between age and LDL-C (r=0.215, p=0.038), and age and the number of leiomyomas (r=0.359, p=0.013). Additionally, a positive correlation has been found between triglyceride levels and the number of leiomyomas (r=0.297, p=0.042).

When the relationship between variables and leiomyoma presence was evaluated by multiple regression analysis, age (odds ratio=0.688, p<0.001), fasting blood glucose value

Table 1. Demographic, laboratory, and electrocardiographic parameters of the patients and comparison of groups.

	Total patients (n=94)	Leiomyoma group (n=47)	Control group (n=47)	p-value
Age (years)	38.86±4.69	41.29±3.56	36.42±4.43	**<0.001**
Gravidity	3 (1–7)	3 (1–7)	4 (1–7)	0.234
Parity	3 (0–6)	2 (0–5)	3 (1–6)	0.126
BMI (kg/m^2)	26.7±2.65	27.46±2.18	25.9±2.87	**0.005**
Abdominal circumference (cm)	88.15±9.81	91.34±9.30	84.97±9.35	**0.001**
Endometrial thickness (mm)	5.81±3.67	5.88±4.46	5.75±2.69	0.867
Uterine volume (mm^3)	144.49±245.47	235.75±323.48	53.24±12.81	**<0.001**
fQRS positivity (n, %)	17 (18.1)	14 (29.8)	3 (6.4)	**0.006**
WBC (mm^3)	8148.01±2812.26	8551.48±3149.11	7744.53±2395.83	0.165
Hemoglobin (g/dL)	12.08±1.61	11.45±1.47	12.7±1.51	**<0.001**
Hematocrit (%)	36.8±4.47	35.15±4.25	38.45±4.1	**<0.001**
Thrombocyte count (1/μL)	279.550±73.515	282.82±81.25	279.27±65.58	0.668
Glucose (mg/dL)	96.93±17.39	104.34±20.19	89.53±9.60	**<0.001**
HgA1c (%)	5.3±0.44	5.29±0.47	5.30±0.42	0.869
Insuline (mIU/mL)	12.60±1.55	13.60±1.94	11.61±10.62	0.538
ALT (U/L)	16.05±7.41	15.55±8.52	16.5±6.16	0.516
AST (U/L)	18.67±3.76	18.19±3.69	19.14±3.82	0.220
BUN (mg/dL)	9.71±3.014	8.66±2.68	10.76±2.98	**0.001**
Creatinine (mg/dL)	0.60±0.090	0.59±0.10	0.60±0.069	0.359
HDL (mg/dL)	51.17±13.03	48.82±13.36	53.51±12.39	0.082
LDL (mg/dL)	107.79±33.26	104.61±32.37	110.97±34.18	0.357
TG (mg/dL)	110.75±56.37	116.34±50.66	105.17±61.61	0.340
Cholesterol (mg/dL)	179.50±40.45	176.32±36.76	182.67±44.01	0.453
VLDL (mg/dL)	21.32±11.13	21.61±9.90	21.04±12.35	0.804
FSH (mIU/mL)	12.22±4.38	17.63±6.15	6.82±6.08	0.237
E2 (pg/mL)	74.072±16.65	90.51±45.77	77.63±23.42	0.141
LH (mIU/mL)	8.98±0.908	8.47±1.003	9.48±8.09	0.594
Heart rate (beat/min)	77.106±9.98	78.74±10.26	75.46±9.52	0.112
QT interval (ms)	373.17±24.14	373.12±28.78	373.21±18.72	0.986
QTc interval (ms)	423.117±27.31	428.06±31.67	418.17±21.34	0.080
HOMA-IR	3.24±3.34	3.85±6.9	2.63±2.61	0.257

ALT: alanine transaminase; AST: aspartate aminotransferase; BMI: body mass index; BUN: blood urea nitrogen; E2: estradiol; FSH: follicle-stimulating hormone; fQRS: fragmented QRS; HDL: high-density lipoprotein; HgA1c: hemoglobin A1c; LDL: low-density lipoprotein; LH: luteinizing hormone. Statistically significant p-values are indicated in bold.

Table 2. Comparison of demographic characteristics of groups according to the presence of fQRS in the myoma group.

	fQRS (-) (n=33)	fQRS (+) (n=14)	p-value
Age (years)	41.03±3.77	41.92±3.04	0.436
Gravidity	2.6±1.29	3.5±1.45	**0.043**
Parity	2.06±0.78	2.85±1.16	**0.009**
BMI (kg/m^2)	27.2±2.33	27.8±1.79	0.427
Abdominal circumference (cm)	91.8±9.11	90.0±9.94	0.548
Endometrial thickness (mm)	5.77±4.51	6.14±4.52	0.798
Uterine volume (mm^3)	259.7±79.91	179.3±100.54	0.442
Leiomyoma volume (mm^3)	303.55±21.50	256.88±18.39	0.482

BMI: body mass index; fQRS: fragmented QRS. Statistically significant p-value are indicated in bold.

(odds ratio=0.893, p=0.003), and the presence of fQRS (odds ratio=11.350, p=0.032) were determined as independent risk factors. The relationship between variables and leiomyoma is presented in Table 3.

DISCUSSION

Among women in their reproductive years, uterine leiomyoma stands out as the most commonly encountered solid pelvic tumor. Utilizing two-dimensional saline contrast sonohysterography has demonstrated remarkable sensitivity in detecting endometrial polyps and submucosal uterine leiomyomas. This positions it as a potential primary diagnostic approach for investigating abnormal uterine bleeding in women[14]. According to the findings of Magalhaes et al., the levonorgestrel-releasing intrauterine system shows promise in managing abnormal uterine bleeding as well as uterine volume associated with leiomyomas and adenomyosis[15]. When addressing symptomatic patients, magnetic resonance-guided high-intensity focused ultrasound emerges as a safe and effective technique in treating uterine fibroids[16].

In this study, the relationship between uterine leiomyoma and the presence of fQRS on electrocardiography was investigated in reproductive-aged women hospitalized preoperatively to hospital. Thus, it was aimed to predict subclinical cardiovascular disease risk by comparing the electrocardiograms of women with and without uterine leiomyoma.

A study by Korkmaz et al., investigated the cardiovascular risk of women with leiomyomas, carotid intima-media thickness (CIMT), insulin resistance, and lipid profile were used. BMI was significantly higher in the study group than in the control group[17]. A study by Uimari et al., investigated the relationship between uterine leiomyomas and cardiovascular risk, and BMI was found similar between the study and control groups[18]. In our study, BMI was found to be statistically significantly higher in the leiomyoma group than in the control group[17,18].

Table 3. Multivariate logistic regression analysis of the variables for leiomyoma.

	Odds ratio (95%CI)	p-value
Age (years)	0.688 (0.569–0.833)	**<0.001**
BMI (kg/m^2)	0.813 (0.610–1.082)	0.156
Waist circumference (cm)	0.942 (0.862–1.029)	0.187
Glucose (mg/dL)	0.893 (0.829–0.963)	**0.003**
fQRS	11.350 (1.235–104.338)	**0.032**

BMI: body mass index; fQRS: fragmented QRS. Statistically significant p-values are indicated in bold.

A study by Tak et al., investigated the relationship between metabolic syndrome and premenopausal women, and the waist circumference was found to be 77 cm (72–83) in the myoma group and 76 cm (71–81) in the control group[19]. In our study, the waist circumference was significantly higher in the leiomyoma group (91.34±9.30 cm) than in the control group (84.97±9.35 cm). Uimari et al., showed that the risk of leiomyoma increased by 1 cm for each 1 cm increase in waist circumference (OR=1.02, 95%CI 1.00–1.04)[18].

Tak et al., have shown that women with uterine leiomyoma have a higher likelihood of being diabetic compared with non-myomatous women and women with three or more leiomyomas have significantly higher fasting plasma glucose than those with one leiomyoma[19]. In our study, consistent with the literature, fasting glucose was significantly higher in the leiomyoma group.

The relationship between uterine leiomyomas and hyperlipidemia has been investigated in the literature. Tak et al., found higher low-density lipoprotein cholesterol (LDL-C) and lower high-density lipoprotein cholesterol (HDL-C) levels among women with uterine leiomyoma. They also found a positive correlation between the number of myomas and triglyceride levels and a negative correlation between the number of myomas and HDL-C levels[19]. Uimari et al., showed that for each 1 mmol/L increase in LDL-C, triglycerides, and total cholesterol levels, there is an increase in the risk of uterine leiomyoma[18]. Takeda et al., were unable to demonstrate a significant relationship between hypertriglyceridemia and uterine leiomyomas[20].

Laughlin-Tommaso et al., aimed to investigate the risk of subclinical cardiovascular disease. Cardiovascular risk factors were found to be slightly more prevalent in women with myomas than in those without myomas. Hypertension was seen to be significantly higher in women with uterine leiomyoma, with an increasing difference at each follow-up. At baseline and 5 years of follow-up, there was no difference in the presence of coronary artery calcification (CAC) between women with and without myomas. After 10 years, the difference in the presence of CAC was significantly higher in women with myomas than in those without myomas (20.0 vs. 14.1%). At the end of the 5-year follow-up, the average CIMT was higher among women with uterine leiomyoma. The authors found that cardiovascular risk factors, especially BMI and hypertension, were higher in women with uterine leiomyomas than in those without myomas. All approximate measurements of subclinical disease, including CAC, mean CIMT, and mean LV mass, were higher in women with uterine leiomyomas. However, multivariate analyses did not show a relationship between leiomyomas and subclinical cardiovascular disease[21]. Aksoy et al., found a significant increase in CIMT in the myoma group compared with the control

group[22]. Haan et al., reported that women with leiomyoma have a worse cardiovascular disease risk profile, including higher blood pressure, higher fasting plasma cholesterol and glucose levels, and more asymptomatic organ damage[23].

Toraman et al., found fQRS to be a predictor of subclinical atherosclerosis in chronic kidney disease patients by looking at fQRS[24]. Fedulaev et al., found that the presence of fQRS in lateral derivations showed that it could be a non-invasive marker of severe coronary atherosclerosis[25]. Karabakan et al., found that fQRS could provide a comprehensive diagnostic approach for underlying cardiovascular diseases[26]. Aksu et al., found that fQRS was found to be associated with important cardiac electrical changes that could indicate an increased risk of atrial and ventricular arrhythmias[27].

One of the limitations of our study was the absence of advanced cardiac evaluation methods such as echocardiography (ECHO). Another important limitation was that long-term follow-up of the evaluated cardiac and hormonal parameters was not performed because all included patients were in the preoperative period. However, despite these limitations, our study was prospective, and the study and control groups were homogeneous. To the best of our knowledge, this is the first study of its kind in the literature, and gynecological and cardiological parameters were independently evaluated by different investigators. Furthermore, the parameters examined in the study were based on objective tests, which are significant strengths of our study.

CONCLUSION

It is shown that uterine leiomyomas and atherosclerosis development may share common mechanisms. In this study, we demonstrated a positive relationship between fQRS which is easily accessible and noninvasive diagnostic methods through ECG and uterine leiomyomas.

INFORMED CONSENT

Written informed consent was obtained from all participants for using data. This study was conducted in accordance with the Declaration of Helsinki.

ETHICS COMMITTEE APPROVAL

The study design was approved by the institutional research ethics committee (Approval number: 31.03.2022-2022/40).

AUTHORS' CONTRIBUTIONS

TKT: Conceptualization, Data curation, Investigation, Writing – review & editing. **NC:** Investigation, Methodology. **BK:** Conceptualization, Data curation, Visualization, Writing – original draft, Writing – review & editing. **CK:** Methodology, Visualization, Writing – original draft, Writing – review & editing. **VK:** Conceptualization, Investigation, Methodology, Supervision.

REFERENCES

1. Donnez J, Dolmans MM. Uterine fibroid management: from the present to the future. Hum Reprod Update. 2016;22(6):665-86. https://doi.org/10.1093/humupd/dmw023
2. Stewart EA, Cookson CL, Gandolfo RA, Schulze-Rath R. Epidemiology of uterine fibroids: a systematic review. BJOG. 2017;124(10):1501-12. https://doi.org/10.1111/1471-0528.14640
3. Lethaby A, Vollenhoven B. Fibroids (uterine myomatosis, leiomyomas). BMJ Clin Evid. 2015;2015:0814. PMID: 26032466
4. Yilmaz BD, Bulun SE. Endometriosis and nuclear receptors. Hum Reprod Update. 2019;25(4):473-85. https://doi.org/10.1093/humupd/dmz005
5. Wallach EE, Vlahos NF. Uterine myomas: an overview of development, clinical features, and management. Obstet Gynecol. 2004;104(2):393-406. https://doi.org/10.1097/01.AOG.0000136079.62513.39
6. Styer AK, Rueda BR. The epidemiology and genetics of uterine leiomyoma. Best Pract Res Clin Obstet Gynaecol. 2016;34:3-12. https://doi.org/10.1016/j.bpobgyn.2015.11.018
7. AlAshqar A, Patzkowsky K, Afrin S, Wild R, Taylor HS, Borahay MA. Cardiometabolic risk factors and benign gynecologic disorders. Obstet Gynecol Surv. 2019;74(11):661-73. https://doi.org/10.1097/OGX.0000000000000718
8. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. Nat Rev Cancer. 2015;15(8):484-98. https://doi.org/10.1038/nrc3967
9. Baird DD, Travlos G, Wilson R, Dunson DB, Hill MC, D'Aloisio AA, et al. Uterine leiomyomata in relation to insulin-like growth factor-I, insulin, and diabetes. Epidemiology. 2009;20(4):604-10. https://doi.org/10.1097/EDE.0b013e31819d8d3f
10. Gkioka E, Msaouel P, Philippou A, Vlaghogiannis NI, Vogkou CT, Margiolis A, et al. Review: the role of insulin-like growth factor-1 signaling pathways in uterine leiomyoma. In Vivo. 2015;29(6):637-49. PMID: 26546520
11. Jain R, Singh R, Yamini S, Das MK. Fragmented ECG as a risk marker in cardiovascular diseases. Curr Cardiol Rev. 2014;10(3):277-86. https://doi.org/10.2174/1573403x10666140514103451
12. Sverdlov AL, Figtree GA, Horowitz JD, Ngo DT. Interplay between oxidative stress and inflammation in cardiometabolic syndrome. Mediators Inflamm. 2016;2016:8254590. https://doi.org/10.1155/2016/8254590
13. Zimmermann A, Bernuit D, Gerlinger C, Schaefers M, Geppert K. Prevalence, symptoms and management of uterine fibroids: an international internet-based survey of 21,746 women. BMC Womens Health. 2012;12:6. https://doi.org/10.1186/1472-6874-12-6
14. Bittencourt CA, Santos Simões R, Bernardo WM, Fuchs LFP, Soares Júnior JM, Pastore AR, et al. Accuracy of saline contrast

sonohysterography in detection of endometrial polyps and submucosal leiomyomas in women of reproductive age with abnormal uterine bleeding: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2017;50(1):32-9. https://doi.org/10.1002/uog.17352

15. Magalhaes J, Ferreira-Filho ES, Soares-Junior JM, Baracat EC. Uterine volume, menstrual patterns, and contraceptive outcomes in users of the levonorgestrel-releasing intrauterine system: a cohort study with a five-year follow-up. Eur J Obstet Gynecol Reprod Biol. 2022;276:56-62. https://doi.org/10.1016/j.ejogrb.2022.06.029
16. Peregrino PFM, Lorenzo Messina M, Santos Simões R, Soares-Júnior JM, Baracat EC. Review of magnetic resonance-guided focused ultrasound in the treatment of uterine fibroids. Clinics (Sao Paulo). 2017;72(10):637-41. https://doi.org/10.6061/clinics/2017(10)08
17. Korkmaz V, Ozkaya E, Özer Kadife S, Kara F, Kucukozkan T. Investigation of cardiovascular disease risk in women with uterine leiomyomas. Ir J Med Sci. 2016;185(3):689-93. https://doi.org/10.1007/s11845-015-1343-0
18. Uimari O, Auvinen J, Jokelainen J, Puukka K, Ruokonen A, Järvelin MR, et al. Uterine fibroids and cardiovascular risk. Hum Reprod. 2016;31(12):2689-703. https://doi.org/10.1093/humrep/dew249
19. Tak YJ, Lee SY, Park SK, Kim YJ, Lee JG, Jeong DW, et al. Association between uterine leiomyoma and metabolic syndrome in parous premenopausal women: a case-control study. Medicine (Baltimore). 2016;95(46):e5325. https://doi.org/10.1097/MD.0000000000005325
20. Takeda T, Sakata M, Isobe A, Miyake A, Nishimoto F, Ota Y, et al. Relationship between metabolic syndrome and uterine leiomyomas: a case-control study. Gynecol Obstet Invest. 2008;66(1):14-7. https://doi.org/10.1159/000114250
21. Laughlin-Tommaso SK, Fuchs EL, Wellons MF, Lewis CE, Calderon-Margalit R, Stewart EA, et al. Uterine fibroids and the risk of cardiovascular disease in the coronary artery risk development in young adult women's study. J Womens Health (Larchmt). 2019;28(1):46-52. https://doi.org/10.1089/jwh.2018.7122
22. Aksoy Y, Sivri N, Karaoz B, Sayin C, Yetkin E. Carotid intima-media thickness: a new marker of patients with uterine leiomyoma. Eur J Obstet Gynecol Reprod Biol. 2014;175:54-7. https://doi.org/10.1016/j.ejogrb.2014.01.005
23. Haan YC, Diemer FS, Woude L, Montfrans GA, Oehlers GP, Brewster LM. The risk of hypertension and cardiovascular disease in women with uterine fibroids. J Clin Hypertens (Greenwich). 2018;20(4):718-26. https://doi.org/10.1111/jch.13253
24. Toraman A, Eren B, Yılmaz I, Duzgun F, Taneli F, Kursat S. Fragmented QRS as a predictor of subclinical cardiovascular disease in patients with chronic kidney disease. Intern Med J. 2020;50(12):1532-7. https://doi.org/10.1111/imj.14743
25. Fedulaev Y, Makarova I, Pinchuk T. Fragmented QRS as a marker of coronary atherosclerosis severity evaluated by the Gensini score. Ann Cardiol Angeiol (Paris). 2021;70(3):143-7. https://doi.org/10.1016/j.ancard.2021.03.001
26. Karabakan M, Bozkurt A, Saylam B, Kucuksu Z, Hirik E, Kagan Aktas B. A new proof for the relationship between erectile dysfunction and atherosclerosis: fragmented QRS? Int J Impot Res. 2020;32(3):302-7. https://doi.org/10.1038/s41443-019-0166-3
27. Aksu E, Berk E, Sökmen A, Sökmen G, Çelik E. Subclinical cardiac structural and electrical abnormalities in fibromyalgia syndrome. Turk J Med Sci. 2020;50(4):885-93. https://doi.org/10.3906/sag-1912-228

ORIGINAL ARTICLE

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Effect of polycystic ovary syndrome on the life quality of young women

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SUMMARY

OBJECTIVE: The study evaluated the opinions of polycystic ovary syndrome on the life quality of women.

METHODS: A total of 249 women with polycystic ovary syndrome participated in this descriptive study between October 2022 and July 2023 in Istanbul, Turkey.

FINDINGS: Polycystic Ovary Syndrome and Quality of Life was significantly correlated with age (p=0.000) and frequent weight loss diets (p=0.000) (p<0.01). Among the Polycystic Ovary Syndrome and Quality of Life total score and polycystic ovary syndrome symptoms, those with hormone imbalance and insulin resistance had the highest mean scores, while those with menstrual irregularity and fatigue had the lowest.

CONCLUSION: Advancing age changes the quality of life of women with polycystic ovary syndrome. To prevent the negative impact of polycystic ovary syndrome on women's quality of life, it is recommended that health professionals develop effective care plans utilizing available evidence.

KEYWORDS: Polycystic ovary syndrome. Quality of life. Lifestyle factors. Women.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a chronic endocrine and metabolic disorder common in women, consisting of polycystic changes in the ovaries, menstrual cycle disorder, insulin resistance, obesity, and hyperandrogenism[1]. Prevalence varies between 6 and 20% depending on the diagnostic criteria[2].

Individual assessments of physical, psychological, and social well-being should be incorporated into the health-related quality of life concept. For example, women with hirsutism are reported to have higher rates of psychological morbidity, social fear, and anxiety than women in the control population, and being infertile has a negative impact on sexual functioning and mood[3]. Therefore, all psychological, social, and cultural needs of young women diagnosed with PCOS should be addressed in a holistic approach[4].

Many studies have shown that PCOS symptoms reduce the quality of life overall as they are painful, uncomfortable, and culturally associated with traits defined as unfeminine and undesirable[5-7]. Despite this, it has been suggested that the negative impact of PCOS symptoms on quality of life has been largely overlooked[8]. Although PCOS has a negative impact on quality of life, this impact varies from culture to culture. In Turkish women with PCOS, irregular menstrual cycles and hirsutism had a major impact on quality of life, whereas, in Iran, menstrual irregularities and infertility were more effective in reducing quality of life[4,8]. For Brazilian women with PCOS, body weight and infertility were reported to have the greatest negative impact on quality of life[9]. In a study on Italian women, PCOS patients with obesity reported significantly deteriorating their quality of life[4]. The impact of PCOS on quality of life may be specific and vary in different cultures. International evidence-based guidelines recommend that health professionals assess and consider the perception of symptoms, impact on quality of life, and personal priorities for care to improve patient outcomes[6].

Health professionals must provide care with a holistic approach that focuses on how the emotional, physical, and social problems experienced by women of different cultures, geographical origins, and traditions due to PCOS symptoms affect their daily lives. In addition, investigating women's perceptions about their treatment and life quality by age is necessary for delivering individualized healthcare services. This research may raise awareness among healthcare professionals and enable them to support women by considering that life quality varies depending on age. Therefore, this study aimed to determine the quality of life of women with PCOS.

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METHODS

This cross-sectional study was conducted between October 2022 and July 2023. The study population consisted of women diagnosed with PCOS aged 18 years and older living in Turkey. In the systematic review study of Deswal et al.[10] the prevalence of PCOS in women of reproductive age was taken with the unknown sampling method, the sample number calculated with a 95% confidence level and 0.05 sensitivity was determined as 249, and the study was completed with 250 women[11]. Ethical and institutional approval for the study (2022.10.19) was obtained (E-71273842-903.07.05-514118). Descriptive Information Form and Polycystic Ovary Syndrome and Quality of Life-50 Scale (PCOSQ-50), prepared in line with the literature, were used as data collection tools. Research data were collected through an online form (Google form). Participants were invited to the study electronically. The women in this study self-reported having PCOS.

The descriptive information form consisted of 25 questions developed by the researchers to determine women's socio-demographics, general health status, and PCOS history[11].

PCOSQ-50 was developed by Nasiri-Amiri et al.[12]. Turkish validation was conducted by Koyutürk[13]. Cronbach's alpha of the PCOSQ-50 scale was reported to be 0.972. In our study, Cronbach's alpha value was 0.926. The data were analyzed with SPSS 21.0 and 95% confidence level. The t-test and ANOVA test were used to analyze the differences in scale scores according to demographic characteristics.

RESULTS

The mean age of the women who participated in the study was 28.47±6.21 years. Notably, 81.5% of the women were university graduates or above. Among the participants, 61.0% were employed, and the most common occupations were civil servants (25.3%) and students (20.5%), respectively.

Among the participants, 30.9% had another disease other than PCOS, and the mean number of years since diagnosis of PCOS was 7.30±5.79. Regarding the symptoms of PCOS, 61.4% of the participants reported hair growth, 72.3% reported menstrual irregularity, 44.2% reported obesity, 49.8% reported acne pimples, 54.2% reported hair loss, 48.6% reported insulin resistance, 52.6% reported hormone imbalance, and 71.1% reported fatigue.

The characteristics of the women who participated in our study and the comparison of PCOSQ total and sub-dimensions are presented in Table 1. For PCOSQ total, those with hormone imbalance and insulin resistance had the highest mean scores for PCOS symptoms, while those with menstrual irregularity and fatigue had the lowest (Table 2).

The logistic regression model established to examine the effect of variables on PCOSQ total was found significant ($p<0.05$). When the results were examined, age had a negative effect on PCOSQ total ($\beta=-0.446$, $p<0.05$), whereas frequency of going to the hospital for PCOS control had a positive effect ($\beta=0.163$, $p<0.05$). Age and frequency of going to the hospital for PCOS control explained 18.6% of the change in PCOSQ total (Table 3).

DISCUSSION

This study showed that the most important symptoms affecting the quality of life of women with PCOS were menstrual irregularity and fatigue, while the least affected areas were hormone irregularity and insulin resistance. At the same time, in this study, it was observed that women were irregular and overfed and neglected their controls. In women, the age variable was found to affect all sub-dimensions of the PCOSQ scale except sexuality. Women aged 26–30 years had higher PCOSQ total scores, and regression analysis showed that the total scores were negatively affected by increasing age.

It is difficult to confirm the diagnosis of PCOS, as it is associated with metabolic dysfunction and is a risk factor for the development of type 2 diabetes cardiovascular diseases and endometrial cancer. Therefore, for diagnosis, according to many studies, anovulation and hyperandrogenism are required[14,15]. In addition, it is necessary to evaluate the clinical, hormonal, and metabolic features to understand the impact of different phenotypes on PCOS[16,17]. There is considerable evidence that women with PCOS have more regular menstrual cycles, decreased serum androgen levels, and improved polycystic ovarian morphology with increasing age[18]. However, another study stated that the decrease in androgen levels over time did not show metabolic improvement and was a risk factor for type 2 diabetes according to phenotypes[16]. A study of women with PCOS between the ages of 31 and 46 years emphasized that these women had significantly lower life quality[19]. In this study, women over 36 years had the lowest life quality scores. This may be due to increased fat accumulation over the years, negative body image, and metabolic problems. When developing treatments to improve quality of life, it is important to understand the factors that reduce quality of life in women with PCOS[20]. Long-term treatments and evaluation of hormonal and metabolic parameters of these women are important for their quality of life[21].

Menstrual irregularity is a common gynecologic condition affecting women with PCOS, especially in early reproductive age. A meta-analysis of studies with women with PCOS

Table 1. Comparison of some characteristics of women and Polycystic Ovary Syndrome and Quality of Life scores (n=249).

		Psychosocial and Emotional		Fertility		Sexual Function		Obesity and Menstrual Disorders		Hair Growth		Coping		PCOSQ Total	
		X	SD	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
Age (years)	≤25	3.13	0.90	2.43	1.03	2.09	0.91	3.11	1.07	3.35	1.36	2.68	1.04	2.90	0.85
	26–30	3.15	0.83	2.76	1.11	2.52	1.03	3.38	1.04	3.44	1.28	2.81	1.11	3.03	0.83
	31–35	2.64	0.83	2.27	0.95	2.13	0.76	2.91	0.88	2.60	1.24	2.23	0.93	2.49	0.68
	≥36	2.52	0.78	1.88	0.77	2.27	0.89	2.88	0.91	2.34	1.27	1.97	0.75	2.35	0.63
	F	7.561		6.395		2.536		2.860		8.879		7.402		8.581	
	p-value	0.000*		0.000*		0.058		0.038*		0.000*		0.000*		0.000*	
Work	Working	2.85	0.88	2.33	0.97	2.18	0.90	3.04	1.01	2.94	1.35	2.42	1.02	2.67	0.80
	Not working	3.15	0.87	2.59	1.13	2.47	0.96	3.26	1.03	3.39	1.35	2.73	1.07	2.98	0.83
	t	-2.669		-1.864		-1.988		-1.656		-2.569		-2.319		-2.964	
	p-value	0.008*		0.064		0.048*		0.099		0.011*		0.021*		0.003*	
Currently not having any disease other than PCOS	Yes	3.11	0.87	2.51	1.05	2.46	0.87	3.36	0.99	3.17	1.31	2.64	1.07	2.92	0.79
	No	2.90	0.89	2.39	1.04	2.19	0.95	3.02	1.02	3.09	1.39	2.50	1.04	2.73	0.83
	t	1.742		0.783		1.831		2.432		0.424		1.010		1.696	
	p-value	0.083		0.435		0.069		0.016*		0.672		0.314		0.091	
Time to diagnosis of PCOS (years)	5 years and less	3.10	0.88	2.49	1.03	2.46	0.90	3.24	1.00	3.17	1.39	2.73	1.08	2.93	0.83
	6–10 years	3.09	0.74	2.54	0.99	2.15	0.98	3.18	0.98	3.42	1.27	2.61	0.93	2.88	0.70
	10 years	3.00	0.86	2.58	1.04	2.44	0.81	3.39	0.85	3.16	1.31	2.57	0.99	2.89	0.71
	F	0.303		0.159		1.929		0.702		0.802		0.556		0.078	
	p-value	0.739		0.853		0.149		0.497		0.45		0.574		0.925	
Family history of PCOS	Yes	3.00	0.94	2.57	1.14	2.26	0.89	3.26	1.01	3.54	1.45	2.54	1.14	2.90	0.87
	No	2.95	0.87	2.39	1.01	2.28	0.94	3.09	1.03	2.99	1.32	2.54	1.03	2.76	0.81
	t	0.366		1.105		-0.125		1.105		2.686		-0.004		1.112	
	p-value	0.715		0.27		0.901		0.27		0.008*		0.997		0.267	
Frequency of hospital visits for PCOS control	36 months	2.87	0.95	2.38	1.10	2.26	0.85	3.10	1.06	3.24	1.38	2.38	1.14	2.75	0.89
	1 year	2.88	0.91	2.23	0.94	2.08	0.85	3.01	1.04	2.96	1.34	2.46	1.03	2.68	0.83
	Depends on the situation	3.06	0.83	2.57	1.02	2.33	0.97	3.22	0.96	3.19	1.36	2.65	1.01	2.89	0.78
	F	1.302		2.165		0.950		0.898		0.619		1.423		1.330	
	p-value	0.274		0.117		0.389		0.409		0.539		0.243		0.267	

X: mean; SD: standard deviation; F: one-way ANOVA; t: Student's t. *p<0.05.

emphasized that hirsutism and menstrual imbalance affect women's quality of life. Up to 90% of women with PCOS report facial hair as a problem. Women with PCOS who experience hirsutism report feeling "unfeminine," "weird," "strange," "weird," and "different"[18]. As a smooth, hairless body or face and regular menstruation are associated with femininity and fertility, women with hair growth/menstrual irregularity symptoms feel "different" and less "feminine" than other women[22,23]. Young women may feel less sexually attractive because of their appearance. Negative affect may be accompanied by anxiety and depression. Women under 25 and over 30 years had a lower quality of life due to menstrual irregularities. Women's long and painful menstrual cycles may have affected their quality of life, and even if they used medication, the side effects may have caused discomfort for them. In addition, childlessness is an important problem in Turkish society. Women's perception that menstrual irregularity would jeopardize their fertility in later life may have caused them anxiety.

Table 3. Evaluation of variables affecting women's Polycystic Ovary Syndrome and Quality of Life total score by logistic regression.

Dependent variable	Independent variable	Non-standardized		Standardized	t	p	R^2
		B	SE	Beta			
PCOSQ total	Fixed	3.589	0.883		4.063	0.000*	0.186
	Age	-0.337	0.066	-0.446	-5.137	0.000*	
	Education status	-0.135	0.144	-0.066	-0.942	0.348	
	Marital status	0.214	0.122	0.139	1.752	0.081	
	Employment status	0.087	0.110	0.055	0.789	0.431	
	Income status	0.081	0.081	0.067	1.009	0.314	
	Currently having any disease other than PCOS	-0.194	0.109	-0.119	-1.782	0.076	
	Diagnosis of PCOS (years)	0.129	0.069	0.134	1.871	0.063	
	Someone in the family diagnosed with PCOS	-0.101	0.121	-0.055	-0.832	0.406	
	Frequency of hospitalization for PCOS controlled	0.163	0.067	0.163	2.447	0.015*	
	Use of nutritional supplements	-0.143	0.116	-0.081	-1.237	0.218	

Model: f=4.622, p=0.000. *p>0.001.

Table 2. Investigation of Polycystic Ovary Syndrome and Quality of Life scale and subscale scores regarding symptoms (n=249).

	Hair growth		Menstrual irregularity		Obesity		Acne-pimples		Hair loss		Insulin resistance		Hormone imbalance		Fatigue	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Psychosocial and emotional	3.22	0.79	3.10	0.84	3.27	0.80	3.31	0.78	3.20	0.85	3.27	0.87	3.30	0.80	3.15	0.84
Fertility	2.55	1.04	2.55	1.02	2.54	0.99	2.61	1.08	2.54	1.10	2.62	1.07	2.71	1.11	2.50	1.06
Sexual function	2.43	0.88	2.35	0.93	2.40	0.95	2.48	0.96	2.42	0.91	2.51	1.01	2.56	0.95	2.36	0.91
Obesity and menstrual disorders	3.34	0.96	3.30	0.96	3.63	0.81	3.34	0.99	3.33	1.01	3.59	0.88	3.45	0.99	3.30	0.98
Hair growth	3.85	1.02	3.25	1.27	3.42	1.36	3.51	1.28	3.51	1.27	3.42	1.34	3.54	1.30	3.32	1.36
Coping	2.84	0.99	2.66	1.01	2.84	1.00	2.87	1.04	2.80	1.05	2.91	1.04	2.87	1.05	2.72	1.03
PCOSQ total	3.08	0.71	2.92	0.76	3.08	0.73	3.08	0.76	3.02	0.79	3.11	0.79	3.12	0.76	2.95	0.79

Sleep disturbances and fatigue are very common in women with PCOS in conditions of inadequate physical activity. In a recent study, women with PCOS reported sleep difficulties and occasional restless sleep. In the same study, women often reported severe fatigue[23]. In another study, it was reported that 29.5% of women with PCOS complained of sleep disorders, and 64% had been struggling with this problem for at least 1 year[20]. Therefore, optimizing sleep may be important to support and maintain healthy lifestyle changes for women with PCOS. In this study, women who reported being tired had a lower quality of life. However, the association between PCOS and diet quality may only be useful if women are able to get enough quality sleep. Overnutrition closure increases with decreased sleep duration. It is, therefore, important to normalize sleep duration through diet and exercise to focus on quality lifestyle behaviors. Insulin resistance may be exacerbated by sleep disturbances in women with PCOS and their energy balance may be disturbed.

CONCLUSION

In women with PCOS, quality of life decreases with age. Menstrual irregularity and fatigue affect women the most. These women eat irregular diets and neglect their check-ups. We recommend utilizing the available evidence and developing appropriate action plans according to age.

Limitations of the study

The diagnosis of menstrual irregularity was based on a self-reported questionnaire, so there may be a risk of information bias in reporting symptoms. Moreover, there was no ovarian ultrasonography to help diagnose PCOS and no clinical assessment of hyperandrogenism.

ETHICS APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University Istanbul Arel (Date: 19.10.2022/ No: E-71273842-903.07.05-514118). Written consent was obtained from all women participating in the study.

AUTHORS' CONTRIBUTIONS

ÖT: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **EYA:** Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. **AA:** Data curation, Formal Analysis, Validation, Writing – original draft. **ÜO:** Supervision, Visualization. **NC:** Project administration, Resources, Validation.

REFERENCES

1. Kocak DY, Ugurlu M. Depression symptoms and quality of life in women with polycystic ovary syndrome. Perspect Psychiatr Care. 2022;58(4):2837-45. https://doi.org/10.1111/ppc.13131
2. Osmanlıoğlu Ş, Omma T. The relationship between polycystic ovary syndrome phenotypes and metabolic dysfunction. J Inonu Univ Health Serv Vocational School. 2023;11(1):1092-100. https://doi.org/10.33715/inonusaglik.1215455
3. Angin P, Yoldemir T, Atasayan K. Quality of life among infertile PCOS patients. Arch Gynecol Obstet. 2019;300(2):461-7. https://doi.org/10.1007/s00404-019-05202-z
4. Panico A, Messina G, Lupoli GA, Lupoli R, Cacciapuoti M, Moscatelli F, et al. Quality of life in overweight (obese) and normal-weight women with polycystic ovary syndrome. Patient Prefer Adherence. 2017;11:423-9. https://doi.org/10.2147/PPA.S119180
5. Açmaz G, Albayrak E, Acmaz B, Başer M, Soyak M, Zararsız G, et al. Level of anxiety, depression, self-esteem, social anxiety, and quality of life among the women with polycystic ovary syndrome. ScientificWorldJournal. 2013;2013:851815. https://doi.org/10.1155/2013/851815
6. Bazarganipour F, Ziaei S, Montazeri A, Foroozanfard F, Kazemnejad A, Faghihzadeh S. Psychological investigation in patients with polycystic ovary syndrome. Health Qual Life Outcomes. 2013;11:141. https://doi.org/10.1186/1477-7525-11-141
7. Benetti-Pinto CL, Ferreira SR, Antunes A, Yela DA. The influence of body weight on sexual function and quality of life in women with polycystic ovary syndrome. Arch Gynecol Obstet. 2015;291(2):451-5. https://doi.org/10.1007/s00404-014-3423-1
8. Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33(9):1602-18. https://doi.org/10.1093/humrep/dey256
9. McCook JG, Reame NE, Thatcher SS. Health-related quality of life issues in women with polycystic ovary syndrome. J Obstet Gynecol Neonatal Nurs. 2005;34(1):12-20. https://doi.org/10.1177/0884217504272945
10. Deswal R, Narwal V, Dang A, Pundir CS. The prevalence of polycystic ovary syndrome: a brief systematic review. J Hum Reprod Sci. 2020;13(4):261-71. https://doi.org/10.4103/jhrs.JHRS_95_18
11. Höbek Akarsu R, İlkin E, Büyükkaplan B. Determination of polycystic over syndrome and affecting factors in young women. Smyrna. 2021;2:1-7.
12. Nasiri-Amiri F, Ramezani Tehrani F, Simbar M, Montazeri A, Mohammadpour RA. Health-related quality of life questionnaire for polycystic ovary syndrome (PCOSQ-50): development and psychometric properties. Qual Life Res. 2016;25:1791-801.
13. Koyutürk G. Polikistik over sendromu yaşam kalitesi-50 ölçeği'nin Türkçe uyarlamasının geçerlik ve güvenirliği [Master thesis]. Fizyoterapi ve Rehabilitasyon Anabilim Dalı, Yıldırım Beyazıt Üniversitesi; 2018.
14. Baracat EC, Baracat MCP, José M SJ Jr. Are there new insights for the definition of PCOS? Gynecol Endocrinol. 2022;38(9):703-4. https://doi.org/10.1080/09513590.2022.2121387
15. Giordano LA, Giordano MV, Célia Teixeira Gomes R, Santos Simões R, Baracat MCP, Giordano MG, et al. Effects of clinical and metabolic variables and hormones on the expression of immune protein biomarkers in the endometrium of women with polycystic ovary syndrome and normal-cycling controls. Gynecol Endocrinol. 2022;38(6):508-15. https://doi.org/10.1080/09513590.2022.2061454
16. Soares-Jr JM, Hayashida SAY, Marcondes JAM, Maciel GAR, Barcellos CRG, Maffazioli GN, et al. Influence of phenotypes on the metabolic syndrome of women with polycystic ovary syndrome over a six-year follow-up in Brazil. Biomedicines. 2023;11(12):3262. https://doi.org/10.3390/biomedicines11123262
17. Baracat MCP, Baracat EC, Simões RS, Simões MJ, Maciel GA, Azziz R, et al. Hormonal and metabolic factors influence the action of progesterone on the endometrium of women with polycystic ovary syndrome. Diagnostics (Basel). 2023;13(3):382.
18. Bazarganipour F, Taghavi SA, Montazeri A, Ahmadi F, Chaman R, Khosravi A. The impact of polycystic ovary syndrome on the health-related quality of life: a systematic review and meta-analysis. Iran J Reprod Med. 2015;13(2):61-70. PMID: 25999994
19. Keizerswaard J, Dietz Loos ALP, Louwers YV, Laven JSE. Changes in individual polycystic ovary syndrome phenotypical characteristics over time: a long-term follow-up study. Fertil Steril. 2022;117(5):1059-66. https://doi.org/10.1016/j.fertnstert.2022.01.014
20. Karjula S, Morin-Papunen L, Franks S, Auvinen J, Järvelin MR, Tapanainen JS, et al. Population-based data at ages 31 and 46 show decreased HRQoL and life satisfaction in women with PCOS symptoms. J Clin Endocrinol Metab. 2020;105(6):1814-26. https://doi.org/10.1210/clinem/dgz256
21. Kitzinger C, Willmott J. "The thief of womanhood": women's experience of polycystic ovarian syndrome. Soc Sci Med. 2002;54(3):349-61. https://doi.org/10.1016/s0277-9536(01)00034-x
22. Nazik H, Özdemir F, Nazik E, Arslan S. Polikistik over sendromu olan kadınların beden imajı ve benlik saygısı. Ank Sağlık Bilim Derg. 2014;3(1):1-12. https://doi.org/10.1501/Asbd_0000000043
23. Mo L, Mansfield DR, Joham A, Cain SW, Bennett C, Blumfield M, et al. Sleep disturbances in women with and without polycystic ovary syndrome in an Australian National Cohort. Clin Endocrinol (Oxf). 2019;90(4):570-8. https://doi.org/10.1111/cen.13922

ORIGINAL ARTICLE

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Coexistence of Hashimoto's thyroiditis and papillary thyroid carcinoma revisited in thyroidology, an experience from an endemic region: fad or future?

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SUMMARY

OBJECTIVE: Papillary thyroid carcinoma, per se, is the most common type of thyroid cancer, and Hashimoto's thyroiditis is the most frequent autoimmune disease of the papillon gland. The liaison between Hashimoto's thyroiditis and thyroid cancers is still an ongoing debate in thyroidology. The aim of the study was to discuss the frequency of the co-occurrence of Hashimoto's thyroiditis and papillary thyroid carcinoma.

METHODS: This study is designed as a retrospective analytical cohort study. The institutional database and archive of histopathology scanning identified the patients who had undergone thyroidectomy between January 2022 and January 2016. The Statistical Package for Social Sciences v21.0 program was used for statistical purposes. Descriptive and chi-square tests were applied, and a $p<0.05$ was considered significant.

RESULTS: Of 498 patients who had undergone thyroidectomy for 4 years, 99 (20%) were male and 399 (80%) were female. Of note, papillary thyroid carcinoma was revealed in 160 (32%) patients, and Hashimoto's thyroiditis was recognized in 178 (35.74%) patients. The prevalence of Hashimoto's thyroiditis in cases with papillary thyroid carcinoma was 43.8%, while the prevalence in patients with Hashimoto's thyroiditis was 41.1%.

CONCLUSION: A debate still remains on the propriety of these two phenomena. Herewith, we recognized a correlation between the presence of papillary thyroid carcinoma and Hashimoto's thyroiditis. Providers should be vigilant about the coexistence of these phenomena. We might postulate the so-called total thyroidectomy for cases with a cytologic diagnosis of Hashimoto's thyroiditis with a papillary thyroid carcinoma. As a matter of fact, this issue merits further investigation.

KEYWORDS: Thyroid gland. Thyroid cancer, papillary. Thyroiditis, pathology. Thyroidology.

INTRODUCTION

Thyroid carcinomas are the most common endocrine tumors, which occupy the ninth most frequent cancer worldwide, according to World Health Organization's 2020 data. However, it is more commonly found in Asian populations and is the fifth most common tumor in Turkey. Papillary thyroid carcinoma (PTC) is the most common thyroid cancer with a commonly good prognosis, although sometimes poor clinical outcomes emerge. As such, tall cell, cribriform, diffuse sclerosing, and hobnail subtypes are considered to have a poor prognosis for PTC[1].

Hashimoto's thyroiditis (HThy), chronic lymphocytic thyroiditis, was first described by a Japanese pathologist and surgeon, Hakaru Hashimoto, in 1912[2]. It is the most common autoimmune thyroid disease, especially in females between the 3rd and 5th decades, possessing a prevalence of HThy of 1–4% and an incidence of 3–6 per 10,000 people. Lymphocyte infiltration and atrophy due to an autoimmune response in the gland are recognized in HThy, which is one of the most common causes of hypothyroidism that develops in 4–5% of cases each year[3]. The associations between PTC and HThy have been reported between the two phenomena[3,4-7], though some publications[8-10] are opposing.

The present study sought to determine the prevalence of thyroid carcinoma and HThy in the cases who had undergone thyroidectomy in the iodine-deficiency endemic region.

METHODS

Study design

This is a retrospective cohort study conducted at the Department of Pathology, Giresun University Education and Research Hospital, incorporating 498 patients with thyroidectomies between January 2016 and January 2022. The patient records

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have been obtained from the institutional database, and archive of histopathology for the purpose of collecting demographic data and histopathologic diagnoses. The tumor size, vascular invasion, multifocality, extrathyroidal extension, nodal metastasis, and subgroup of PTC have been evaluated. To this end, the patients were categorized into two groups: those with HThy and those with PTC. Afterward, the incidence of PTC was determined among the cases with HThy, and subsequently, the occurrence of HThy in patients with PTC was analyzed. Of note, the multifocality, lymphovascular invasion, and tumor diameter were assessed in cases with both HThy and PTC.

Statistical analysis

All the patients' data were entered into a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 (SPSS, IBM Inc., Chicago, IL, USA) software in order to evaluate the present retrospective analytical cohort study. The data were statistically evaluated using the chi-square test and Fisher's exact test. A $p<0.05$ was considered statistically significant.

RESULTS

In total, 99 (20%) of cases were male and 399 (80%) were female in 498 patients who had undergone thyroidectomy for six decades. The mean age was 51 (21–81) years, and the PTC was revealed in 160 (32%) and HT in 178 (35.74%) cases. However, 343 (68%) cases had thyroid follicular nodular diseases, while 14 (2.8%) had diffuse hyperplasia, 48 (9.6%) had follicular adenoma, 28 (5.5%) had Hurtle cell adenoma, 4 (0.8%) had follicular carcinoma, 4 (0.8%) had Hurtle cell carcinoma, and 13 (2.6%) had non-invasive follicular thyroid neoplasm with papillary-like nuclear features.

Histopathologic diagnosis was HThy and/or PTC in 268 of a total of 468 patients included in the study; 42 (15.7%) of them were male and 226 (84.3%) were female. Herein, tumor size was smaller than 10 mm in 104 (45.5%) and 10 mm or larger in 56 (20.9%) cases with PTC, in which the tumor was found multifocal in 44. Both PTC and HThy were present in 70 cases (Figure 1), and the prevalence of HThy in patients with PTC was 43.7% and that of PTC in HThy was revealed to be 39.3%. In this case, tumor size was smaller than 10 mm in 48 (68.6%) and multifocal in 22 (31.4%) (Table 1). Lymphovascular invasion was detected in six cases with PTC, and HThy was exhibited in half of them without significance ($p=0.52$). However, statistical significance was recognized in the case of coexistence of HThy with PTC cases, regardless of tumor size ($p<0.001$) (Table 2), in which tumors were multifocal in 22 of 70 cases. A statistical correlation was observed between multifocality and HThy ($p=0.019$). In addition, the odds ratio was calculated to be 2.3 in the coexistence of PTC and HThy.

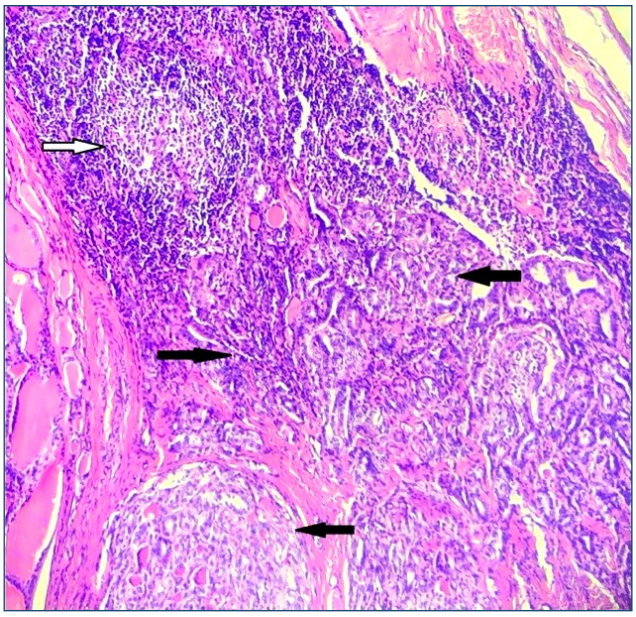

Figure 1. Papillary thyroid carcinoma in the background of Hashimoto's thyroiditis (the white arrow indicates a lymphoid follicle with a germinal center in Hashimoto's thyroiditis, while the black ones indicate papillary microcarcinoma near the lymphoid infiltration).

Table 1. Demographic, sonographic, and histopathologic features of papillary thyroid carcinoma and Hashimoto's thyroiditis.

	PTC (n=160)	HThy (n=178)
Male	31 (19.4%)	23 (12.9%)
Female	129 (80.6%)	155 (87.1%)
Median age	51 (21–84)	51 (21–81)
HThy with PTC	70 (43.7%)	70 (39.3%)
Multifocality	44 (27.5%)	22 (31.4%)
Tumor size (cm)		
≥1	58 (36.3%)	22 (31.4%)
<1	102 (63.7%)	48 (68.6%)
Lymphovascular invasion	6 (8.6%)	3 (4.3%)
Lymph node metastasis	6 (8.6%)	3 (4.3%)

DISCUSSION

In this study, it was concluded that there is a correlation between HThy and PTC in the patient group examined. The size of tumors was mostly less than 10 mm in cases with HThy, and

Table 2. Statistical outcomes of the cases with papillary thyroid carcinoma and/or Hashimoto's thyroiditis (n=268).

			HThy		Total
			Absent	Present	
PTC	Absent	Count	0	108	110
		% within PTC	0.0%	100.0%	100.0%
		% within HThy	0.0%	60.7%	40.3%
	Present	Count	90	70*	160
		% within PTC	56.2%	43.8%	100.0%
		% within HThy	100.0%	39.3%	59.7%
Total		Count	90	178	268
		% within PTC	33.6%	66.4%	100.0%
		% within HThy	100.0%	100.0%	100.0%

Odds ratio	Values	95% Confidence interval	
		Lower	Upper
For cohort HThy present	2.324	1.942	2.780
No. of valid cases	268		

*p<0.001 (Fisher's exact test).

a tendency toward multifocal disease was observed. The presence of multiple small tumor islands in the background of HThy suggests that it might provoke the development of PTC. To date, various hypotheses have been put forward in studies on the subject. The Cappelli et al.'s[11] study has linked high thyroid stimulating hormone (TSH) levels with an increased risk of malignancy. Based on this research, it may be thought that increased TSH secondary to hypothyroidism in thyroiditis might lead to increased follicular epithelial cell proliferation and thyroid papillary carcinoma. On the contrary, some studies argue that thyroid autoimmunity might emerge against antigens released by cancerous thyrocytes[7]. Most recent studies have reported correlations between the two diseases, whose outcomes support the results of this study. Of these, Liang et al., reported a significantly higher risk of PTC development in patients with HThy[12]. Uhliarova et al.[13] stated that HThy causes a significantly increased risk of developing thyroid carcinoma, especially papillary thyroid microcarcinoma. Moreover, Molnár et al.[14] reported that HThy is a promoter of thyroid carcinogenesis. The authors also described a correlation with tumoral multifocality[14]. In addition, a high rate of multicentricity in tumors in HThy has been reported in some studies in our country[15-19]. Although HThy is a possible risk factor for PTC, it has been suggested that it reduces progression[20], especially in thyroidology[21-25], a vital and substantial field in order to provide optimal thyroid health by thyroidologists; however, no such results were found in our study. Poor prognostic factors such as lymph node metastasis and lymphovascular invasion in the cases were not statistically different when compared to other cases. There have been reported associations between the studies and thyroidectomy specimens, but in some studies that were performed with fine needle aspiration cytology (FNAC) no correlation was reported between the two phenomena[8,9].

CONCLUSION

There has been an increase in the prevalence of PTC in HThy, although a debate is still ongoing about the provocation of these two phenomena. A non-negligible frequency of HThy and PTC coexistence and a noteworthy risk of multifocal disease should be considered in the management and follow-up of the phenomenon. As such, HThy should be followed up for the development of PTC, and we might postulate that the so-called surgical procedure of total thyroidectomy should be planned in patients with HThy with a PTC diagnosis in FNAC. As a matter of fact, this issue merits further investigation.

AUTHORS' CONTRIBUTIONS

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

REFERENCES

1. Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. Lancet. 2016;388(10061):2783-95. https://doi.org/10.1016/S0140-6736(16)30172-6
2. Hashimoto H. Zur kenntniss der lymphomatosen verandderung der schilddruse (struma lymphomatosa). Arch fur Klin Chir. 1912;97(219):219-48.
3. Hanege FM, Tuysuz O, Celik S, Sakallıoglu O, Solmaz OA. Hashimoto's thyroiditis in papillary thyroid carcinoma: a 22-year study. Acta Otorhinolaryngol Ital. 2021;41(2):142-5. https://doi.org/10.14639/0392-100X-N1081
4. Feldt-Rasmussen U. Hashimoto's thyroiditis as a risk factor for thyroid cancer. Curr Opin Endocrinol Diabetes Obes. 2020;27(5):364-71. https://doi.org/10.1097/MED.0000000000000570
5. Graceffa G, Patrone R, Vieni S, Campella S, Calamia S, Laise I, et al. Association between Hashimoto's thyroiditis and papillary thyroid carcinoma: a retrospective analysis of 305 patients. BMC Endocr Disord. 2019;19(1):4-9. https://doi.org/ 10.1186/s12902-019-0351-x
6. Cappellacci F, Canu GL, Lai ML, Lori E, Biancu M, Boi F, et al. Association between Hashimoto thyroiditis and differentiated thyroid cancer: a single-center experience. Front Oncol. 2022;12:959595. https://doi.org/10.3389/fonc.2022.959595
7. Nagayama Y. Thyroid autoimmunity and thyroid cancer - the pathogenic connection: a 2018 update. Horm Metab Res. 2018;50(12):922-31. https://doi.org/10.1055/a-0648-4593
8. Anil C, Goksel S, Gursoy A. Hashimoto's thyroiditis is not associated with increased risk of thyroid cancer in patients with thyroid nodules: a single-center prospective study. Thyroid. 2010;20(6):601-6. https://doi.org/10.1089/thy.2009.0450
9. Mateša-Anić D, Mateša N, Dabelić N, Kusić Z. Coexistence of papillary carcinoma and Hashimoto's thyroiditis. Acta Clin Croat Suppl. 2009;48(1):9-12. PMID: 19623865.
10. Rio P, Montana Montana C, Cozzani F, Rossini M, Loderer T, Dall'Aglio E, et al. Is there a correlation between thyroiditis and thyroid cancer? Endocrine. 2019;66(3):538-41. https://doi.org/10.1007/s12020-019-01935-8
11. Cappelli C, Pirola I, Gandossi E, Lombardi D, Rotondi M, Casella C, et al. Could serum TSH levels predict malignancy in euthyroid patients affected by thyroid nodules with indeterminate cytology? Int J Endocrinol. 2020;2020:7543930. https://doi.org/ 10.1155/2020/7543930
12. Liang J, Zeng W, Fang F, Yu T, Zhao Y, Fan X, et al. Analisi clinica dell'associazione fra tiroidite di Hashimoto e carcinoma papillare della tiroide in 1392 pazienti. Acta Otorhinolaryngol Ital. 2017;37(5):393-400. https://doi.org/10.14639/0392-100X-1709
13. Uhliarova B, Hajtman A. Hashimoto's thyroiditis – an independent risk factor for papillary carcinoma. Braz J Otorhinolaryngol. 2018;84(6):729-35. https://doi.org/10.1016/j.bjorl.2017.08.012
14. Molnár C, Molnár S, Bedekovics J, Mokanszkil A, Gyory F, Nagy E, et al. Thyroid carcinoma coexisting with Hashimoto's thyroiditis: clinicopathological and molecular characteristics clue up pathogenesis. Pathol Oncol Res. 2019;25(3):1191-7. https://doi.org/10.1007/s12253-019-00580-w
15. Taşlı F, Vardar E, Özkok G. Tiroid neplazileri ve Hashimoto tiroiditi birlikteliği. ADÜ Tıp Fakültesi Derg. 2013;14(2):1-4. http://hdl.handle.net/11607/1892
16. Çayır D, Külah B, Bozkurt M, Taşkın Türkmenoğlu T. Factors influencing clinical outcomes in coexistence of chronic lymphocytic thyroiditis with papillary thyroid carcinoma. Turkiye Klin J Intern Med. 2020;5(1):8-14. https://doi.org/10.5336/intermed.2019-70935
17. Sengul D, Sengul I. Are there any variation in neutrophil lymphocyte ratio, mean platelet volume, and platelet count between papillary thyroid cancer and benign nodular thyroid diseases? Sanamed. 2018;13(1):11-6.
18. Sengul D, Sengul I. Can neutrophil lymhocyte ratio, mean platelet volume, and platelet count be determined as the diagnostic variables between papillary thyroid cancer and benign nodlar thyroid diseases? 41st annual meeting of the European Thyroid Association (ETA), Newcastle Upon Tyne, Newcastle, England, September, 15-18, 2018. Eur Thyroid J. 2018;7(Suppl. 1):68. https://doi.org/10.1159/000491542
19. Sengul I, Sengul D, Ozen O. A patient with conventional and follicular variant papillary thyroid microcarcinoma and Eagle's syndrome, Langlais type III, with hyoid bone variation. Ann Ital Chir. 2019;90:S2239253X19030895.
20. Xu J, Ding K, Mu L, Huang J, Ye F, Peng Y, et al. Hashimoto's thyroiditis: a "Double-Edged Sword" in thyroid carcinoma. Front Endocrinol (Lausanne). 2022;13:801925. https://doi.org/10.3389/fendo.2022.801925
21. Sengul D, Sengul I. Reassessing combining real-time elastography with fine-needle aspiration biopsy to identify malignant thyroid nodules. Am J Med Case Rep. 2021;9(11):552-3. https://doi.org/10.12691/ajmcr-9-11-9
22. Sengul I, Sengul D. Hermeneutics for evaluation of the diagnostic value of ultrasound elastography in TIRADS 4 categories of thyroid nodules. Am J Med Case Rep. 2021;9(11):538-9. https://doi.org/10.12691/ajmcr-9-11-5
23. Sengul I, Sengul D. Proposal of a novel terminology: minimally invasive FNA and thyroid minimally invasive FNA; MIFNA and thyroid MIFNA. Ann Ital Chir. 2021;92:330-1. PMID: 34312332
24. Sengul I, Sengul D. Delicate needle with the finest gauge for a butterfly gland, the thyroid: is it worth mentioning? Sanamed. 2021;16(2):173-4. https://doi.org/10.24125/sanamed.v16i2.515
25. Dailey ME, Lindsay S, Skahen R. Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. AMA Arch Surg. 1955;70(2):291. https://doi.org/10.1001/archsurg.1955.01270080137023

ORIGINAL ARTICLE

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Teaching and mental health in medical students

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SUMMARY

OBJECTIVE: The objective of this study was to evaluate the relationship between quality of life, perceived stress, anxiety, and depression in medical students and the university teaching method: traditional method versus active methodology.

METHODS: Four questionnaires were administered to volunteer students (n=361) enrolled in two institutions that employ active (Universidade Tiradentes) or traditional (Faculdade de Medicina do ABC) teaching methodology: socioeconomic level; brief quality of life (World Health Organization Quality of Life-Bref); perceived stress scale (PSS10); and depression and anxiety scale (hospital anxiety and depression scale).

RESULTS: Of the students who responded to the questionnaires (226 UNIT and 135 FMABC), 70% were female and 67% were White. The majority did not use medication for depression (90%), anxiety (81%), and stress management (91%). Regarding anxiety, it was found: absence in the traditional method and moderate anxiety in the active methodology (26% UNIT×13% FMABC) ($p<0.001$). Regarding quality of life, it was found to be better quality of life in the environment domain at FMABC (78.12%) versus 71.88% at the UNIT ($p<0.001$). There was no difference between the institutions in relation to depression and perceived stress, and in quality of lifethere was only a difference in the environmental domain ($p<0.001$). In relation to gender, stress was higher in females (93.7%) than males (79.6%) with $p<0.001$.

CONCLUSION: Differences were recorded between the groups regarding anxiety, with a predominance in UNIT students (active methodology), and no differences were recorded in relation to depression, perceived stress, and quality of life in all domains, except for the environment domain, which was higher in the traditional methodology, although about one-third of participants used medication for anxiety/depression.

KEYWORDS: Students, medical. Quality of life. Emotional stress. Depression. Anxiety. Education.

INTRODUCTION

Concerns about the mental health of university students have become relevant because studies have shown a high occurrence of psychological and psychiatric disorders in the general population. It is estimated that 15–25% of university students have psychiatric disorders, with depression and anxiety being the most common among medical students, whose rates of depression and suicidal ideation are higher than in the general population[1-3].

Quality of life is a complex and subjective concept, influenced by the individual's physical health, psychological state, level of independence, living conditions, and social relationships[4]. Stress corresponds to a series of neuroendocrinological changes suffered by an individual, called as "general adaptation syndrome," resulting from sources internal or external to the organism[5].

Anxiety is defined as feelings of apprehension, anguish, worry, and intense restlessness and may lead to systemic cardiorespiratory changes and oppression[6]. Depression is characterized as a mental disorder that determines changes in mood or affect, with functional disability and decreased quality of life[7].

In Brazil, two teaching methodologies are adopted in medical schools: traditional, where teaching is based on the student/teacher approach, and active, when the student is the center and responsible for building their learning. Several publications address aspects related to mental health and quality of life in medical students. However, there is a gap in the approach to these aspects in relation to teaching methods, justifying this study.

METHODS

After approval by the FMABC Ethics Committee (CAE no. 13152119.2.0000.0082), a cross-sectional, observational study was carried out with a quantitative approach, with analysis of self-administered questionnaires answered online by medical students of both genders who aged at least 18 years and

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enrolled in the second, third and fourth years of the course, constituting two groups, according to the pedagogical political plan of each institution, both private: GROUP 1: UNIT (UNIT)—Aracaju, SE, active methodology of teaching and GROUP 2: FMABC (FMABC)—Santo André, SP, traditional teaching methodology. Students in the first, fifth, and sixth years were excluded, due to the additional stress they are subjected to (entering college, change in course dynamics, social environment, being a better professional, studying for the residency exam, and finding a job, among others). The recruitment and data collection period was between April and August 2021. Inclusion criteria were medical students, over the age of 18 years, of both sexes, enrolled in the second, third, and fourth years of the course.

The following questionnaires were used: (1) demographic and socioeconomic issues to characterize the sample; (2) WHOQOL questionnaire—abbreviated, the WHOQOL-Brief, (3) perceived stress scale (PSS-10); and (4) anxiety and depression scale (hospital anxiety and depression scale—HADS). All questionnaires used were adapted, translated, and validated for the Brazilian population. For the sample calculation, the Barbetta formula was used (95% reliability).

Of the 490 enrolled at UNIT and 290 at FMABC, excluding students in the first, fifth, and sixth years, 361 questionnaires were completed appropriately, with 226 from UNIT and 135 from FMABC, after signing the consent form. The exploratory analysis was carried out by calculating simple frequency and percentage for qualitative variables, and median and minimum and maximum interquartile range for quantitative variables, using a Microsoft Excel spreadsheet, and statistical analyses were carried out in the R software, version 4.1.0 (The R Core Team, 2021). The significance level adopted was 5%.

RESULTS

Of the total of 361 students (226 UNIT and 135 FMABC) who responded to the survey, 70% were female, aged between 18 and 39 years (mean=22 years), White (92.2% FMABC, 52.2% UNIT), Niger (0.7% FMABC, 8% UNIT), and Brown (1.5% FMABC, 37.6% UNIT). The majority declared themselves to be Catholic (30% FMABC, 54.4% UNIT), to have no religion (51.1% FMABC), to live at home with their parents (65.6% FMABC, 64.2% UNIT), to share house in shares home with one or more colleagues (19.3% FMABC, 12.4% UNIT), and to stay alone (11.9% FMABC, 16.4% UNIT).

Most students lived less than 30 min from the institution (UNIT—90.7% versus FMABC—40.4%, p<0.001), the car was the most used means of transport to college (74% FMABC, 66.4% UNIT) (p=0.116), and 27% had student financing at UNIT versus 10.4% at FMABC (p<0.001).

Table 1 shows the percentage of students who used medication for health and for mental disorders and stress. It was found that 36.3 and 34.9% of FMABC and UNIT students used medication, respectively.

All FMABC students had stress and, at the UNIT, 96% of them reported some degree of stress, but there was no statistical difference. In relation to gender, stress (moderate and high) was higher in females (93.7%) than males (79.6%) with p<0.001. Anxiety was reported with p<0.001: absence, in the traditional method (51.1%) versus (45.1%) in UNIT; more frequent moderate anxiety, in the active methodology (26.5%) versus traditional method (13.3%). On the contrary, depression was mostly not reported (70.4% FMABC and 76.5% UNIT) and the other degrees (mild, moderate, and severe) with no statistical difference in both HEIs, as we can observe in Table 2.

In relation to the year of teaching, there were no statistical differences in the population studied. However, the second year

Table 1. Frequency of medication consumption among medical students at two private educational institutions (FMABC and UNIT) (n=361).

Variables		FMABC	UNIT	p-value
		135 (%)	226 (%)	
Use of medicines for health	No	84 (62.2)	157 (69.5)	0.194[1]
	Yes	51 (37.8)	69 (30.5)	0.194[1]
Use of medicines for depression	No	115 (85.2)	211 (93.4)	<0.001[1]
	Yes	20 (14.8)	15 (6.6)	<0.001[1]
Use of medicines for anxiety	No	109 (80.7)	184 (81.4)	0.984[1]
	Yes	20 (19.3)	42 (18.6)	0.984[1]
Use of medicines for stress control	No	126 (93.3)	204 (90.3)	0.416[1]
	Yes	9 (6.7)	22 (9.7)	0.416[1]

[1]Significance level of the chi-square test.

presented a low risk of stress on average (5.5%) compared with the fourth year (13%). Anxiety in the form of mild or moderate was higher in the second year (46.5%) compared with the fourth year (36.2%), without statistical differences. It was observed that there was a higher risk of mild depression in the second year (18.1%) and fourth year (13%) of teaching and a moderate risk for depression in 9% of students in the second year compared with those in the fourth year (12%), but without statistical difference.

Table 3 shows the results of the questionnaires applied to identify stress, anxiety, depression, and QoL. Statistical differences were recorded regarding the anxiety aspect: absence in FMABC, more frequent moderate anxiety in UNIT (26% UNIT×13% FMABC; p<0.001), and better QoL in the environment domain at FMABC [78.12 vs. 71.88% UNIT (p<0.001)].

Table 2. Responses to the perceived stress scale and HDA questionnaires by medical students from two private institutions (FMABC and UNIT) (n=361).

Variables	FMABC	UNIT	p-value
	135 (%)	226 (%)	
PSS-10 questionnaire			
Low	17 (12.6)	21 (9.3)	0.511[1]
(Perceived stress)			
Moderate	72 (53.3)	118 (52.2)	0.511[1]
High	46 (34.1)	87 (38.5)	0.511[1]
HAD-A questionnaire			
No	65 (51.1)	102 (45.1)	<0.001[1]
(Anxiety)			
Mild	27 (20)	37 (16.4)	<0.001[1]
Moderate	18 (13.3)	60 (26.5)	<0.001[1]
Severe	21 (15.6)	27 (11.9)	<0.001[1]
HAD-D questionnaire			
No	95 (70.4)	173 (76.5)	0.246[2]
(Depression)			
Mild	18 (13.3)	32 (14.2)	0.246[2]
Moderate	19 (14.1)	19 (8.4)	0.246[2]
Severe	3 (2.2)	2 (0.9)	0.246[2]

[1]Significance level of the chi-square test. [2]Significance level of the Fisher's exact test.

DISCUSSION

The analysis of the QoL questionnaires showed that students from both schools did not differ in the different domains, except the environment, which could be justified by the difference in the Human Development Index (HDI) between the cities (0.815—Santo André and 0.79—Aracaju)[8]. The lowest values occurred in the psychological domain, in agreement with the results of Jesus et al[9]. In females, QL levels were lower in both groups, which is repeated in the literature consulted[10].

All students reported some degree of stress, with the sum of moderate and severe levels being high (87.4% FMABC and 90.7% Unit). During the course, stress increases due to the demands of academic life and interpersonal and emotional conflicts, such as situations of death and suffering experienced, especially in recent years, during the internship period, when students maintain direct and continuous contact with patients[11,12]. In this study, it was observed that advancing the course increased stress and reduced anxiety, coinciding with the literature consulted[13-16]. The need to improve cognitive performance, concentration, and memory and to increase study time, experimentation, and the influence of friends are factors that can lead to an increase in the use of psychotropic drugs by students[17].

The prevalence of depression in medical students ranges from 5.60 to 45.70%[18], and almost 30% of these use some medication for anxiety or depression[13].

The medical course causes stress, due to students being subjected to fear of failure, self-demand, demands from parents, extensive workload, curricular and extracurricular activities at the college, and situations capable of causing greater susceptibility to various psychiatric disorders[14]. All participants in this research reported some degree of stress, with no difference between the groups, a result greater than that reported in

Table 3. Responses to the WHOQOL and its domains by medical students enrolled in two private educational institutions (FMABC and UNIT) (n=361).

Variables	FMABC	UNIT	p-value
	135 (%)	226 (%)	
Age	22	22	0.957[1]
Domain 1—physical domain	67.86	67.86	0.81[1]
Domain 2—psychological domain	58.33	62.5	0.378[1]
Domain 3—social relations	66.67	66.67	0.691[1]
Domain 4—environment	78.12	71.88	**<0.001**[1]

[1]Significance level of the Mann-Whitney test. The statistically significant p-value is indicated in bold.

the literature (stress levels ranging from 40.95 to 84.30%) but higher in females, coinciding with the values of this research[15,16].

In this study, some degree of anxiety was observed in the institution that adopts the traditional methodology and a greater degree in the active methodology. A possible explanation for the greater tendency toward anxiety in individuals who use the active methodology would be the fact that, in this, the student needs to go in search of knowledge. Several authors report a higher frequency of stress, anxiety, and depression in students using the active methodology, when compared with the traditional method, due to the pace imposed by the former, with the need for greater active participation by the student in research, meetings, and discussions[19,20]. A direct relationship between stress and depression has been found in several studies, with a high degree of stress being related to moderate and severe depression, in agreement with other authors[21-23].

CONCLUSION

The differences observed between the groups point to a higher frequency of anxiety in students undergoing the active methodology than in the traditional one, with no differences regarding depression and perceived stress. One-third of the participants used medication for anxiety/depression and the score obtained in the psychological domain was low in both universities, suggesting possible psychological distress in students. Monitoring mental health and QoL in medical students is necessary regardless of the teaching method.

AUTHORS' CONTRIBUTIONS

HFO: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **MRDA:** Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. **JRSS:** Formal Analysis, Methodology, Validation. **HAF:** Supervision, Writing – review & editing. **NFW:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. ROSS: Project administration, Resources, Supervision, Validation, Writing –original draft, Writing – review & editing.

REFERENCES

1. Bassols AM, Okabayashi LS, Silva AB, Carneiro BB, Feijó F, Guimarães GC, et al. First- and last-year medical students: is there a difference in the prevalence and intensity of anxiety and depressive symptoms? Braz J Psychiatry. 2014;36(3):233-40. https://doi.org/10.1590/1516-4446-2013-1183
2. Al-Maashani M, Al-Balushi N, Al-Alawi M, Mirza H, Al-Huseini S, Al-Balushi M, et al. Prevalence and correlates of depressive symptoms among medical students: a cross-sectional single-centre study. East Asian Arch Psychiatry. 2020;30(1):28-31. https://doi.org/10.12809/eaap1882
3. Bert F, Lo Moro G, Corradi A, Acampora A, Agodi A, Brunelli L, et al. Prevalence of depressive symptoms among Italian medical students: the multicentre cross-sectional "PRIMES" study. PLoS One. 2020;15(4):e0231845. https://doi.org/10.1371/journal.pone.0231845
4. Dias AF, Junior CSC, Moreira VES. Stress and academic performance in students of UNEC Anais do VIII Encontro de Iniciação Científica - ENIC UNEC 2018 - Caratinga-MG, 25 a 27 de outubro de 2018 - ISSN 2237-8618
5. Schönhofen FDL, Neiva-Silva L, Almeida RBD, Vieira MECD, Demenech LM. Generalized anxiety disorder among pre-university students. J Bras Psi. 2020;69(3):179-86. https://doi.org/10.1590/0047-2085000000277
6. Silva NR, Bolsoni-silva AT, Loureiro SR. Burnout and depression in elementary school teachers: a correlational study. Rev Bras Educ. 2018;23(1):e230048. https://doi.org/10.1590/S1413-24782018230048
7. Moreira EEP, Oliveira SMC, Oliveira JVP. Contributes and challenges of problem-based learning in medical training. Res Soc Dev. 2020;9(8):1-18. https://doi.org/10.33448/rsd-v9i8.5361
8. IBGE. Cities. 2020. [cited on 2023 Oct 13]. Avaiable from: https://l1nk.dev/QSJVF
9. Jesus AMV, Oliveira HF, Azevedo MRD, Santos RGA, Nogueira HG. Analysis of medical student's life quality: a systematic review. Res Soc Dev. 2022;11(8):1-9. https://doi.org/10.33448/rsd-v11i8.30548
10. Silveira DJ, Bomfim Neto R, Silveira KM, Santos Júnior EL, Ferro Neto PM, Pimentel D. Mental disorders and academic impact on medical students subjected to the problem-based learning method. Braz J Dev. 2021;7(8):83040-56. https://doi.org/10.34117/bjdv7n8-488
11. Luna IS, Dominato AAG, Ferrari F, Costa AL, Pires AC, Silva Ximendes G. Consumption of psychotropic drugs among first and sixth year medical students at a university in the state of São Paulo. Colloq Vitae. 2018;10(1):22-8. https://doi.org/10.5747/cv.2018.v10.n1.v216
12. Cruz PE, Caramona M, Guerreiro, MP. A reflection on self-medication and non-prescription medicines in Portugal. Rev Port Farmacoter. 2015;7(2):83-90. https://doi.org/10.25756/rpf.v7i2.2
13. Brito JR, Silva PR. Consumption of anxiolytics and antidepressants: an analysis of their use among medical students. 2020. Available from: https://repositorio.pucgoias.edu.br/jspui/bitstream/123456789/2092/1/UMA%20AN%C3%81LISE%20SOBRE%20O%20CONSUMO%20DE%20ANSIOL%C3%8DTICOS%20E%20ANTIDEPRESSIVOS%20ENTRE%20ESTUDANTES%20DE%20MEDICINA%20%28corrigido%29.pdf
14. Silveira DJAS, Bomfim Neto R, Silveira KMAS, Santos Júnior EL, Ferro Neto PM, Pimentel D. Mental disorders and the academic impact on medical students submitted to the problem-based learningmethod. Braz J Dev. 2021;7(8):83040-56. https://doi.org/10.34117/bjdv7n8-488
15. Aragão JA, Freire MRM, Nolasco Farias LG, Diniz SS, Sant'anna Aragão FM, Sant'anna Aragão IC, et al. Prevalence of depressive symptoms

among medical students taught using problem-based learning versus traditional methods. Int J Psychiatry Clin Pract. 2018;22(2):123-8. https://doi.org/10.1080/13651501.2017.1383438

16. Machado SLM, Sirico NDS, Barbosa PF, Rosa RRM. Anxiety and depression in medical students. 2019. Available from: http://revistas.famp.edu.br/revistasaudemultidisciplinar/article/view/74
17. Medeiros JM, Barbosa AG. Nonprescription use of methylphenidate hydrochloride among college students. Rev Int. 2019;15:2(2). http://doi.org/10.24219/rpi.v2i2.0.275
18. Mendes TC, Dias ACP. Symptoms of depression, anxiety, stress and associated factors in Brazilian medical students: integrative review. Res Soc Dev. 2021;10(4):e14910414033. http://doi.org/10.33448/rsd-v10i4.14033
19. Ferreira JL, Rossi SV, Araujo KF, Klein MP, Miranda NR, Campos VR. Prevalence of common mental disorders and associated factors in medical students: a comparative study. Rev Bras Educ Med. 2016;40(2):268-77. https://doi.org/10.1590/1981-52712015v40n2e02812014
20. Ariño DO, Bardagi MP. Relationship between academic factors and the mental health of university students. Psicol Pesq. 2018;12(3):44-52. http://doi.org/10.24879/2018001200300544
21. Ribeiro CF, Lemos CMC, Alt NN, Marins RLT, Corbiceiro WCH, Nascimento MI. Prevalence of and factors associated with depressionand anxiety in Brazilian medical students. Rev Bras Educ Med. 2020;44(1):1-8. https://doi.org/10.1590/1981-5271v44.1-20190102.ING
22. Costa DS, Medeiros NSB, Cordeiro RA, Frutuoso ES, Lopes JM, Moreira SNT. Symptoms of depression, anxiety and stress in medical students and institutional coping strategies. Rev Bras Educ Med. 2020;44(1):1-10. https://doi.org/10.1590/1981-5271v44.1-20190069
23. Solis AC, Lotufo-Neto F. Predictors of quality of life in Brazilian medical students: a systematic review and meta-analysis. Braz J Psychiatry. 2019;41(6):556-67. https://doi.org/10.1590/1516-4446-2018-0116

ORIGINAL ARTICLE

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Mortality related to sickle cell disease and COVID-19 in Brazil, 2020

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SUMMARY

OBJECTIVE: The ability to cause death is the definitive measure of an infectious disease severity, particularly one caused by a novel pathogen like severe acute respiratory syndrome-CoV-2 (COVID-19). This study describes sickle cell disease-related mortality issues during the COVID-19 pandemic in Brazil.

METHODS: The provisional 2020 mortality data originated from the public databases of the Mortality Information System and were investigated using the multiple-cause-of-death methodology.

RESULTS: In 2020, 688 sickle cell disease-related deaths occurred, of which 422 (61.3%) had an underlying cause of death and 266 (38.7%) had an associated cause of death. Furthermore, 98 COVID-19-related deaths occurred, of which 78 were underlying cause of death among sickle cell disease associated (non-underlying) cause of death. Sickle cell disease-related deaths occurred mostly among young adults aged 25–49 years. COVID-19 deaths occurred at ages older than among sickle cell disease-related deaths. Majority of deaths happened in the southeast (42.3%) and northeast regions (34.0%), while COVID-19 deaths prevailed in the northeast region (42.9%). Regarding overall deaths, the leading underlying cause of death was sickle cell disease itself, followed by infectious and parasitic diseases (14.8%), owing to COVID-19 deaths, and diseases of the circulatory system (8.9%). Next, in males, diseases of the digestive system (4.8%) occurred, while, in females, maternal deaths succeeded, included in the chapter on pregnancy, childbirth, and the puerperium, accounting for 5.9% of female deaths. The leading overall associated (non-underlying) cause of deaths were septicemias (29.4%), followed by respiratory failure (20.9%), pneumonias (18.3%), and renal failure (14.7%).

CONCLUSION: In Brazil, COVID-19 deaths produced trend changes in sickle cell disease-related causes of death, age at death, and regional distribution of deaths in 2020.

KEYWORDS: Sickle cell disease. COVID-19. Multiple-cause-of-death. Mortality. Cause of death. Brazil.

INTRODUCTION

The ability to cause death is the definitive measure of an infectious disease severity, particularly one caused by a novel pathogen like severe acute respiratory syndrome (SARS)-CoV-2 (COVID-19). Mortality rates improve the knowledge about the character of a disease, identify at-risk population, and evaluate the quality of health care[1]. Epidemiological findings suggest that persons with sickle cell disease (SCD) who are infected with COVID-19 have a higher risk of severe disease course and higher mortality rates compared with COVID-19 infections in the general population of similar ages[2]. The higher risk for serious illness from COVID-19 results from the chronic inflammation linked with enhanced risk of thrombosis, especially during a vaso-occlusive crisis. Patients with SCD are also immunocompromised due to spleen autoinfarction or surgical splenectomy often due to red-cell splenic sequestration. Furthermore, comorbidities and the cumulative impact of acute and chronic complications lead to progressive organ damage in SCD patients which increases the mortality risk from COVID-19-related SARS.

This paper aims to describe national mortality related to SCD during the COVID-19 pandemic in 2020 in Brazil using the methodology of multiple causes of death.

METHODS

The provisional 2020 annual mortality data were extracted from the public multiple-cause-of-death databases of the Mortality Information System (Sistema de Informações Sobre Mortalidade – SIM) located at the Brazilian Unified Health System Information Technology Department (Departamento de Informática do Sistema Unico de Saúde – DATASUS), Ministry of Health (MH)[3]. All deaths were included in which SCD, three-character category D57 of the International Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10), as a cause of death, was listed on any line or in either part of the WHO International Form of Medical Certificate of Cause-of-Death (the medical certification section of the death certificate), irrespective of whether they were characterized as the underlying cause of death (UCOD)

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or as an associated (non-underlying) cause of death (ACOD). Complications of the underlying cause (part I of the medical certification section) and contributing causes (part II of the medical certification section) were jointly designated as associated (non-underlying) causes of death[4-6]. We employed the 2020 mid-year estimates of the population for Brazil, discriminated by year, sex, age group, and Brazilian regions.

The World Health Organization (WHO), and the Ministry of Health, in Brazil, defined the guidelines to standardize the medical certification on ICD-10 mortality processing and UCOD selection for COVID-19-related deaths[7-9]. As a cause of death, COVID-19 should be identified using ICD-10 four-character subcategory B34.2 with pandemic markers U07.1 (COVID-19, virus identified) and U07.2 (COVID-19, virus not identified). The markers would help the investigation of the cause of death process, specifying if the diagnosis of COVID-19 was confirmed or not. Throughout the first semester of 2020, the code U04.9 was used to refer to SARS to identify deaths from COVID-19. In July 2020, a note or the MH revised the use of code U04.9 and addressed the use of code J98.8 instead or in conjunction[10]. The U04.9 code would only remain in the SIM if the SARS were part of the events chain that led to death.

Using mortality rates and proportions, we studied the distributions of the following variables: sex, age at death (in 5-year age groups), race/color, year of death, UCOD, associated (non-underlying) cause(s) of death, total mentions of each cause of death, mean number of causes listed per death certificate, and geographical distribution of deaths. SCD mortality rates (per one million population) as UCOD and ACOD for males and females were calculated. The expected number of deaths in 2020 was found by applying 2019 age-specific SCD-death rates to the 2020 population estimates. Standardized mortality ratios (SMRs) between 2019 and 2020 deaths were calculated. Medical and demographic variables were processed using the following software: dBASE III Plus, version 1.1, dBASE IV (Ashton-Tate Corporation, Torrance, CA), and Epi Info, version 6.04d (Centers for Disease Control and Prevention, Atlanta, GA), Excel 2016 (Microsoft Corporation, Redmond, WA). The Multiple Causes Tabulator (Tabulador de Causas Múltiplas for Windows) (TCMWIN, version 1.6) program (DATASUS, Ministério da Saúde, Faculdade de Saúde Pública, Universidade de São Paulo, Brazil) processed ICD-10 codes in the presentation of the associated causes and of the mean number of causes per death certificate[11]. The sex and age-adjusted crude and average mortality rates for the study period were standardized, by the direct method, to the new WHO Standard Population[12]. Crude and standardized rates were calculated according to the 5-year age groups.

We used analysis of variance to compare the mean number of causes mentioned on the death certificate and the Kruskal-Wallis H test to compare the mean age at death between groups. Statistical significance was set at $p<0.05$.

The study was waived by the author's institutional review boards because it exclusively uses large public domain national mortality databases of secondary data that are anonymous, without nominal personal identification, and therefore carries no individual risk whatsoever. The ethical principles contained in the Resolution of the National Health Council (CNS) n. 466 of December 12, 2012, were observed.

RESULTS

The provisional data show 1,552,740 deaths recorded in Brazil during 2020. Of these, 688 were related to SCD, of which 422 (61.3%) identified as the UCOD and 266 (38.7%) as the ACOD (Table 1). Furthermore, among sickle cell-related deaths, 98 COVID-19-related deaths occurred as follows: 78 COVID deaths as UCOD along with 266 ACOD sickle cell; 6 deaths as ACOD cause with sickle cell UCOD; 6 deaths as ACOD with other UCOD causes, and 8 deaths coded as U04.9 with sickle cell as UCOD. In Brazil, all mentioned COVID-19 deaths occurred 14.2% among overall SCD-related deaths, of which 11.3% were the UCOD.

Male and female 2020 standardized mortality rates were 2.02 and 1.92 per million for UCOD and 1.10 and 1.24 per million for ACOD (non-underlying), respectively. Figure 1 updates the mortality SCD-related trend since 2000, including 2019 and 2020 data. The SMRs resulting from the comparison between 2020 observed with expected SCD-related deaths, for males and females, as UCOD, were 0.76 and 0.89, and as ACOD, 1.45 and 1.18, respectively.

Around 82% of deaths occurred among brown and black persons. Majority of SCD-related deaths happened in the southeast region, mostly in São Paulo (18.5%), Rio de Janeiro (12.2%), and Minas Gerais (10.5%), followed by the northeast region, in the states of Bahia (18.6%). However, COVID-19-related deaths occurred in the northeast region, in Bahia (25.5%), and southeast, in Rio de Janeiro (16.3%) and São Paulo (13.3%). Otherwise, higher COVID-19-specific proportional mortality was observed in the northeast, center-west, and north regions. SCD-related deaths occurred mostly among young adults aged 25–49 years (Table 1).

The UCOD on 688 certificates for males and females in which SCD was listed as a cause of death is presented in Table 2, according to the ICD structure. SCD occurred in 61.3% of overall deaths. The second major UCOD was included in the ICD

chapter on infection and parasitic diseases, tallying 14.8% of deaths, owing to 78 deaths caused by the coronavirus infection, followed by the circulatory system diseases. In males, gastrointestinal system diseases were observed, while, in females, maternal deaths, included in the chapter on pregnancy, childbirth, and the puerperium, accounted for 5.9% of female deaths.

The leading associated (non-underlying) causes of death listed on UCOD death certificates with SCD and other selected conditions for 2020 include septicemias, occurring in 29.4% of overall deaths, followed, among sickle cell UCOD, by pneumonia, respiratory failure, and renal failure, whereas, among other UCODs, by respiratory failure, shock, renal failure, pneumonia, and hypertensive diseases. The main ACODs related to COVID-19 deaths were respiratory failure (33.7%), SARS (26.5%), pneumonia (26.5%), septicemias (23.5%), and renal failure (12.2%).

DISCUSSION

This study describes the mortality related to SCD in Brazil in 2020 looking for the impact of the COVID-19 pandemic on its course and characteristics by means of the comparison with 2000–2018 and 2019 data. As in the previous study, the multiple-cause-of-death methodology was used to profit from its benefits considering all mentioned death certificates where SCD was listed as a cause of death[13]. Note that the Ministry of Health emphasizes the use of multiple causes as mandatory to the study of the COVID-19 mortality[8].

Overall, in 2020, SCD was identified as the UCOD in 61.3% of death certificates. This proportion is the lowest ever observed since the start of multiple-cause methodology use in Brazil. The average percentage of SCD as UCOD from 2000 to 2018 was 70.5%, and in 2019, it reached 71.6% among 714 SCD-related deaths. Therefore, SCD deaths were relocated

Table 1. Deaths related to sickle cell disease and COVID-19 according to the qualification of the cause of death, sex, age at death, race/color, and Brazilian regions, Brazil, 2020.

	SCD causes of death						COVID-19 total deaths		
	Underlying		Associated		Total				
	n	%	n	%	n	%	n	%	SPM%
All deaths	422	61.3	266	38.7	688	100.0	98	100.0	14.2
Sex									
Male	211	50.0	122	45.9	333	48.4	48	49.0	14.4
Female	211	50.0	144	54.1	355	51.6	50	51.0	14.1
Age at death (years)									
00–04	20	62.9	4	37.1	24	3.5	2	2.0	8.3
05–24	134	77.2	52	22.8	186	27.0	25	25.5	13.4
25–49	177	73.5	129	26.5	306	44.5	46	46.9	15.0
50–74	79	62.4	61	37.6	140	20.3	19	19.4	13.6
75 and more	12	69.0	20	31.0	32	4.7	6	6.1	18.8
Race/color*									
White	65	15.9	46	18.1	111	16.7	14	14.7	12.6
Black	120	29.3	80	31.5	200	30.1	26	27.4	13.0
Yellow	1	0.2	3	1.2	4	0.6	1	1.1	25.0
Brown	221	53.9	125	49.2	346	52.1	54	56.8	15.6
Indigenous	3	0.7	0	0.0	3	0.5	0	0.0	0
Brazilian regions									
North	42	79.2	11	20.8	53	7.7	7	7.1	13.2
Northeast	136	58.1	98	41.9	234	34.0	42	42.9	17.9
Southeast	178	61.0	114	39.0	292	42.4	33	33.7	11.3
South	20	57.1	15	42.9	35	5.1	4	4.1	11.4
Center_West	46	62.2	28	37.8	74	10.8	12	12.2	16.2

Source: Ministry of Health, Unified Health System Information Technology Department (provisional data). SPM% = Specific proportional mortality. *Race/color available data on death certificates not totally complete.

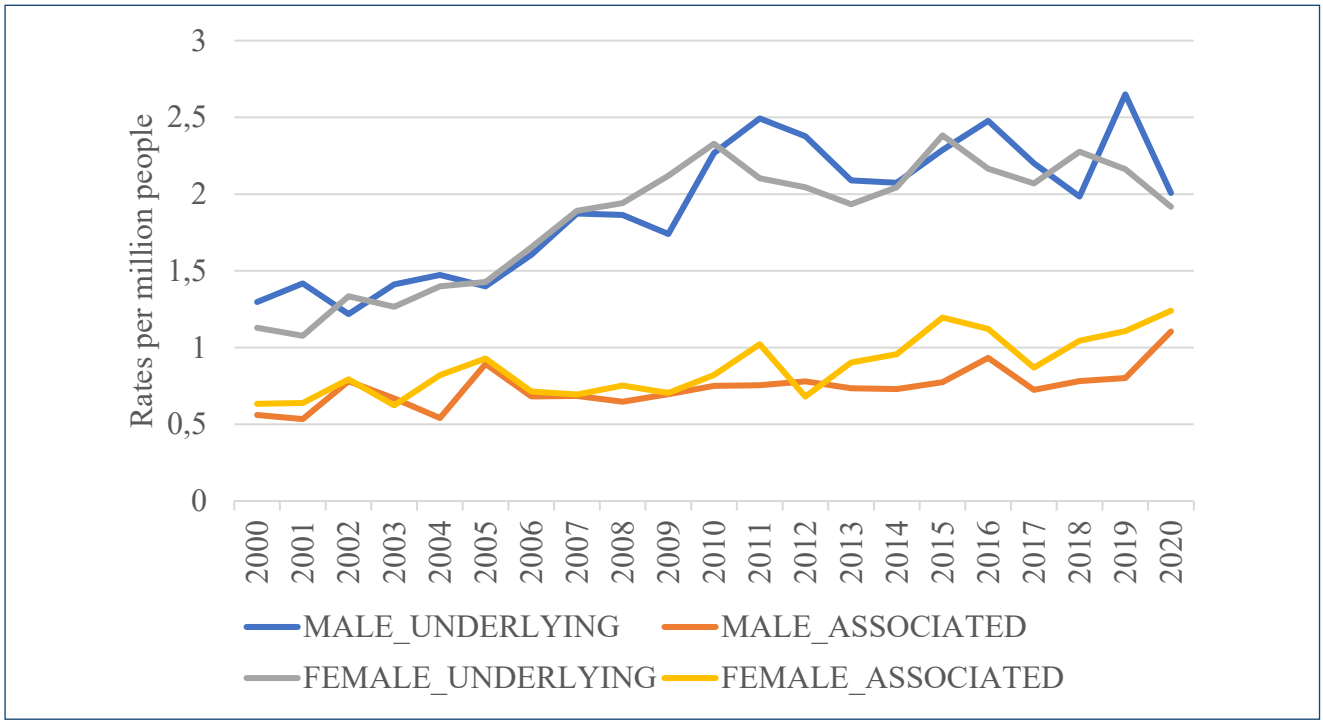

Figure 1. Age-standardized death rates related to sickle cell disease according to causes of death and sex in Brazil, 2000–2020.

as ACOD (non-underlying), reaching 38.7% among all mentioned ones. SCD ACOD attained then the highest value in the historical series, greater than 28.4% verified among 2019 related-SCD deaths. COVID-19 deaths, 29.3% among ACOD, are accountable for this result.

The description of SCD mortality trends was presented before[13]. The highest UCOD mortality rates were verified for females in 2015, followed by leveling, and for males in 2019, dropping in 2020 (Figure 1). Owing to COVID-19 deaths, the highest ACOD mortality rates occurred in 2020. Compared with the 2019 deaths, the SMRs confirmed the drop in UCOD and the increase in ACOD. The investigation of SCD-related mortality in the United States in 2020, compared with previous years, also identified an increase in deaths during the pandemic considering all mentions of SCD-related mortality[14].

The distribution of deaths among Brazilian regions in 2020 in comparison with 2000–2018 and 2019 deaths shows less non-significant proportional increases in the north, northeast, and south regions and a decrease in the southeast region. Otherwise, the predominance of COVID-19 deaths in the northeast region, in the state of Bahia, is a subject of concern and further studies. The state of Bahia concentrates the largest proportion of hemoglobinopathies, particularly SCD, in Brazil[15]. Additionally, a survey of COVID-19 deaths in Belo Horizonte, Salvador, and Natal in 2020 observed that Salvador concentrated 57.7% of COVID-19 deaths and whose proportion in the total number of deaths was much higher than in the other two capitals[9], such as also observed among SCD-related deaths.

The distinct outline of UCOD distribution among SCD-related mortality during 2020, in comparison with former years, points to the severity of COVID-19 deaths. Secondary only to SCD as the most frequent specific UCOD, COVID-19 deaths displaced even the cerebrovascular diseases and, considering ICD-10 chapters, the infectious and parasitic diseases replaced the circulatory system diseases. Among female deaths, the increase in the maternal proportional mortality of 5.9% in comparison with the 4.6% average verified in 2000–2018[14] must be emphasized owing to three COVID-19 as ACOD.

While in Brazil, COVID-19 deaths occurred 14.2% with SCD-related deaths, of which 11.3% were identified as the UCOD, in the United States, COVID-19 deaths were associated with 8.4% of SCD-related deaths[14]. To the best of our knowledge, the United States and the Brazilian are the only ones available published country mortality studies over this time.

The overall outline of ACOD (non-underlying causes) studied in 2020 follows an equivalent composition as in the 2000–2018 period when related to SCD UCOD or other UCOD[13]. Septicemias, respiratory failure, pneumonia, renal failure, shock, hypertensive, and cerebrovascular diseases stand for the most frequently associated causes. Identified as an ad hoc cause of death owing to the COVID-19 pandemic, SARS is mentioned as ACOD among others UCOD, split according

Table 2. Underlying causes on death certificates that listed sickle cell disease as a cause of death, Brazil, 2020.

Underlying causes of death (ICD-10 chapters and rubrics)	Male		Female		Total	
	n	%	n	%	n	%
Certain infectious and parasitic diseases (A00–B99)	**52**	**15.6**	**50**	**14.1**	**102**	**14.8**
Intestinal infectious disease (A00–A09)	1	0.3	4	1.1	5	0.7
Tuberculosis (A15–A19, B90)	3	0.9	0	0.0	3	0.4
Dengue and dengue hemorrhagic fever (A90–A91)	3	0.9	2	0.6	5	0.7
Viral hepatitis (B15–B19)	4	1.2	2	0.6	6	0.9
Human immunodeficiency virus (HIV) disease (B20–B24)	1	0.3	2	0.6	3	0.4
Coronavirus infection, unspecified (B34.2)	39	11.7	39	11.0	78	11.3
Neoplasm (C00–D48)	**3**	**0.9**	**5**	**1.4**	**8**	**1.2**
Diseases of blood and blood-forming organs (D50–D890)	**215**	**64.6**	**217**	**61.1**	**432**	**62.8**
Sickle-cell disorders (D57)	211	63.4	211	59.4	422	61.3
Endocrine nutritional and metabolic diseases (E00–E90)	**5**	**1.5**	**6**	**1.7**	**11**	**1.6**
Diabetes mellitus (E10–E14)	3	0.9	5	1.4	8	1.2
Diseases of the circulatory system (I00–I99)	**30**	**9.0**	**31**	**8.7**	**61**	**8.9**
Hypertensive diseases (I10–I13)	7	2.1	4	1.1	11	1.6
Ischemic heart diseases (I20–I25)	1	0.3	2	0.6	3	0.4
Pulmonary heart diseases and diseases of pulmonary circulation (I26–I28)	2	0.6	4	1.1	6	0.9
Heart failure (I50–I51)	2	0.6	3	0.8	5	0.7
Cerebrovascular diseases (I60–I69)	11	3.3	13	3.7	24	3.5
Diseases of the respiratory system (J00–J99)	**3**	**0.9**	**1**	**0.3**	**4**	**0.6**
Diseases of the digestive system (K00–K93)	**16**	**4.8**	**13**	**3.7**	**29**	**4.2**
Disease of liver (K70–K76)	7	2.1	6	1.7	13	1.9
Disorders of gall bladder, biliary tract, and pancreas (K80–K86)	4	1.2	2	0.6	6	0.9
Other diseases of the digestive system (K90–K92)	1	0.3	2	0.6	3	0.4
Diseases of the genitourinary system (N00–N99)	**3**	**0.9**	**5**	**1.4**	**8**	**1.2**
Renal failure (N17–N19)	2	0.6	4	1.1	6	0.9
Pregnancy, childbirth, and the puerperium (O00–O99)	**–**	**–**	**21**	**5.9**	**21**	**3.1**
Other underlying causes of death	**6**	**1.8**	**6**	**1.7**	**12**	**1.7**
Total	**333**	**100.0**	**355**	**100.0**	**688**	**100.0**

Source: Ministry of Health, Unified Health System Information Technology Department Rubrics and codes of the International Statistical Classification of Diseases and Related Health Problems, Tenth Revision. Bold values refer to the titles of ICD-10 Chapters, while the indented causes of death refer to its included rubrics.

to the proposed codes (U04.9 and J98.8), following previous paper approach[9].

This study has many limitations. Without doubt, the provisional mortality data for 2020 is the main question. COVID-19 is a new epidemic disease and its record may be hampered by the lack of knowledge by the medical community. Otherwise, some deaths may be under revision by the surveillance system until their inclusion or removal from mortality data. Otherwise, the official guidance on COVID-19 coding as a cause of death occurred in May 2020; as before the U04.9 was used to identify SARS meant for COVID-19 during the first semester of 2020. Notice that, in this paper, deaths including U04.9 are identified as ACOD and remain with their original UCOD. Therefore, despite the severity of the pandemic, COVID-19 deaths were underreported as a cause of death in the first semester of 2020. Conversely, the crude mean number of 3.67 causes per overall death certificate and the unprecedented crude mean number of 5.02 per COVID-19 UCOD certificates stand for the quality of death certification of SCD-and COVID-19-related deaths, as referred elsewhere[9]. The COVID-19 mean number results from its double codification as B34.2 with its mark numbers U07.1 or U07.2.

CONCLUSION

In Brazil, the COVID-19 pandemic produced outlined changes in SCD-related causes of death, age at death, and regional distribution of deaths in 2020. SCDs as UCOD were relocated to ACOD due to the severity of COVID-10. The prevalence of COVID-19 deaths in the northeast region, especially in the city of Salvador, deserves further research, as well as the infectious and parasitic diseases as the second most frequent UCOD and the proportional rise of maternal deaths in females.

AUTHORS' CONTRIBUTIONS

AHS: Writing – original draft.

REFERENCES

1. World Health Organization. Estimating mortality from COVID-19. Scientific brief. 4 August 2020. Geneva: World Health Organization; 2020.
2. Panepinto JA, Brandow A, Mucalo L, Yusuf F, Singh A, Taylor B, et al. Coronavirus disease among persons with sickle cell disease, United States, March 20-May 21, 2020. Emerg Infect Dis. 2020;26(10):2473-6. https://doi.org/10.3201/eid2610.202792
3. Ministério da Saúde. Departamento de Informática do SUS. Acesso à Informação. Serviços. transferência-download-de-arquivos. arquivos-de-dados. Available at Transferência de Arquivos – DATASUS. 2022. Available from: https://www.gov.br/saude/pt-br
4. World Health Organization. International statistical classification of diseases and related health problems. Tenth Revision. vol. 1. Geneva: World Health Organization; 1993.
5. World Health Organization. International statistical classification of diseases and related health problems. Tenth Revision. vol. 2. Instruction Manual. Geneva: World Health Organization; 1993.
6. Santo AH. Causas múltiplas de morte: formas de apresentação e métodos de análise. [Multiple causes of death: presentation forms and analysis methods] [Tese de Doutorado]. São Paulo (SP): Faculdade de Saúde Pública, Universidade de São Paulo; 1988.
7. World Health Organization. International guidelines for certification and classification (coding) of COVID-19 as cause of death. Based on ICD. International Statistical Classification of Diseases (16 April 2020). Geneva: World Health Organization; 2020.
8. Ministério da Saúde. Secretaria de Vigilância em Saúde Departamento de Análise em Saúde e Vigilância de Doenças não Transmissíveis. Orientações para codificação das causas de morte no contexto da COVID-19. Brasília/DF Versão 1 • Publicada em 11/05/2020. Brasília: Ministério da Saúde; 2020.
9. Nogales Vasconcelos AM, Ishitani L, Abreu DMX, França E. Covid adult mortality in Brazil: an analysis of multiple causes of death. Front Public Health. 2022;9:788932. https://doi.org/10.3389/fpubh.2021.788932
10. Ministério da Saúde. Secretaria de Vigilância em Saúde Departamento de Análise em Saúde e Vigilância de Doenças não Transmissíveis. Orientações sobre causes de mortes no contexto da COVID-19. Respostas às perguntas mais frequentes. Brasília: Ministério da Saúde; 2021.
11. Santo AH, Pinheiro CE. Tabulador de causas múltiplas de morte [Multiple causes-of-death tabulador]. Rev Brasil Epidemiol. 1999;2:90-7.
12. Ahmad OB, Boschi-Pinto C, Lopez AD, Murray CJL, Lozano R, Inoue M. age standardization of rates: a new WHO standard. Geneva: World Health Organization; 2001.
13. Santo AH. Sickle cell disease related mortality in Brazil, 2000-2018. Hematol Transfus Cell Ther. 2022;44(2):177-85. https://doi.org/10.1016/j.htct.2020.09.154
14. Payne AB, Schieve LA, Abe K, Hulihan M, Hooper WC, Hsu LL. COVID-19 and sickle cell disease-related deaths reported in the United States. Public Health Rep. 2022;137(2):234-8. https://doi.org/10.1177/00333549211063518
15. Ministério da Saúde. Secretaria de Atenção Especializada à Saúde. Departamento de Atenção Especializada e Temática. Perfil das hemoglobinopatias. Doença Falciforme. Dados 2016. Brasília: Ministério da Saúde; 2021.

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Autoimmunity-related *LINC01934* and *AP002954.4* lncRNA polymorphisms may be effective in pediatric celiac disease: a case-control study

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SUMMARY

OBJECTIVE: Various studies have reported that certain long non-coding RNA levels are unusually low in the intestines of celiac disease patients, suggesting that this may be associated with the inflammation observed in celiac disease. Despite these studies, the research aimed at uncovering the potential role of long non-coding RNAs in the pathogenesis of autoimmune diseases like celiac disease remains insufficient. Therefore, in this study, we plan to assess long non-coding RNA polymorphisms associated with autoimmunity in children diagnosed with celiac disease according to the European Society for Paediatric Gastroenterology Hepatology and Nutrition criteria.

METHODS: DNA was isolated from paraffin tissue samples of 88 pediatric celiac disease patients and 74 healthy pediatric individuals. Single-nucleotide polymorphism genotyping of five long non-coding RNA polymorphisms associated with autoimmunity (*LINC01934-rs1018326*, *IL18RAP-rs917997*, *AP002954.4-rs10892258*, *UQCRC2P1-rs6441961*, and *HCG14 rs3135316*) was conducted using the TaqMan single-nucleotide polymorphism genotyping assays with the LightCycler 480.

RESULTS: In our study, the genotypic and allelic frequency distribution of *LINC01934-rs1018326* and *AP002954.4-rs10892258* polymorphisms was found to be statistically significant in the comparison between the two groups ($p<0.05$). According to the multiple genetic model analyses, the *LINC01934-rs1018326* polymorphism was observed to confer a 1.14-fold risk in the recessive model and a 1.2-fold risk in the additive model for pediatric celiac disease. Similarly, the *AP002954.4-rs10892258* polymorphism was found to pose a 1.40-fold risk in the dominant model and a 1.7-fold risk in the additive model.

CONCLUSION: Our study results draw attention to the *LINC01934-rs1018326* and *AP002954.4-rs10892258* polymorphisms in celiac disease and suggest that these polymorphisms may be associated with inflammation in autoimmune diseases like celiac disease.

KEYWORDS: Celiac disease. lncRNA. Polymorphism. Autoimmunity. Epigenetics.

INTRODUCTION

Celiac disease (CeD) is a food-related small intestine disorder that is also named gluten-sensitive enteropathy and is observed in approximately 1–2% of people. Active CeD is characterized by villous atrophy, crypt hyperplasia, and lymphocytic infiltration in the intestinal epithelium. While it is known that gluten proteins serve as environmental triggers in CeD, the genetic risk factors have not yet been fully defined. It is known that the human leukocyte antigen (HLA) genes are responsible for approximately 40% of the genetic risk for developing CeD, and the majority of CeD patients carry HLA-DQ2 or HLA-DQ8 risk alleles[1-4]. However, both genetic and epigenetic variants within and outside the HLA region are also associated with the risk of developing CeD[3,4]. In this regard, the results of genome-wide association studies (GWAS) have shown that over 85% of the single nucleotide polymorphisms (SNPs) associated with diseases are found in the non-coding parts of the genome. It has been shown that the SNPs found in these regions can regulate the expression of many genes[4]. Therefore, illuminating the disease-associated functional effects of non-coding variants will assist in clarifying the role of immune-related SNPs in disease susceptibility[3].

Non-coding RNAs over 200 bases are classified as long non-coding RNAs (lncRNAs) and do not encode proteins[5-7]. The lncRNAs can influence processes related to the passage of gluten peptides through the intestinal barrier and the activation of both innate and adaptive immune responses in the pathogenesis of CeD[3]. Many studies have identified lncRNAs associated with CeD. The *AC104820.2* lncRNA was reported as upregulated in the intestinal mucosa of active CeD patients[8].

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Another report identified lnc13 as being associated with susceptibility to CeD and demonstrated its functional role[9]. A recent study has shown that gliadin induces the expression of two lncRNAs (*TUG1* and *NEAT1*) in biopsies taken from CeD patients on a gluten-free diet[10]. Plaza-Izurieta et al. and Trynka et al. found an association of *LINC01934-rs1018326* with CeD risk in their studies[8,11]. Additionally, a meta-analysis conducted in 2015 provides strong predictions that *IL18RAP-rs917997* and *UQCR2P1-rs6441961* may be potential risk factors for CeD in European populations[12]. In light of the research conducted in this field, some lncRNAs have been associated with CeD, but there is still a lack of sufficient experimental evidence regarding their effects on the development of this disease. Therefore, in this study, lncRNA polymorphisms related to autoimmunity were investigated in pediatric CeD (pCeD).

METHODS

Cases and ethics

The study included 88 pCeD patients who presented to the Pediatric Gastroenterology Clinic of the Department of Pediatrics at Haseki Education Research Hospital in Istanbul. These patients were suspected of having CeD based on the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) criteria, and their duodenal biopsy samples were histopathologically evaluated as Marsh stage 3[13]. Additionally, 84 healthy children who underwent upper gastrointestinal endoscopy for various reasons and had normal duodenal biopsy results were included in the study as the control group.

The study was conducted upon approval from the Institutional Ethics Board (#2023/254). Informed consent of all participants was obtained before the study.

SNP selection

In the study, the lncRNA polymorphism associated with five autoimmune diseases to be investigated (*LINC01934-rs1018326*, *lL18RAP-rs917997*, *AP002954.4-rs10892258*, *UQCRC2P1-rs6441961*, and *HCG14-rs3135316*) was determined through a literature review.

Genomic DNA isolation, concentration, and purity

In our research, genomic DNA isolation from biopsy samples embedded in paraffin blocks was carried out using a commercially available DNA FFPE isolation kit (GeneRead™ FFPE kit, Qiagen, Hilden, Germany). The quality and concentration of the isolated DNA samples were assessed with a spectrophotometer (NanoDrop 1000 V3.7, Thermo Scientific, USA).

Genotyping

Five SNPs in five different lncRNAs were examined in isolated DNA samples from biopsy specimens. The screening of the lncRNA SNPs was conducted using quantitative real-time polymerase chain reaction (RT-qPCR) with a TaqMan SNP Genotyping Assays (Thermo Fisher Scientific, Waltham, MA). A volume of 10 µL qPCR reaction mixture was prepared, consisting of 0.5 µL TaqMan SNP Genotyping Assay, 5 µL LightCycler 480 Probes Master (Roche Diagnostics KK), 2.5 µL RNase-free water, and 2 µL DNA (50 ng/µL). The qPCR procedure was carried out on a LightCycler480 system (Roche, Germany) with the following conditions: 95°C for 10 min, followed by 45 cycles of 95°C for 15 s, and 60°C for 1 min. Data analysis was conducted using the LightCycler 480 software in the Tm calling mode or with melting curve genotyping.

Statistical analyses

The chi-squared test was applied for the comparison of categorical variables between the two groups. Student's t-test was employed to compare continuous independent variables. The Hardy-Weinberg equilibrium (HWE) was assessed by comparing the genotype distribution of the subjects with those of the controls, utilizing Fisher's exact test. Multiple genetic model analyses were applied using the Cochran-Amitage trend test to assess the association between SNPs and pCeD. All statistical tests were two-tailed, and the results were considered significant at $p<0.05$.

RESULTS

Clinical and demographic features of the groups

The clinical and demographic characteristics of the groups are presented in Table 1. The age profile of the children in the pCeD group (11.55±6.62) (mean±standard deviation) and the control group (11.76±4.92) did not show a statistically significant difference (p=0.56). When we looked at the gender distribution in the cases, the pCeD group consisted of 59.3% female and 39.7% male children, while the control group had 61% female and 39% male children, and there was no statistically significant difference between them (p=0.09).

When examining the Marsh classification of pCeD cases, it was found that 19% had Marsh 1–2, 22% had Marsh 3a, 28% had Marsh 3b, and 31% had Marsh 3c classification.

Table 1. Demographic and clinical features of the groups.

Demographic and clinical features	pCeD Group (n=88)	Control group (n=84)	OR (95% CI)	p-value
Gender				
Female	59.3%	61%	1.4 (0.72–2.73)	0.09
Male	39.7%	39%		
Age (M±SD)	11.55±6.62	11.76±4.92	0.37 (0.17–0.79)	0.56
Marsh classes				
Marsh 1-2	19%	–	–	–
Marsh 3a	22%	–	–	–
Marsh 3b	28%	–	–	–
Marsh 3c	31%	–	–	–
Symptoms				
Abdominal pain	79%	–	–	–
Inability to gain weight	58%	–	–	–
Anemia		–	–	–
Short height	54%	–	–	–
Constipation	20.6%	–	–	–
Diarrhea	15.1%	–	–	–
Vomiting	12.9%	–	–	–
Abdominal pain	8%	–	–	–

OR (95% CI): odds ratio (95% confidence interval).

In the pCeD group, 79% of the cases had abdominal pain complaints, 58% had inadequate growth, 54% had iron-deficiency anemia, 20% had short stature, 15% had constipation, 12.9% had diarrhea, and 8% had vomiting.

In the cases classified as Marsh 3c according to the Marsh classification, pCeD cases exhibited statistical significance with respect to having iron-deficiency anemia and growth retardation (p=0.004 and p=0.03, respectively).

Genotyping analyses

The potential relationships between the pCeD risk and the lncRNA polymorphisms (*LINC01934-rs1018326*, *IL18RAP-rs917997*, *AP002954.4-rs10892258*, *UQCRC2P1-rs6441961*, and *HCG14-rs3135316*) were investigated by comparing the genotype and allele frequency distributions of the listed polymorphisms between the groups. Genotype distributions of the polymorphisms follow the HWE. The genotype and allele frequency distributions, as well as the HWE values of the polymorphisms in the groups, are shown in Table 2.

The genotypes and allele frequency distributions of the *LINC01934-rs1018326* polymorphism were statistically significant between the groups (p=0.007 and p=0.05, respectively). The AA, AG, and GG genotype distributions in the pCeD and control groups were as follows: 30.68, 51.14, and 18.18% in pCeD and 23.81, 38.10, and 38.10% in the control group, respectively. Additionally, the frequencies of A and G alleles were determined as 56.25 and 43.75% in pCeD and 42.85 and 57.15% in the control group, respectively.

The genotype and allele frequency distributions of the *IL18RAP-rs917997* variant were not found to be statistically significant between the two groups (p=0.39 and p=0.50, respectively). Distributions of TT, TC, and CC genotypes in the pCeD group were 12.50, 45.45, and 42.05%, respectively, while in the control group, they were 11.90, 55.95, and 32.14%, respectively. The frequencies of the T and C alleles were determined as 35.23 and 64.77% in pCeD and 39.88 and 60.12% in the control group, respectively.

The distributions of genotype and allele frequencies of the *AP002954.4-rs10892258* polymorphism were found to be statistically significant between the groups (p=0.01 and p=0.05, respectively). In the pCeD group, the GG, GA, and AA genotype distributions were 71.59, 22.73, and 5.68%, respectively, while in the control group, they were 51.19, 40.48, and 8.33%, respectively. The G and A allele frequency distribution in the

Table 2. Genotype and allele frequency comparison of long non-coding RNA polymorphisms between the pCeD and control groups.

SNP and genotypes	pCeD group (n=88)	Control group (n=84)	X^2	df	p-value
LINC01934-rs1018326					
AA	27 (30.68%)	20 (23.81%)	9.82	2	0.007*
AG	45 (51.14%)	32 (38.10%)			
GG	16 (18.18%)	32 (38.10%)			
HWE p-value	0.714	0.041*			
IL18RAP-rs917997					
TT	11 (12.50%)	10 (11.90%)	2.42	2	0.39
CT	40 (45.45%)	47 (55.95%)			
CC	37 (42.05%)	27 (32.14%)			
HWE p-value	0.970	0.126			
AP002954.4-rs10892258					
GG	63 (71.59%)	43 (51.19%)	8.88	2	0.01*
GA	20 (22.73%)	34 (40.48%)			
AA	5 (5.68%)	7 (8.33%)			
HWE p-value	0.065	0.611			
UQCRC2P1-rs6441961					
TT	11 (12.50%)	14 (16.67%)	1.53	2	0.47
CT	42 (47.73%)	43 (51.19%)			
CC	35 (39.77%)	27 (32.14%)			
HWE p-value	0.769	0.653			
HCG14-rs3135316					
GG	78 (88.64%)	69 (82.14%)	1.98	2	0.37
AG	7 (7.95%)	9 (10.71%)			
AA	3 (3.41%)	6 (7.14%)			
HWE p-value	0.000*	0.000*			
Allele frequencies	**pCeD group (n=88)**	**Control group (n=84)**	**X^2**	**df**	**p-value**
LINC01934-rs1018326					
T	56.25%	42.85%	3.59	1	0.05*
C	43.75%	57.15%			
IIL18RAP-rs917997					
T	35.23%	39.88%	0.46	1	0.50
C	64.77%	60.12%			
AP002954.4-rs10892258					
G	82.95%	71.43%	3.77	1	0.05*
A	17.05%	28.57%			
UQCRC2P1-rs6441961					
T	36.36%	42.26%	0.73	1	0.39
C	63.64%	57.74%			
HCG14-rs3135316					
G	92.62%	87.50%	1.46	1	0.23
A	7.38%	12.50%			

*Significant p<0.05. HWE: Hardy-Weinberg equilibrium.

patient group was 82.95 and 17.05%, while in the control group, it was 71.43 and 28.57%, respectively.

The genotype and allele frequency distributions of the *UQCRC2P1-rs6441961* polymorphism were not found to be statistically significant between the groups (p=0.47 and p=0.39, respectively). In the pCeD and control groups, the TT, TC, and CC genotype distributions were as follows: 12.50, 47.73, and 39.77% in pCeD and 16.67, 51.19, and 32.14% in the control group, respectively. Also, the frequencies of T and C alleles were determined as 36.36 and 63.64% in pCeD and 42.26 and 57.74% in the control group, respectively.

The genotype and allele frequency distributions of the *HCG14-rs3135316* variant were not found to be statistically significant between the two groups (p=0.37 and p=0.23, respectively). In the pCeD group, the GG, GA, and AA genotype distributions were 88.64, 7.95, and 3.41%, respectively, while in the control group, they were 82.14, 10.71, and 7.14%, respectively. Additionally, the G and A allele frequencies were determined as 92.62 and 7.38% in pCeD and 87.50 and 12.50% in the control group, respectively.

Genetic model analyses

The *LINC01934-rs1018326* and *AP002954.4-rs10892258* polymorphisms were evaluated further by genotyping test models that include "dominant," "recessive," and "additive" to investigate the association of genotype and phenotype of the genes and the risk of pCeD. The *LINC01934-rs1018326* polymorphism is observed to create a 1.14-fold risk (OR: 1.14, 95%CI 0.60–2.17, p=0.004) for pCeD in the recessive model and a 1.2-fold risk (OR: 1.2, 95%CI 0.54–2.62, p=0.018) in the additive model. Similarly, the *AP002954.4-rs10892258* polymorphism is found to create a 1.40-fold risk (OR: 1.40, 95%CI 0.72–2.73, p=0.005) for pCeD in the dominant model and a 1.7-fold risk (OR: 1.7, 95%CI 1.08–2.77, p=0.015) in the additive model (Table 3).

DISCUSSION

Literature studies have shown that lncRNAs act as key regulators in inflammatory pathways. Furthermore, studies have been conducted on lncRNAs in CeD, which is also triggered by autoimmune mechanisms. The heterodimeric IL-18R formed by *IL-18R1* and the *IL-18* receptor accessory protein (*IL-18RAP*) is structurally expressed in the innate immune system cells. On the contrary, the *IL-18R1* is expressed in T cells. The *IL-18RAP* is necessary in the signaling process and its expression is increased during activation, especially in *IL-12* presence. Recently, GWAS have revealed that the

Table 3. The hereditary model risk of pCeD in different genotypes of the long non-coding RNA polymorphisms.

lncRNA SNPs	Inheritance model	Genotype	OR (95%CI)	p-value
LINC01934-rs1018326	Recessive	AA+AG	Ref.	0.004*
		GG	1.14 (0.60–2.17)	
	Dominant	AA	Ref.	0.312
		GG +AG	0.65 (0.85–3.46)	
	Additive	AA	Ref.	0.018*
		AG	1.04 (0.50–2.17)	
		GG	1.2 (0.54–2.62)	
AP002954.4-rs10892258	Recessive	GG+GA	Ref.	0.495
		AA	0.49 (0.15–1.64)	
	Dominant	GG	Ref.	0.005*
		AA+GA	1.40 (0.72–2.73)	
	Additive	GG	Ref.	0.015*
		GA	1.03 (0.55–1.92)	
		AA	1.7 (1.08–2.77)	

*Significant p<0.05. OR (95%CI): odds ratio (95% confidence interval).

IL18RAP-rs917997 is protective in type 1 diabetes but confers susceptibility to CeD[12]. In our study, unlike the literature, no statistical association was observed between pCeD and the *IL18RAP-rs917997* polymorphism. This is believed to be attributed to the limited sample size, the possibility that different SNPs within *IL18RAP* could be responsible for CeD autoimmunity, and population variability.

The preliminary data in this study draw attention to the *LINC01934-rs1018326* polymorphism in CeD. *rs1018326* has been described as a localized SNP in the non-MHC susceptibility locus identified in ankylosing spondylitis. Plaza-Izurieta et al. and Trynka et al. in their studies found an association between *rs1018326* and CeD risk[8,11]. This study also reports a similar relationship between pCeD and the *LINC01934-rs1018326* polymorphism, suggesting that this polymorphism may be a risk factor for pCeD. Therefore, this polymorphism may have a role in inflammation in autoimmune diseases like CeD.

Ricaño-Ponce et al. identified genes near autoimmune-associated SNPs, and these SNPs were found to be particularly associated with two lncRNAs (*AP002954.4* and *AC104820.2*)[14]. In our study, similar to the literature, a statistically significant difference in genotype distribution was observed between pCeD and the *AP002954.4-rs10892258* polymorphism. Genetic model analyses revealed that this polymorphism confers a 1.40 and 1.70 times increased risk in pCeD patients.

To identify risk variants contributing to CeD susceptibility outside of the HLA-DQ region, Hunt et al. and Heel et al. determined the genotypes of the most strongly associated non-HLA markers identified in studies involving 1.643 CeD cases and 3.406 controls. The *rs6441961* polymorphism has been determined to be associated with a broad cluster of chemokine receptor genes, including *CCR1*, *CCR2*, *CCRL2*, and *CCR3*, located on chromosome 3p21[15,16]. In a study conducted by Dema et al. involving 722 Spanish CeD patients and ethnically matched 794 controls, they confirmed the association of the "A" risk allele of *rs6441961*[17]. However, in an Italian cohort comprising 538 CeD patients and 593 healthy controls, Romanos et al. did not find any association with the *rs6441961* SNP, as previously reported by Hunt et al.[15,18] Additionally, a meta-analysis study conducted in 2015 provides strong predictions that *IL18RAP-rs917997*, *CCR3*, or *UQCR2P1-rs6441961* may be potential risk factors for CeD in European populations[12]. In our study, similar to the results of Romanos et al., no association was found between the *rs6441961* polymorphism and pCeD. This alignment has been attributed to the fact that the patient population selected in this study is from the same European cohort. It has been suggested that discrepancies in other studies may arise from population differences across Europe in terms of loci contributing to CeD.

Santin et al. conducted a study in which they performed high-resolution SNP genotyping in the MHC region. They compared the CeD subjects with homozygous *HLA-DR3* with healthy heterozygous controls that carry one copy of preserved and extended *B8-DR3-DQ2*. Their study identified two linked SNPs. One of them was present in the *TRIM27* gene, and the other one is *rs3135366* located in the non-coding *HCG14* gene. Through the stratification studies, the *HCG14* gene demonstrated a significant correlation, which is independent of the *HLA-DR-DQ* loci. In the analysis of duodenal biopsies of the CeD patients, the epithelial *HCG14* expression was slightly downregulated. The potential associations between the downregulated expression of *NOD1* in duodenum and the polymorphisms in the *HCG14* region were suggested by the eQTL analysis[19]. In our study, unlike Santin et al., no association was detected between the *HCG14-rs3135366* polymorphism and pCeD in the analysis we conducted on duodenal biopsy samples. This situation may once again be attributed to population differences and inadequate sample size.

CONCLUSION

The results of this study highlight the significance of the *LINC01934-rs1018326* and *AP002954.4-rs10892258* polymorphisms in pCeD and suggest that these polymorphisms might be linked to inflammation in autoimmune diseases such as CeD.

AUTHORS' CONTRIBUTIONS

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

REFERENCES

1. Iversen R, Sollid LM. The immunobiology and pathogenesis of celiac disease. Annu Rev Pathol. 2023;18:18:47-70. https://doi.org/10.1146/annurev-pathmechdis-031521-032634
2. Rubio-Tapia A, Hill ID, Semrad C, Kelly CP, Greer KB, Limketkai BN, et al. American College of Gastroenterology guidelines update: diagnosis and management of celiac disease. Am J Gastroenterol. 2023;118(1):59-76. https://doi.org/10.14309/ajg.0000000000002075
3. Gnodi E, Meneveri R, Barisani D. Celiac disease: from genetics to epigenetics. World J Gastroenterol. 2022;28(4):449-63. https://doi.org/10.3748/wjg.v28.i4.449
4. Aboulaghras S, Piancatelli D, Oumhani K, Balahbib A, Bouyahya A, Taghzouti K. Pathophysiology and immunogenetics of celiac disease. Clin Chim Acta. 2022;528:74-83. https://doi.org/10.1016/j.cca.2022.01.022
5. Ali T, Grote P. Beyond the RNA-dependent function of LncRNA genes. Elife. 2020;9:e60583. https://doi.org/10.7554/eLife.60583
6. Bridges MC, Daulagala AC, Kourtidis A. LNCcation: lncRNA localization and function. J Cell Biol. 2021;220(2):e202009045. https://doi.org/10.1083/jcb.202009045
7. Nojima T, Proudfoot NJ. Mechanisms of lncRNA biogenesis as revealed by nascent transcriptomics. Nat Rev Mol Cell Biol. 2022;23(6):389-406. https://doi.org/10.1038/s41580-021-00447-6
8. Plaza-Izurieta L, Fernandez-Jimenez N, Irastorza I, Jauregi-Miguel A, Romero-Garmendia I, Vitoria JC, et al. Expression analysis in intestinal mucosa reveals complex relations among genes under the association peaks in celiac disease. Eur J Hum Genet. 2015;23(8):1100-5. https://doi.org/10.1038/ejhg.2014.244
9. Castellanos-Rubio A, Fernandez-Jimenez N, Kratchmarov R, Luo X, Bhagat G, Green PH, et al. A long noncoding RNA associated with susceptibility to celiac disease. Science. 2016;352(6281):91-5. https://doi.org/10.1126/science.aad0467
10. Gnodi E, Mancuso C, Elli L, Ballarini E, Meneveri R, Beaulieu JF, et al. Gliadin, through the activation of innate immunity, triggers lncRNA NEAT1 expression in celiac disease duodenal mucosa. Int J Mol Sci. 2021;22(3):1289. https://doi.org/10.3390/ijms22031289
11. Trynka G, Hunt KA, Bockett NA, Romanos J, Mistry V, Szperl A, et al. Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. Nat Genet. 2011;43(12):1193-201. https://doi.org/10.1038/ng.998
12. Yang G, Zhang B, Huang W, Zhang N, Dong F, Jing L, et al. Systematic review and meta-analysis of the association between IL18RAP rs917997 and CCR3 rs6441961 polymorphisms with celiac disease risks. Expert Rev Gastroenterol Hepatol. 2015;9(10):1327-38. https://doi.org/10.1586/17474124.2015.1075880
13. Husby S, Koletzko S, Korponay-Szabó I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European society paediatric gastroenterology, hepatology and nutrition guidelines for diagnosing coeliac disease 2020. J Pediatr Gastroenterol Nutr. 2020;70(1):141-56. https://doi.org/10.1097/MPG.0000000000002497
14. Ricaño-Ponce I, Zhernakova DV, Deelen P, Luo O, Li X, Isaacs A, et al. Refined mapping of autoimmune disease associated genetic variants with gene expression suggests an important role for non-coding RNAs. J Autoimmun. 2016;68:62-74. https://doi.org/10.1016/j.jaut.2016.01.002
15. Hunt KA, Zhernakova A, Turner G, Heap GA, Franke L, Bruinenberg M, et al. Newly identified genetic risk variants for celiac disease related to the immune response. Nat Genet. 2008;40(4):395-402. https://doi.org/10.1038/ng.102
16. Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M, et al. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nat Genet. 2007;39(7):827-9. https://doi.org/10.1038/ng2058
17. Dema B, Martínez A, Fernández-Arquero M, Maluenda C, Polanco I, Concha EG, et al. Association of IL18RAP and CCR3 with coeliac disease in the Spanish population. J Med Genet. 2009;46(9):617-9. https://doi.org/10.1136/jmg.2009.067041
18. Romanos J, Barisani D, Trynka G, Zhernakova A, Bardella MT, Wijmenga C. Six new coeliac disease loci replicated in an Italian population confirm association with coeliac disease. J Med Genet. 2009;46(1):60-3. https://doi.org/10.1136/jmg.2008.061457
19. Santin I, Jauregi-Miguel A, Velayos T, Castellanos-Rubio A, Garcia-Etxebarria K, Romero-Garmendia I, et al. Celiac diasease-associated lncRNA named HCG14 regulates NOD1 expression in intestinal cells. J Pediatr Gastroenterol Nutr. 2018;67(2):225-31. https://doi.org/10.1097/MPG.0000000000001970

ORIGINAL ARTICLE

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Reduced expression of miRNAs as potential biomarkers in axial spondyloarthritis

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SUMMARY

OBJECTIVE: This study aimed to investigate the value of miR-29a-3p, miR-27a, miR126-3p, miR-146a-5p, miR-625-3p, miR-130a, miR-32, miR-218, miR-131, and miR5196 in the diagnosis of axial spondyloarthritis and to determine whether there is a difference in miRNA expression levels between radiographic axial spondyloarthritis and non-radiographic axial spondyloarthritis, as well as the relationship between miRNA expression levels, disease activity, and uveitis history.

METHODS: This study included 50 patients with axial spondyloarthritis (25 with radiographic axial spondyloarthritis and 25 with non-radiographic axial spondyloarthritis) and 25 healthy individuals. The fold change of miRNA expression for each miRNA was calculated using the $2^{-\Delta\Delta Ct}$ method.

RESULTS: The expression of all miRNAs except miR-130a was downregulated in axial spondyloarthritis patients (miR-27a: fold regulation: -11.21, p<0.001; miR-29a-3p: fold regulation: -2.63, p<0.001; miR-32: fold regulation: -2.94, p=0.002; miR-126-3p: fold regulation -10.94, p<0.001; miR-132: fold regulation: -2.18, p<0.001; miR-146-5p: fold regulation: -9.78, p<0.001; miR-218: fold regulation: -2.65, p<0.001; miR-625-3p: fold regulation: -2.01, p=0.001; miR-5196-3p: fold regulation: -7.04, p<0.001). The expression levels of these miRNAs did not differ significantly between non-radiographic axial spondyloarthritis and radiographic axial spondyloarthritis patients (p>0.05 for all).

CONCLUSION: Particularly, miR-27a, miR-126-3p, miR-146-5p, and miR-5196-3p were found to be substantially downregulated in both non-radiographic axial spondyloarthritis and radiographic axial spondyloarthritis patients, suggesting their potential as diagnostic biomarkers for axial spondyloarthritis.

KEYWORDS: Axial spondyloarthritis. microRNA. Uveitis.

INTRODUCTION

Axial spondyloarthritis (AxSpA) is a chronic inflammatory disease that mainly involves the axial joint and significantly affects the quality of life and functionality[1]. Although the exact pathogenesis of AxSpA remains elusive, many genetic, environmental, and immunological factors contribute to its pathogenesis[2].

MiRNAs are known to have a role in various processes, including the regulation of inflammation, bone resorption, and formation in AxSpA[3]. There is still debate about whether non-radiographic AxSpA (nr-AxSpA) should be considered a distinct condition from radiographic AxSpA (r-AxSpA) or an early stage of r-AxSpA. Although nr-AxSpA resembles r-AxSpA regarding disease activity, health status, and functionality, it differs from nr-AxSpA in the degree of inflammation and the lack of structural damage in most patients, even after many years[4]. This suggests that nr-AxSpA may have a distinct miRNA expression profile. Despite the increasing amount of research on miRNA expression in r-AxSpA patients, few studies have focused on the difference between miRNA profiles of nr-AxSpA and r-AxSpA[5].

The aim of this study was to investigate the expression levels of target 10 miRNAs in AxSpA patients and determine whether there was a difference between r-AxSpA and nr-AxSpA. Furthermore, the association between miRNA expression levels and the presence of uveitis history and the correlation between inflammatory markers and disease activity was also evaluated.

METHODS

Study participants

This cross-sectional study included a total of 50 patients diagnosed with AxSpA (25 with nr-AxSpA and 25 with r-AxSpA) and 25 age and gender-matched healthy controls (HCs). The study only included participants who were naïve to biological treatments. Healthcare professionals who visited the

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general internal medicine outpatient clinic for periodic control and had no known diseases comprised the HC group.

The Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and the Ankylosing Spondylitis Disease Activity Score-C-reactive protein (ASDAS-CRP) scores were used to assess the disease activity of AxSpA patients.

The Institutional Clinical Research Ethics Committee of Mersin University approved the study (Approval No: 2019/322, Date: 24/07/2019). All participants provided informed, written consent before the study.

miRNA analysis

For miRNA analysis, the samples were centrifuged at 2,000 *g* for 10 min without waiting for more than 2 h at room temperature. The aliquoted plasma samples were stored at -80°C in a freezer. MiRNA isolation was performed from plasma samples using a miRNA isolation kit (Roche Diagnostics, GmbH, Mannheim, Germany). After isolation, the samples were converted to cDNA and stored at -20°C until the analysis. The expression analysis of the 10 target miRNAs was performed using a real-time polymerase chain reaction (RT-PCR) device (Roche LightCycler 480). The Ct values of the miRNAs were normalized using the reference RNU6B. The fold change of miRNA expression for each miRNA was calculated as the $2^{-\Delta\Delta Ct}$ method[6].

Statistical analysis

The statistical software program SPSS (Statistical Package for Social Sciences) version 22.0 for Windows (IBM Inc., Chicago, IL, USA) was used to analyze the data. To test the normal distribution of data, the Shapiro-Wilk test was used. Independent-samples Student's t-test or Mann-Whitney U test was used to compare the two groups. Comparisons between more than two groups were performed using the analysis of variance (ANOVA) test or the Kruskal-Wallis test. For the correlation between the parameters, Pearson's or Spearman's correlation test was used. Receiver operating characteristics (ROC) analysis was used to evaluate the potential of each miRNA to differentiate between AxSpA patients and HCs. $p<0.05$ was considered statistically significant.

RESULTS

The demographic characteristics and clinical and laboratory data of 25 nr-AxSpA, 25 r-AxSpA, and 25 HCs are presented in Table 1.

The expression levels of 10 miRNAs in nr-AxSpA and r-AxSpA patients and HCs and the differences between the groups are presented in Table 2.

No significant correlation was found between miRNA expression levels and CRP, ESR, BASDAI, and ASDAS-CRP levels of AxSpA patients for any of the 10 miRNAs ($p>0.05$ for all parameters).

The expression of all miRNAs except miR-130a was downregulated in all AxSpA patients [miR-27a: Fold Regulation (FR): -11.21, $p<0.001$; miR-29a-3p: FR: -2.63, $p<0.001$; miR-32: FR: -2.94, $p=0.002$; miR-126-3p: FR: -10.94, $p<0.001$; miR-132: FR: -2.18, $p<0.001$; miR-146-5p: FR: -9.78, $p<0.001$;

Table 1. Differences between demographic characteristics, laboratory data, and disease activity scores of axial spondyloarthritis patients and healthy controls.

	nr-AxSpA (n=25) (mean+SD)	r-AxSpA (n=25) (mean+SD)	HC (n=25) (mean+SD)	p-value
Age (years)	39.72±6.71	41.68±9.99	40.55±6.43	0.664
Sex (n/%) (female/male)	14/11 (56/44%)	10/15 (40/60%)	12/13 (48/52%)	0.399
ESR, mm/h	16.12±9.97	19.22±16.62	5.42±4.08	<0.001†
				<0.001‡
				0.922§
CRP, mg/L	9.06±10.75	13.39±13.39	2.26±1.65	<0.001†
				<0.001‡
				0.265§
Disease duration (months)	51.76±35.78	115.2±103.53	N/A	0.032
HLA-B27 positivity (n/%)	13 (52%)	17 (68%)	N/A	0.253
BASDAI	3.79±1.77	4.26±1.20	N/A	0.317
ASDAS-CRP	2.85±1.13	3.29±0.89	N/A	0.133

SD: standard deviation; N/A: not assessed; †p-value between HC and Nr-AxSpA; ‡p-value between HC and r-AxSpA; §p-value between nr-AxSpA and r-AxSpA. $p<0.05$ is considered statistically significant.

Table 2. Expression levels of plasma miRNAs in patients with non-radiographic axial spondyloarthritis, radiographic axial spondyloarthritis, and healthy controls.

	HC ΔCt (mean±SD)	nr-AxSpA ΔCt (mean±SD)	r-AxSpA ΔCt (mean±SD)	p-value
miR-27a	1.88±1.69	5.99±2.49	5.50±1.54	<0.001†
				<0.001‡
				0.649§
miR-29a-3p	5.93±1.35	8.42±2.01	7.23±1.60	<0.001†
				0.009‡
				0.066§
miR-32	2.97±1.82	5.06±25	4.60±1.70	0.001†
				0.011‡
				0.684§
miR-126-3p	0.94±1.85	5.19±2.59	4.42±1.86	<0.001†
				<0.001‡
				0.204§
miR-130a	-2.02±1.73	-0.71±2.44	-2.18±1.95	0.06
miR-132	3.06±1.38	5.75±2.55	5.07±1.69	<0.001†
				<0.001‡
				0.510§
miR-146-5p	-3.18±4.57	4.57±3.24	3.07±2.58	<0.001†
				<0.001‡
				0.177§
miR-218	-4.93±1.37	-2.39±2.39	-3.20±2.20	<0.001‡
				0.005‡
				0.438§
miR-625-3p	2.43±2.94	5.13±2.78	4.67±2.15	0.005†
				0.010‡
				0.789§
miR-5196-3p	4.02±1.92	6.81±1.52	6.55±1.64	<0.001†
				<0.001‡
				0.859§

ΔCt: delta cycle threshold; SD: standard deviation; †p-value between HC and Nr-AxSpA; ‡p-value between HC and r-AxSpA; §p-value between nr-AxSpA and r-AxSpA. p<0.05 is considered statistically significant.

miR-218: FR: -2.65, p<0.001; miR-625-3p: FR: -2.01, p=0.001; miR-5196-3p: FR: -7.04, p<0.001]. miR-130a expression was not different between HCs and AxSpA patients (FR: -1.14, p=0.402). The expression levels of these nine miRNAs did not differ significantly between nr-AxSpA and r-AxSpA patients (p>0.05, for all).

Table 3 presents AUC, sensitivity, specificity, and optimal cutoff values for 10 miRNAs.

The expressions of miR-29a-3p and miR-146-5p were significantly lower in AxSpA patients with a history of uveitis compared with those without uveitis (miR-29a-3p: FR: -2.56, p=0.001 and miR-146-5p: FR: -2.56, p=0.034, respectively).

DISCUSSION

Our results revealed that miR-27a, miR-29a-3p, miR-32, miR-126-3p, miR-132, miR-146-5p, miR-218, miR-625-3p, and miR-5196-3p were found to be significantly downregulated in AxSpA patients compared with healthy individuals and might be a promising diagnostic biomarker for AxSpA. MiR-29a-3p

Table 3. Receiver operating characteristics analysis for target miRNAs to diagnose axial spondyloarthritis.

	AUC	95%CI	Optimal cutoff	Sensitivity	Specificity	p-value
miR-27a	0.933	0.866–0.999	3.57	90%	88%	<0.001
miR-29a-3p	0.828	0.724–0.931	6.43	78%	84%	<0.001
miR-32	0.756	0.639–0.872	3.79	70%	76%	<0.001
miR-126-3p	0.892	0.819–0.965	2.6	74%	76%	<0.001
miR-132	0.836	0.740–0.931	3.65	76%	80%	<0.001
miR-146-5p	0.962	0.914–1.000	0.24	94%	92%	<0.001
miR-218	0.816	0.713–0.920	-4.46	74%	76%	<0.001
miR-625-3p	0.749	0.637–0.860	3.05	72%	68%	<0.001
miR-5196-3p	0.846	0.742–0.949	5.33	78%	80%	<0.001

AUC: area under curve; CI: confidence interval; statistically significant at p<0.05.

and miR-146-5p were also associated with a history of uveitis in AxSpA patients. Thus, these miRNAs may play a role in the pathophysiology of AxSpA and clinical features.

The progression of immune dysfunction and autoimmunity appears to be highly associated with changes in miRNA regulation[7]. Profiles of microRNA expression in AxSpA may serve as biomarkers for pathogenesis, diagnosis, prognosis, or treatment monitoring.

Despite nr-AxSpA and r-AxSpA being similar in terms of clinical presentation and disease activity, there is ongoing controversy as to whether nr-AxSpA represents a distinct disease entity or an early stage of ankylosing spondylitis (AS)[8]. The identification of the miRNA expression signatures of both forms of the disease, as well as the similarities and variances in expression levels, may give further objective evidence for points of view on this issue.

Diagnosis of patients in the non-radiographic stage can be challenging due to the lack of specific diagnostic tests for the disease, the fact that currently used tests such as ESR and CRP can be normal in some patients, and the high cost and limited accessibility of magnetic resonance imaging[9]. MiRNAs, which are known to have a role in the pathogenesis of the disease, may have the potential to serve as diagnostic biomarkers for AxSpA.

The same miRNA tested for an identical disease may produce conflicting results. This is assumed to be due to the fact that various factors, such as the origin of tissue samples, ethnic variations, and disease severity, may influence miRNA expression differently[10]. For instance, in some studies, miR-146 expression has been shown to be upregulated in AS patients. Chen et al.[11] found no statistically significant difference between the expression levels of miR-146a-5p in Th17 cells of HCs and AS patients. In contrast, Fogel et al.[12] reported downregulation of miR-146a-5p in the monocytes of AxSpA patients and a negative correlation between its level and ASDAS and CRP. Similarly, miR-146-5p was substantially downregulated in our study compared with HC in AxSpA patients. However, no correlation was found between miR-146-5p expression levels and disease activity or acute phase reactants.

In a study comparing the expression of miR-625-3p and miR-29a-3p in patients with nr-axSpA, AS, and HC, lower levels of expression were found in patients with AS than in patients with nr-AxSpA and HCs. However, in patients with nr-AxSpA, only miR-625-3p showed lower expression levels than in HC. miR-29a-3p expression levels were reported to be similar in patients with nr-AxSpA and HCs. The authors attributed this inverse relationship between radiographic bone formation and circulating miRNA levels of miR-29a-3p to its association with bone formation[13]. However, in our study, miR-625-3p and miR-29a-3p showed similarly low expression in both nr-AxSpA and r-AxSpA patients compared with HCs. Although these results suggest that miR-29a-3p may indicate the risk of radiographic progression in patients with nr-AxSpA, studies evaluating disease progression in these patients over time are needed.

Jiang et al.[14] examined the relationship between miRNA-130a and its targets tumor necrosis factor (TNF)-1α and histone deactylase (HDAC)3 in AS patients' peripheral blood mononuclear cells. Decreased miRNA-130a levels and increased HDAC3 and TNF-1 were observed in AS patients. However, miR-130a expression in AxSpA patients was similar to that in the healthy population in our study.

The most prevalent extra-articular manifestation of AxSpA is uveitis. Recurrent uveitis is a significant cause of morbidity that may lead to synechia and vision loss. No biomarker has yet been identified to predict the development of uveitis. Recent studies have indicated that a subset of microRNAs may serve as a biomarker for uveitis.

miR-146a, miR-146a-5p, miR-155, miR-182, and miR-223-3p were identified as potential biomarkers for uveitis in a systematic review[15]. In our study, miR29a-3p and miR-146-5p were found to be associated with uveitis.

The small sample size and single-center study are the main limitations of this study. As biological treatment-naive patients were included, which are substantially effective inhibitors of inflammation, the effect of these agents on miRNA signature could not be evaluated. Furthermore, patients were categorized only regarding sacroiliac joint involvement, and spinal involvement was not analyzed. In addition, the effect of geographic and ethnic differences on the expression profiles of miRNAs and the effect of disease progression over time on miRNA expression in nr-AxSpA patients were not evaluated in this study.

CONCLUSION

Our study showed that especially miR-27a, miR-126-3p, miR-146-5p, and miR-5196-3p were significantly downregulated in both nr-AxSpA and r-AxSpA patients and had a high predictive value for AxSpA. It suggests that they have the potential to be used as a biomarker for the diagnosis of AxSpA. Furthermore, the similarity of all miRNA expression levels in individuals with nr-AxSpA and r-AxSpA supports the notion that these two disease types are the same disease entity. Additionally, lower expression of miR-29a-3p and miR-146-5p was associated with uveitis history in AxSpA patients. Investigating the expression and function of miRNAs in AxSpA may provide new insights into the underlying pathophysiology of AxSpA and could lead to the development of novel diagnostic biomarkers and treatment strategies.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethics Committee of Mersin University (Decision No: 2019/322, Date: 24.07.2019). Written informed consent was obtained from all participants.

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AUTHORS' CONTRIBUTIONS

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

REFERENCES

1. Zhu W, He X, Cheng K, Zhang L, Chen D, Wang X, et al. Ankylosing spondylitis: etiology, pathogenesis, and treatments. Bone Res. 2019;7(1):22. https://doi.org/10.1038/s41413-019-0057-8
2. Liao HT, Tsai CY, Lai CC, Hsieh SC, Sun YS, Li KJ, et al. The potential role of genetics, environmental factors, and gut dysbiosis in the aberrant non-coding RNA expression to mediate inflammation and osteoclastogenic/osteogenic differentiation in ankylosing spondylitis. Front Cell Dev Biol. 2022;9:748063. https://doi.org/10.3389/fcell.2021.748063
3. Motta F, Carena MC, Selmi C, Vecellio M. MicroRNAs in ankylosing spondylitis: function, potential and challenges. J Transl Autoimmun. 2020;3:100050. https://doi.org/10.1016/j.jtauto.2020.100050
4. Kiltz U, Baraliakos X, Karakostas P, Igelmann M, Kalthoff L, Klink C, et al. Do patients with non-radiographic axial spondylarthritis differ from patients with ankylosing spondylitis? Arthritis Care Res (Hoboken). 2012;64(9):1415-22. https://doi.org/10.1002/acr.21688
5. Li X, Lv Q, Tu L, Zhao M, Zhang P, Li Q, et al. Aberrant expression of microRNAs in peripheral blood mononuclear cells as candidate biomarkers in patients with axial spondyloarthritis. Int J Rheum Dis. 2019;22(7):1188-95. https://doi.org/10.1111/1756-185X.13563
6. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 2001;25(4):402-8. https://doi.org/10.1006/meth.2001.1262
7. Mohammadi H, Hemmatzadeh M, Babaie F, Gowhari Shabgah A, Azizi G, Hosseini F, et al. MicroRNA implications in the etiopathogenesis of ankylosing spondylitis. J Cell Physiol. 2018;233(8):5564-73. https://doi.org/10.1002/jcp.26500
8. Rudwaleit M, Haibel H, Baraliakos X, Listing J, Märker-Hermann E, Zeidler H, et al. The early disease stage in axial spondylarthritis: results from the German spondyloarthritis inception cohort. Arthritis Rheum. 2009;60(3):717-27. https://doi.org/10.1002/art.24483
9. Rosa Neto NS, Carvalho JF, Shoenfeld Y. Screening tests for inflammatory activity: applications in rheumatology. Mod Rheumatol. 2009;19(5):469-77. https://doi.org/10.1007/s10165-009-0211-z
10. Esteller M. Non-coding RNAs in human disease. Nat Rev Genet. 2011;12(12):861-74. https://doi.org/10.1038/nrg3074
11. Chen L, Al-Mossawi MH, Ridley A, Sekine T, Hammitzsch A, Wit J, et al. miR-10b-5p is a novel Th17 regulator present in Th17 cells from ankylosing spondylitis. Ann Rheum Dis. 2017;76(3):620-5. https://doi.org/10.1136/annrheumdis-2016-210175

12. Fogel O, Bugge Tinggaard A, Fagny M, Sigrist N, Roche E, Leclere L, et al. Deregulation of microRNA expression in monocytes and CD4+ T lymphocytes from patients with axial spondyloarthritis. Arthritis Res Ther. 2019;21(1):51. https://doi.org/10.1186/s13075-019-1829-7

13. Prajzlerová K, Grobelná K, Hušáková M, Forejtová Š, Jüngel A, Gay S, et al. Association between circulating miRNAs and spinal involvement in patients with axial spondyloarthritis. PLoS One. 2017;12(9):e0185323. https://doi.org/10.1371/journal.pone.0185323

14. Jiang Y, Wang L. Role of histone deacetylase 3 in ankylosing spondylitis via negative feedback loop with microRNA-130a and enhancement of tumor necrosis factor-1α expression in peripheral blood mononuclear cells. Mol Med Rep. 2016;13(1):35-40. https://doi.org/10.3892/mmr.2015.4494

15. Pockar S, Globocnik Petrovic M, Peterlin B, Vidovic Valentincic N. MiRNA as biomarker for uveitis - a systematic review of the literature. Gene. 2019;696:162-75. https://doi.org/10.1016/j.gene.2019.02.004