

SÃO PAULO Medical Journal

E V I D E N C E F O R H E A L T H C A R E

Medline, LILACS,
SciELO, Science
Citation Index Expanded,
Journal Citation Reports/
Sciences Edition
(impact factor 1.838),
EBSCO Publishing and
PubMed Central
(PMC)

July 2 - Volume 144 - Number 4

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Aerial view of São Paulo's illuminated skyscrapers at night, State of São Paulo.
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EM MEDICINA E CIRURGIA

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Founded in 1932, a bimonthly publication of the Associação Paulista de Medicina e-mail: revistas@apm.org.br

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Proofreading: Editage.
Desktop publishing: Zeppelini Publishers (www.zeppelini.com.br).
Listed in: Medline, Lilacs, SciELO, Science Citation Index Expanded and Journal Citation Reports/Sciences Edition, EBSCO publishing and PubMed Central.
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Academic Leagues and the Formation of Physicians in Brazil

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Medical education has traditionally emphasized the acquisition of theoretical knowledge and procedural competence as the central attributes of physician training. However, contemporary practice increasingly demands abilities that extend beyond technical expertise, including communication, teamwork, leadership, ethical responsibility, and engagement with healthcare systems and research. This gap between formal curricula and the broader professional role expected of physicians has become a persistent challenge in medical education worldwide.

The CanMEDS competency framework proposed by the Royal College of Physicians and Surgeons of Canada formalized this expanded understanding of the physician's role, defining the doctor not only as a medical expert but also as a communicator, collaborator, leader, scholar, health advocate, and professional. Competency-based medical education therefore requires learning environments in which these roles can be experienced in practice rather than only taught in theory.^{1,2}

Within Brazilian medical education, academic leagues have emerged as a distinctive response to this need. These student-led organizations complement formal curricula by integrating teaching activities, research participation, and supervised clinical exposure. Their widespread presence across medical schools reflects both student demand and institutional recognition of their educational relevance. In this context, leagues function not merely as extracurricular groups, but as environments in which students transition from passive recipients of information to active participants in professional formation.

A comprehensive survey revealed that in the setting of 126 Medical Schools, only four didn't have information about AL. All public medical schools and 96.2% of private institutions host academic leagues, with 74.4% maintaining specialized leagues in high-demand areas such as oncology. This widespread adoption reflects genuine student demand and institutional recognition of their educational value. The digital presence of these organizations is substantial, with the majority maintaining active social media profiles and discussion forums, demonstrating their adaptation to contemporary educational technologies.³

Evidence supports this educational role. Studies evaluating pain education in Brazil demonstrated that many institutions lack structured curricular coverage of important clinical topics, whereas leagues provide organized teaching activities, supervised patient care, and research participation. International literature similarly shows that structured academic extracurricular activities are associated with leadership development, increased self-confidence, improved academic satisfaction, and greater postgraduate engagement. These findings suggest that such initiatives can partially compensate for curricular limitations while fostering early professional identity formation.⁴

The experience of the Cardiothoracic Surgery Academic League at the University of São Paulo Medical School illustrates this model in practice. Operating continuously for more than two decades, the league integrates lectures, research activities, and clinical observation. Its annual introductory course, organized primarily by students and attracting approximately 60 applicants each year, functions both as an educational event and as the selection process for new members. The preparation of this course requires coordination, communication, and responsibility among students, transforming the organizational process itself into a learning experience. Beyond clinical exposure, participation has been associated with scientific production and influence on specialty choice, demonstrating its impact on professional development.

In a article published in 2010 the league showed that they had 47 scientific publications with league members as authors or co-authors. Beyond publications, the league has successfully integrated teaching, research, and community engagement through structured activities including theoretical lectures, scientific meetings, and practical observation in the operating room and post-operative recovery units. Student satisfaction with this model was exceptionally high. Among current members, 78.5% responded to evaluation surveys, with 98% recommending the league to colleagues. Notably, 49% of league participants engaged in scientific research projects directly linked to the league, demonstrating its capacity to foster research engagement.⁵

Early and sustained contact with a specialty increases the likelihood that students will pursue it during postgraduate training. This effect is not merely the result of increased exposure, but of meaningful engagement: students encounter mentors, observe professional identities in practice, and begin to envision their own place within the field. In this sense, academic leagues function as more than educational initiatives. They contribute to workforce formation by attracting motivated students to areas that require long training and early commitment, such as surgical specialties. By facilitating mentorship and guided participation, leagues help align professional development with healthcare needs, reinforcing both individual vocation and social responsibility.

Academic leagues therefore represent more than complementary activities; they constitute practical environments of competency-based education. By promoting mentorship, teamwork, research engagement, and responsibility for collective tasks, they anticipate principles later formalized in modern educational frameworks. Rather than being viewed as informal student initiatives, such structures should be recognized as valuable components of medical training. Their experience suggests that meaningful professional formation may depend not only on curricular reform, but also on preserving learning spaces in which students actively participate in the practice, organization, and social role of medicine.

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What happens next: The lingering effects of child abuse in a Mexican cohort

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KEYWORDS (MeSH terms):

Child abuse.
Cohort studies.
Sports.
Protective factors.

AUTHOR KEYWORDS:

Substance abuse.
Mood disorders.
Survivors.

ABSTRACT

BACKGROUND: Understanding the lingering effects of child abuse may improve healthcare pathways with preventive and supportive strategies.

OBJECTIVE: To compare adverse outcomes between survivors of child abuse and non-exposed subjects.

DESIGN AND SETTING: This is a cohort study conducted at the Pediatric Emergency Department of the Nuevo Hospital Civil de Guadalajara, Guadalajara, Mexico.

METHODS: This cohort study of Mexicans compared adverse outcomes between survivors of child abuse and non-exposed individuals from 2011 to 2018. Additionally, we contacted survivors in 2020 to collect additional data, including sociodemographic and study variable-related information.

RESULTS: This study included 300 survivors of child abuse, paired by age with 300 non-exposed subjects. Neglect and sexual abuse were the most frequent types of abuse. Among the survivors, there was a higher risk of hyperactivity with attention deficit [Relative Risk (RR) = 4.65(2.06–10.51); P = 0.0001], drug abuse [RR = 3.09(1.25–7.63); P = 0.0001], mood disorders [RR = 2.97(1.81–4.86); P = 0.0001], tobacco use [RR = 2.79(1.60–4.88); P = 0.0001], and engagement with substance abuse and sexual intercourse at a younger age. Non-exposed subjects had a higher level of education [RR = 0.65(0.49–0.87); P = 0.0001] and were more engaged in sports [RR = 0.31(0.18–0.52); P = 0.0001].

CONCLUSION: Survivors of child abuse are at risk for psychological problems and substance abuse. Increasing sports engagement and school attendance may protect against adverse outcomes.

INTRODUCTION

Child abuse refers to any type of maltreatment that causes physical, emotional, sexual, or negligent harm and, consequently, affects a child's health, dignity, development, or survival.^{1,2} In addition to the traditional four types of child abuse (physical, emotional/psychological, sexual, and neglect), there are several other forms of maltreatment. These include peer-on-peer abuse (bullying), domestic violence, abandonment, harm to the unborn child (fetal abuse), Munchausen syndrome by proxy, ill-treatment of migrant and displaced children due to armed conflicts and violence, and children recruited as soldiers, hitmen, or assassin by drug cartels armed forces.^{2,3}

In the United States, the prevalence of child abuse by a father or caregiver was nine per 1000 children.⁴ In Western Mexico, one hospital reported 318 cases per year.² The real magnitude of child abuse is unknown, mainly because violence against children is often concealed. Family members are frequently involved, and survivors are afraid to speak out. Furthermore, in some regions, physical maltreatment is regarded as discipline. "Beating and scolding are the emblems of love" is an oriental proverb.⁵ Additionally, identifying child abuse without physical signs, such as psychological or emotional abuse, is challenging.¹

The harm inflicted on a child has health and other consequences, including death. The World Health Organization estimates that deaths secondary to child abuse are more prevalent in children aged five years or younger, probably because this age group is entirely dependent on the caretaker—parent or family member. For children who survived child abuse, the consequences remain long after physical injuries have healed, negatively impacting neurologic development, emotional responses, cognition, and overall health.¹ To provide comprehensive care and support, recognizing the long-term effects of child abuse is essential.

OBJECTIVE

This study aimed to compare adverse outcomes between survivors of child abuse and non-exposed subjects.

METHODS

This cohort study included patients aged < 16 years, suspected of child abuse, who were admitted to the Pediatric Emergency Department of the Nuevo Hospital Civil de Guadalajara from January 2011 to April 2018. Our Institution is a University Hospital with 3,500 to 4,000 pediatric emergencies a year. Patients were evaluated by a multidisciplinary group, the Programa de Atención Multiple al Maltrato Infantil (PAMMI, Comprehensive Care Program for Child Abuse), to determine if they were survivors of child abuse and, in confirmed cases, to provide care, including medical and legal aspects. The PAMMI database, first established in 2008, contains information on child abuse cases, including physical, emotional/psychological, sexual, negligent, abandonment, fetal, Munchausen syndrome by proxy, or bullying, and their follow-up.

In 2020, we conducted personal interviews with patients who were evaluated by PAMMI. The patients were divided into two groups: confirmed survivors of child abuse (exposed) and patients with maltreatment excluded (non-exposed), and compared. From both groups, we collected information concerning actual sociodemographic variables, psychological/emotional problems, school attendance and difficulties, nutritional profile, substance abuse, initial sexual experience, pregnancy, sports engagement, and rebellious behavior or delinquency. Exposed and non-exposed subjects were paired by age. Psychological/emotional problems were confirmed in the medical records. Prescribed medications were also documented.

As only some patients were contactable in this cohort study, potential bias was considered. A sample size was calculated using a proportions formula for cohort studies, with a result of 150 exposed and 150 non-exposed. The number included in the study was doubled.

Statistical analysis is presented with means and standard deviation (SD) for continuous variables, with frequencies and percentages for categorical variables, and associations were determined using the Student's t-test and chi-square test. Relative risk (RR) with 95% confidence intervals (95%CI) were calculated. Significance was set at $P \leq 0.05$. We used logistic regression with the enter method and forward conditional selection for the final model to analyze independent risk factors. Sports engagement was considered a protective factor, and substance abuse, along with psychological/emotional problems, were risk outcomes. We present RR as crude RR (cRR) and logistic regression as adjusted RR (aRR).

The Nuevo Hospital Civil de Guadalajara Dr. Juan I Menchaca Research and Ethics Committee (CONBIOÉTICA-14-CEI-008-20161212) approved the study protocol (authorization and registration 00156) on August 18, 2020, and confirmed its

compliance with the guiding framework of the Helsinki Declaration (1975, rev. 2013). Before the interviews, informed consent was obtained from patients aged > 16 years or from the parents or tutors in the case of younger patients. Patients aged ≥ 6 years also provided their written assent to participate.

RESULTS

From January 2011 to April 2018, 3,238 survivors of child abuse were admitted to our Pediatric Emergency Department, which represented 11.7% of the 27,566 total admissions. Abused children ranged from one to 16 years of age, with a mean age of 11 years [± 4 years].

In 2020, we attempted to interview all survivors. However, we could only contact 300 [9.2%]. We also found that 16 [0.04%] patients were no longer alive, although their deaths were unrelated to child abuse. The main causes of death were cancer and related complications (e.g., leukemia, astrocytoma, neurofibrosarcoma, and sepsis), and chronic renal failure.

The age of the survivors at the time of the interview ranged from 4 to 25 years, with a mean of 17 years [± 4 years]. The most frequent types of abuse were negligence, sexual, and psychological. **Table 1** presents the types of abuse by age and sex of the 300 interviewed child abuse survivors admitted to our Pediatric Emergency Department between 2011 and 2018.

School attendance was lower among survivors of child abuse. Consequently, a significant number of survivors were illiterate or had incomplete primary-level education compared to the non-exposed group, who generally had over nine years of education. School fights occurred more frequently among survivors of child abuse, and delinquency was found only within that group. Interestingly, nutritional status was not significantly different between the two groups (**Table 2**).

Sociodemographic variables and parental education were similar in both groups. The bivariate analysis did not reveal statistically significant differences. Therefore, these variables were not included in the multivariate model.

Attention-Deficit/Hyperactivity Disorder (ADHD) and mood disorders were significantly more prevalent in survivors of child abuse ($P = 0.0001$). Sadness, sleep disturbances, depression, and anxiety were some of the problems mentioned. Medical treatment with sertraline, risperidone, fluoxetine, olanzapine, alprazolam, or clonazepam was necessary for 41% [123] of the affected survivors. Recurrent substance abuse was also more likely among survivors of child abuse, especially drug use ($P = 0.0001$). Cannabis was the preferred psychoactive drug for both groups, alone or in combination with cocaine, inhalants, or amphetamines. Drug use also increased among the parents of the survivors ($P = 0.0008$). Sports engagement was less common in survivors of child abuse [$P = 0.0001$], while non-exposed subjects practiced soccer, gymnastics, cycling, basketball, and swimming for health and leisure,

but not as high-performance athletes. The significance of these outcomes persisted after adjustment with logistic regression. (Table 3). The starting age for the risk outcomes (substance abuse, tobacco

use, sexual intercourse, and pregnancy), defined in this study was younger among survivors, and a significantly larger number had offspring [69, 23% versus 40, 13%; $P = 0.002$] (Table 4).

Table 1. Type of abuse by age and gender of the interviewed survivors (Guadalajara, 2011-2018)

Type of abuse	Female, n = 200 (66%)				Male, n = 100 (34%)				Total
	Age (years)				Age (years)				
	£ 2	3-5	6-12	13-16	£ 2	3-5	6-12	13-16	n (%)
Negligence	2	11	40	34			32	27	146 (48.5%)
Sexual	1	5	20	40		1	1	4	72 (24%)
Psychological			11	23			4	2	40 (14%)
Physical		1	4	6		4	8	2	25 (8%)
Abandonment					2	6			8 (2.6%)
Bullying			2				5		7 (2.3%)
Munchausen syndrome by proxy							2		2 (0.6%)
Total	3	17	77	103	2	11	52	35	300

Table 2. Education, behavior and nutritional profile of survivors of child abuse versus non-exposed

Variable	Child abuse (n = 300)	Non-exposed (n = 300)	RR (95%CI)	P
Education				
Illiterate	38	1	2.09 (1.88-2.31)	0.0001
Primary incomplete	60	2	2.17 (1.95-2.41)	0.0001
Primary complete	16	1	1.93 (1.67-2.23)	0.0001
Secondary complete	19	8	1.43 (1.11-1.86)	0.03
High school complete	33	63	0.65 (0.49-0.87)	0.0001
School fighting	20	6	2.22 (1.09-4.4)	0.008
Delinquency				
Stealing	18	0	1.96 (1.72-2.23)	0.0001
Street fighting	5	0		
Nutritional profile				
Severe malnutrition	13	0	1.34 (0.60-2.98)	0.34
Mild malnutrition	30	15	1.37 (1.10-1.71)	0.02
Overweight	33	37	0.94 (0.72-1.22)	0.26
Morbid obesity	13	13	1.00 (0.68-1.48)	0.1

RR = relative risk; 95%CI = confidence interval at 95%; P = Chi square test statistically significant ≤ 0.05 .

Table 3. Logistic regression of risk variables among survivors of child abuse

Variable	cRR (95%IC)	P*	aRR (95%IC)	P**
ADHD	1.69 (1.42-2.02)	0.001	4.65 (2.06-10.51)	0.0001
Mood disorder	2.08 (1.52-2.86)	0.0001	2.97 (1.81-4.87)	0.0001
Tobacco use	2.10 (1.50-2.96)	0.0001	2.79 (1.60-4.88)	0.0001
Alcohol use	1.2 (0.98-1.58)	0.06	0.79 (0.45-1.41)	0.43
Drug use	4.05 (2.02-8.12)	0.0001	3.09 (1.25-7.63)	0.0001
Drug use in parents	12.4 (1.8-85)	0.0001	15.7 (2.04-121.7)	0.008
Sports engagement	0.56 (0.49-0.65)	0.001	0.31 (0.18-0.52)	0.0001

ADHD = attention-deficit/hyperactivity disorder; cRR = crude relative risk; 95%IC = confidence interval of 95%; P* = statistically significant ≤ 0.05 according to the chi-square test; aRR = adjusted relative risk, logistic regression with the Hosmer-Lemeshow goodness-of-fit test and R^2 (Cox-Snell, Nagelkerke); P** = ≤ 0.05 according to the adjusted chi-square test.

Table 4. Start age for substance use, initial sexual experience and pregnancy

Start age (years)	Child abuse (n = 300)	Non-exposed (n = 300)	P*
Tobacco use	14 (7-22)	18 (16-20)	0.0001
Alcohol use	14 (7-18)	18 (15-20)	0.30
Drug use	13 (9-17)	18 (17-20)	0.0001
Initial sexual experience	14 (7-19)	17 (16-21)	0.0001
First pregnancy	16 (11-24)	19 (17-23)	0.0001

Results are presented in mean (range); P* = Chi square test statistically significant ≤ 0.05 .

DISCUSSION

Approximately one in eight children admitted to our Pediatric Emergency Department between January 2011 and April 2018, were survivors of child abuse. This high frequency is reflective of referrals to the PAMMI group at our hospital.

The follow-up interviews revealed that Mexican survivors of child abuse have significantly lower educational levels and additional challenges, such as ADHD and school fighting. Child abuse impairs normal developmental processes, including cognitive, emotional, and social aspects. Maltreated children are at a higher risk of substandard academic achievement, decreased intelligence in childhood, and inferior educational levels. Notably, these risks may persist into adulthood.⁶⁻⁸

Child abuse affects memory, emotional regulation, and neurological connectivity of the inhibitory pathway. Similarly, these neurocognitive traits are compromised in ADHD. While child abuse and ADHD are significantly related, there is no consensus on the degree, type, or duration of maltreatment that could lead to ADHD.⁶

Other associations have also been identified. For example, milder forms of child abuse, such as inadequate infant attachment, have been related to school underachievement and mental health problems during adolescence.⁹ Emotional/psychological abuse and neglect have been associated with obesity in adulthood.¹⁰ Sexual abuse has been mostly linked to drug abuse during adolescence.¹¹ Nevertheless, the extent of the neurological effects of child abuse during childhood and later in life has not been clearly determined.

Maltreatment survivors more often participate in violent acts or delinquency during adolescence and adulthood.^{4,12,13} We also found that stealing, street fighting, and school fights leading to expulsion or dropping out of school were only identified among the survivors. Commonly, abused children develop protective strategies that heighten their awareness of hostility. Moreover, survivors of abuse are vigilant of danger.⁸ These result from being hurt by the people who are supposed to care for them the most, often their parents and family.⁴ The constantly vigilant state could hinder stress management among the survivor, and increase the risk of recurrent violence.⁸

Young adults who have suffered child abuse are also at risk of mood disorders, such as suicidal behaviors, self-harm, depression,

anxiety, and, to a lesser degree, bipolar disorder and psychosis. Therefore, they often require psychiatric or psychological services and medications, which over time could lead to the misuse or abuse of psychotropic drugs.^{5,14}

Mills et al.¹⁵ found that Australian survivors of child abuse were at an increased risk of cannabis use during adolescence, which often continued into adulthood. Cannabis is the most commonly used drug worldwide. However, when cannabis is used before the age of 17 years, it is associated with alcohol consumption and smoking.¹⁵ In this study, early exposures (7-9 years of age) to drugs (e.g., cannabis, cocaine, amphetamines, alcohol, and tobacco) was demonstrated among the survivors of abuse. In contrast, non-exposed individuals had a start age of 15 to 17 years. In both groups, the start age for substance abuse was early, perhaps because, unlike in Australia, Mexico is a country involved in drug production and transit, which facilitates access for its residents.

Child abuse has also been associated with risky sexual behaviors, including early intercourse, transactional sex, multiple sexual partners, or unprotected sex, and with these, the associated risk of pregnancy and sexually transmitted diseases.¹¹⁻¹³ In our study, the onset of intercourse and first pregnancy among survivors occurred during the adolescent years, starting as early as nine and 11 years, respectively, while in the non-exposed group, the earliest ages were 16 and 17 years. In Mexico, adolescent pregnancy is a public health problem with multifactorial causes, including gender inequity, social disparity, lack of education, early unions, and sexual abuse. Adolescent pregnancy in survivors of child sexual abuse is associated with a higher risk of miscarriage, postpartum depression, substance abuse, and unhealthy diets during pregnancy.¹⁶

Protective factors against the consequences of child maltreatment, including mood disorders, substance abuse, and aggressive behavior, have been explored. A high-quality relationship with a parental figure (e.g., mother or father) and school connection, along with neighborhood collective efficacy, are generally protective against offending behaviors. These factors could prevent unfavorable outcomes by fostering social engagement and supportive relationships with family, teachers, friends, and the community. We also found that non-abused subjects participated in sports significantly more than survivors. The bonds developed with their peers, along

with involvement in a structured and supervised activity offer positive buffers. As Hirschi's theory suggests, stronger social connections diminish the probability of antisocial behaviors.⁴

A comprehensive program that includes home visits, health-care, and family support is essential for the prevention of child abuse.¹⁷ In Mexico, the Sistema Nacional para el Desarrollo Integral de la Familia (DIF, National System for Comprehensive Family Development) is the responsible institution for providing follow-up care to child abuse survivors. Unfortunately, available resources are insufficient to provide home visits, and the institution is not integrated with the healthcare system, which places child abuse survivors at a disadvantage. The social burden of this inadequate care includes a high prevalence of adolescent pregnancy, frequent school dropout, increased substance abuse, and involvement with drug cartels and organized crime. It is imperative to enhance the care pathways for child abuse survivors.

CONCLUSION

Long-term consequences of child abuse include psychological issues, substance abuse, and at-risk adolescent sexual behaviors. Comprehensive programs that incorporate sports engagement and enhance school attendance could help mitigate these adverse outcomes in a cost-effective way.

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Authors contributions: Romero Martínez E: conceptualization, writing – original draft; Robledo Aceves M: conceptualization, methodology, data curation formal analysis, supervision, writing – original draft, writing – review and editing; Barrón Balderas A: conceptualization, data curation, supervision, validation; Corona Gutiérrez AA: formal analysis, validation,

supervision; Camarena Pulido EE: formal analysis, validation, supervision; Magallón Picazo D: data curation, supervision, validation. All authors reviewed and approved the final version submitted for publication

Sources of funding: None

Conflicts of interest: None

Data availability statement:

Data supporting the findings of this study are available upon request from the corresponding author, Mireya Robledo Aceves

Declaration of generative AI in scientific writing:

The authors did not use generative AI or AI-assisted technologies during the preparation of this study.

First submission: November 25, 2024

Last received: June 18, 2025

Accepted: May 21, 2026

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Vitamin D supplementation and central adiposity in male shift workers: a randomized clinical trial on metabolic health

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KEYWORDS (MeSH terms):

Shift Work Schedule.
Circadian rhythm.
Calcitriol.
Obesity.

AUTHOR'S KEYWORDS:

Vitamin D.
Calciferol.
Adiposity.
Clinical trial.

ABSTRACT

BACKGROUND: Vitamin D deficiency is common among individuals with obesity; however, the association between low 25-hydroxyvitamin D [25(OH)D] concentrations and adiposity remains unclear. Shift workers are at increased risk for both vitamin D deficiency and metabolic disorders, making this population particularly relevant for investigating this relationship.

OBJECTIVES: This study aimed to evaluate the effect of cholecalciferol supplementation on central adiposity, assessed by waist circumference (WC) and waist-to-height ratio (WHtR), among male shift workers.

DESIGN AND SETTING: Randomized, double-blind, placebo-controlled clinical trial involving 198 male shift workers employed by an iron ore extraction company in Minas Gerais, Brazil.

METHODS: Participants with 25(OH)D concentrations <30 ng/mL and at least one metabolic abnormality, including metabolic syndrome, impaired fasting glucose, dyslipidemia, elevated blood pressure, or increased WC, were randomly assigned to receive placebo, cholecalciferol 14,000 IU/week, or cholecalciferol 28,000 IU/week for 4 months. Plasma 25(OH)D concentrations, WC, and WHtR were evaluated at baseline and post-intervention.

RESULTS: After 4 months, no significant differences in WC or WHtR were observed between the vitamin D supplementation groups (14,000 IU/week and 28,000 IU/week) and the placebo group. Nevertheless, serum 25(OH)D concentrations increased significantly in the supplementation groups: +6.23 ng/mL (p₂₅ = 0.58; p₇₅ = 10.61) in the 14,000 IU/week group and +15.25 ng/mL (p₂₅ = 10.57; p₇₅ = 19.68) in the 28,000 IU/week group. In contrast, the placebo group showed a decrease of -0.50 ng/mL (p₂₅ = -1.03; p₇₅ = 2.71). Despite these increases, supplementation produced no significant effect on central adiposity outcomes (p > 0.05).

CONCLUSION: Although vitamin D supplementation effectively increased serum 25(OH)D concentrations, it did not reduce central adiposity among male shift workers. These findings indicate that vitamin D supplementation alone is unlikely to improve obesity-related outcomes in this high-risk occupational population and underscore the need for comprehensive strategies targeting metabolic health in shift workers.

CLINICAL TRIAL OR SYSTEMATIC REVIEW REGISTRATION: This study was registered in the Brazilian Registry of Clinical Trials (ReBEC) under identifier RBR-6CS352 on October 25, 2017.

INTRODUCTION

Obesity is a chronic, multifactorial disease characterized by excessive adipose tissue accumulation and is among the most significant global public health challenges. It contributes to millions of deaths each year and is strongly associated with comorbidities such as hypertension, type 2 diabetes, and cardiovascular disease.¹ In Brazil, the burden is similarly substantial, with 39.6% of the population classified as overweight and 23.8% as obese.² The rising prevalence of obesity highlights the need to identify modifiable risk factors and develop effective interventions.

Vitamin D deficiency has emerged as a potential factor associated with obesity and is now recognized as a worldwide health concern affecting all age groups.³ Cross-sectional studies have consistently demonstrated an inverse relationship between serum 25(OH)D concentrations and adiposity, as well as associations with metabolic abnormalities, including insulin resistance, type 2 diabetes, and dyslipidemia.⁴ Experimental evidence suggests that vitamin D may regulate leptin expression, a hormone involved in appetite control and energy homeostasis, indicating a possible role in adipogenesis and obesity-related mechanisms.⁵ Nevertheless, despite these findings, the causal relationship between vitamin D deficiency and obesity remains uncertain.^{6,7} Additional high-quality evidence is needed to clarify the contribution of vitamin D to obesity

management and related metabolic disorders. Furthermore, occupational factors have been identified as important determinants of vitamin D status.

Shift workers are particularly vulnerable because reduced sunlight exposure limits endogenous vitamin D synthesis. Studies have shown that shift workers have an 80% higher risk of hypovitaminosis D than individuals in other occupational groups.⁸ In this population, Batista et al.⁹ reported a high prevalence of hypovitaminosis D (73%) and a significant association with increased visceral fat area (odds ratio [OR]: 2.4; 95% confidence interval [CI]: 1.1–5.2). These findings highlight the importance of targeted strategies to address vitamin D deficiency among shift workers.

OBJECTIVE

This study aimed to evaluate the effect of cholecalciferol supplementation on central adiposity, measured by changes in waist circumference (WC) and waist-to-height ratio (WHtR), among male shift workers.

METHODS

Study design and population

This study was conducted within the *Fatigue Management Project*, an ongoing research program investigating metabolic and occupational health among rotating shift workers in Brazilian mining companies since 2012 through cross-sectional assessments performed in 2012, 2015, and 2018. The present analysis derives from a randomized, double-blind, placebo-controlled clinical trial conducted in 2016 and nested within the *Fatigue Management Project*. The trial was designed to assess the effects of vitamin D supplementation on biochemical and body composition risk factors related to fatigue, cardiovascular disease, and cognitive function.¹⁰ The intervention was implemented during the summer.

The study included male employees of an iron ore mining company located in the Quadrilátero Ferrífero (Iron Quadrangle) region of Minas Gerais, Brazil. Eligible workers were truck drivers and heavy machinery operators who used automated, climate-controlled equipment and worked rotating shifts. The schedule followed a clockwise rotation (morning, afternoon, and night shifts) consisting of 6 hours of work followed by 12 hours of rest. After four consecutive shifts, workers received 1 day off, resulting in a weekly workload of 36 hours.

Sample size calculations were based on the assumptions that hypertriglyceridemia would be present in 40% of participants not receiving vitamin D supplementation and in 22% of those receiving supplementation, with a likelihood ratio of 2.3.¹⁰ Considering 80% statistical power and a 95% CI, 106 participants were required per group. After accounting for an anticipated

10% attrition rate, the minimum target sample was 350 participants. Sample size estimation was performed using OpenEpi (Open Source Epidemiologic Statistics for Public Health, United States), version 3.01.

The analysis included participants who completed the 4-month intervention period. Eligibility was restricted to individuals with baseline 25(OH)D concentrations < 30 ng/mL and complete socioeconomic and anthropometric data collected before and after the intervention. A total of 362 workers were initially recruited. Of these, 44 did not receive the intervention because of withdrawal, transfer, or employment termination. Following randomization, 120 additional participants were excluded because their 25(OH)D concentrations exceeded 30 ng/mL or because of incomplete data. Consequently, 198 participants were included in the final analysis (**Figure 1**).

According to the study protocol, primary and secondary outcomes were planned for analysis using both intention-to-treat (ITT) and per-protocol (PP) populations.¹ The ITT population comprised all randomized participants with available outcome data, analyzed according to their assigned groups. The PP population excluded individuals with protocol deviations or those who failed to meet all biochemical inclusion criteria identified after allocation. The present manuscript reports findings from the PP population (n = 198) to evaluate the clinical efficacy of cholecalciferol supplementation among participants with confirmed baseline 25(OH)D insufficiency (< 30 ng/mL).

The study was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines. A completed CONSORT checklist is provided as supplementary material.

Intervention

Participants were randomly assigned to one of three groups: placebo, cholecalciferol 14,000 IU/week, or cholecalciferol 28,000 IU/week. The intervention groups received oral cholecalciferol (vitamin D3) supplementation at the assigned dosage, whereas the placebo group received identical capsules without cholecalciferol. All participants received bottles containing 10 capsules and were instructed to take one capsule weekly.

To promote adherence and reduce missed doses, the research team conducted regular follow-up contacts, providing individualized reminders on scheduled administration days. Supplement distribution was coordinated through prearranged workplace visits, during which participants received vitamin D or placebo bottles and brief reinforcement regarding protocol compliance.

Blinding procedures were implemented by a study statistician who was not involved in data collection or participant contact. This investigator generated the randomization sequence and assigned participants to the study groups. Placebo and active-treatment capsules were identical in appearance and packaging.

All capsules were labeled with participant codes to conceal treatment allocation from participants, intervention personnel, and outcome assessors. Group assignments remained masked until completion of data verification and statistical analyses. A post-trial evaluation of participants' allocation guesses supported the effectiveness of the blinding procedures.

Eligibility criteria were established according to the primary objective of the clinical trial, which was to assess the effects of cholecalciferol supplementation on cardiometabolic risk factors. Participants were required to have serum 25(OH)D concentrations < 30 ng/mL and at least one of the following conditions: metabolic syndrome, impaired fasting glucose, dyslipidemia, elevated blood pressure, or increased WC. Exclusion criteria included malabsorption syndrome; renal, hepatic, or thyroid disease; use of anticonvulsants, corticosteroids, hormones, vitamin supplements, or mineral supplements; and abnormal serum concentrations of creatinine, parathyroid hormone, calcium, or albumin.

Stratified randomization was used to ensure balanced distribution of vitamin D status and cardiovascular risk factors across the two intervention groups and the placebo group (Figure 1), consistent with the primary objectives of the parent clinical trial.⁹

Adverse events were monitored throughout the study via weekly reminder messages and follow-up assessments conducted at months

4, 8, and 12.. Participants were instructed to report symptoms suggestive of vitamin D toxicity, such as dizziness, nausea, or constipation, as well as any other health-related changes experienced during the trial.

Data collection and definitions

Data were collected between January and July 2016 at five mining facilities operated by the company. Biological samples were processed at the Pilot Clinical Analysis Laboratory (LAPAC) and the Laboratory of Epidemiology of Parasitic Diseases (LEPI) of the Escola de Medicina, Universidade Federal de Ouro Preto (UFOP). A trained research team conducted all study procedures, including administration of sociodemographic questionnaires, anthropometric assessments, and collection and analysis of biological samples.

Sociodemographic information was obtained using a standardized questionnaire administered by trained personnel. Variables included sex, age (years), marital status (single, married, separated/divorced, or widowed), and educational attainment (primary, secondary, or higher education). Skin color was self-reported according to the classification adopted by the Brazilian Institute of Geography and Statistics (white, black, brown, yellow, or indigenous). Duration of shift work was determined from the total number of years worked in rotating shifts.

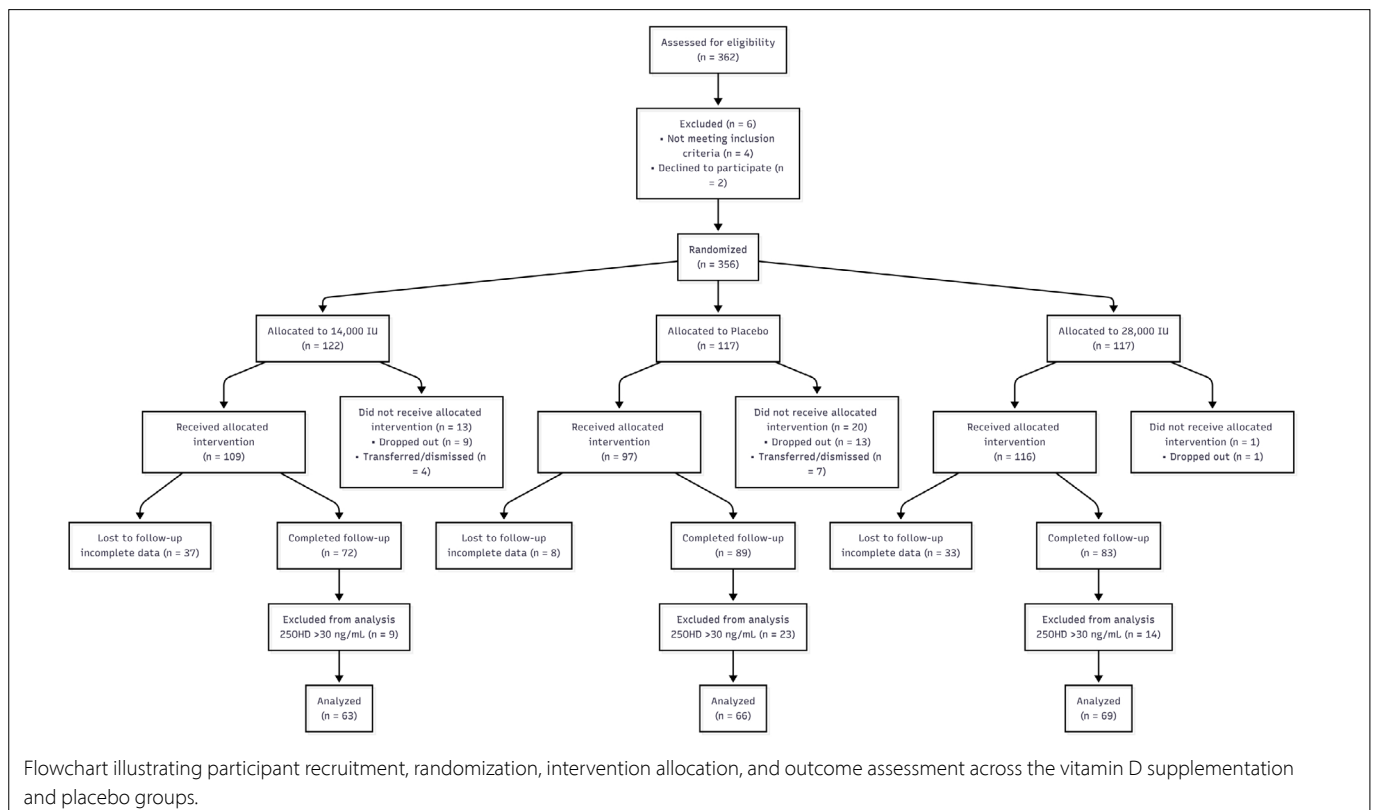


Figure 1. Study flowchart.

ADIPOSIITY MARKERS

WC was measured using a flexible, nonelastic tape positioned midway between the iliac crest and the lowest rib margin.¹¹ Participants removed their shirts and stood upright with a relaxed abdomen, arms at their sides, and feet together. Measurements were obtained in triplicate while participants breathed normally to avoid abdominal muscle contraction.¹² WC was classified according to International Diabetes Federation criteria,¹³ with values ≥ 90 cm considered elevated. Height was measured using a portable AlturExata® stadiometer (AlturExata, Belo Horizonte) with 1-mm precision. The WHtR was calculated by dividing WC by height. WHtR values ≥ 0.5 were classified as elevated.¹⁴

Vitamin D

Serum vitamin D concentrations were determined by chemiluminescence using Beckman Coulter® commercial kits (Beckman Coulter, Fullerton, California, United States). Vitamin D status was classified according to the Brazilian Society of Endocrinology and Metabolism guidelines: deficiency (< 20 ng/mL), insufficiency (20–29 ng/mL), and sufficiency (≥ 30 ng/mL).¹⁵

Statistical analysis

Data analysis was performed using Stata/MP version 15.0 and R version 3.5.0 for Linux (The R Foundation for Statistical Computing). Categorical variables are presented as absolute frequencies (n) and percentages (%). Continuous variables are reported as mean $\pm$ standard deviation or median (p25–p75), according to data distribution assessed with the Kolmogorov–Smirnov test.

Statistical analyses were conducted in three stages. First, baseline comparability among the three randomized groups was evaluated using Pearson's chi-square (χ^2) test. Second, intervention effects after 4 months were assessed using McNemar's chi-square test for categorical variables and paired Student's t test or the Wilcoxon signed-rank test for continuous variables, as appropriate. Third, post-intervention differences among groups were examined using the Kruskal–Wallis test followed by Bonferroni-adjusted post hoc comparisons.

Changes in WC and WHtR were additionally assessed using percentage variation between baseline and 4 months. Percentage change was calculated as: $[(\text{final value} - \text{initial value}) / \text{final value}] \times 100$. This measure represented the proportional change relative to the final value.

Participants with incomplete outcome data were excluded from the analysis, resulting in a per-protocol approach. Baseline characteristics of completers and non-completers were compared to evaluate potential attrition bias (data not shown).

Ethical considerations

The study protocol was approved by the Research Ethics Committee of the Universidade Federal de Ouro Preto (CEP 1.381.376) on

December 17, 2015. The trial was subsequently registered in the Brazilian Registry of Clinical Trials (RBR-6CS352) under the title “Fatigue Management Project.” The study was conducted under the responsibility of the Escola de Medicina, Universidade Federal de Ouro Preto. All participants provided written informed consent prior to enrollment.

RESULTS

The study included 198 rotating shift workers aged 24–57 years. The largest age group was 30–39 years (51.5%), followed by 40–49 years (35.9%). Most participants (93.9%) had worked rotating shifts for >5 years. More than half had completed high school and self-identified as non-White (**Table 1**). The prevalence of abdominal obesity was 72.2% (n = 143) based on WC and 83.3% (n = 165) based on the WHtR (data not shown).

No significant baseline differences were observed between treatment groups for WC (p = 0.487), WHtR (p = 0.973), age (p = 0.563), ethnicity (p = 0.694), shift work duration (p = 0.794), or baseline plasma vitamin D levels (p = 0.083) (**Table 2**).

Among participants receiving 14,000 IU/week of cholecalciferol, insufficient and deficient vitamin D levels decreased by 16.2% and 3%, respectively, whereas sufficient levels increased by 19.3%. In the 28,000 IU/week group, insufficient and deficient vitamin D levels decreased by 21.8% and 7.1%, respectively, while sufficient levels increased by 28.8%. In contrast, the placebo group exhibited a 1.5% increase in insufficient vitamin D levels, a 3.0% decrease

Table 1. Sociodemographic profile of shift workers in a mining company in the Iron Quadrangle region, Minas Gerais

Variables	n	%
Age group		
24–39 years	110	55.5
40–57 years	88	44.5
Years of shift work		
<10 years	71	35.9
≥ 10 years	121	64.1
Marital status		
Married	167	84.3
Single	31	15.6
Education level		
Up to primary school	7	3.5
High school/Technical	180	90.9
Higher education	11	5.6
Skin color		
White	58	29.3
Non-white	140	70.7

Data represent the sociodemographic profile of shift workers.

in deficient levels, and a 1.5% increase in sufficient levels between pre- and post-intervention assessments.

No significant changes in WC or WHtR were detected following supplementation in any intervention group (Table 3). However, WC and WHtR adequacy rates increased by 2.5% and 0.5%, respectively, in the 14,000 IU/week vitamin D3 group. In the 28,000 IU/week group, the proportion of participants with adequate WC decreased by 2%, whereas WHtR remained unchanged (Figure 2). In the placebo group, WC adequacy decreased by 1%, whereas WHtR adequacy increased by 1% (Table 3).

Plasma 25(OH)D levels increased substantially in both supplementation groups. The 14,000 IU/week group demonstrated a median increase of 6.23 ng/mL ($p_{25} = 0.58$; $p_{75} = 10.61$), whereas the 28,000 IU/week group showed a median increase of 15.25 ng/mL ($p_{25} = 10.57$; $p_{75} = 19.68$). Conversely, the placebo group exhibited a median decrease of 0.50 ng/mL ($p_{25} = -3.73$; $p_{75} = 2.71$) in plasma 25(OH)D levels (Table 3).

As illustrated in Figure 3, both vitamin D supplementation groups demonstrated significantly greater increases in 25(OH)D levels than the placebo group. No adverse events or unintended effects associated with supplementation were reported or observed during the 4-month follow-up period.

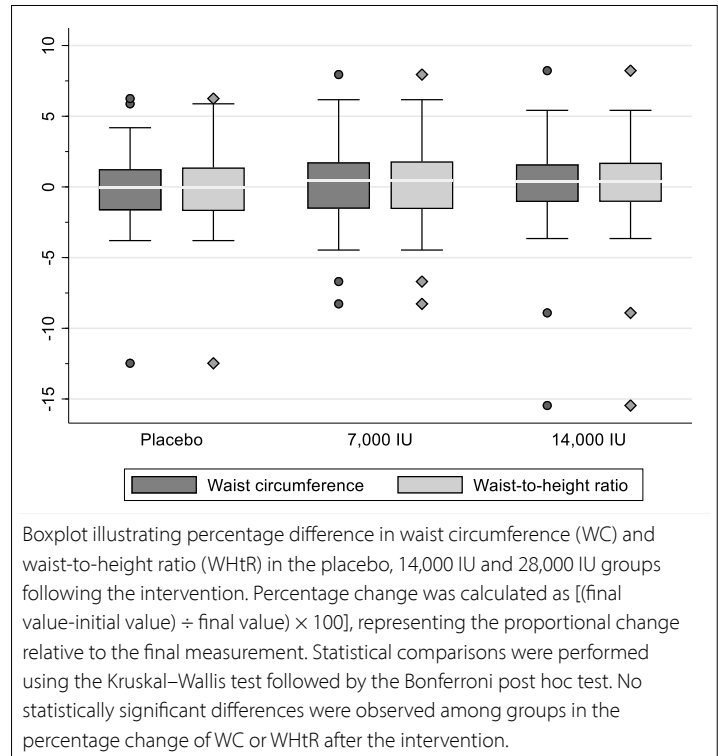


Figure 2. Percentage difference in waist circumference (WC) and waist-to-height ratio (WHtR) after the intervention.

Table 2. Sociodemographic, anthropometric, and vitamin D characterization of the alternating shift workers sample at baseline

	Placebo (n = 63)	14,000 IU (n = 66)	28,000 IU (n = 69)	p value
Age group				
24–39 years	32 (16.2%)	35 (17.7%)	43 (21.6%)	0.363
40–57 years	31 (15.7%)	31 (15.7%)	26 (13.1%)	
Skin color				
White	21 (10.6%)	18 (9.1%)	19 (9.6%)	0.694
Non-white	42 (21.2%)	48 (24.2%)	50 (25.3%)	
Waist circumference				
<90 cm	18 (9.1%)	15 (7.6%)	22 (11.1%)	0.487
≥90 cm	45 (22.7%)	51 (25.8%)	47 (23.7%)	
Waist-to-height ratio				
<0.5	11 (5.6%)	11 (5.6%)	11 (5.6%)	0.973
≥0.5	52 (26.2%)	55 (27.8%)	58 (29.2%)	

Pearson's chi-square test was used to verify homogeneity between intervention groups.

Table 3. Difference in vitamin D levels and waist circumference after 4 months between the intervention and placebo groups.

	Placebo	14,000 IU	28,000 IU
25(OH)D (ng/mL)	−0.5 (−3.73; 2.71) ^a	6.23 (0.58; 10.61) ^b	15.25 (10.57; 19.68) ^c
WC (cm)	−0.1 (−1.8; 1.4) ^a	0.37 (−1.25; 1.6) ^a	0.8 (−1; 1.83) ^a
* WHtR (e ^{−1})	0.01 (−1.03; 0.08) ^a	−0.07 (0.02; 0.09) ^a	0.04 (−0.05; 1.03) ^a

Data are presented as median (p₂₅–p₇₅). Comparisons were performed using the Kruskal–Wallis test followed by the Bonferroni post hoc test. Superscript letters (a, b, c) indicate statistically significant differences between groups. Groups sharing the same letter do not differ significantly ($p > 0.05$), whereas groups with different letters differ significantly ($p < 0.05$). * WHtR raised to −1.

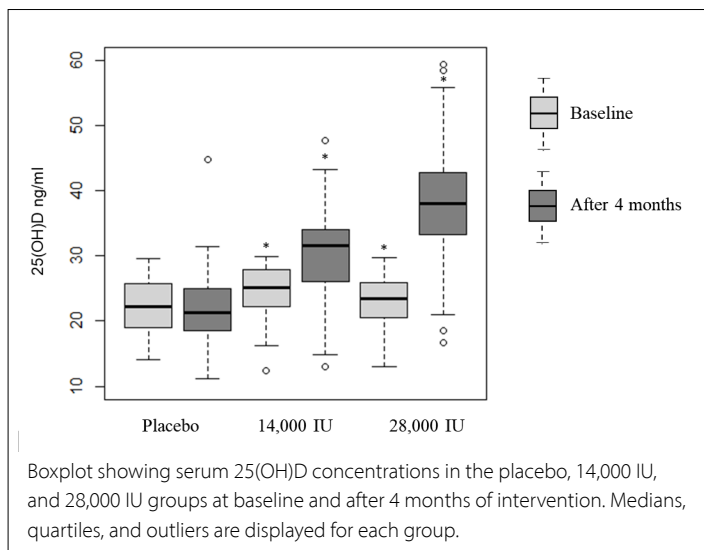


Figure 3. Serum vitamin D [25(OH)D] levels before and after the intervention.

DISCUSSION

This randomized, placebo-controlled trial evaluated the effects of weekly oral cholecalciferol supplementation at doses of 14,000 IU and 28,000 IU for 16 weeks in individuals with baseline 25(OH)D concentrations < 30 ng/mL. Although supplementation significantly increased 25(OH)D levels in both intervention groups ($p < 0.05$), no significant changes were observed in WC or WHtR compared with placebo.

Cross-sectional studies have consistently reported an inverse association between 25(OH)D concentrations and adiposity.^{16–18} In a study of 250 adults in New Zealand, serum vitamin D levels decreased by 0.12 ng/mL for each 1-cm increase in WC ($p = 0.01$) and by 0.30 ng/mL for each 1 kg/m² increase in body mass index (BMI) ($p = 0.002$).¹⁹ However, studies designed to evaluate causality have not produced similar findings. A randomized clinical trial involving 328 participants reported that vitamin D supplementation of up to 4,000 IU/day for 3 months had no significant effect on BMI (mean difference [MD], -0.06 kg/m²; 95% CI, -0.14 to 0.03), body weight (MD, -0.05 kg; 95% CI, -0.32 to 0.23), or fat mass (MD, -0.43 kg; 95% CI, -1.69 to 0.84) compared with placebo.²⁰ These findings are consistent with the present study.

Although BMI, WC, and WHtR are widely used because of their simplicity, low cost, and established associations with disease risk, these measures do not directly quantify body fat. Bioelectrical impedance analysis (BIA) offers a validated alternative for estimating body fat in clinical settings.²¹ In a study of 1,105 adults aged 20–70 years, Zhang et al.²² reported an inverse association between serum 25(OH)D concentrations and visceral fat area (VFA) estimated by BIA. Higher VFA was associated with an increased likelihood of vitamin D insufficiency or deficiency, demonstrating a significant dose-response relationship ($p < 0.001$).

Nevertheless, both anthropometric measures and BIA have limited ability to distinguish abdominal adipose tissue from retroperitoneal fat, potentially weakening estimates of the association between vitamin D status and adiposity. More precise assessments can be obtained using dual-energy X-ray absorptiometry (DXA), computed tomography (CT), and magnetic resonance imaging (MRI).²³ A randomized, double-blind, placebo-controlled trial involving 52 participants aged 18–50 years used DXA and MRI to assess body composition and found that daily supplementation with 7,000 IU of cholecalciferol for 26 weeks significantly increased 25(OH)D concentrations but produced no changes in total body fat, subcutaneous adipose tissue, or visceral fat relative to placebo.⁷

Obesity is a multifactorial disease influenced by genetic, socioeconomic, cultural, and behavioral determinants.²⁴ Consequently, vitamin D supplementation alone may be insufficient to modify obesity-related outcomes. This interpretation is supported by an analysis of 21 adult cohorts showing that higher BMI leads to lower concentrations of 1,25(OH)₂D, whereas no evidence indicated that vitamin D deficiency contributes to obesity development.⁴ Similarly, randomized controlled trials, including the present study, have not supported a causal effect of vitamin D deficiency on adiposity.^{7,20,25,26}

Physical inactivity is another factor associated with obesity and reduced vitamin D status because it may limit sun exposure. Approximately 90% of vitamin D is synthesized endogenously through exposure to sunlight.²⁹ Therefore, conditions that restrict outdoor activity, including sedentary behavior and shift work, may contribute to lower circulating 25(OH)D concentrations. A study of 5,409 workers in South Korea aged 20–65 years reported a significantly greater risk of vitamin D deficiency among shift workers than among daytime workers (OR: 1.456; 95% CI: 1.089–1.946).²⁸ Additional studies have similarly demonstrated higher serum vitamin D concentrations among outdoor workers than among indoor workers.²⁹

Although dietary sources contribute to vitamin D intake, diet is generally not a major determinant of circulating 25(OH)D levels. Dietary intake was not assessed during the present intervention; however, previous research has documented inadequate vitamin D intake across diverse populations. In a cross-sectional study of 1,222 adults, dietary inadequacy reached 100% among both males and females, with a median intake of 3.4 µg/day, less than half the Estimated Average Requirement.³⁰ These findings suggest that dietary intake alone is unlikely to substantially influence 25(OH)D concentrations, particularly in regions with abundant sunlight.

Although no causal effect of vitamin D supplementation on adiposity was identified, individuals with excess weight consistently exhibit lower vitamin D concentrations. Monitoring vitamin D status in overweight individuals and promoting adequate sun exposure, healthy dietary habits, and regular physical activity

may help improve vitamin D status and reduce the adverse effects of excess adiposity on this parameter.

Despite its methodological strengths, this study has several limitations. Information on sun exposure habits was not collected, preventing assessment of their influence on 25(OH) D concentrations. However, the absence of increased 25(OH) D levels in the placebo group suggests limited variation in sun exposure within this relatively homogeneous population. Future studies should incorporate assessments of sun exposure and dietary intake and use advanced body composition techniques such as DXA and MRI to further clarify the relationship between vitamin D and adiposity.

Furthermore, a substantial number of participants were excluded after randomization because baseline vitamin D concentrations exceeded 30 ng/mL or because of incomplete data, resulting in a per-protocol analysis. Although both ITT and PP analyses were specified in the study protocol to reduce bias,¹ post-randomization exclusions may have introduced attrition bias. However, restricting the analysis to participants with confirmed vitamin D insufficiency or deficiency was necessary to evaluate the metabolic effects of supplementation in the target population and remained consistent with the study objectives.

CONCLUSION

No significant differences in body adiposity parameters were observed between the cholecalciferol-supplemented groups and the placebo group, despite a significant increase in plasma 25(OH)D concentrations among participants receiving vitamin D. The association between vitamin D and obesity, the underlying mechanisms of this relationship, and the increase in vitamin D levels accompanying reductions in adiposity remain incompletely understood. Furthermore, whether obesity contributes causally to low vitamin D levels or whether vitamin D deficiency promotes obesity has yet to be fully established. Additional studies are needed to clarify the relationship between vitamin D status and excess body weight in this worker population. The present findings support the hypothesis that adiposity is more likely to influence vitamin D concentrations than vitamin D status is to influence adiposity.

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Authors' contributions: Menezes-Júnior L, Fajardo VC, Freitas SN, Pimenta FAP, Machado Coelho GLP, Oliveira FLP, Nascimento RM Neto: conception and design of the study, data acquisition, analysis and interpretation, and preparation of the manuscript draft. Menezes L Jr, Fajardo VC: contributed to data analysis, interpretation, and manuscript development. All authors critically reviewed the manuscript and approved the final version for publication.

Acknowledgments: The authors thank the Escola de Medicina and Escola de Nutrição of the Universidade Federal de Ouro Preto for providing transportation for data collection activities. The authors also acknowledge the Laboratório Piloto de Análises Clínicas (LAPAC) and the Laboratório de Epidemiologia de Doenças Parasitárias of the Universidade Federal de Ouro Preto (UFOP) for providing facilities and equipment for laboratory procedures and biochemical analyses. The authors further thank the Grupo de Pesquisa e Ensino em Nutrição e Saúde Coletiva (GPENSC), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their support.

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: Data supporting the findings of this study are available from the corresponding author, Luiz Menezes Júnior, upon reasonable request.

Declaration of generative AI in scientific writing: The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the writing, analysis, or production of this manuscript.

Date of first submission: March 8, 2025

Last received: March 5, 2026

Accepted: March 18, 2026

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Editor responsible for the evaluation process:

Marianne Yumi Nakai, MD, PhD (AE)
Paulo Manuel Pêgo-Fernandes, MD, PhD (EIC)



Assessment of thyroid hormone profiles and derived ratios for predicting thyroid nodule malignancy using the Bethesda system: thyroid hormone ratios in malignancy risk stratification of thyroid nodules: a cross-sectional study

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KEYWORDS (MeSH terms):

Thyroid Nodule.
Biopsy.
Thyroid Function Tests.
Thyroid Cancer.

AUTHOR'S KEYWORDS:

Cytodiagnosis.
Thyrotropin.
Thyroid Neoplasms.
Fine Needle Aspiration Biopsy.
Hormonal Ratio Biomarkers.
Preoperative Risk Assessment.

ABSTRACT

BACKGROUND: Thyroid nodules are common, and although most are benign, a proportion may be malignant. Fine-needle aspiration biopsy (FNAB), interpreted using the Bethesda System, is the standard diagnostic method. However, in euthyroid patients, the predictive value of thyroid function tests and their derived ratios in relation to Bethesda categories remains unclear.

OBJECTIVES: To evaluate thyroid function parameters and their association with Bethesda classification in euthyroid patients with thyroid nodules, and to identify potential predictors of malignancy.

DESIGN AND SETTING: This retrospective cross-sectional study was conducted at the University of Health Sciences Haydarpaşa Numune Training and Research Hospital and included a convenience sample of eligible euthyroid patients who underwent FNAB between January 2020 and December 2023.

METHODS: A total of 828 euthyroid patients were included. Demographic data, pre-biopsy TSH, fT3, fT4, and derived ratios (TSH/T4, TSH/T3, and T3/T4) were analyzed for correlations with Bethesda categories and compared between the benign and malignant groups.

RESULTS: The mean age was 52.85 ± 12.53 years, and 80.8% of participants were female. Benign cytology was found in 54.6% of cases, and malignant cytology was found in 2.8%. Cases with malignant cytology were younger ($p = 0.003$) and more frequently male ($p = 0.021$). No significant differences were observed in hormone levels or ratios between the benign and malignant groups. However, TSH and the TSH/T4 ratio showed positive correlations with Bethesda category ($p = 0.034$ and $p = 0.033$, respectively).

CONCLUSION: The TSH level and TSH/T4 ratio were correlated with Bethesda categories; however, thyroid function tests and derived ratios showed no diagnostic value for predicting malignancy in euthyroid nodules.

INTRODUCTION

Thyroid nodules are common thyroid disorders frequently encountered in clinical practice and are highly prevalent in the general population. The prevalence of thyroid nodules varies depending on the studied population and the diagnostic method used, ranging from 2–6% by palpation, 19–35% by ultrasound, and 8–65% in autopsy studies.¹

Most thyroid nodules are benign; however, approximately 5–15% are malignant.² Accurate evaluation and management of nodules are crucial for both clinical decision-making and public health. Therefore, the primary goal in the management of patients with thyroid nodules is to assess the risk of malignancy. A definitive diagnosis of malignancy or benign disease can be established only through histopathological examination. Thyroid fine-needle aspiration biopsy (FNAB) should be performed on nodules with suspicious malignant features based on history, physical examination, laboratory findings, and ultrasonographic characteristics.³ The Bethesda System for Reporting Thyroid Cytopathology was developed to standardize FNAB cytology and provide clinicians with guidance on malignancy risk estimation and patient management. According to this system, FNAB results are categorized as follows: Bethesda 1 – non-diagnostic, Bethesda 2 – benign, Bethesda 3 – atypia of undetermined significance, Bethesda 4 – follicular neoplasm, Bethesda 5 – suspicious for malignancy, and Bethesda 6 – malignant.⁴ Bethesda 3 and 4 are collectively referred to as indeterminate cytology.

Elevated serum thyroid-stimulating hormone (TSH) levels have been suggested to increase the risk of thyroid malignancy in patients with nodular thyroid disease. Several studies have demonstrated that even within the normal range, higher TSH values are associated with a greater frequency and more advanced stages of thyroid cancer.^{5,6} Free triiodothyronine (T3) and free thyroxine (T4) levels have also been investigated in the literature, with conflicting results. A meta-analysis indicated that higher T3 levels were associated with a 14% reduction in thyroid cancer risk.⁷ However, other studies suggest that T3 and other thyroid hormones may influence cancer cell growth, proliferation, and metastasis.^{8,9} In addition, another study demonstrated that elevated free T4 and free T3 levels are associated with the development of thyroid cancer in both sexes.¹⁰

Although TSH levels are thought to be associated with malignancy in thyroid nodules, there are insufficient data on the relationship between TSH levels and preoperative cytopathological findings. Therefore, in this study, we aimed to investigate the correlations between Bethesda classification and TSH, free T3, free T4, the T3/T4 ratio, the TSH/T3 ratio, and the TSH/T4 ratio in euthyroid individuals. Our literature review indicates that studies comprehensively examining the relationship between the Bethesda classification and thyroid function parameters are limited. The objective of this study was to identify a readily applicable, non-invasive laboratory parameter with a potential cutoff value for predicting thyroid malignancy.

OBJECTIVE

The primary objective of this study was to investigate potential associations between thyroid function parameters and the Bethesda classification in patients with euthyroid thyroid nodules. Specifically, this study aimed to determine whether serum levels of thyroid-stimulating hormone (TSH), free thyroxine (fT4), and free triiodothyronine (fT3), as well as their derived ratios (TSH/fT4, TSH/fT3, and fT3/fT4), had a statistically significant relationship with cytopathological findings obtained through fine-needle aspiration biopsy (FNAB). By comparing these parameters between patients classified as having benign (Bethesda category II) and malignant (Bethesda category VI) nodules, the study sought to identify accessible and cost-effective biochemical predictors that may aid pre-biopsy risk stratification and clinical decision-making in thyroid nodule management.

METHODS

Study design and population

This retrospective cross-sectional study was conducted with the approval of the University of Health Sciences Haydarpaşa Numune Training and Research Hospital Ethics Committee

(approval no. HNEAH-KAEK 2024/2 24/110) and adhered to the principles outlined in the Declaration of Helsinki. A convenience sampling method was employed. All patients aged 18 years or older who underwent thyroid fine-needle aspiration biopsy (FNAB) between January 1, 2020, and December 31, 2023, at the University of Health Sciences Haydarpaşa Numune Training and Research Hospital, and whose cytopathological results were available in the hospital records, were included in the study. The final sample size was determined by the number of patients who met the inclusion and exclusion criteria within the specified study period. This sample was considered representative of the institution's real-world patient population during this timeframe.

Given the retrospective design of the study and the availability of comprehensive electronic medical records, this approach enabled the inclusion of a broad, unselected patient population, thereby enhancing the external validity and generalizability of the findings. Moreover, the temporal scope of data collection allowed diagnostic and clinical patterns to be captured over a multi-year period, which may further strengthen the robustness of the observed associations.

Data collection and definitions

Demographic variables (age and sex), cytopathological results of FNAB, and serum levels of thyroid-stimulating hormone (TSH), free thyroxine (fT4), and free triiodothyronine (fT3), measured within the three months preceding the biopsy, if available, were recorded. Derived ratios, including TSH/fT4, TSH/fT3, and fT3/fT4, were then calculated. The relationships between these parameters and Bethesda classification categories were analyzed. In addition, patients classified as benign (Bethesda category II) and malignant (Bethesda category VI) were compared with respect to these variables.

Exclusion criteria

The study excluded individuals younger than 18 years of age and those with known primary thyroid diseases, including hypothyroidism, subacute thyroiditis, thyrotoxicosis, Hashimoto's thyroiditis, or Graves' disease; advanced-stage hepatic or renal disease; pituitary disorders; or treatment with antithyroid medications, levothyroxine sodium, or triiodothyronine. Additional exclusion criteria included the use of medications known to interfere with thyroid function, such as estrogen, androgen, lithium, or glucocorticoids; the presence of active infections or malignancies other than thyroid cancer; and incomplete or missing data.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 21.0 (Armonk, New York: IBM Corp.) and MS Excel 2007. The normality of continuous variables was assessed both graphically and using the Shapiro–Wilk test. Descriptive statistics were

presented as mean $\pm$ standard deviation (SD) and median (minimum–maximum) values.

To compare sex distribution between the benign and malignant groups, cross-tabulations were created, and the number (n), percentage (%), and Fisher exact test statistic were reported. The Mann–Whitney U test was used to compare age, TSH, free T4, free T3, TSH/T4, TSH/T3, and T3/T4 values between the benign and malignant groups. To identify potential risk factors, receiver operating characteristic (ROC) analysis was conducted for TSH, free T4, free T3, TSH/T4, TSH/T3, and T3/T4 values, with the benign–malignant classification as the reference. The ROC curve was plotted, and the area under the curve (AUC), together with its 95% confidence interval (95% CI) was determined. For correlation analysis between the Bethesda classification and TSH, free T4, free T3, TSH/T4, TSH/T3, and T3/T4 values, Spearman's non-parametric correlation coefficient was calculated. Bethesda categories were treated as ordinal variables in the correlation analyses to assess monotonic trends across increasing malignancy risk; however, it is acknowledged that, clinically, the Bethesda classification represents a categorical and non-linear risk stratification system. A p value of < 0.05 was considered statistically significant.

RESULTS

Data from 1,719 individuals were initially collected, and analyses were conducted on 828 individuals who met the inclusion criteria. Of these, 80.8% (n = 669) were female, and 19.2% (n = 159) were male. The mean age of the participants was 52.85 ± 12.53 years.

Regarding the Bethesda classification, 24.5% (n = 203) of cases were categorized as Bethesda 1, while 54.6% (n = 453) were categorized as Bethesda 2. Bethesda 3 accounted for 14.9% (n = 123), Bethesda 4 for 1.0% (n = 8), Bethesda 5 for 2.2% (n = 18), and Bethesda 6 for 2.8% (n = 23).

The mean TSH level was 1.74 ± 1.06 , while the mean free T3 and free T4 levels were 3.06 ± 0.43 and 1.16 ± 0.18 , respectively. Additionally, the mean TSH/T4, TSH/T3, and T3/T4 ratios were 1.55 ± 1.01 , 0.56 ± 0.36 , and 2.69 ± 0.51 , respectively (Table 1). The mean age of individuals with benign cytology was 53.73 ± 12.05 years, whereas that of individuals with malignant cytology was 45.78 ± 12.56 years. A statistically significant difference in age was observed between the benign and malignant groups ($z = 2.927$, $p = 0.003$).

In the benign group, 81.5% (n = 369) were female and 18.5% (n = 84) were male, whereas in the malignant group, 60.9% (n = 14) were female and 39.1% (n = 9) were male. Sex distribution differed significantly between the benign and malignant groups ($p = 0.021$).

No statistically significant differences were found between the benign and malignant groups in terms of TSH, T3, T4, TSH/T4, TSH/T3, or T3/T4 values ($p > 0.05$) (Table 2). ROC analysis based on the benign versus malignant classification revealed that the area under the curve (AUC) values for TSH, T3, T4, TSH/T4,

TSH/T3, and T3/T4 were not statistically significant ($p > 0.05$) (Table 3, Figure 1).

Table 1. Demographic and clinical characteristics of the participants

Participants (n = 828)		
Sex, n (%)		
Male		159 (19.2)
Female		669 (80.8)
Age (years)	Mean $\pm$ SD	52.85 ± 12.53
	Median (min–max)	53.0 (19–85)
Bethesda, n (%)		
1		203 (24.5)
2		453 (54.6)
3		123 (14.9)
4		8 (1)
5		18 (2.2)
6		23 (2.8)
TSH	Mean $\pm$ SD	1.74 ± 1.06
	Median (min–max)	1.42 (0.454–4.99)
T3	Mean $\pm$ SD	3.06 ± 0.43
	Median (min–max)	3.05 (2.08–4.33)
T4	Mean $\pm$ SD	1.16 ± 0.18
	Median (min–max)	1.15 (0.81–1.7)
TSH/T4	Mean $\pm$ SD	1.55 ± 1.01
	Median (min–max)	1.24 (0.30–5.8)
TSH/T3	Mean $\pm$ SD	0.56 ± 0.36
	Median (min–max)	0.46 (0.12–1.9)
T3/T4	Mean $\pm$ SD	2.69 ± 0.51
	Median (min–max)	2.66 (1.39–4.55)

TSH: thyroid-stimulating hormone; SD: standard deviation.

Table 2. Comparison of demographic and clinical characteristics between benign and malignant groups

	Benign (n = 453)	Malign (n = 23)	z	p
	Mean $\pm$ SD Median (Min–Max)	Mean $\pm$ SD Median (Min–Max)		
Age	53.73 ± 12.05 54 (19–85)	45.78 ± 12.56 44 (20–68)	2.927	0.003
Sex, n (%)				
Male	84 (18.5)	9 (39.1)	–	0.021*
Female	369 (81.5)	14 (60.9)		
TSH	1.68 ± 1.06 1.37 (0.45–4.99)	1.82 ± 1.1 1.54 (0.64–4.53)	0.937	0.349
	3.04 ± 0.41 3.03 (2.11–4.19)	3.01 ± 0.56 2.98 (2.09–4.24)		
T3	1.16 ± 0.18 1.16 (0.81–1.7)	1.14 ± 0.18 1.1 (0.9–1.49)	0.834	0.404
	1.49 ± 1.03 1.16 (0.3–5.8)	1.63 ± 1.01 1.23 (0.56–3.91)		
TSH/T4	0.55 ± 0.37 0.43 (0.13–1.9)	0.59 ± 0.36 0.47 (0.21–1.54)	0.886	0.376
	2.65 ± 0.51 2.63 (1.39–4.55)	2.62 ± 0.52 2.6 (1.4–3.4)		
TSH/T3			0.089	0.929
T3/T4			0.089	0.929

Z: Mann–Whitney U test; *Fisher exact test. TSH: thyroid-stimulating hormone; SD: standard deviation.

A weak but statistically significant positive correlation was found between Bethesda classification and both TSH ($\rho = 0.085$; $p = 0.034$) and TSH/T4 values ($\rho = 0.086$; $p = 0.033$). However, the strength of these correlations was very low, indicating limited practical or clinical significance despite statistical significance. As the Bethesda category increased (from 2 to 6), TSH and TSH/T4 values showed corresponding increases. No statistically significant correlations were observed between Bethesda classification and T4, T3, TSH/T3, or T3/T4 values ($p > 0.05$) (Table 4, Figure 2).

DISCUSSION

In this study, we compared thyroid function test parameters between patients with benign and malignant cytology (Bethesda 6) and examined the correlations of TSH, free T3, T4, TSH/T4, TSH/T3, and T3/T4 ratios with Bethesda classification. In the current literature, few studies have investigated the relationship between Bethesda classification and thyroid function tests. In this context, our findings contribute to this underexamined area of research, with the additional strength of a relatively large participant cohort compared with those of existing studies.

Our study identified a statistically significant difference in mean age between the benign and malignant groups. The malignant group had a lower mean age than the benign group (45.78 ± 12.56 vs. 53.73 ± 12.05 years), suggesting that thyroid cancer may occur more frequently in younger age groups. This observation aligns with existing literature reporting a higher prevalence of malignant thyroid nodules in young adults, particularly in the 20–40-year age range, with increasing incidence rates.¹¹ A cohort study further supports this pattern, demonstrating that while thyroid nodule prevalence increases with age, malignancy risk shows an inverse relationship.¹² These findings underscore the clinical importance of thorough evaluation for malignancy in younger patients presenting with thyroid nodules.

Our analysis of sex distribution revealed a female predominance in the benign group (81.5%), while males had significantly higher representation in the malignant group (39.1%, $p = 0.021$). This finding suggests that male sex may be a potential risk factor for thyroid nodule malignancy. The literature presents conflicting evidence regarding sex-specific thyroid cancer risk. Although studies report a higher prevalence of nodules in women, the association

between sex and malignancy risk remains inconsistent. Some studies indicate higher rates of differentiated thyroid cancers in females, potentially due to the proliferative effects of sex hormones (estrogen and progesterone) on thyroid cells.^{13–15} A recent cohort study found that female sex was specifically associated with increased papillary thyroid carcinoma risk, while other histological subtypes showed comparable incidence between sexes.¹⁶ However, most reports suggest that although male thyroid cancers are less common, they tend to demonstrate more aggressive behavior and are often diagnosed at advanced stages.^{13,14} Our results align with these observations: while thyroid nodules were more prevalent in women, male sex emerged as a potential malignancy risk factor.

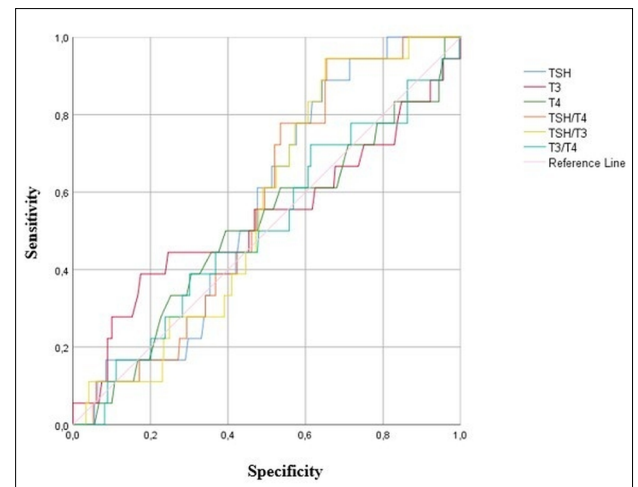


Figure 1. ROC analyses for TSH, T4, T3, TSH/T4, TSH/T3, and T3/T4.

Table 4. Relationships between Bethesda classification and thyroid parameters

	Bethesda	
	ρ	p
TSH	0.085	0.034
T3	0.015	0.768
T4	−0.018	0.648
TSH/T4	0.086	0.033
TSH/T3	0.055	0.285
T3/T4	0.013	0.802

R: Spearman's correlation coefficient; TSH: thyroid-stimulating hormone.

Table 3. Area under the curve (AUC) analysis for malignancy prediction

	AUC $\pm$ SE	95% CI	Cut.	p	Sensitivity (%)	Specificity (%)
TSH	0.558 ± 0.053	0.454–0.662	–	0.349	–	–
T3	0.531 ± 0.083	0.368–0.694	–	0.659	–	–
T4	0.552 ± 0.064	0.427–0.676	–	0.404	–	–
TSH/T4	0.567 ± 0.052	0.466–0.668	–	0.279	–	–
TSH/T3	0.562 ± 0.054	0.456–0.668	–	0.376	–	–
T3/T4	0.506 ± 0.071	0.366–0.646	–	0.929	–	–

AUC: area under the curve; SE: standard error; CI: confidence interval; TSH: thyroid-stimulating hormone.

Recent research has revealed that TSH not only regulates thyroid hormone synthesis but may also influence the development and progression of thyroid cancer. As a major growth factor for thyroid cells, TSH activates signaling pathways known to contribute to tumorigenesis.¹⁷ Differentiated thyroid cancer cells express TSH receptors, and TSH stimulation may enhance proliferation through increased expression of thyroid-specific proteins.⁴ However, the precise mechanisms remain unclear because of limited clinical research. Although multiple meta-analyses have associated elevated TSH levels with increased thyroid cancer risk,^{5,18} the evidence remains inconsistent. Boelaert et al.¹⁹ demonstrated that euthyroid patients with TSH levels of 1.0–1.7 mU/L had a higher malignancy risk than those with TSH < 0.4 mU/L, with a significantly increased risk at TSH > 1.8 mU/L. Baser et al.²⁰ identified ≥ 1.24 mIU/mL as a potential TSH cutoff for malignancy prediction. However, a meta-analysis conducted in 2012 that evaluated surgically treated patients with nodular goiter found no significant association between TSH levels and thyroid cancer incidence.²¹ Another meta-analysis revealed that elevated TSH levels were associated with increased malignancy risk when compared with benign thyroid tumors. However, in the limited number of studies that used healthy individuals as controls, no statistically significant

difference in malignancy risk was observed in relation to TSH levels.⁷ These discrepancies may reflect methodological differences across studies. In our study, although we found no significant difference in TSH levels between the benign and malignant groups ($p > 0.05$), correlation analysis revealed a weak but statistically significant positive association between Bethesda classification and both TSH and TSH/T4 ratios. This aligns with literature reporting progressively higher TSH levels from Bethesda 2 to 4 categories.^{20,22} Consequently, some researchers propose that elevated TSH levels could function as a supplementary biomarker in cases with suspicious or malignant cytology, although their definitive prognostic significance requires validation through larger-scale studies.²⁰ However, the very weak correlation coefficients observed in our study suggest that these associations should be interpreted with caution and are unlikely to be of meaningful clinical utility on their own.

In addition to TSH, recent studies have highlighted the potential direct effects of thyroid hormones on thyroid cell proliferation. The T3 receptor has been identified as a regulator of thyroid fibroblast growth factor expression, and murine studies demonstrate that mutated T3 receptors may promote thyroid carcinogenesis by upregulating the phosphatidylinositol 3-kinase signaling pathway.²³ Meta-analyses suggest that elevated free T3 levels are associated with reduced thyroid cancer risk, while free T4 levels show no significant correlation.⁷ Another study investigating the relationship between thyroid function and differentiated thyroid carcinomas found that lower free T3 and T4 concentrations within normal ranges independently predicted increased cancer risk, regardless of sex or nodule type.²⁴ However, other studies report paradoxical findings. For instance, Kim et al.¹⁰ associated higher free T3 and T4 levels with incident thyroid cancer, whereas large-scale studies detected no significant relationship between free thyroid hormones and malignancy risk. In our cohort, free T3 and T4 levels showed no statistically significant association with malignancy when comparing the benign and malignant groups. However, we identified a potential link between the TSH/T4 ratio and Bethesda classification, warranting further investigation.

This study has several notable limitations that should be considered when interpreting the results. The primary limitation is the absence of histopathological confirmation for cytological findings, as fine-needle aspiration biopsy carries inherent risks of both false-positive and false-negative results, as acknowledged in current guidelines.⁴ In addition, the limited sample size of the malignant group may have reduced the statistical power of our analyses. This is particularly relevant for ROC and correlation analyses, where the small number of malignant cases ($n = 23$) increases the risk of type 2 error and may weaken the reliability of predictive performance estimates. Furthermore, all laboratory markers were measured only once within three months before biopsy,

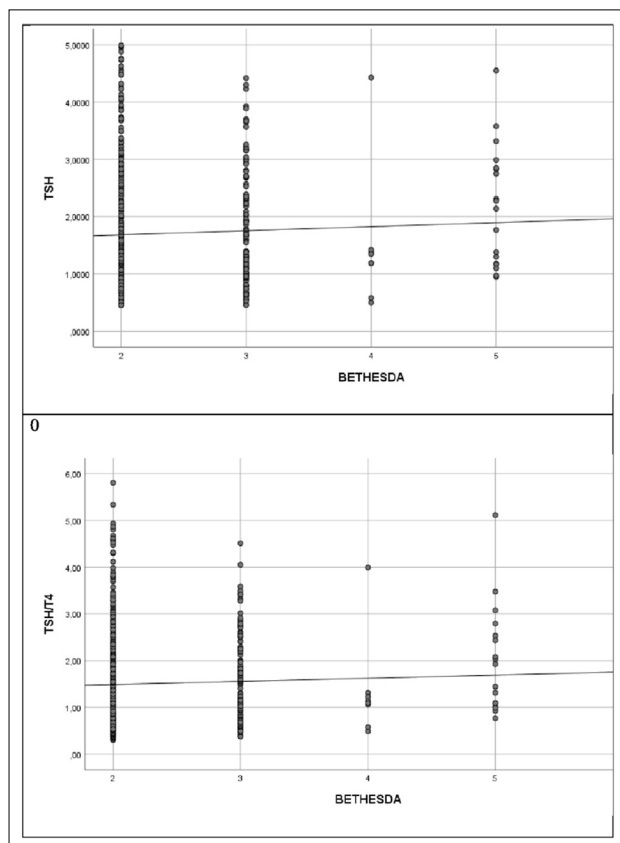


Figure 2. Correlation between Bethesda classification and TSH and the TSH/T4 ratio.

which may not capture hormonal fluctuations or adequately reflect patients' long-term thyroid status. Taken together, these limitations should be considered when interpreting the observed associations. Importantly, the absence of significant findings in ROC analysis indicates that these biochemical parameters lack sufficient discriminatory power to be used as standalone diagnostic tools for malignancy prediction.

In conclusion, both biochemical markers and cytopathological findings serve as critical tools for assessing malignancy risk in thyroid nodules. Conventional thyroid function tests (TSH, free T3, and free T4) did not differentiate between the benign and malignant groups in our study, but the observed association of both TSH and the TSH/T4 ratio with advancing Bethesda categories suggests potential diagnostic utility. Although elevated TSH levels consistently correlate with increased malignancy risk across studies, the prognostic significance of thyroid hormones remains indeterminate. This unresolved relationship underscores the need for rigorously controlled, large-scale investigations. Findings from such research could help clinicians use laboratory tests more effectively to predict malignancy risk and guide the management of patients with thyroid nodules.

CONCLUSION

The TSH level and TSH/T4 ratio showed weak correlations with Bethesda categories, but no parameter demonstrated diagnostic value for malignancy prediction in ROC analysis. Therefore, these biochemical markers appear to have limited clinical utility in differentiating benign from malignant thyroid nodules. These findings suggest that thyroid function tests have limited utility in differentiating cytological malignancy, while supporting further exploration of TSH as a potential biomarker. Future studies with larger malignant cohorts are required to validate these findings and clarify any potential clinical role.

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Authors' contributions: Şenoymak İ: conceptualization, methodology, writing – original draft preparation, and visualization. Şenoymak MC: data curation, formal analysis, investigation, resources, and writing. Egici MT: statistical analysis, software, data analysis, validation, and writing – review and editing. Kahveci İ: data curation, formal analysis, data analysis. Oğuz İ: data curation, formal analysis, statistical analysis, software, data analysis, validation. Gökçel A: statistical analysis, software, data analysis, validation, and writing – review and editing. All authors reviewed and approved the final version of the manuscript for publication.

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: Data supporting the findings of this study are available upon request from the corresponding author, İrem Şenoymak.

Declaration of generative AI in scientific writing: Generative AI was used solely for language editing and improvement of readability during the preparation of this manuscript.

Date of first submission: July 25, 2025

Last received: May 4, 2026

Accepted: June 8, 2026

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Editor responsible for the evaluation process:

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Paulo Manuel Pêgo-Fernandes, MD, PhD (EIC)



Depression and sleep disturbances in children and adolescents with familial Mediterranean fever: a prospective case-control study

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KEYWORDS (MeSH terms):

Familial Mediterranean Fever.
Depression.
Sleep Wake Disorders.
Sleep Quality.

AUTHOR'S KEYWORDS:

Pediatric Autoinflammation.
Psychological Burden.
Sleep Problems

ABSTRACT

BACKGROUND: Familial Mediterranean fever (FMF) is a hereditary autoinflammatory disorder characterized by recurrent febrile episodes and serositis.

OBJECTIVES: The aim of this study was to assess the impact of FMF on sleep quality in children and adolescents and to explore the relationship between depressive symptoms and sleep disturbances in this patient population.

DESIGN AND SETTING: A prospective case-control study was conducted at a secondary hospital in İstanbul between July 2024 and January 2025.

METHODS: Children and adolescents diagnosed with FMF who were followed at a pediatric rheumatology clinic were included. Sleep disturbances were assessed using the Sleep Disturbance Scale for Children (SDSC), and depressive symptoms were evaluated using the Beck Depression Inventory (BDI). Correlations between these scores were analyzed.

RESULTS: The mean age of the patient group ($n = 97$) was significantly higher than that of the control group ($n = 90$) (10.6 ± 2.9 years vs. 9.3 ± 2.4 years; $p < 0.001$). The median SDSC and BDI scores were significantly higher in the patient group than in the control group ($p < 0.05$). Analysis of the SDSC subscales indicated that patients with FMF experienced difficulties in sleep initiation and maintenance, sleep-related breathing problems, hyperhidrosis, arousal disturbances, sleep-wake transitions, and excessive daytime somnolence ($p < 0.05$). No significant differences were found between patient subgroups categorized by MEFV gene mutations in total BDI scores, total SDSC scores, all SDSC subscale scores, or depression severity classes ($p > 0.05$). Furthermore, no significant correlations were observed between total BDI scores and total SDSC scores or any of the SDSC subscale scores ($p > 0.05$).

CONCLUSION: Children and adolescents with FMF had significantly higher levels of depressive symptoms and sleep disturbances than controls.

INTRODUCTION

Familial Mediterranean fever (FMF) is the most common hereditary autoinflammatory disease and is typically diagnosed in the first two decades of life.¹ The disease is particularly prevalent among specific ethnic groups, including Jewish, Arabic, Armenian, and Turkish populations.^{2,3} FMF can significantly impair physical and psychosocial well-being, especially in cases of poorly controlled or refractory disease.¹

Sleep is fundamental to maintaining quality of life and physical and mental health.¹ The relationships between chronic inflammatory conditions, mood, and sleep disturbances are well established.^{1,4} Several studies have demonstrated that higher disease activity in FMF is negatively associated with sleep quality in children and adolescents.^{1,4} Furthermore, anxiety and depressive symptoms in children and adolescents with FMF have been strongly linked to sleep disturbances, underscoring the importance of addressing psychopathology in this population.^{2,5}

However, the complex relationship between chronic inflammation and sleep problems remains controversial, particularly regarding cause-and-effect dynamics.^{2,6}

OBJECTIVE

In view of the foregoing, this study was conducted to assess the impact of FMF on sleep quality in children and adolescents and to explore the relationship between depressive symptoms and sleep disturbances in this patient population.

METHODS

Study design and population

This prospective, single-center, case-control study was conducted at the Pediatric Rheumatology Department of Başakşehir Çam and Sakura City Hospital, a secondary care hospital, in Istanbul, Turkey, between July 2024 and January 2025. The study protocol was approved by the Ethics Committee of Başakşehir Çam and Sakura City Hospital (KA EK 11/29.05.2024.14). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participating children and adolescents.

Data collection and definitions

The study population consisted of consecutive children and adolescents diagnosed with and followed up for FMF. FMF diagnoses were made based on the Tel Hashomer/Turkish Pediatric FMF criteria.^{7–9} The inclusion criteria were an age between 2 and 18 years and treatment with colchicine or a combination of colchicine and an interleukin-1 inhibitor for at least 6 months. The exclusion criteria were chronic comorbidities and inability to complete the questionnaires. The sample size calculation was performed using G*Power 3.1.9.6 software. The required sample size was determined based on a comparison of the mean total BDI scores between two independent groups, the patient and control groups. Assuming a moderate effect size (Cohen's $d = 0.5$), $\alpha = 0.05$, and 80% power, the minimum number of participants required in each group was calculated as 64. The dropout rate was set at 15% to account for potential sample attrition. Consequently, the target sample size was set at a minimum of 74 participants per group to ensure adequate statistical power and robustness of the study findings. Ultimately, the patient group consisted of 97 patients with FMF who met the study's inclusion criteria. To ensure adequate comparability, the number of participants in the control group was planned to be at least half the number of patients with FMF. Therefore, 90 children and adolescents, matched by sex with the patient group, were randomly selected from those who presented to the outpatient clinic with complaints of extremity pain of unknown etiology.

Data collection

The participants' demographic characteristics (age and sex) and *MEFV* gene mutations were retrieved from their medical records and recorded, along with their BDI and SDSC scores, in a pre-designed worksheet.

Beck Depression Inventory (BDI)

The 21-item BDI was used to assess participants' behavioral symptoms and the severity of depressive symptoms.⁶ Each item

is assigned a score of zero to three points. Therefore, the highest score that can be obtained from the BDI is 63 points. BDI scores in the ranges of 0–9, 10–16, 17–29, and 30–63 points indicate none/minimal, mild, moderate, and severe depression, respectively. Validity studies of the Turkish version of the BDI were conducted by Hisli Şahin et al.¹⁰

Sleep Disturbance Scale for Children (SDSC)

Participants' sleep disturbances were assessed using the SDSC, which has been validated for use in children aged 6–16 years.^{11–14} The 26-item, 5-point Likert-type SDSC evaluates sleep over the past six months. Each item is assigned a score of 1 to 5 points. Therefore, the total score that can be obtained from the SDSC ranges from 26 to 130 points. The SDSC includes six subscales concerning the types of common sleep disturbances: disorders of initiating and maintaining sleep (DIMS), sleep breathing disorders (SBD), disorders of arousal (DA), sleep-wake transition disorders (SWTD), disorders of excessive somnolence (DOES), and sleep hyperhidrosis (HDY). A total score of ≥ 40 indicates sleep disturbance.^{11,12} The validity studies of the Turkish version of the SDSC were conducted by Ağca et al.¹⁵

Statistical analysis

Statistical analyses were performed using Jamovi Project 2.3.28 (Jamovi, version 2.3.28.0, 2023, retrieved from <https://www.jamovi.org>) and JASP 0.19.2 (Jeffreys' Amazing Statistics Program, version 0.19.2, 2024, retrieved from <https://jasp-stats.org>) software packages. Probability (p) values of ≤ 0.05 were deemed to indicate statistical significance. The results of the statistical analyses were expressed using descriptive statistics, including mean $\pm$ standard deviation values for continuous variables determined to conform to a normal distribution, median and minimum and maximum values for continuous variables determined not to conform to a normal distribution, and numbers (n) and percentage (%) values for categorical variables. The normal distribution characteristics of numerical variables were analyzed using the Shapiro–Wilk test, and the homogeneity of variances between the study groups was assessed using Levene's test. To compare differences in numerical variables between two independent groups, the independent samples t -test was used for numerical variables determined to conform to a normal distribution, and the Mann–Whitney U test was used for numerical variables determined not to conform to a normal distribution.

Additionally, the chi-square test was used to compare differences in categorical variables between the study groups. In cases where the expected frequencies were < 5 in $> 20\%$ of the cells, the Fisher–Freeman–Halton test was used. The patients' *MEFV* gene

mutation and zygosity statuses were determined by calculating the percentages of patients with positive test results for each mutation and compound heterozygosity.

The Kruskal–Wallis test was used to compare *MEFV* mutation status groups (homozygous, heterozygous, and compound heterozygous groups) in terms of depression and sleep parameters for continuous variables. To compare categorical variables between depression severity groups, the Fisher–Freeman–Halton test was used in cases where the expected frequencies were < 5% in > 20% of the cells, and Fisher’s exact test was used for 2 × 2 contingency tables. The relationships between BDI scores and sleep parameters were assessed using Spearman’s correlation coefficients (ρ), given that these parameters did not meet the normality assumptions.

RESULTS

The mean age of the patient group was significantly higher than that of the control group (10.6 ± 2.9 years vs. 9.3 ± 2.4 years; $p < 0.001$). No significant difference in terms of sex distribution was observed between the groups ($p = 0.999$).

Analysis of *MEFV* gene mutations revealed that M694V was the predominant pathogenic variant (63.5%), comprising 60.7% heterozygous ($n = 37$) and 39.3% homozygous ($n = 24$) genotypes, followed by the M680I mutation (18.6%), comprising 77.8% heterozygous ($n = 14$) and 22.2% homozygous genotypes. The distribution

of patients according to their *MEFV* mutation status revealed that the most common *MEFV* mutation status was heterozygosity, seen in 42 (43.3%) patients, followed by homozygosity (28 [28.9 %]), and compound heterozygosity (27 [27.8 %]).

The median total BDI score (15 [6–28] vs. 10 [0–17]) and total SDSC score (73 [46–94] vs. 36 [31–50]) were significantly higher in the patient group than in the control group ($p < 0.001$ for both) (Table 1). The proportion of children with poor sleep quality (SDSC score ≥ 40) was significantly higher in the patient group than in the control group (100% vs. 22.2%; $p < 0.001$). Similarly, all SDSC subscale scores were significantly higher in the patient group ($p < 0.001$ for all subscales). The distribution of depression severity categories showed significant differences between groups, with a higher proportion of moderate depression in the FMF group (37.1% vs. 1.1%) and a lower proportion of none/minimal depression (10.3% vs. 48.9%) compared with the control group ($p < 0.001$) (Table 1).

No significant differences were found between the patient subgroups created according to *MEFV* mutation status in terms of total BDI scores, total SDSC scores, all SDSC subscale scores, or depression severity classes ($p > 0.05$) (Table 2). When examining the correlation between BDI scores and sleep parameters, no significant correlations were found between the total BDI score and total SDSC score ($p = 0.01$, $p = 0.925$) or any of the SDSC subscale scores (Table 3).

Table 1. Comparison of depression and sleep disturbance scores between groups

Parameters	Control Group (n = 90)	FMF Group (n = 97)	p values
Beck Depression Inventory [†]	10 [0–17]	15 [6–28]	< 0.001**
Depression severity categories [†]			
None/minimal	44 (48.9)	10 (10.3)	< 0.001***
Mild	45 (50)	51 (52.6)	
Moderate	1 (1.1)	36 (37.1)	
Total SDSC Score [†]	36 [31–50]	73 [46–94]	< 0.001**
Poor sleep quality (SDSC score ≥ 40)	20 (22.2)	97 (100)	< 0.001**
Subscales [†]			
DIMS	10 [7–15]	21 [10–30]	< 0.001**
SBD	5 [3–10]	8 [4–15]	< 0.001**
DA	5 [3–7]	7 [3–11]	< 0.001**
SWTD	7 [6–10]	18 [6–25]	< 0.001**
DOES	7 [5–10]	14 [5–22]	< 0.001**
SHY	3 [2–6]	5 [2–8]	< 0.001**

[†] Data are presented as median (minimum–maximum) for non-normally distributed variables. [‡] n (%).

* Independent samples t-test.

** Mann–Whitney U test.

*** Fisher Freeman Halton test. Bold p values indicate statistical significance ($p < 0.05$).

FMF, Familial Mediterranean fever; SDSC, Sleep Disturbance Scale for Children; DIMS, Disorders of Initiating and Maintaining Sleep; SBD, Sleep Breathing Disorders; DA, Disorders of Arousal; SWTD, Sleep–Wake Transition Disorders; DOES, Disorders of Excessive Somnolence; SHY, Sleep Hyperhidrosis.

Table 2. Impact of *MEFV* mutation status on depression and sleep parameters in children and adolescents with FMF (n = 97)

Parameters	Homozygous (n = 28)	Heterozygous (n = 42)	Compound heterozygous (n = 27)	p values
Beck Depression Inventory [†]	15 [8–28]	15 [6–27]	16 [8–28]	0.780
Depression severity categories [†]				
None/minimal	3 (10.7)	3 (7.1)	4 (14.8)	0.851
Mild	14 (50)	24 (57.1)	13 (48.1)	
Moderate	11 (39.3)	15 (35.7)	10 (37)	
Total sleep score [†]	75 [46–90]	73.5 [47–94]	70 [47–93]	0.469
Sleep disturbance subscales [†]				
DIMS	22.5 [10–30]	22 [12–30]	21 [13–28]	0.168
SBD	8 [4–12]	8.5 [4–13]	8 [4–15]	0.376
DA	8 [4–11]	7 [3–10]	7 [3–11]	0.198
SWTD	17 [6–25]	18 [7–25]	18 [10–22]	0.572
DOES	14.5 [7–20]	14 [5–22]	13 [6–21]	0.350
SHY	4.5 [3–7]	5 [2–8]	5 [3–8]	0.565

[†] Data presented as median [minimum–maximum].

[‡] n (%)

Abbreviations: FMF, familial Mediterranean fever; DIMS, disorders of initiating and maintaining sleep; SBD, sleep breathing disorders; DA, disorders of arousal; SWTD, sleep-wake transition disorders; DOES, disorders of excessive somnolence; SHY, sleep hyperhidrosis; NA, not applicable.

Note: Kruskal–Wallis test was used for continuous variables and the Fisher Freeman Halton test for categorical variables.

Table 3. Correlation analysis between Beck Depression Inventory and sleep disturbance scores in children and adolescents with FMF (n = 97)

Sleep disturbance parameters	Spearman's ρ	p value
Total sleep score	0.01	0.925
Sleep disturbance subscales		
DIMS	–0.042	0.685
SBD	0.071	0.491
DA	0.022	0.833
SWTD	0.111	0.281
DOES	–0.031	0.762
SHY	0.187	0.067

Note: Spearman's correlation coefficient was used for analysis.

Abbreviations: FMF, familial Mediterranean fever; DIMS, disorders of initiating and maintaining sleep; SBD, sleep breathing disorders; DA, disorders of arousal; SWTD, sleep-wake transition disorders; DOES, disorders of excessive somnolence; SHY, sleep hyperhidrosis.

DISCUSSION

Children and adolescents with FMF had significantly higher total BDI and SDSC scores than controls, indicating greater depressive symptoms and sleep disturbances. A comparative analysis of SDSC subscale scores between the patient and control groups revealed that children and adolescents with FMF are more likely to experience disorders of sleep initiation and maintenance, sleep-related breathing problems, hyperhidrosis, arousal disturbances, sleep-wake transition disorders, and excessive daytime somnolence.

However, our analysis did not reveal significant correlations between total BDI scores and total SDSC scores or any of the SDSC subscale scores, suggesting that depressive symptoms and sleep disturbances may represent independent manifestations of FMF rather than directly related outcomes. Although this study could not establish causality among FMF, depressive symptoms, and sleep disorders, these findings suggest that both conditions may be independently associated with FMF rather than having a direct relationship with each other.

Sleep quality is affected by personal, societal, and environmental factors, which are reflected in individual well-being in a multidimensional way.¹ Although FMF typically causes intermittent physical symptoms, sleep disturbance is common among patients with chronic illnesses.^{1,2,4,16–19} Incesu et al.¹ reported a significantly higher prevalence of sleep disturbances in children and adolescents with FMF than in healthy controls. Similarly, Makay et al.⁴ reported that sleep quality was impaired in patients with FMF, mainly due to sleep delay, anxiety, night awakenings, and sleep-disordered breathing. They also demonstrated a significant positive correlation between the frequency of FMF attacks and these sleep-related impairments. In parallel, several other studies have reported significantly higher depression rates and poorer sleep quality in both pediatric and adult FMF populations.^{2,3,6}

Children and adolescents with FMF included in this study had significantly higher scores on all SDSC subscales than controls, indicating a range of sleep problems. Although the mechanisms

underlying poor sleep quality in FMF remain unclear, these problems may be linked to chronic inflammation and its systemic effects. Further research is necessary to elucidate these mechanisms and develop targeted interventions to enhance the quality of life of patients with FMF.

Several studies have suggested that FMF-related sleep disturbances may result from inflammation and acute disease exacerbations. Acute FMF attacks can impair quality of life by causing symptoms such as difficulty falling asleep, night awakenings, and sleep-disordered breathing. Frequent FMF attacks are also negatively correlated with sleep quality.^{1,2,4} Durcan et al.² specifically highlighted that FMF attacks experienced in the last month were associated with elevated depression and sleep disturbance scores. Although we did not assess the relationship between the frequency of FMF attacks and sleep quality, the elevated SDSC scores among children and adolescents with FMF included in our study suggest that FMF-related sleep disturbances are likely multifactorial, involving chronic inflammation and mood changes.

The interplay between sleep disturbances and mood disorders, such as depression and anxiety, in the context of various chronic diseases, including FMF, has been well documented.²⁰ Celen Yoldas et al.²⁰ reported that preschool-age children with chronic rheumatic diseases are more vulnerable to developmental and behavioral challenges. Similarly, in another study, sleep disturbances in children were associated with lower self-esteem, poorer academic performance, and elevated depressive symptoms.²¹ Additionally, depression has been reported to increase with disease severity. It should be noted that short-lived and self-limiting FMF attacks may explain why depressive symptoms in FMF are less severe and frequent than in other chronic diseases.²²

The number of participants with moderate and severe depression was significantly higher in the patient group than in the control group. Despite the higher prevalence of both depressive symptoms and sleep disturbances in the FMF group than in the controls, we did not find significant correlations between the BDI and sleep disturbance scores. This contrasts with previous findings on the relationship between these two entities. Durcan et al.² did not find any significant difference in depression and anxiety scores between children with FMF and healthy control subjects. However, they noted that children with FMF who had sleep problems had higher depression and anxiety scores. Therefore, periodic assessment of sleep quality in children with chronic illnesses, such as FMF, may help assess disease progression and improve their general well-being.

Although homozygous, heterozygous, and compound heterozygous mutations are often associated with greater disease severity, we found that mutation status was not significantly associated with sleep disturbances or depressive symptom scores in patients with FMF, consistent with the literature.^{1,2} These findings emphasize the need for further research to clarify the role of genetic

and disease-related factors in FMF-associated sleep and mood disturbances.

Various tools are available for evaluating sleep disturbances, including the Children's Sleep Habits Questionnaire (CSHQ),^{2,4} the Pittsburgh Sleep Quality Index (PSQI),^{1,3,17,19} and the CDCS.^{11,23}

Although few studies to date have assessed sleep problems in children with rheumatic diseases using the SDSC, no studies have used the SDSC to assess sleep problems in children and adolescents with FMF.²³

A recent study evaluating children with juvenile idiopathic arthritis (JIA), another pediatric rheumatological disease, using both the Beck Depression Inventory (BDI) and the Sleep Disturbance Scale for Children (SDSC) reported significantly higher BDI and SDSC scores in patients with JIA than in healthy controls. In that study, BDI and SDSC scores were positively correlated in the JIA group, suggesting a possible interaction between depressive symptoms and sleep disturbances. Interestingly, both BDI and SDSC scores were negatively correlated with leukocyte and neutrophil counts in patients with JIA, indicating that the relationship between psychological symptoms, sleep problems, and inflammatory parameters may be complex and disease-specific. In contrast, the present study found higher BDI and SDSC scores in children and adolescents with FMF than in controls, but no significant correlation was observed between BDI and SDSC scores. This difference may reflect variations in disease course, inflammatory burden, attack patterns, and chronicity between FMF and JIA. While JIA represents a more persistent inflammatory rheumatological condition, FMF is characterized by recurrent, self-limited inflammatory attacks, which may explain why depression and sleep disturbances appear to be independent manifestations in our FMF cohort. These findings support the need for further comparative studies using standardized tools, such as the BDI and SDSC, across pediatric rheumatological diseases to clarify the shared and disease-specific mechanisms underlying sleep disturbances and depressive symptoms.²⁴

To our knowledge, this study is the first to assess the effect of FMF on sleep quality in children and adolescents and to investigate the relationship between depressive symptoms and sleep disturbances in this patient population. However, further studies are needed to validate the use of the SDSC in assessing sleep problems in children and adolescents with FMF.

Limitations of the study

This study has certain limitations. First, the study design precluded establishing causality among FMF, sleep disturbances, and depressive symptoms. Longitudinal studies are needed to elucidate the directionality of these relationships and potential mediating or moderating factors. Second, the assessment of sleep disturbances relied solely on a subjective self-report questionnaire, the SDSC. Although the SDSC is a validated and

widely used tool, objective measures, such as polysomnography or actigraphy, could provide more precise data on sleep architecture and disturbances. Finally, we did not evaluate potential confounding factors that may influence sleep quality, such as socioeconomic status, comorbidities, medication adherence, and family dynamics, which are likely to contribute to both sleep and mood disturbances in children and adolescents with FMF.

CONCLUSION

The findings of this study highlight the significant impact of FMF on sleep quality and mood in children and adolescents. Children and adolescents with FMF had significantly higher BDI and SDSC scores than controls, indicating a strong relationship between FMF, sleep disturbances, and depressive symptoms. Despite the higher prevalence of both depressive symptoms and sleep problems in patients with FMF, our study did not find significant correlations between these conditions, suggesting that they may be independent manifestations of FMF rather than directly related to each other. Future longitudinal studies with larger and more diverse populations and objective sleep assessments are needed to clarify the causal relationships and underlying mechanisms. Additionally, tailored interventions targeting both sleep and mood disturbances may enhance the quality of life of children and adolescents with FMF. Periodic assessment of sleep quality and mental health in patients with FMF should be considered a critical component of routine clinical care.

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Authors' contributions: Doğanatan Ş: conceptualization, formal analysis, methodology, resources, software, writing – original draft, writing – review and editing; Bahadır El: conceptualization, investigation; Yücel BB: data curation, validation; Bekece E: data curation, software. All authors reviewed and approved the final version of the manuscript for publication.

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: The data supporting the findings of this study are available upon reasonable request from the corresponding author, Şeyda Doğanatan.

Declaration of generative AI in scientific writing: During the preparation of this work, the authors used ChatGPT (OpenAI) solely for language editing and to improve grammar, readability, and academic clarity. The authors reviewed and revised all AI-generated suggestions and take full responsibility for the content of the manuscript.

Date of first submission: August 19, 2025

Last received: April 30, 2026

Accepted: May 13, 2026

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Editor responsible for the evaluation process:

Marianne Yumi Nakai, MD, PhD (AE)
 Paulo Manuel Pêgo-Fernandes, MD, PhD (EIC)



Effect of aminomix-1 solution as an in vitro scolicalidal agent on the bile ducts and liver in rats: a controlled experimental animal study

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KEYWORDS (MeSH terms):

Parenteral Nutrition Solutions.
Anticestodal Agents.
Hydatid Cyst.

AUTHOR'S KEYWORDS:

Bile ducts.
Cystobiliary Fistula.
Echinococcosis.

ABSTRACT

BACKGROUND: Many scolicalidal agents are used to treat hydatid cysts. However, most of these have been discontinued because of complications. Currently, no safe, effective, and useful scolicalidal agent are available for the treatment of hydatid cysts in humans. Therefore, novel agents with greater efficacy, fewer side effects, and lower costs are required. In this study, we evaluated the effects of aminomix-1 solution on the liver and bile ducts of rats.

MATERIALS AND METHODS: Twenty-two male Wistar-Albino rats were divided into two groups. A 0.5 mL solution of aminomix-1 was injected into the common bile duct with a 27-gauge syringe without pressure in the experimental group and normal saline (0.9% isotonic) in the control group. Blood samples for hepatic function tests and liver and bile duct tissues were examined histopathologically 4 weeks after the procedure. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0. Statistical significance was set at $p < 0.05$.

RESULTS: The aminomix-1 group showed significantly higher aspartate aminotransferase and alkaline phosphatase levels than the control group. No significant differences were observed in the alanine aminotransferase and amylase levels. Total and direct bilirubin and gamma-glutamyl transferase levels were below the detection limits. No fibrosis or confluent necrosis was observed in either group. The aminomix-1 group showed moderate necrosis in the spotty (focal) necrosis analysis, which was borderline significant ($p \approx 0.08$).

CONCLUSION: Although aminomix-1 caused mild biochemical and histopathological changes, it did not cause advanced or irreversible liver damage or sclerosing cholangitis.

INTRODUCTION

Despite recent advances, hydatid cyst disease remains an important public health issue globally.¹ The World Health Organization has called for new, effective scolicalidal agents with fewer complications.² Surgery remains the basis of the treatment for the hydatid cyst disease.³ Many chemical scolicalidal agents have been used to eliminate cyst scoleces; however, most cause unwanted complications that limit their use, such as sclerosis and chemical cholangitis.⁴ Thus, surgeons urgently require novel scolicalidal agents with fewer side effects, lower cost, and higher efficacy.⁵ We previously demonstrated that aminomix-1 solution is an effective scolicalidal agent in vitro. In this study, we investigated the effects of aminomix-1 solution on the liver and bile ducts of rats.

MATERIALS AND METHODS

Aminomix-1 formula

Malic acid 1 (4.598 g), threonine 2 (4.4 g), L-methionine 2 (4.3 g), L-histidine 2 (3 g), L-lysine mono hydrochloride 1 (6.188 g), L-proline 1 (11.25 g), L-alanine 1 (11.25 g), L-methionine 3 (2.15 g), L-phenylalanine 3 (2.55 g), L-threonine 3 (2.20 g), L-tryptophan 3 (2.2 g), L-valine 3 (3.1 g), arginine 3 (6 g), L-valine 2 (6.2 g), L-isoleucine 3 (2.25 g), L-isoleucine 1 (3.75 g), L-leucine 3 (3.7 g), glycine 3 (7 g), L-phenylalanine 2 (5.1 g), L-lysine mono hydrochloride 3 (4.125 g), L-leucine 1 (5.55 g), L-histidine 1 (2.25 g), L-methionine 1 (3.23 g), L-phenylalanine 1 (3.83 g), L-threonine 1 (3.3 g), L-tryptophan (1.5 g), L-valine 1 (4.65 g), L-lysine mono hydrochloride 2 (8.25 g), glycine 1 (10.5 g), arginine 2 (12 g), L-histidine 3 (1.5 g), malic acid 3 (3.065 g),

L-proline 2 (15 g), L-alanine 2 (15 g), malic acid 2 (6.13 g), L-leucine 2 (7.4 g), L-isoleucine 2 (5 g), glycine 2 (14 g), arginine 1 (9 g), L-tryptophan 2 (2 g), L-alanine 3 (7.5 g), L-proline 3 (7.5 g); electrolytes (mmol/L): sodium (Na^+), 35.5; potassium (K^+), 25; magnesium (Mg^{++}), 2.5; chloride (Cl^-), 53.4; acetate (CH_3COO^-), 25; serum osmolality, 718 mOsmol/L. Aminomix-1 was used for total parenteral nutrition and electrolyte and fluid replacement at 20 mL/kg/day.

Twenty-two Wistar-Albino male rats (200 ± 15 g) were used. The rats were fasted for 12 h before anesthesia but had free access to water until 2 h before anesthesia. Preoperative enteral antibiotics and analgesics were administered. Animals were housed individually at $21^\circ\text{C} \pm 2^\circ\text{C}$ with a 12-h light–dark cycle. Two rats that died postoperatively in the experimental and control groups were excluded from the study. Upon review, the deaths were due to perioperative complications rather than the administered solution; however, a definitive cause was not established. Owing to the exploratory nature, no *a priori* power analysis was performed. This study was approved by the Ethics Committee on the Care of Animals at the Mersin University Faculty of Medicine Experimental Animals Research Laboratory (Ethics Committee No. 2024/23).

Surgical procedure

The rats were randomly divided into two groups. Anesthesia was induced using intramuscular ketamine hydrochloride (40 mg/kg) and xylazine (5 mg/kg). All animals were allowed to breathe spontaneously.

After shaving and cleaning the abdomen with povidone-iodine, midline laparotomy was performed. In the experimental group, 0.5 mL of aminomix-1 solution was injected into the common bile duct using a 27-gauge syringe without pressure. The control group received normal saline (0.9% isotonic). Immediately after the injection, the common bile duct was clamped for 3 min using a non-traumatic vascular clamp (bulldog) and then allowed to drain.

A second midline laparotomy was performed under ketamine hydrochloride anesthesia 4 weeks postoperatively. Blood samples were drawn to assess the levels of bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and amylase. The liver and bile ducts were excised en bloc, fixed in 10% formalin, and embedded in paraffin. The sections were stained with hematoxylin and eosin and examined under a light microscope for histopathological changes. Hepatic and bile duct injuries were assessed histomorphologically. Confluent necrosis, interface hepatitis, portal inflammation, bile duct injury, fibrosis, and necrosis were graded independently. Histopathological evaluations were performed by a pathologist who was blinded to the group allocation. An autoanalyzer (Siemens ADVIA) was used for biochemical tests.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows version 21.0 (IBM Corp., Armonk, NY, USA). The distribution of numerical variables was evaluated using the Shapiro–Wilk test. The results are reported as mean $\pm$ standard deviation (mean $\pm$ SD) for normally distributed data and median (minimum–maximum) for non-normally distributed data. An independent-samples Mann–Whitney U test was used to compare the distribution characteristics between the two groups.

The Mann–Whitney U test was used for the ordinal data. Categorical variables are expressed as numbers (percentages) and were compared using Fisher’s exact test between the two groups. Statistical significance was set at $p < 0.05$.

RESULTS

Biochemical results

As not all parameters met the criteria for normal distribution (Shapiro–Wilk test), the groups were compared using the Mann–Whitney U test for biochemical variables. The Welch test results were provided for parameters with near-normal distributions (ALP). The threshold for statistical significance was set at $p < 0.05$. The effect size for the parametric distributions was calculated using Cohen’s *d* coefficient.

AST and ALP levels were significantly higher in the aminomix-1 group than in the control group (Mann–Whitney $p = 0.0046$ and 0.0022 , respectively). No significant differences were observed in ALT and amylase levels. The effect size for ALP was high (Cohen’s $d \approx 1.85$), indicating a significant difference between the groups. As the total and direct bilirubin levels and GGT levels were below the detection threshold in all rats (< 0.1 mg/dL and < 6 U/L), no statistical comparison was performed (Table 1).

Histopathological results

Pathological findings were scored semi quantitatively as absent (0), mild (+), moderate (++), or severe (+++). Nonparametric tests are recommended for small samples and nominal or ordinal data. Fisher’s exact test was used to compare categorical variables in two independent groups when the expected frequencies of the cells were < 5 .

Fibrosis and confluent necrosis were absent in both groups (all values were 0); therefore, no comparison was performed. Inflammation and bile duct interface findings were analyzed in a binary manner as “present/absent” (0 or +). Portal inflammation and spotty (focal) necrosis scores were quantified as $+$ = 1, $++$ = 2; none of the samples showed a $+++$ value (Figures 1–4).

Seven rats in the aminomix-1 group showed mild spotty (focal) necrosis, and three showed moderate necrosis. This finding showed borderline significance when compared with that of the control

group (Mann–Whitney U test, $p = 0.08$). This may reflect an aminomix-1 effect but requires verification in a larger sample (Table 2).

DISCUSSION

Currently, no ideal non-invasive treatment exists for hydatid cyst disease. Surgery is the first-line therapy; however, despite the advantages of surgical techniques, there is a risk of leaks and secondary infections. Current treatment methods include conventional medicine, percutaneous aspiration, and surgery.⁶ Among them, the puncture–aspiration–injection–reaspiration (PAIR) technique as a percutaneous aspiration treatment has drawn interest as a minimally invasive technique, particularly for the treatment of Gharbi type 1,2 hydatid cysts.⁷ Thus, checking for parasitic seedlings during surgery is important. Many surgeons use scolical agents to prevent parasitic seedlings during surgery. However, leakage of cyst fluid containing scoleces during surgery may cause disease recurrence at a rate of 3–30%.⁸

Savlon, hypertonic saline, silver nitrate, chlorhexidine, formaldehyde, ethyl alcohol, hydrogen peroxide, *Eucalyptus globulus*, *Mesobuthus eupeus* toxin, and betadine are used as scolical agents worldwide, though many were discontinued due to early- and late-term complications.^{9–12} Although the WHOIWGE (Informal Working Group on Echinococcosis) recommends the use of 20% hypertonic saline as a scolical agent during surgery or 95% ethyl alcohol during PAIR, comparative studies are required.^{13,14}

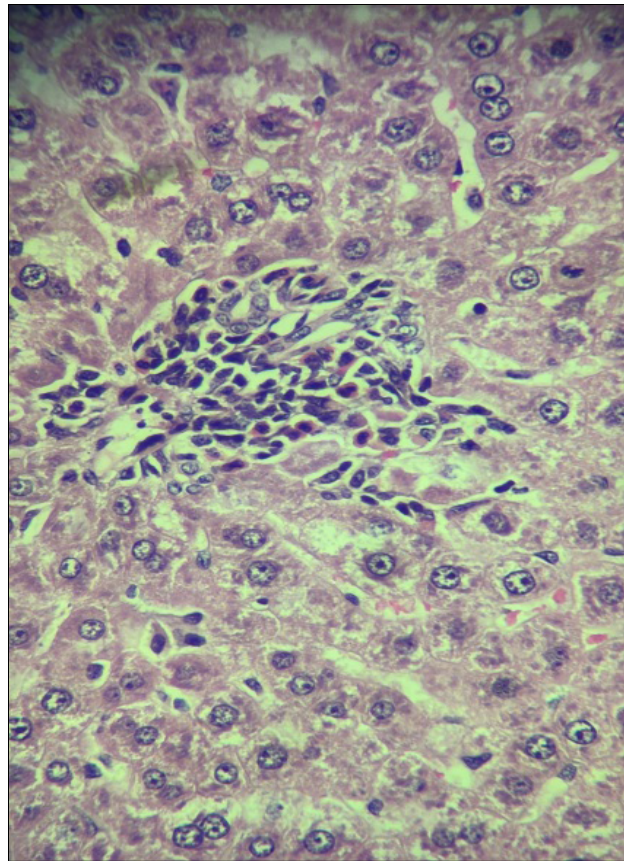


Figure 1. Interface

Table 1. Biochemical findings and statistical test results

Parameter	Group (n = 10)	Median [IQR]	Mean $\pm$ SD	Mann–Whitney p	Welch test p	Effect size (Cohen's d)
AST (U/L)	Aminomix	155 [55.75]	182.6 $\pm$ 66.1	0.0046	0.0716	0.86
	Control	110 [15.75]	129.9 $\pm$ 56.4			
ALT (U/L)	Aminomix	84 [81.5]	105.1 $\pm$ 51.7	0.3443	0.155	0.68
	Control	73.5 [20.25]	78.6 $\pm$ 18.9			
ALP (U/L)	Aminomix	149 [57.25]	163.8 $\pm$ 37.5	0.0022	0.0021	1.85
	Control	112.5 [10.25]	113.5 $\pm$ 8.7			
Amylase (U/L)	Aminomix	1451.5 [107.25]	1437.9 $\pm$ 75.9	0.8501	0.7717	–0.13
	Control	1420.5 [98.25]	1452.1 $\pm$ 131			

Table 2. Pathological findings and statistical test results

Pathological finding	Control distribution (n = 10)	Aminomix distribution (n = 10)	Test	p value	Interpretation
Bile duct inflammation	5 no inflammation, 5 mild	3 no inflammation, 7 mild	Fisher's exact	0.65	Not significant
Portal inflammation	10 mild	9 mild, 1 moderate	Mann–Whitney U	0.37	Not significant
Spotty (focal) necrosis	10 mild	7 mild, 3 moderate	Mann–Whitney U	0.08	Borderline; trend toward higher scores
Interface	10 absent	9 absent, 1 mild	Fisher's exact	10	Not significant

Note: Fisher's exact test was used for binary variables (presence/absence), and the Mann–Whitney U test was used for ordinal variables.

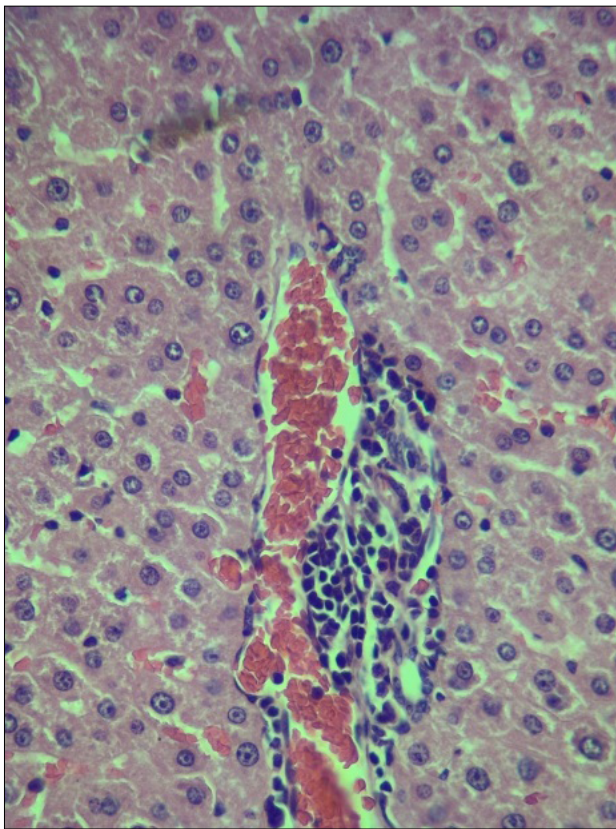


Figure 2. Portal inflammation

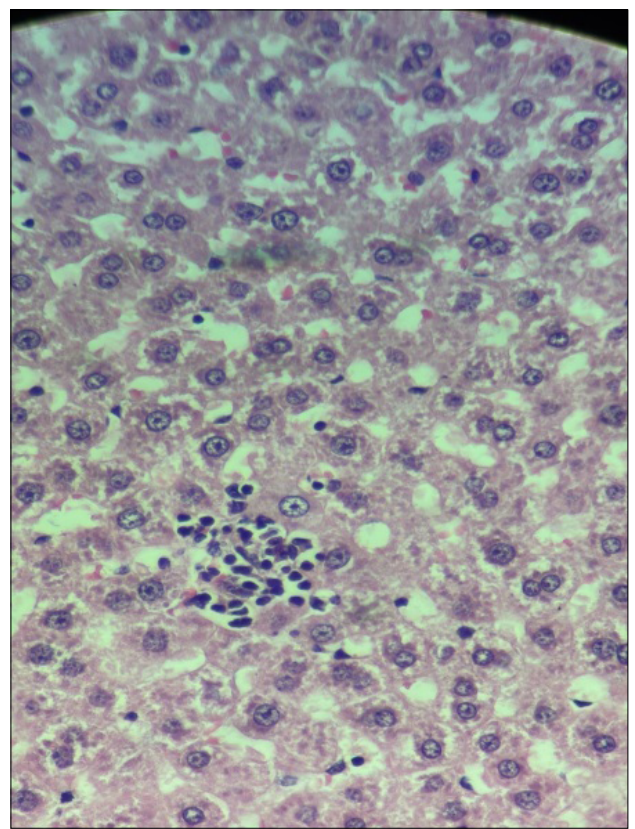


Figure 4. Spotty necrosis

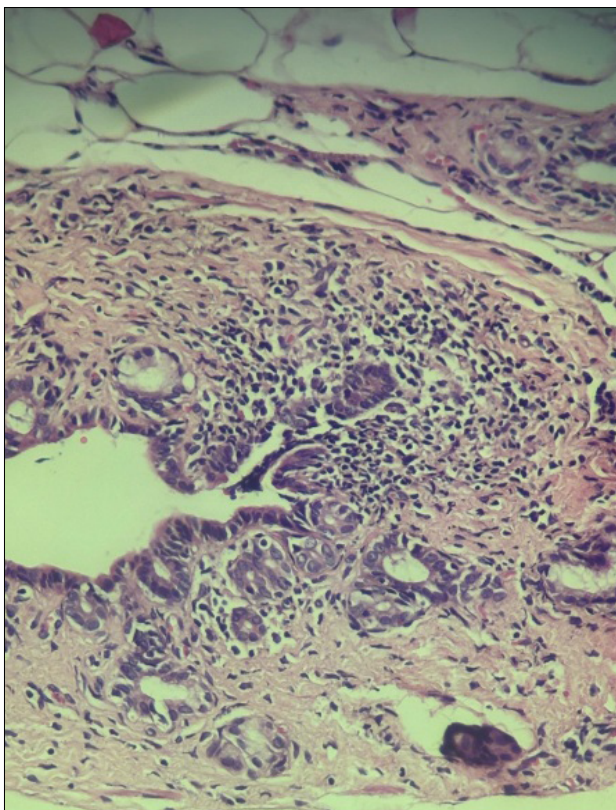


Figure 3. Main bile duct inflammation

Sclerosing cholangitis (SC) is the most common and feared complication following cyst leakage and dissemination of various scolicidal agents. Immunological reactions drive the pathogenesis of SC. Direct bile duct exposure to scolicidal agents is a primary cause of secondary SC.^{15,16} Several studies have revealed the scolicidal effects of Ag-nanoparticles synthesized from the liquid volatile extract of *Penicillium aculeatum*, methanolic extract of *Sambucus ebulus* fruit, nitric oxide metabolites, and endophytic *Pestalotiopsis* against scoleces.^{17–20} Malekifard et al.²¹ reported that gold nanoparticles at a concentration of 1 mg/mL killed all scoleces within 60 min. In our previous in vitro study, we showed that silver nitrate, dextrose, isotonic saline, and mannitol demonstrated 100% scolicidal activity after 20, 30, 45, and 45 min, respectively.⁷ Mahmoudvand et al.²² showed that biogenic selenium nanoparticles were effective at 500 and 250 µg/mL within 10 and 20 min, respectively. Eskandarian et al.,²³ demonstrated that the chloroform extract of garlic could kill 98% of the scoleces at 50 mg/mL and after a 20 min.

Most hydatid cysts are connected to the bile ducts, known as cystobiliary fistulas. Thus, it is important that scolicidal agents do not cause SC. If a cystobiliary fistula and cyst fluid is bile-colored, hypertonic saline and silver nitrate should be avoided.^{17,18} Hosseini et al. showed that 5% hypertonic saline and 1% eucalyptus

essential oil caused intra- and extrahepatic bile duct injury and portal inflammation, potentially causing long term SC.²⁴ Houry et al.²⁵ showed that 5% hypertonic saline solution had minimal or no effect on SC induction.

Hypertonic saline solution, a common scolical agents, may cause hypernatremia, as diagnosed in studies using 20% and 30% solutions.^{26,27} Ethyl alcohol may cause SC in patients with cystobiliary fistula, and this effect is concentration-dependent.²⁸ Betadine and povidone-iodide may cause complications, such as depot disease, kidney failure, sterile peritonitis, and sclerosing serositis.²⁹ Cetrimide causes complications, such as sclerosing peritonitis, metabolic acidosis, and methemoglobinemia.^{30,31}

In our study, AST and ALP levels were significantly higher in the aminomix-1 group than in the control group (Mann–Whitney U test, $p = 0.0046$ and 0.0022 , respectively). No significant differences were observed in ALT, amylase, total and direct bilirubin, and GGT levels.

Histopathologically, no fibrosis or confluent necrosis was observed in the aminomix-1 and control groups. No significant differences were observed between the groups in terms of bile duct, interface, or portal inflammation.

Mild spotty (focal) necrosis occurred in seven rats and moderate spotty (focal) necrosis in three rats in the aminomix-1 group. This finding showed borderline significance compared to the control group (Mann–Whitney U test, $p \approx 0.08$), potentially indicating a necrotic effect of aminomix-1; however, this should be confirmed using a larger sample.

Aminomix-1 is a hyperosmolar solution used for total parenteral nutrition. Hyperosmolar formulations exert osmotic stress on the biliary epithelium, potentially leading to cellular irritation, altered bile composition, and impaired bile flow. These effects may contribute to cholestatic changes and elevated cholestatic enzymes, such as ALP.³²

In this study, the significantly higher ALP levels in the aminomix-1 group and more frequent mild bile duct inflammation may be partially explained by the osmolar characteristics of the administered solution.

CONCLUSION

To date, no safe, effective, or useful scolical agent has been developed for hydatid cysts in humans. As many scolical agents cause SC in bile ducts, their use is limited. In conclusion, aminomix-1 administration in this experimental model did not result in advanced fibrosis or severe bile duct injury. However, elevated liver enzymes and mild-to-moderate hepatocellular necrosis suggest hepatic stress. These findings indicate that while aminomix-1 may be relatively well tolerated, its safety profile requires investigation in studies with larger sample sizes and longer follow-up periods.

Limitations of the Experimental Model

1. The relatively small sample size may have limited statistical power, increasing the risk of Type II errors, particularly for histopathological outcomes. This may explain the borderline significance of spotty necrosis ($p \approx 0.08$).
2. The experimental model (direct bile duct injection with temporary clamping) does not fully replicate the physiological or clinical conditions. This approach may introduce non-physiological pressure changes and localized exposure effects that differ from those of systemic administration. Future studies using models that closely mimic clinical administration routes are required to define the hepatobiliary effects of these formulations.
3. Inability to adequately assess cholestatic injury: total and direct bilirubin and GGT levels were below the detection threshold in all rats.
4. Two animals died postoperatively and were excluded. After review, these deaths were likely related to perioperative complications rather than the administered solution; however, a definitive cause could not be established.

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Authors' contributions: Çağlar R: conception and design of the study; Pülüt H: data collection and drafting of the manuscript; Tekingündüz E: histopathology of the study; Aksak T: interpretation and analysis of the data. All authors have reviewed and approved the final version of the manuscript for publication.

Acknowledgments: We thank veterinarian Tamer Yener for his contributions and support in this study.

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: The data supporting the findings of this study are available upon reasonable request from the corresponding author, Recep Çağlar.

Declaration of generative AI in scientific writing: During the preparation of this study, the authors did not use generative AI or AI-assisted technology.

Date of first submission: October 18, 2025

Last received: April 21, 2026

Accepted: May 13, 2026

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Editor responsible for the evaluation process:

Marianne Yumi Nakai, MD, PhD (AE)
Paulo Manuel Pêgo-Fernandes, MD, PhD (EIC)



Temporal trends in birthweight and its association with overweight/obesity in schoolchildren from a Brazilian city: a cross-sectional panel study (2002–2019)

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KEYWORDS (MeSH terms):

Infant, Low Birth Weight.
High Birth Weight Infant.
Child.
Nutritional Status.
Cross-Sectional Surveys.

AUTHOR'S KEYWORDS:

Birth weight.
Overweight.
Obesity.
Associated Factors.
Cross-Sectional Panels.
Temporal Trend.

ABSTRACT

BACKGROUND: Long-term metabolic complications in childhood are associated with birthweight.

OBJECTIVES: This study aimed to examine trends in the prevalence of low and high birthweight over a 16-year period and to investigate the association between birthweight and overweight/obesity among schoolchildren aged 7 to 10 years.

DESIGN AND SETTING: The analyses included four school-based cross-sectional surveys conducted in Florianópolis.

METHODS: The probabilistic panels were conducted in 2002, 2007, 2012–2013, and 2018–2019. Multiple logistic regression models were used to assess the associations between low and high birthweight and childhood overweight/obesity. Adjusted models included biological, socioeconomic, survey-period, and dietary-intake variables.

RESULTS: Over the 16-year period, low birthweight showed a progressive and statistically significant increase, rising by 58.3% from 2002 to 2018–2019. In contrast, the prevalence of high birthweight was 9.1% in 2002 and declined significantly over the ten-year period from 2002 to 2012–2013, decreasing by 40.7%. In the adjusted model, high birthweight was significantly associated with overweight/obesity across all study waves (OR = 1.63; 95% CI = 1.32–2).

CONCLUSION: A statistically significant upward trend in the prevalence of low birthweight was observed among schoolchildren in Florianópolis from 2002 to 2018–2019. Despite a reduction in high birthweight over time, high birthweight remained directly associated with overweight/obesity, independently of food intake and other confounding factors.

INTRODUCTION

Birthweight is an important indicator of maternal and early childhood health. Regardless of gestational age, newborns weighing less than 2,500 g are classified as having low birthweight (LBW),¹ whereas those weighing 2,500 g to 2,999 g are considered to have insufficient birthweight.² Causes of LBW include prematurity, maternal undernutrition, and a range of intrauterine complications.^{1–3} Data from 158 countries and regions indicated that the prevalence of low birthweight in Latin America and the Caribbean was 9.6% in 2020.⁴ Estimated trends for Latin America and the Caribbean between 2000 and 2015 indicated only a marginal decline, from 8.8% to 8.7%.⁵ In Brazil's capital and its 26 states, the prevalence of low birthweight remained stable between 1996 and 2011, averaging approximately 8% annually.⁶

High birthweight (HBW), defined as birthweight greater than 3,999 g,⁷ is also of concern, as it reflects the presence of maternal metabolic risk factors such as gestational diabetes mellitus,⁸ pregestational obesity,⁹ or obesity during pregnancy.¹⁰ The reported prevalence of HBW in Northern Hemisphere countries ranges from 5.1% in Spain to 19.2% in Ireland,¹¹ highlighting substantial geographical variation. In Brazil, estimates of high birthweight have also varied across municipalities, with rates of 11.9% in Recife¹² and 7.3% in Florianópolis.¹³ This heterogeneity underscores the influence of regional demographic, nutritional, and healthcare factors on HBW prevalence. Trends in high birthweight have been only scarcely documented in the scientific literature. A study conducted in Southern India reported that the prevalence of newborns classified as large for gestational age (LGA) remained stable, with rates of 8.6% in 1996 and 8.8% in 2010.¹⁴

Long-term metabolic complications associated with low birthweight may manifest as early as childhood and include hypertriglyceridemia, dyslipidemia, fasting hyperglycemia, and an increased risk of type 2 diabetes mellitus,^{1,15,16} conditions commonly linked to obesity and excessive adiposity. Conversely, obesity and excessive adiposity are adverse long-term outcomes directly associated with high birthweight,^{11,17–18} in addition to heightened risks of breast cancer, psychiatric disorders, hypertension, and both type 1 and type 2 diabetes.¹⁹ The literature search identified no studies that had examined temporal trends in the prevalence of low or high birthweight and their association with overweight or obesity later in life. Existing analyses have primarily focused either on trends in birthweight alone or on associations between birthweight and overweight/obesity or other metabolic complications, typically using cross-sectional designs that do not account for temporal variation.

OBJECTIVE

This study aimed to examine temporal trends in the prevalence of low and high birthweight and their association with overweight/obesity from 2002 to 2019 among schoolchildren aged 7 to 10 years in Florianópolis. The study hypothesized that: (i) the prevalence of low and high birthweight remained stable over the 16-year period; and (ii) high birthweight was positively associated with overweight/obesity across the study period.

METHODS

Study design and population

Data were collected from schools located in Florianópolis, Southern Brazil. In 2024, the city had an estimated population of 576,361 inhabitants²⁰ and a very high Municipal Human

Development Index (HDI_m = 0.847).²¹ Data were obtained from the Study on the Prevalence of Obesity in Children and Adolescents (Estudo da Prevalência da Obesidade em Crianças e Adolescentes, EPOCA), which comprises four school-based cross-sectional surveys conducted in 2002, 2007, 2012–2013, and 2018–2019. EPOCA is a probabilistic study designed to investigate temporal trends in obesity and associated factors among children and adolescents aged 7 to 14 years.^{22–24} For the present analysis, only data from students aged 7 to 10 years were included.

The sampling parameters were consistent across the four survey waves. In the first stage of sampling, schools were randomly selected from clusters stratified by geographic area (Centre, Continent, North, East, and South) and by school type (public or private). The second stage varied by survey year. In 2002, all classes within each selected school were included, and all children enrolled in the 2nd to 5th grades were invited to participate.^{23,24} In 2007, the sample was drawn proportionally to the distribution of students registered in the 2004 School Census of Florianópolis (53,595 individuals), stratified by residential area, school type, sex, and age group. In the 2012–2013 and 2018–2019 surveys, sample sizes were calculated using data from the 2010 and 2017 School Censuses, respectively, and based on expected overweight/obesity prevalences of 38% in 2012–2013 and 39% in 2018–2019. Sampling details for each wave are summarized in **Table 1**, with further methodological information available in previous publications.^{25,26}

Data collection and definitions

The inclusion criteria were: (i) attendance at school on the day of data collection; (ii) provision of written informed consent by parents or guardians; and (iii) provision of written informed assent by the student at the time of data collection (applicable only to

Table 1. Sampling details of the EPOCA surveys in 2002, 2007, 2012–2013, and 2018–2019, Florianópolis

Parameters	2002	2007	2012–2013	2018–2019
Population of 7–10-year-old schoolchildren in the municipality	28.395	25.619	19.172	27.110
Total number of elementary schools in the municipality	122	87	85	82
Total number of schools included in the survey†	16	17	30	30
Prevalence of overweight/obesity (%)	10	10	38	39
Sampling error (%)	2	2	3.5 (two-tailed) with a 95% CI	3.5 (two-tailed) with a 95% CI
Design effect	2	1.3	1.8	1.8
Additional (losses/refusals)	–	10%	10%	10%
Expected final sample	–	1.210	1.440	1.430
Total number of invited students	3.313	1.350	2.234	3.098
Total number of students investigated	2.936	1.232	1.423	922
Participation rate	88.6%	91.2%	63.7%	29.8%

† School units were drawn based on geographic region, type of administration (public and private), and number of students.

the 2018–2019 wave). The exclusion criteria were missing data on birthweight, weight, or height.

In all four waves, birthweight data were reported by parents or guardians. This data collection strategy has been widely used in scientific literature.^{27,28} Shenkin et al.²⁷ analyzed studies in which birthweight data were available from both parental recall and birth records. The comparison of 19 studies showed a high correlation (0.9; 95% confidence interval [95% CI] = 0.83–0.93), particularly in samples from high-income countries. In addition, the absolute effect size for the difference between recalled and recorded birthweight was 1.4 g, which did not represent a statistically significant difference in the pooled data.²⁷ Similarly, Moreno-Galarraga et al.²⁸ compared recalled data provided by 241 parents in Spain, when their children were 4 to 6 years old, with birth data from medical records and found an intraclass correlation coefficient of 0.95 (95% CI = 0.94–0.96). Even in older samples, self-reported birthweight data have been shown to be reliable in Norwegian participants aged 29 and 63 years. The kappa values between self-reported data and the medical birth registry were 0.63 and 0.71 for the respective age groups, indicating substantial agreement.²⁹ Birthweight was categorized according to internationally recognized standards as follows: low (< 2,500 g), insufficient (2,500–2,999 g), adequate (3,000–3,999 g),² or high ($\geq$ 4,000 g).⁷ This categorization was applied to enable the evaluation of temporal trends over the 16-year study period.

In all four cross-sectional panels, weight and height were measured by a trained team following the World Health Organization (WHO) protocol.³⁰ Children's weight status was classified according to body mass index (BMI)-for-age z-scores into two categories: "not overweight or obese" (z-score $\leq$ +1) and "overweight or obese" (z-score > +1).³¹ Data on age, sex, and school type were obtained from school records across all survey waves.

Food consumption was evaluated using qualitative, illustrated questionnaires. Researchers administered these questionnaires in all four seasons of each year and on all school days. In the 2002 cross-sectional panel, children completed the Typical Day Food Questionnaire (TDFQ), a paper-based pictorial instrument printed in color on A4 sheets. The questionnaire included 16 food illustrations distributed across five meals in chronological order (breakfast, morning snack, lunch, afternoon snack, and dinner), and children were instructed to mark the items they typically consumed in a day. This instrument was improved based on validation studies carried out in this age group. First, the food questionnaire was validated against the 24-hour recall method and showed moderate agreement in a sample of schoolchildren from Florianópolis, as assessed by concordance analysis,³² suggesting that this instrument provides information similar to that obtained from a 24-hour recall. Second, four food items were added to each meal, and the guiding question on recall time was changed to the previous day.

Third, an additional meal was added: the evening snack before bed. Therefore, in the 2007 and 2012–2013 waves, food consumption data were collected using this new version, renamed the Previous Day Questionnaire (PDQ), which included 21 food items and/or groups.³² This version showed acceptable reproducibility and validity, with average sensitivity and specificity values of 70.2% and 96.2%, respectively, for 12 food items in three combined meals.³³ In the 2018–2019 wave, food consumption data were obtained using the Web-CAAFE (Consumo Alimentar e Atividade Física de Escolares, or Food Consumption and Physical Activities of Schoolchildren) questionnaire. The Web-CAAFE was developed from the earlier paper-based instruments and has been tested for reproducibility, usability, and validity.³⁴ The food consumption section comprised the six previously defined meals, with an animated character (avatar) assisting children in identifying the meal being assessed. For each meal, children could select from 31 images representing specific food items or groups displayed on a computer screen. **Table 2** provides a comparison of the food items investigated in each wave.

A child was considered to have consumed a given food group if they reported consuming at least one item from that group in the survey instruments. Variables were grouped according to markers of healthy and unhealthy food consumption outlined in the Brazilian Dietary Guidelines.³⁵ This resulted in the following binary variables (yes/no): (i) healthy food consumption—fruit intake and intake of legumes or vegetables; and (ii) unhealthy food consumption—intake of sweets and soft drinks.

Birthweight was considered the main independent variable, while overweight/obesity was treated as the outcome variable. Because biological characteristics and individual behaviors may directly influence weight status, variables related to food consumption and sex were included as control variables. With respect to socioeconomic factors, social determinants are known to shape access to health services and health-related information. Previous evidence indicates that 80% of Brazilian children from wealthier families attend private schools and that monthly family income is directly associated with the likelihood of enrolment in a private school.³⁶ Given this characteristic of Brazilian schoolchildren, and because the "family income" variable had limited responses in our study, "school type" was incorporated into the multivariate model both as a control variable and as a proxy for family income.

Statistical analysis and ethical approval

Statistical analyses were conducted using STATA version 18.0. Variables describing the sample—sex, type of school, birthweight, and weight status (BMI-for-age and sex)—were summarized as absolute and relative frequencies using the "svy" command, which accounts for the sampling weight of each cross-sectional wave.

Table 2. Food and beverage items by survey year, Florianópolis, 2002 to 2018–2019

Food and beverage		Survey year			
Item or food group		2002	2007	2012–2013	2018–2019
Dairy products	Milk/cheese	■	■	■	
	Milk		■	■	■
	Cheese		■	■	■
	Coffee with milk		■	■	■
	Yogurt	■	■	■	■
Fruits and vegetables	Fruits	■	■	■	■
	Green leaves	■	■	■	■
	Legumes				■
	Vegetable soup		■	■	■
	Fresh juice	■	■	■	■
Fast food and processed meat	Hamburger/pizza	■	■	■	
	French fries	■	■	■	■
	Sausages				■
	Fried snacks/hamburger/pizza/hot dogs				■
<i>In natura</i> animal protein	Beef/chicken	■	■	■	■
	Fish	■			
	Fish/seafood		■	■	■
	Eggs	■			■
Major sources of carbohydrates	Cheese bread				■
	Pasta/bread/crackers	■	■	■	
	Pasta				■
	Bread				■
	Breakfast cereal				■
	Rice	■	■	■	■
	Beans	■	■	■	■
	Manioc flour				■
	Corn/potato/mashed potato				■
	Cake without icing				■
Sweets	Cake with icing				■
	Candies	■	■	■	■
	Stuffed biscuits				■
	Chocolate				■
	Ice cream				■
Sugary drinks	Sodas/soft drinks	■	■	■	■
	Chocolate milk	■	■	■	■
Highly processed food	Packaged chips-type snacks		■	■	■
	Instant pasta		■	■	■
	Water				■
Total		16	21	21	34

The four databases were merged into a single database to analyze temporal trends in birthweight categories, which were described using prevalence estimates with 95% CIs for each survey year. Differences across years were assessed through visual inspection of the overlap of 95% CIs, interpreted as a conservative, descriptive approach rather than a formal statistical test of trend. These methodological procedures are similar to those used in previous studies, including those by Spanholi et al.,³⁷ Nascimento et al.,³⁸ and Medeiros et al.³⁹

To assess the association between exposure and outcome variables, birthweight categories collapsed. Low birthweight and insufficient birthweight were combined into a single category (LBW/IBW). Bivariate logistic regression analyses were then performed to examine associations between birthweight, control variables, and weight status within each wave and across all waves combined (using the variable “year of study” as a control variable). Subsequently, multivariable logistic regression was used to estimate

the adjusted association between birthweight and overweight/obesity across the study period.

The four waves (2002, 2007, 2012–2013, and 2018–2019) were approved by the Human Research Ethics Committee of the Universidade Federal de Santa Catarina (UFSC) under protocol no. 037/2002, 028/2006, CAAE 02713312000000121, and 87539718.1.0000.0121, respectively.

RESULTS

The final sample comprised 5,694 observations. Details on missing data and exclusions are shown in **Figure 1**.

The composition of each wave’s sample was similar, with most participants being female and enrolled in public schools, and approximately one-third of the children having overweight/obesity. In 2007, the statistically significant reduction in the proportion of students from private schools required greater inclusion of students from public schools (**Table 3**).

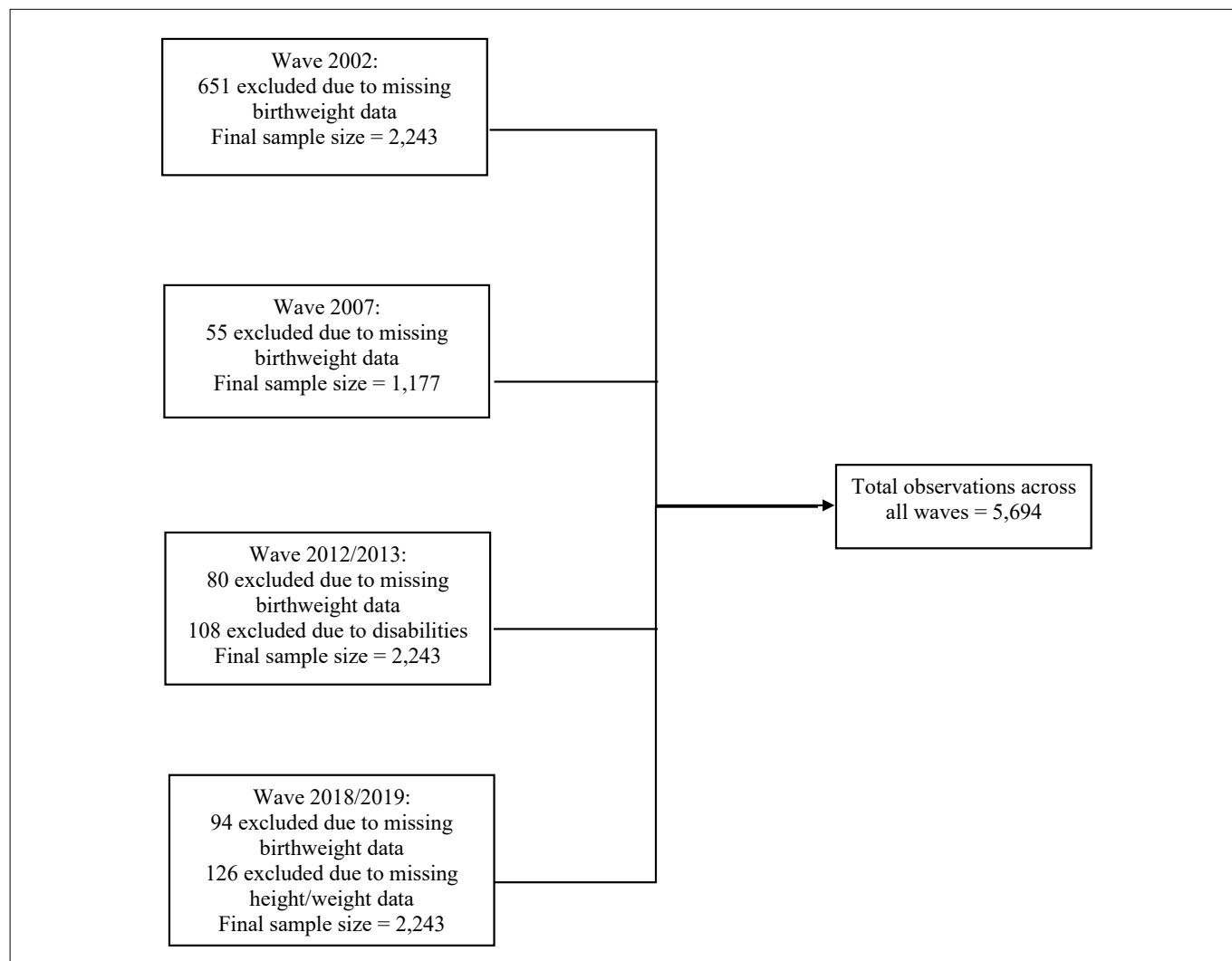


Figure 1. Excluded schoolchildren and the total sample in each study wave.

The rate of low birthweight increased significantly, from 6% in 2002 to 9.7% in 2012–2013, representing a 61.7% increase. Although prevalence declined slightly in 2018–2019, it remained significantly higher than in 2002, with a 58.3% increase. In contrast, the prevalence of high birthweight declined significantly between 2002 and 2012–2013, with a 40.7% reduction. The prevalence of overweight/obesity rose from 2002 to 2012–2013, although this increase was not statistically significant (**Table 3**).

Bivariate analyses (data not shown) revealed a significant association between HBW and overweight/obesity, even when the variable “year of study” was included (OR = 2.21; 95% CI = 1.77–2.77). The strongest association was observed in the 2002 sample (OR = 2.4; 95% CI = 1.53–3.75). In addition, food consumption was significantly related to overweight/obesity, suggesting a direct influence on schoolchildren’s weight status. For example, fruit consumption was inversely associated with overweight/obesity in two waves (2002 and 2018–2019) and when all waves were analyzed collectively (OR = 0.89; 95% CI = 0.80–0.98). Other variables, such as sex and school type, were also associated with the outcome. Therefore, all variables associated with overweight/obesity were included as control variables in the multivariate analysis.

Table 4 shows that, although the model was adjusted for food consumption variables, the association between HBW and overweight/obesity remained statistically significant across all waves (OR = 1.63; 95% CI = 1.32–2). Conversely, LBW showed an inverse

association with the outcome, indicating that children born with low or insufficient birthweight had a reduced likelihood of having overweight/obesity (OR = 0.74; 95% CI = 0.65–0.85).

DISCUSSION

The prevalence of low birthweight among schoolchildren from Florianópolis in 2002 was lower than the Brazilian rate (6% versus 8%). However, the temporal trend in the city showed a progressive and statistically significant increase over the following 10 years, with a 61.7% rise between 2002 and 2012–2013, exceeding national indicators. Although prevalence decreased slightly in 2018–2019, it remained statistically higher than in 2002, with a 58.3% increase. In contrast, the prevalence of high birthweight in Florianópolis was lower than that reported in other Brazilian cities and showed a statistically significant decline over the same period (2002 to 2012–2013), falling by 40.7%. Regarding overweight/obesity, approximately one-third of schoolchildren had this outcome over the 16-year period, with no statistically significant changes. The analysis of associations between birthweight and overweight/obesity revealed a statistically significant relationship between high birthweight and the outcome over the 16-year period, even after accounting for variables related to food consumption. Conversely, children born with low/insufficient birthweight were less likely to have overweight/obesity in childhood over the same period.

Table 3. Sample characteristics in 2002, 2007, 2012–2013, and 2018–2019, Florianópolis

Characteristic	2002 (n = 2.243)			2007 (n = 1.177)			2012–2013 (n = 1.446)			2018/ 2019 (n = 828)			Final dataset (n = 5.694)		
	n	%/mean	95% CI ^a	n	%/mean	95% CI ^a	n	%/mean	95% CI ^a	n	%/mean	95% CI ^a	n	%/mean	95% CI ^a
Sex															
Male	1.094	48.8	46.7–50.8	572	48.6	45.7–51.4	680	47	44.5–49.6	366	44.2	40.9–47.6	2712	47.6	46.3–48.9
Female	1.149	51.2	49.2–53.3	605	51.4	48.5–54.3	766	53	50.4–55.5	462	55.8	52.4–59.2	2982	52.4	51.1–53.7
School type															
Public	1.406	62.7	60.7–64.7	902	76.6*	74.1–79	930	64.3	61.8–66.7	498	60.1	56.8–63.4	3736	65.6	64.4–66.8
Private	837	37.3	35.3–39.3	275	23.4*	21–25.9	516	35.7	33.3–38.2	330	39.9	36.6–43.2	1958	34.4	33.2–35.6
Birthweight (g)	2.243	3.313,3	3.229,2–3.397,5	1.177	3.300,4	3.211,7–3.389,2	1.446	3.220,7	3.178,1–3.263,2	828	3.197,2	3.232,1–3.262,3	5.694	3.264,8	3.250,2–3.279,3
Birthweight ^c															
LBW	134	6*	5.1–7	97	8.2	6.8–10	141	9.7*	8.3–11.4	79	9.5*	7.7–11.7	451	7.9	7.2–8.6
IBW	417	18.6	17–20.3	226	19.2	17.1–21.1	309	21.4	19.4–23.6	168	20.3	17.7–23.2	1120	19.7	18.6–20.7
ABW	1.488	66.3	64.4–68.3	772	65.6	62.8–68.3	918	63.5	61–65.9	526	63.5	60.2–66.7	3704	65	63.8–66.3
HBW	204	9.1*	8–10.4	82	7	5.6–8.6	78	5.4*	4.3–6.7	55	6.6	5.1–8.6	419	7.4	6.7–8
Weight status ^d															
Without OW/OB	1.534	68.4	66.4–70.3	782	66.4	63.7–69.1	916	63.7	61.2–66.2	539	65.1	61.8–68.3	3707	66.3	65–67.5
With OW/OB	709	31.6	29.7–33.6	395	33.6	30.9–36.3	521	36.3	33.8–38.8	289	34.9	31.7–38.2	1886	33.7	32.5–35

^a 95% CI: 95% confidence interval. ^c LBW: low birthweight; IBW: insufficient birthweight; ABW: adequate birthweight; HBW: high birthweight; ^d OW: overweight; OB: obesity; * bold values indicate prevalence estimates that are statistically significant according to confidence intervals.

When compared with South American and Brazilian prevalence estimates in 2012, children in Florianópolis had a higher rate of low birthweight (8.6% and 8.3%, respectively, versus 9.7%). The higher prevalence and its increase in Florianópolis highlight the importance of determining whether greater efforts have been made to ensure the survival of small and preterm newborns. Pinheiro et al.⁴⁰ analyzed survival rates in the first year of life among 90,153 newborns in Florianópolis and São José, finding a survival rate of 98.8%, which is high compared with national and international standards. Data from the National System of Live Births

support this hypothesis: despite the increase in LBW prevalence in Florianópolis, mortality rates among children aged one year declined continuously between 2002 and 2018–2019 (from 9.8 deaths per 1,000 live births in 2002 to 6.6 per 1,000 in 2018–2019).⁴¹ Nonetheless, the underlying cause of rising low birthweight should not be overlooked and calls for strengthened monitoring of maternal health during pregnancy. Such care should be aligned with the principles of the Brazilian Policy for Integral Attention to Child Health Care within the Unified Health System, which includes a strategic focus on improving obstetric and neonatal care.⁴²

The significant reduction in the prevalence of high birthweight in Florianópolis over ten years (from 9.1% to 5.4%) may indicate that maternal health during pregnancy has increasingly focused on glycemic control and maternal body weight. Data from 9,047,145 singleton births in South Korea between 2000 and 2020 also demonstrated a reduction, from 3.7% to 2.5%.⁴³ Although the prevalence of HBW in Florianópolis is higher than that observed in South Korea, the rate recorded in 2018 remains considerably lower than national data from the United States of America (8.9%).⁴⁴ In Brazil, pregnant women with diabetes are classified as being at risk within the public healthcare system and are eligible for more frequent and specialized treatment.⁴⁵ In a study of 12,712 recent mothers in Brazilian hospitals, Rocha et al.⁴⁶ found that women with diabetes received “more than adequate prenatal care” more frequently than women in other risk categories (74.1% versus 65%, respectively).

The association between HBW and overweight/obesity has also been reported in other Brazilian and international studies. In Santa Cruz do Sul, Rio Grande do Sul, birthweight greater than 3,000 g increased the likelihood of overweight/obesity between the ages of 7 and 14 years by 18%, even after adjustment for biological, socioeconomic, and behavioral factors.⁴⁷ In another sample from Santa Cruz do Sul, Brand et al.⁴⁸ evaluated 1,562 children and adolescents aged 6 to 17 years and identified the same positive association between birthweight and overweight ($\beta = 0.84$; 95% CI = 0.08–1.6) in models adjusted for both child and maternal characteristics. Among schoolchildren aged 9 to 11 years in São Caetano do Sul, São Paulo, Santos et al.⁴⁹ found a positive and statistically significant association between birthweight and body mass index, as well as between birthweight and body fat ($\beta = 0.001$; 95% CI: 0.001–0.002, and $\beta = 0.002$; 95% CI: 0.001–0.003, respectively), in models that were also adjusted for physical activity levels. The findings on temporal trends indicate a statistically significant reduction in the prevalence of high birthweight over ten years, alongside stable rates of overweight/obesity among schoolchildren aged 7 to 10 years. Nevertheless, the association between high birthweight and overweight/obesity remained statistically significant in adjusted models across the 16 years. This suggests that the reduction in high birthweight prevalence over time was insufficient to lower the outcome at a population level. Public health

Table 4. Associations between birthweight and overweight/obesity from 2002 to 2018–2019, Florianópolis

Variables	Overweight/obesity ^a		
	OR	95% CI	p value
Birthweight			
Adequate birthweight	Ref		
Low birthweight	0.8	0.64–1	0.053
Insufficient birthweight	0.72	0.61–0.83	< 0.001
High birthweight	1.62	1.32–2	< 0.001
Sex			
Male	Ref		
Female	0.73	0.65–0.82	< 0.001
Type of school			
Public	Ref		
Private	1.15	1.02–1.3	0.021
Fruit intake			
No	Ref		
Yes	0.93	0.82–1.04	0.238
Legumes or vegetables intake			
No	Ref		
Yes	0.93	0.83–1.05	0.305
Soft drinks intake			
No	Ref		
Yes	0.96	0.85–1.09	0.573
Sweets intake			
No	Ref		
Yes	0.8	0.71–0.91	< 0.001
Survey year			
2002	Ref		
2007	1.08	0.93–1.27	0.285
2012–2013	1.22	1.05–1.42	0.008
2018–2019	1.05	0.87–1.28	0.548

^a“With overweight/obesity” is the reference category; OR: Odds ratio; 95% CI: 95% confidence interval; Ref: reference category. All variables had $p < 0.20$ in the simple models. Bold values denote statistical significance at the $p < 0.05$ level.

efforts to prevent and manage the causes of birthweight $\geq 4,000$ g are therefore essential. Future research to inform policy and practice in this field should consider trends in maternal health to identify the need for targeted interventions to manage glycemia and maternal weight status during pregnancy.

Despite the inverse association between low/insufficient birthweight and overweight/obesity, children born weighing more than 3,000 g remain at risk of developing other chronic conditions. In a meta-analysis of 28 studies, Martín-Calvo et al.⁵⁰ reported that children born small for gestational age had a 2.33-fold higher risk of developing type 2 diabetes (95% CI = 1.05–5.17). The same meta-analysis also demonstrated that both LBW and being born small for gestational age were associated with increased insulin resistance.

This study has some limitations. First, the participation rate in the 2018–2019 wave was lower, mainly due to difficulties in obtaining parental consent and the length of the parental questionnaire; this could have introduced selection bias and may limit the representativeness of recent findings. Second, cross-sectional design does not allow for the assessment of cause-and-effect relationships. Third, the findings are context-specific and may not be generalizable to other settings, including Brazil as a whole or other countries. Notwithstanding these limitations, a comparison of non-response and response rates by school type in the third wave showed no differences between public and private schools,³⁷ suggesting that the sample was homogeneous, at least with respect to socioeconomic factors. The study also has several strengths, including the representativeness of the data in the first waves, objective anthropometric measurements, and data collection by a trained team of anthropometrists. Since Florianópolis is a highly developed city, the results of this study may reflect the realities of other developed regions or countries. Regarding reported birthweight data, the city's developmental characteristics may be comparable to those observed in validation studies from high-income countries, which show high correlations between parental recall and birth records. Such maternal and neonatal health outcomes are likely connected to the implementation of, and population access to, high-quality healthcare services.

CONCLUSION

This study identified a 61.7% increase in the prevalence of low birthweight over a 10-year period (2002 to 2012–2013) among schoolchildren in Florianópolis. Although a 3.4% decline was observed in the subsequent six years, the overall rise in low birthweight prevalence from 2002 to 2018–2019 remained statistically significant. In contrast, the prevalence of high birthweight showed a statistically significant reduction between 2002 and 2012–2013.

The association between birthweight and overweight/obesity remained statistically significant, even after accounting for food consumption variables and the temporal decline in HBW.

These findings underscore the need for strengthened public health strategies to prevent macrosomia, given the association between HBW and the occurrence of overweight and obesity in childhood.

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Authors' contributions: Rossi CE: conceptualization (equal), investigation (equal), methodology (equal), formal analysis (equal), writing – original draft (equal), and writing – review and editing (equal); Barboza BP: data curation (equal), formal analysis (equal), and writing – review and editing (equal); Hinnig PF: project administration (equal), resources (equal), validation (equal), and supervision (equal); Vasconcelos FAG: project administration (equal), resources (equal), validation (equal), and supervision (equal). All authors reviewed and approved the final version of the manuscript for publication.

Acknowledgments: We thank the Florianópolis municipal government for agreeing to the development of the four waves of this research, as well as the private schools that participated in them. Special acknowledgment is given to the schoolchildren's families, who agreed to participate and provided all necessary data.

Sources of funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant no. 014/2011 – CNPq nº. 483955/2011-6; CNPq nº. 303817/2023-2. Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC), grant no. 2017TR1759.

Conflicts of interest: None.

Data availability statement: The dataset generated for the analysis of the four waves of this study is not publicly available due to confidentiality and security restrictions, but is available from the corresponding author on reasonable request.

Declaration of generative AI in scientific writing: The authors declare that they used the Grammarly AI tool to correct the English grammar, sentence structure, and semantics of the manuscript.

Date of first submission: October 23, 2025

Last received: October 23, 2025

Accepted: April 24, 2026

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Editor responsible for the evaluation process:

Marianne Yumi Nakai, MD, PhD (AE)
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From diagnosis to refusal—causes and barriers to organ donation after brain death: a cross-sectional study

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KEYWORDS (MeSH terms):

Brain Death.
Organ Transplantation.
Public Health.
Tissue and Organ Procurement.
Critical Care.

AUTHOR'S KEYWORDS:

Transplantation.
Bioethics.
Multiprofessional Team.

ABSTRACT

BACKGROUND: Brain death is the essential condition for organ procurement, yet donation rates in Brazil remain insufficient to meet national transplant needs. Understanding both the clinical determinants of brain death and the social or institutional barriers to organ donation is crucial for improving donation outcomes and informing public health strategies.

OBJECTIVES: To identify the main causes of brain death and analyze the factors associated with organ non-donation at a university hospital in São Paulo State.

DESIGN AND SETTING: This cross-sectional study with a quantitative approach was conducted at a university hospital in São Paulo State, using data from the Organ Procurement Organization affiliated with the Universidade de São Paulo.

METHODS: The medical records of all patients diagnosed with brain death between 2001 and 2015 were reviewed. Sociodemographic variables, clinical causes of brain death, and factors related to donation outcomes were assessed using descriptive statistics and chi-square tests.

RESULTS: In total, the data of 1,688 cases were analyzed, including those of 574 donors (34%) and 1,114 non-donors. Traumatic brain injury and hemorrhagic stroke were the predominant neurological causes of brain death. No significant association was observed between sex or cause of brain death and donation outcome. Public initiatives such as the 2001 consent law and the 2014 “Green September” campaign showed limited short-term impact on donation rates.

CONCLUSION: Although neurological causes account for most cases of brain death, donation outcomes are strongly influenced by social and institutional factors—particularly family refusal and trust in the healthcare system.

INTRODUCTION

The shortage of organs for transplantation constitutes one of the main challenges facing health systems worldwide, despite advances in public policies and donation and transplantation programs. The World Health Organization recognizes the chronic insufficiency of organs as a global problem, highlighting that the demand for transplants significantly exceeds supply in nearly all countries.¹ In Brazil, organ donation depends, among other factors, on the recognition and reporting of brain death, the appropriate implementation of the diagnostic protocol, and family authorization for donation.²

Although the country has one of the largest public transplantation networks worldwide, with national coverage and regulation under the Unified Health System, Brazil still has a considerable rate of loss of potential donors, with family refusal representing one of the main causes for non-effectuation of donation. In 2024, nearly half (46%) of family interviews resulted in refusal to donate organs.³ Other factors also contribute to the low donation rates, such as late identification of brain death and insufficient training of healthcare teams for the appropriate management of potential donors.^{4,5}

Brazilian legislation on organ donation has undergone significant transformations over the past decades, reflecting the maturation of public policies and the confrontation of complex ethical dilemmas. The initial milestone was Law No. 5479/1968, which authorized the removal of organs and tissues for therapeutic purposes. In 1997, the enactment of Law No. 9434—known as the “Transplant Law”—established specific rules for organ removal and donation, adopting

the presumed consent model. According to this model, all citizens would be considered donors unless they had expressed otherwise in an official document. Although this measure sought to expand the availability of organs, it generated controversies related to individual autonomy and lack of public awareness.⁶

In response to these criticisms, Law No. 10.211 came into effect in 2001, revoking presumed consent and instituting the explicit consent model, in which express family authorization is required for donation.⁷ This change represented an advance in respecting the will of donors and their families, but it also increased ethical and communicational demands, making family approach a central factor in the donation process.⁶

The regulatory framework was further consolidated with the publication of Conselho Federal de Medicina (CFM) Resolution No. 2.173/2017, in which the clinical and ethical criteria for the diagnosis of brain death were updated. This resolution promoted greater standardization and safety by more precisely defining the technical and scientific parameters required to confirm neurological death.² It also granted physicians greater autonomy by allowing the withdrawal of therapeutic support after the confirmation of brain death, except in cases of potential donation.⁶

These legal milestones highlight the effort to balance respect for individual autonomy with the principles of beneficence, justice, and human dignity. At the same time, they impose growing responsibilities on healthcare professionals, requiring technical expertise, ethical sensitivity, and communication skills to conduct the donation process safely, legally, and respectfully.

The factors leading to family refusal have received increasing attention in the literature, as understanding these factors is essential for planning interventions aimed at increasing donation rates. Strategies such as strengthening the relationship between healthcare teams and families, improving communication in situations of grief, and providing training to the Intra-Hospital Commissions for Organ and Tissue Donation for Transplantation as well as the organ procurement organizations (OPOs) have proven to be fundamental in reaching this goal.^{8–10}

Considering this context, this study aimed to evaluate the causes of brain death and the factors contributing to non-donation of organs in a quaternary hospital in São Paulo State.

METHODS

Study design

This cross-sectional study was conducted using data obtained from both institutional medical records and the database of the OPO of a Brazilian university hospital. The study included patients diagnosed with brain death during the study period, which enabled the evaluation of clinical, demographic, and organizational factors associated with organ donation outcomes.

Setting

The research was based on the analysis of data collected from the medical records of patients reported with a diagnosis of brain death between 2001 and 2015, obtained from the archives of the OPO affiliated with the Hospital das Clínicas of the Faculdade de Medicina de Ribeirão Preto of the Universidade de São Paulo. Access to the data was granted through institutional authorization.

Participants

All patients declared brain-dead during the study period were included. The selection of records was conducted on a census basis, including all available and duly registered cases in the OPO archive. No probabilistic sampling criteria were applied, thus characterizing the sample as one of convenience.

VARIABLES

The variables analyzed included sociodemographic characteristics (sex, age), clinical factors (etiology of brain death: traumatic brain injury, hemorrhagic stroke, other neurological causes), and variables related to organ donation, such as family decision (authorization or refusal) and organs effectively donated (heart, lungs, liver, pancreas, kidneys, skin, corneas, and bones).

Data sources and measurement

Information was extracted exclusively from the medical records archived by the OPO. Data collection was carried out manually by a nurse researcher using a structured protocol developed by the principal investigator. The instrument included both closed and open-ended items, and the data were recorded in electronic spreadsheets (Microsoft Excel®) to ensure standardization and systematic organization. Definitions of variables were consistently maintained throughout the collection process, minimizing measurement variability.

Potential donors were defined as patients diagnosed with brain death and eligible for organ donation according to institutional criteria. Donors were defined as patients from whom at least one organ was successfully procured. Non-donors were defined as eligible patients from whom donation did not occur.

Bias

Because this was a retrospective study based on medical records, the main potential limitation was the incompleteness or inconsistency of medical documentation, which could introduce information bias. To mitigate this risk, only complete and legible records were included, and data collection was conducted by a trained and qualified professional.

Quantitative variables

Continuous quantitative variables, such as age, were analyzed using measures of central tendency and dispersion.

Categorical variables, such as cause of brain death or family decision, were expressed as absolute and relative frequencies.

Statistical methods

Descriptive statistics were used to summarize the data, with categorical variables presented as frequencies and percentages, and continuous variables as mean $\pm$ standard deviation or median (interquartile range), as appropriate. Associations between categorical variables were assessed using Pearson's chi-square test or Fisher's exact test when applicable. To examine the relationship between the cause of brain death and organ donation decision, causes were grouped into the Neurological, Cardiac Arrest, and Other categories to ensure adequate cell counts and compliance with test assumptions.

The association between donor sex and donation actualization was evaluated through bivariate analysis using the `epi2by2()` function from the `epiR` package, with estimation of odds ratios, relative risks, and attributable risks, along with their respective 95% confidence intervals (95% CIs). Comparisons involving continuous variables such as age were performed using Student's t-test or the Mann–Whitney U test, depending on data distribution. All analyses were conducted using R software (R Core Team, 2023).

Ethical approval

The study was approved by the Research Ethics Committee of the Faculdade de Medicina de Ribeirão Preto of the Universidade de São Paulo, under CAAE No. 50811615.4.3001.5440, in compliance with all ethical principles established by Resolution No. 466/2012 of the Brazilian National Health Council.

RESULTS

In this study, the data from 1,688 medical records, comprising 574 donors and 1,114 non-donors, were analyzed. The sociodemographic characteristics are presented in **Table 1**, whereas the distribution between donors and non-donors is illustrated in **Figure 1**. With respect to **Figure 1**, the year 2008 had the highest donation rate (9.8%), followed by 2009 (9.6%) and 2014 (8.5%). Regarding non-donors, we observed that the highest rate of non-donation (9.2%) was recorded in the very year that the new law (Law No. 10.211/2001) was implemented. This outcome may be related to the lack of recognition of the new law's provisions by society, as well as insecurity arising from limited knowledge of the subject and distrust in the public health system.

Conversely, the lowest rate of non-donations (5%) was recorded in 2014; this may be attributed to substantial federal government incentives made known through awareness campaigns and, particularly, to the enactment of Law No. 15.463 in that same year, through which "Organ Donation Month," known as Setembro Verde (Green September), was instituted in the State of São Paulo.

This most recent legislation represents a historical milestone for transplantation in Brazil, because it encourages activities for raising awareness and fosters community engagement with organ and tissue donation (**Figure 1**).

With regard to donors, 59.9% were male, 77.9% self-identified as White, and the majority were aged 50 to 59 years (24.4%). Similar values were identified among non-donors, of whom 60%

Table 1. Sociodemographic characteristics of donors and non-donors

Sociodemographic data	Donors (n)	Donors (%)	Non-donors (n)	Non-donors (%)
Sex				
Female	230	40.1	443	40
Male	344	59.9	671	60
Not specified	–	–	–	–
Race/Color				
White	447	77.9	721	65
Black	64	11.1	111	10
Mulatto	23	4	55	5
Brown	34	5.9	67	6
Asian	1	0.2	–	–
Not specified	5	0.9	160	14
Age group				
0–10 years	22	3.8	71	6
10–19 years	64	11.1	89	8
20–29 years	76	13.2	153	14
30–39 years	93	16.2	201	18
40–49 years	134	23.3	230	21
50–59 years	140	24.4	216	19
≥ 60 years	43	7.5	126	11
Not specified	2	0.3	28	3
Total	574	100	1,114	100

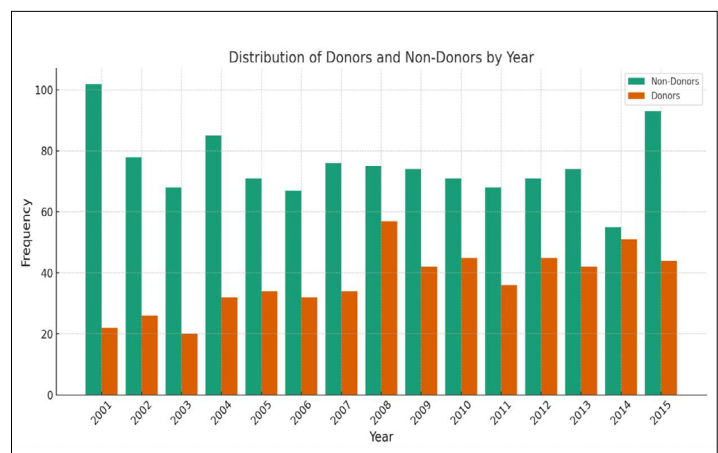


Figure 1. Distribution of donors and non-donors by year.

were male and 65% were White, although the predominant age group was 40 to 49 years (21%), as shown in **Table 1**.

The distribution between sex and actualization of donation did not demonstrate statistical significance. The odds ratio was 0.98 (95% CI: 0.80–1.21), and the relative risk was 0.99 (95% CI: 0.92–1.07), indicating that male and female individuals had similar chances of effectuating donation. The results of the chi-square test showed no significance ($\chi^2(1) = 0.033$; $p = 0.856$), and those of the Fisher's exact test confirmed the absence of association ($p = 0.874$). Similarly, Pearson's chi-square analysis indicated no statistically significant association between the cause of brain death and organ donation ($\chi^2 = 4.25$; $df = 2$; $p = 0.1196$).

Among non-donor patients, the main causes of brain death were neurological conditions, such as hemorrhagic stroke (33%), ischemic stroke (6%), subarachnoid hemorrhage (12%), and traumatic brain injury (26%). The most frequent reasons for non-donation of organs included cardiopulmonary arrest (40%), family refusal (32%), medical decision (11%), and positive serology (8%). Regarding the legal next of kin responsible for consent, spouses and children were the most frequent decision-makers.

DISCUSSION

In our study, the conversion rate of patients with suspected or confirmed brain death into effective donors was 34.2%. This value is similar to that observed in a cohort of 79 patients diagnosed with brain death in Turkey, where only 32.9% became donors despite 43% of families having given consent.¹¹ Furthermore, a study conducted across 10 public hospitals in the State of São Paulo reported similar results, with organ donation realized in only 236 cases (33.1%).¹²

More recent data confirm that, at approximately 46% in the past year, family adherence to organ donation after brain death remains low in Brazil.³ Between 2021 and 2022, approximately 45% of families refused donation, even in the presence of potential donors with confirmed diagnoses of brain death.¹² Among the main reasons for refusing organ donation are religious factors, mistrust of the healthcare system, lack of information about the brain death and organ donation process, as well as difficulties in decision-making by family members because of bereavement.¹³

Studies indicate that the main factors contributing to non-donation include lack of knowledge about the concept of brain death, inadequate communication by healthcare teams, insufficient technical and emotional preparation of professionals for approaching families, as well as cultural and religious beliefs.^{13,14} In many cases, the absence of prior conversations about donation, coupled with the difficulty families face in accepting brain death—particularly in the presence of artificial vital signs—significantly contributes to refusal.^{14,15}

Family disputes over whether to authorize or refuse donation, even when the potential donor had expressed their wishes in life, may be addressed through the Electronic Authorization for Organ Donation, a tool that remains little known and underutilized in the country. In Brazil, despite the implementation of the Electronic Authorization for Organ Donation, the process of organ donation remains legally contingent upon family consent.¹⁶

These findings demonstrate that, despite advances in diagnostic protocols for brain death and improvements in technical training, the realization of organ donation continues to face significant challenges. These challenges are primarily related to the subjective and emotional dimension of families, as well as structural and institutional limitations affecting the process.

Influence of public policies and legal frameworks

The enactment of Law No. 10.211/2001 marked an important change in Brazil's organ donation framework by establishing the requirement for explicit family consent. In this study, an increase in refusal rates was observed in 2001, which may be temporally associated with the introduction of this legal requirement and the level of public familiarity with the policy at that time. Although the requirement for family consent preserves autonomy, its implementation highlights the importance of ongoing health education and communication strategies to improve public understanding of brain death and organ donation.

In 2014, the establishment of Setembro Verde (Law No. 15.463) in São Paulo State represented a public awareness initiative aimed at promoting organ donation. In the present analysis, no significant reduction in refusal rates was observed following its implementation. This finding may be related to the limited duration of exposure within the study period. Awareness campaigns typically require sustained implementation, broad outreach, and culturally adapted strategies to influence family decision-making over time. Overall, the findings of this study suggest that isolated initiatives, even when supported by legislation, should be interpreted within the broader context of long-term public policies focused on continuous population education and professional training.

Sociodemographic profile of donors and non-donors

The sociodemographic data revealed a predominance of male and White individuals among both donors and non-donors in this sample. This pattern may reflect the epidemiological profile of the causes of brain death—more prevalent in male individuals, and particularly from traumatic brain injury—as well as social inequalities regarding access to healthcare and information. The underrepresentation of Black and mixed-race individuals raises important questions about equity within the health system.

The predominant age group among donors (50–59 years) and non-donors (40–49 years) aligns with the epidemiology of

cerebrovascular diseases and trauma, the leading causes of brain death. These findings highlight the need for primary prevention strategies in public health, especially targeting urban violence and cardiovascular risk factors.

Causes of brain death and implications for donation

The primary causes of brain death were neurological, with traumatic brain injury a notable contributor. An analysis of 504 records in Florianópolis found that 29% of cases of brain death had neurological causes, with cerebral trauma and hemorrhagic stroke being the most prevalent.¹⁷ Paixão et al.,¹⁸ analyzing a 3-year period in a trauma and emergency hospital in Belém, found traumatic brain injury in 93% of cases. These findings support the results observed in this study, conducted in a municipality in São Paulo State.

Traumatic brain injury can lead to brain death through multiple mechanisms, including focal and diffuse injuries that cause neural disconnection and deep coma.¹⁹ Furthermore, increased cerebral edema may result in herniation, compromising the brainstem and inhibiting vital functions such as respiratory control. In this population, additional care—including avoidance of hypothermia, sedation, metabolic disorders, or hypotension—is required to prevent confounding factors in the diagnosis of brain death. The apnea test confirms the absence of respiratory reflexes even under strong neural drive because of carbon dioxide accumulation, which is common in individuals with neurological trauma who progress to brain death.²⁰

Determinant factors for non-donation

Over the 15-year study period, the most frequent reason for non-donation was cardiopulmonary arrest (40%), followed by family refusal (32%). In Brazil, between 2001 and 2005, the main reason for non-donation was medical contraindication, also followed by family refusal.²¹ However, between 2009 and 2015, family refusal became the most frequent cause, reaching up to 47% of cases;²¹ it remains the leading cause to this day, accounting for 46% in 2024.¹² In the present study, however, cardiopulmonary arrest was identified as the main reason for non-donation during the period analyzed.

This discrepancy may be related to inadequate management of potential donors by healthcare teams, in addition to the prolonged interval required for clinical examinations, which—according to the former CFM resolution²²—mandated a minimum 6-hour interval between clinical evaluations. With the current resolution,² this interval was reduced to only 1 hour, which may significantly influence donation outcomes.

Regarding family refusal, several factors may contribute to its increase over time. The decision to authorize organ donation is strongly linked to trust—both in the healthcare team and in the

institutions involved in the process. Lack of rapport with professionals, communication failures, and doubts regarding medical conduct at the end of life weaken acceptance of donation. Simultaneously, perceptions of injustice, corruption, and lack of transparency in the allocation system fuel concerns about whether the process is ethically conducted. Institutional insecurity and interpersonal mistrust constitute additional barriers that must be addressed through qualified communication strategies, process audits, and greater transparency.^{13,23}

Healthcare teams play a fundamental role in the identification and management of potential organ donors. Professionals must recognize patients with suspected brain death at an early stage, perform diagnostic tests in accordance with legislation, and manage hemodynamic changes until the protocol is completed. At the same time, it is essential to provide support and clear information to families throughout the process. However, many professionals report difficulties in performing this task, attributed to several factors, such as gaps in academic training, lack of specific education on diagnosing brain death, insufficient preparation for family discussions about organ donation, and insecurity in managing physiological changes during the process.²⁴

The lack of professional training in donor management, combined with family insecurity and the absence of rapport with healthcare teams, may compromise the donation process, leading to increased family refusals or loss of potential donors because of clinical conditions that could otherwise be adequately managed.

Limitations

This study had limitations inherent to an observational and retrospective design, which allowed only the identification of associations without establishment of causality, making it subject to uncontrolled biases and residual confounding from unmeasured variables. Moreover, its cross-sectional nature precluded the analysis of temporal changes, and the generalizability of the findings should be interpreted with caution, as they reflect the specific reality of a single-center study.

CONCLUSION

In this study, we sought to evaluate the causes of brain death and the factors associated with non-donation of organs. The findings suggest that although neurological causes predominate in brain death, the realization of donation is more strongly conditioned by social and organizational aspects—such as family refusal and trust in the healthcare system—than by demographic and clinical characteristics. The temporal variation observed indicates that public policies and awareness campaigns, such as Setembro Verde, may play a decisive role in raising societal awareness.

Thus, expanding public access to information, strengthening institutional transparency, and fostering dialogue with families are

central strategies to overcoming barriers to donation. Prospective and interventional studies are needed to deepen the understanding of these determinants and to test approaches capable of increasing the availability of organs for transplantation.

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Authors' contributions: Victorino JP: conceptualization, data collection, data analysis, drafting of the manuscript; Campos VP Jr: statistical analysis and preparation of tables and figures; Monteiro VNV: manuscript editing and literature review; Mendes KD: critical review of intellectual content; Brito ES: literature review, critical review of intellectual content; Ventura CA: project administration, supervision, manuscript revision, and approval of the final version for submission. All authors reviewed and approved the final version of the manuscript for publication.

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: Data supporting the findings of this study are available upon request from the corresponding author, Vagner Pires de Campos Junior.

Declaration of generative AI in scientific writing: During the preparation of this study, the authors did not use generative AI or AI-assisted technologies.

Date of first submission: November 16, 2025

Last received: March 24, 2026

Accepted: March 27, 2026

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Serum irisin levels in women with polycystic ovary syndrome: a cross-sectional case-control study

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KEYWORDS (MeSH terms):

Polycystic Ovary Syndrome.

Myokines.

Biomarkers.

ROC Curve.

AUTHOR'S KEYWORDS:

Insulin Resistance.

Hyperandrogenism.

Adiposity.

Rotterdam Criteria.

Diagnostic Threshold.

ABSTRACT

BACKGROUND: Polycystic ovary syndrome (PCOS) is a common endocrine–metabolic disorder. Irisin is an exercise-related myokine involved in energy homeostasis; however, prior studies have reported inconsistent associations between irisin and PCOS.

OBJECTIVES: To compare serum irisin levels between women with PCOS and non-PCOS controls and to assess the discriminatory performance of irisin for PCOS.

DESIGN AND SETTING: Cross-sectional, case–control study conducted at a tertiary referral center.

METHODS: We enrolled 144 women, 72 with PCOS and 72 non-PCOS as controls. Serum irisin levels were measured using an enzyme-linked immunosorbent assay. Adjusted comparisons were performed using a general linear model, and the association between irisin and PCOS was further evaluated using multi-variable binary logistic regression. Receiver operating characteristic (ROC) analysis was used to assess the diagnostic performance of irisin.

RESULTS: Serum irisin levels in the PCOS group were significantly lower than those in the control group ($p = 0.006$). In the age and body mass index (BMI) adjusted ANCOVA model, PCOS status remained significantly associated with lower log-transformed irisin ($p = 0.002$). In a multivariable logistic regression analysis adjusted for age and BMI, log-transformed irisin levels remained independently associated with PCOS ($p = 0.006$). In ROC analysis, the area under the ROC curve for irisin was 0.632; an irisin cut-off of ≤ 16.2 ng/mL resulted in 87.5% sensitivity and 38.89% specificity.

CONCLUSIONS: Women with PCOS had lower circulating irisin levels than controls, and this association persisted after adjusting for age and BMI. Discriminatory performance was modest, and specificity was low at the selected cutoff. Larger studies are warranted to validate these findings.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a prevalent endocrine-metabolic disorder, affecting 5–20% of women of reproductive age depending on the diagnostic criteria used.^{1,2} The syndrome is characterized by irregular menstruation, hyperandrogenism, and polycystic ovarian morphology.³ Insulin resistance is common in PCOS and plays a significant role in its pathophysiology, contributing to increased risks of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular complications.^{4,5}

Recent studies have highlighted the role of irisin, a myokine induced by exercise, in regulating energy balance and metabolism. Primarily synthesized in skeletal muscle, irisin promotes the conversion of white to brown adipose tissue, thereby enhancing thermogenesis.^{6,7} In women with PCOS, altered irisin levels may be an adaptive response to insulin resistance; however, findings on irisin levels in patients with PCOS are contradictory.^{8,9}

OBJECTIVE

The primary objective of this study was to determine serum irisin levels in patients with PCOS compared to non-PCOS controls, evaluate irisin's potential as a diagnostic marker, and examine its relationship with insulin resistance. By proposing a cutoff value through receiver operating characteristic (ROC) analysis, we sought to fill a notable gap in the literature and contribute to the ongoing debate on whether irisin can serve as a clinically useful marker in the management of PCOS.

METHODS

Study design and ethical approval

This study was designed as an observational, cross-sectional, case-control investigation. It was approved by the Ankara City Hospital Clinical Research Ethics Committee No. 1 on September 2, 2020 (Approval No. E1–20–1039). Written informed consent was obtained from all participants prior to enrollment.

Study population and sample size

A total of seventy-two women with PCOS, diagnosed based on the Rotterdam criteria, were consecutively recruited from the infertility/PCOS clinic, while seventy-two non-PCOS women as control were selected from those visiting the reproductive endocrinology clinic for benign conditions. An a priori sample size calculation was performed for the primary outcome (difference in serum irisin levels between PCOS and controls). Based on the between-group difference reported by Abali et al.³ (158.5 ± 123.3 vs. 222.9 ± 152.2 ng/mL), the targeted effect corresponded to an expected mean difference of 64.4 ng/mL (Cohen's $d \approx 0.47$). With a two-sided significance level of 0.05 and 80% power ($\beta = 0.20$) at a 1:1 allocation ratio, the required sample size was approximately seventy-one participants per group, which was rounded up to seventy-two per group (total $n = 144$).

The PCOS group included women aged 20–49 years who satisfied at least two of the following three Rotterdam criteria: oligo-anovulation, clinical or biochemical hyperandrogenism, or polycystic ovarian morphology observed by transvaginal ultrasonography (ovarian volume > 10 mL or at least one ovary with ≥ 12 follicles measuring 2–9 mm).¹⁰ Controls were aged 20–49 years, with regular menstrual cycles, and did not meet the Rotterdam diagnostic criteria for PCOS. Isolated PCOS-related features, such as hirsutism, biochemical hyperandrogenism, or polycystic ovarian morphology, were allowed provided that the full diagnostic criteria for PCOS were not met.

The exclusion criteria were as follows: refusal to participate, age below twenty or above forty-nine years, pregnancy, chronic systemic diseases, cardiovascular risk, liver disease, rheumatologic conditions, recent infections, previous ovarian surgery, hormonal therapy in the previous six months, smoking, and medications affecting insulin metabolism.

Clinical and biochemical assessments

Menstrual irregularity was defined as cycle length of less than twenty-one or greater than thirty-five days,¹ and hirsutism was assessed using the modified Ferriman–Gallwey score, with a score of ≥ 8 . Biochemical hyperandrogenism was defined as total testosterone > 59 ng/dL, androstenedione > 15 nmol/L, free androgen index > 5, or free testosterone > 4.2 pg/mL.¹¹

Insulin resistance was calculated using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula (fasting glucose [mg/dL] $\times$ fasting insulin [mIU/mL]/405), with HOMA-IR ≥ 3.2 considered indicative of insulin resistance.¹² Definitions for dyslipidemia and body mass index (BMI) classifications followed the American Diabetes Association, World Health Organization, and established guidelines.^{13–15}

Biochemical measurements

Fasting blood samples (10 mL) were collected on the second or third day of each participant's menstrual cycle. Samples were centrifuged at 4,500 rpm for 15 minutes, and the resulting serum was stored at -80°C until analysis. Serum irisin concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Human Irisin ELISA, Sunred Biological Technology, China, Catalog No. 201–12–5328) with a detectable concentration range of 0.2–60 ng/mL. The sensitivity (limit of detection) of the assay was 0.157 ng/mL.

Assay validation was conducted using standard curve measurements. The intra-assay coefficient of variation (CV) ranged from 0% to 8% for low to medium concentrations and up to 16% for higher concentrations. The inter-assay CV was within an acceptable range, ensuring assay reliability. All measurements were performed in duplicate, and optical density (OD) readings were obtained at 450 nm. The precision of the assay depended on the position of the measurement on the dose-response curve, and calibration was conducted using seven standards ranging from 0 to 64 ng/mL.

Additional parameters, including total testosterone, DHEA-S, SHBG, HbA1c, fasting glucose, fasting insulin, 25-hydroxy vitamin D, FSH, LH, estradiol, AMH, and lipid profiles, were obtained from blood samples collected on the same day as serum irisin sampling and were retrieved from hospital records.

Statistical analysis

Statistical analyses were performed using NCSS (NCSS LLC, Utah) and IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Shapiro–Wilk and box plot analyses assessed normality. For normally distributed variables, group comparisons utilized Student's *t*-test; non-normally distributed variables were assessed with the Mann–Whitney *U* test. Chi-square, Fisher's Exact, and Fisher–Freeman–Halton tests compared categorical data. Because serum irisin values were right-skewed, they were log-transformed prior to multivariable modeling. To address potential confounding by age and BMI, adjusted group differences in log-transformed irisin were assessed using a general linear model (ANCOVA) with age and BMI as covariates; homogeneity of variances was evaluated using Levene's test. In addition, a multivariable binary logistic regression model was used to examine the association between log-transformed irisin and PCOS after adjustment for age and

BMI. Results were reported as adjusted estimates and odds ratios (OR) with 95% confidence intervals (95% CI). ROC curve analysis was used to evaluate the discriminatory performance of irisin for PCOS, reporting the area under the curve (AUC), sensitivity, specificity, and the selected cutoff value. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Comparisons of demographic, anthropometric, biochemical, and hormonal parameters between the PCOS and control groups are shown in **Table 1** and **2**. The mean age was significantly lower in the PCOS group (24.01 vs. 28.22 years; $p = 0.001$), and BMI was higher ($p = 0.002$). The PCOS group had a significantly higher

Table 1. Comparison of demographic, anthropometric and metabolic parameters between the PCOS and non-PCOS control groups

	PCOS (n = 72) Mean $\pm$ SD	Non-PCOS Control (n = 72) Mean $\pm$ SD	p
Age (years)	24.01 $\pm$ 3.66	28.22 $\pm$ 4.65	0.001
BMI (kg/m ²)	27.02 $\pm$ 6.11	24.04 $\pm$ 5.31	0.002
Fasting Blood Glucose (mg/dL)	88.54 $\pm$ 9.19	87.42 $\pm$ 10.22	0.382
HbA1c (%)	5.5 $\pm$ 0.4	5.60 $\pm$ 1.2	0.692
Fasting Insulin (μ u/L)	23.47 $\pm$ 37.39	13.23 $\pm$ 11.2	0.001
HOMA-IR	4.39 $\pm$ 3.53	3.2 $\pm$ 3.02	0.003
Total Cholesterol (mg/dL)	160.46 $\pm$ 27.18	168.09 $\pm$ 45.2	0.171
LDL (mg/dL)	89.73 $\pm$ 24.78	92.03 $\pm$ 27.5	0.645
HDL (mg/dL)	46.35 $\pm$ 12.49	58.46 $\pm$ 16.2	0.001
Triglycerides (mg/dL)	112.54 $\pm$ 57.93	120.16 $\pm$ 128.96	0.107
25(OH) Vitamin D (ng/mL)	14.89 $\pm$ 13.02	15.87 $\pm$ 7.78	0.103

Abbreviations: BMI, Body Mass Index; PCOS, Polycystic Ovary Syndrome; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

Table 2. Comparison of hormonal parameters and Polycystic Ovary Syndrome-related clinical features between the PCOS and non-PCOS control groups

		PCOS (n = 72) Mean $\pm$ SD	Non-PCOS Control (n = 72) Mean $\pm$ SD	p
Hormonal Parameters				
Anti-Müllerian Hormone (μ g/L)		6.39 $\pm$ 2.43	3.76 $\pm$ 2.53	0.001
Total Testosterone (ng/dL)		38.97 $\pm$ 11.38	31.10 $\pm$ 13.08	0.001
LH/FSH ratio		1.39 $\pm$ 0.85	1.04 $\pm$ 0.75	0.008
SHBG (nmol/L)		35.81 $\pm$ 20.02	66.22 $\pm$ 39.5	0.001
DHEA-S (μ g/dL)		262.66 $\pm$ 110.25	214.15 $\pm$ 100.01	0.007
Free Androgen Index (FAI)		5.46 $\pm$ 4.42	2.36 $\pm$ 1.81	0.001
PCOS-related Clinical Features* (n(%))				
Hirsutism	Absent	8 (11.1)	67 (93.1)	0.001
	Present	64 (88.9)	5 (6.9)	
Biochemical Hyperandrogenism	Absent	45 (62.5)	53 (73.6)	0.153
	Present	27 (37.5)	19 (26.4)	
Menstrual Irregularity	Absent	7 (9.7)	72 (100)	0.001
	Present	65 (90.3)	0 (0)	
Polycystic Ovarian Morphology	Absent	6 (8.3)	68 (94.4)	0.001
	Present	66 (91.7)	4 (5.6)	

* Control participants could have isolated PCOS-related features; however, none fulfilled the Rotterdam diagnostic criteria for PCOS.

Abbreviations: PCOS, Polycystic Ovary Syndrome; LH, Luteinizing Hormone; FSH, Follicle Stimulating Hormone; SHBG, Sex Hormone-Binding Globulin; DHEA-S, Dehydroepiandrosterone Sulfate; FAI, Free Androgen Index; AMH, Anti-Müllerian Hormone.

LH/FSH ratio, total testosterone, DHEA-S, and free androgen index (all $p < 0.01$) and lower SHBG levels ($p = 0.001$).

The PCOS group showed higher fasting insulin and HOMA-IR levels ($p = 0.001$; $p = 0.003$) and a higher mean AMH level ($6.39 \mu\text{g/L}$ versus $3.76 \mu\text{g/L}$, $p = 0.001$). HDL was significantly lower in the PCOS group (46.35 mg/dL versus 58.46 mg/dL , $p = 0.001$). Hirsutism, menstrual irregularity, and polycystic ovarian morphology were significantly more prevalent in the PCOS group (all $p = 0.001$); hirsutism was observed in 88.9%, menstrual irregularity in 90.3%, and polycystic morphology in 91.7% of the PCOS group.

Serum irisin levels were significantly lower in the PCOS group (median, 4.61 ng/mL) than in the control group (median, 9.18 ng/mL ; $p = 0.006$). **Table 3** and **Figure 1** show irisin levels in both groups.

In unadjusted analyses, no statistically significant association was found between age and irisin levels ($p > 0.05$). However, a weak but statistically significant negative correlation was observed between BMI and irisin levels ($\rho = -0.299$; $p = 0.001$) and between body weight and irisin levels ($\rho = -0.268$; $p = 0.001$), indicating that irisin levels decreased as BMI and body weight increased.

Given the between-group differences in age and BMI and the observed inverse association between BMI and irisin, additional multivariable analyses were performed using natural log-transformed irisin values. In ANCOVA adjusting for age and BMI, PCOS status remained significantly associated with lower log-transformed irisin ($F(1,140) = 10.338$, $p = 0.002$; partial $\eta^2 = 0.069$). At the mean covariate values (age = 26.12 years; BMI = 25.53 kg/m^2), the estimated marginal mean of log-transformed irisin was 2.181 (95% CI, 1.953–2.409) in controls and 1.619 (95% CI, 1.391–1.847) in the PCOS group, corresponding to an adjusted mean difference of 0.562 (95% CI, 0.216–0.908; $p = 0.002$). In the same model, age ($p = 0.002$) and BMI ($p = 0.048$) were also associated with log-transformed irisin, and homogeneity of variances was not violated (Levene's $p = 0.278$).

In the multivariable binary logistic regression including log-transformed irisin, age, and BMI, log-transformed irisin remained independently associated with PCOS status ($B = -0.638$, $p = 0.006$; OR = 0.529, 95% CI, 0.336–0.831). BMI was positively associated with PCOS ($B = 0.083$, $p = 0.033$; OR = 1.086, 95% CI, 1.007–1.172), whereas age showed an inverse association ($B = -0.284$, $p < 0.001$; OR = 0.753, 95% CI, 0.676–0.839). The overall model was significant ($\chi^2(3) = 51.645$, $p < 0.001$; Nagelkerke $R^2 = 0.402$) and correctly classified 75.7% of participants (77.8% of the non-PCOS controls and 73.6% of the PCOS group).

ROC analysis was performed to determine a cutoff value for irisin in the diagnosis of PCOS, based on the significant difference in irisin levels between the PCOS and control groups. The optimal cutoff value was calculated as 16.2 ng/mL , with a sensitivity of 87.5% and a specificity of 38.89%. The positive predictive value was 58.9%, and the negative predictive value was 75.7%. The AUC was 0.632 (95% CI, 0.541–0.723, standard error: 0.046), and the association was statistically significant ($p = 0.006$). Individuals with irisin levels of 16.2 ng/mL or lower had a 4.45-fold higher odds of PCOS (95% CI, 1.91–10.36) (**Table 4**, **Figure 2**).

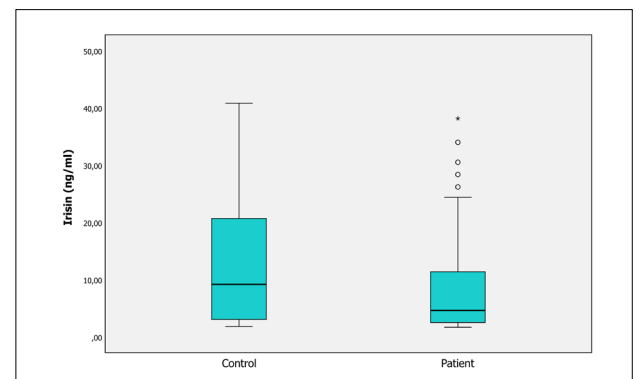


Figure 1. Distribution of Irisin Levels in PCOS and non-PCOS Control Groups

Table 3. Evaluation of irisin levels in the PCOS and non-PCOS control groups

		PCOS (n = 72)	Non-PCOS Control (n = 72)	p
Irisin Level (ng/mL)	Median (Q1–Q3)	4.61 (2.5–11.38)	9.18 (3.04–20.67)	0.006

Abbreviations: PCOS: Polycystic Ovary Syndrome

Values are presented as median (IQR). Groups were compared using the Mann–Whitney U test.

Table 4. Diagnostic screening tests and Receiver operating characteristic curve results for irisin by group

	Diagnostic Screening					ROC Curve		<i>P</i>
	Cut-off	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)	Area	95% CI	
Irisin	≤ 16.2	87.5	38.89	58.9	75.70	0.632	0.541–0.723	0.006

Abbreviations: ROC, Receiver Operating Characteristic.

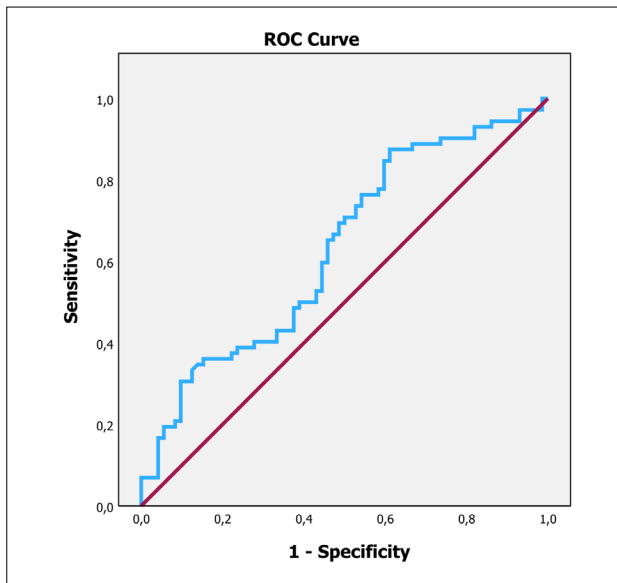


Figure 2. Receiver Operating Characteristic Curve Graph for Irisin by Groups.

Among all participants ($n = 144$), significant but weak correlations were found between irisin levels and HOMA-IR (negative, $p = -0.184$; $p = 0.038$), 25(OH) vitamin D (positive, $p = 0.185$; $p = 0.042$), SHBG (positive, $p = 0.176$; $p = 0.044$), total cholesterol (positive, $p = 0.190$; $p = 0.044$), and HDL (positive, $p = 0.271$; $p = 0.004$).

Irisin levels were significantly lower in women with hirsutism (8.16 ng/mL) than in those without (12.5 ng/mL; $p = 0.002$). Additionally, participants with polycystic ovarian morphology had significantly lower irisin levels (7.19 ng/mL) than those without this feature (13.49 ng/mL; $p = 0.002$). No statistically significant differences were observed in irisin levels in relation to insulin resistance, biochemical hyperandrogenism, or menstrual irregularity ($p > 0.05$).

DISCUSSION

Recent literature has shown increasing interest in investigating irisin levels in patients with PCOS; however, study results remain contradictory.⁹ Given the conflicting evidence on the association between irisin and PCOS, our aim was to evaluate irisin as a candidate biomarker for PCOS and explore its discriminatory performance by establishing an ROC-based cutoff.

Our results demonstrated that irisin levels were significantly lower in the PCOS group than in the control group. Importantly, this difference persisted after adjusting for age and BMI in both the ANCOVA model and multivariable logistic regression. This finding is consistent with the studies by Abali et al.³ and Wang et al.,¹⁶ which reported reduced irisin levels in patients with PCOS. However, conflicting results exist, with some studies documenting

elevated irisin levels in PCOS, and others reporting no significant difference.^{17–19} These discrepancies may reflect differences in population characteristics, PCOS diagnostic criteria, and laboratory assay methods.

Reduced peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) signaling, which regulates fibronectin type III domain-containing protein 5 (FNDC5) transcription in skeletal muscle, may explain the lower irisin levels observed in women with PCOS.²⁰ Although we did not directly assess PGC-1 α /FNDC5 in this study, prior evidence of impaired mitochondrial/oxidative pathways in PCOS provides a plausible biological context for reduced irisin.²¹

Because a higher BMI is observed in women with PCOS,²² and because BMI may influence irisin secretion, we also examined the relationship between adiposity and irisin. We observed a significant negative correlation between BMI and irisin levels, suggesting that irisin levels decrease as BMI increases. This finding contrasts with that of Bostanci et al.,²³ who reported a positive correlation between BMI and irisin levels; however, it aligns with that of Wang et al., who also observed a negative correlation between irisin levels and body weight and BMI.¹⁶ Similarly, a meta-analysis by Cai et al.⁹ proposed that BMI may modulate irisin via insulin resistance and physical activity. These mixed findings suggest that BMI, adiposity distribution, and lifestyle factors collectively influence circulating irisin.

Regarding insulin resistance, irisin did not significantly correlate with fasting glucose or fasting insulin, diverging from some reports in which higher insulin or glucose levels were correlated with elevated irisin levels.^{9,16} Interestingly, we detected a weak but negative correlation between irisin and HOMA-IR ($p = -0.184$; $p = 0.038$), unlike Wang et al.¹⁶ who reported a positive association. Furthermore, we did not observe a significant difference in irisin levels when comparing participants with and without insulin resistance defined by HOMA-IR ≥ 3.2 , suggesting that insulin resistance alone may not fully dictate irisin dynamics in PCOS.

In the ROC analysis, irisin showed modest discrimination for PCOS (AUC = 0.632). The selected cut-off (≤ 16.2 ng/mL) provided high sensitivity but low specificity, limiting its value as a standalone diagnostic test. Therefore, this threshold should be considered exploratory requiring external validation in independent cohorts.

Although preclinical studies suggest that irisin may influence metabolic and reproductive pathways, its clinical implications in humans remain unclear.^{24–26} Further translational studies are warranted before therapeutic conclusions can be drawn.

Several limitations should be considered when interpreting our findings. First, this was a single-center, cross-sectional case-control study conducted in a tertiary referral setting, which limits generalizability and precludes causal inference. Second, the PCOS and control groups differed in age and BMI and although we

addressed these differences using age- and BMI-adjusted models, residual confounding cannot be excluded, particularly from unmeasured factors, such as physical activity, diet, and body composition/fat distribution. These unmeasured factors, together with heterogeneity in irisin assay methods across studies, may partly explain the conflicting results reported in the PCOS literature.

CONCLUSION

Circulating irisin levels were lower in women with PCOS than in non-PCOS controls, and this association persisted after adjusting for age and BMI. Irisin demonstrated modest discriminatory performance with low specificity at the selected cutoff; therefore, the results should be considered exploratory requiring external validation. Larger, well-characterized cohorts are needed to confirm these findings and clarify whether irisin adds value alongside established clinical and biochemical markers.

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Authors' contributions: Toyran A: conceptualization, methodology, writing – original draft, validation, data curation, formal analysis; Minareci Y: writing – review and editing; Gulerman C: project administration, supervision, investigation. All authors reviewed and approved the final version of the manuscript for publication.

Notice: This study is derived from the postgraduate thesis *The significance of irisin levels in patients with polycystic ovary syndrome* by Dr. Atahan Toyran, under the supervision of Prof. Dr. Cavidan Gulerman, and completed at the University of Health Sciences Türkiye, Ankara Hospital, Ankara, Türkiye.

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: Data supporting the findings of this study are available upon request from the corresponding author, Atahan Toyran.

Declaration of generative AI in scientific writing: During the preparation of this manuscript, the authors did not use generative AI or AI-assisted technologies.

Date of first submission: January 4, 2026

Last received: March 30, 2026

Accepted: April 8, 2026

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Editor responsible for the evaluation process:

Marianne Yumi Nakai, MD, PhD (AE)

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Validation of the thermal stability of EMAIS-SR autonomous packaging for preservation of porcine lungs: cooling curve in an experimental model

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KEYWORDS (MeSH terms):

Models, Animals.
 Monitoring, Physiologic.
 Cold Ischemia.
 Organ Preservation.

AUTHOR'S KEYWORDS:

Experimental Study.
 Lung Transplantation.
 Thermal Stability.
 Controlled Hypothermic Storage
 EMAIS-SR.

ABSTRACT

BACKGROUND: Intermediate temperatures (10°C) better preserve mitochondrial function and extend ischemic time, which are fundamental for lung preservation during transplantation. The EMAIS-SR device was developed as a reusable, cost-effective alternative to maintain the 10°C set-point, thereby overcoming logistical and economic barriers in organ transport.

OBJECTIVE: This study aimed to validate the thermal stability of the EMAIS-SR system in a swine model by evaluating the organ-cooling curve and the time spent within the target temperature range.

DESIGN AND SETTING: Validation study using experimental surgery on porcine organs.

METHODS: Lung blocks were harvested from two Landrace swine in independent experiments (P1, P2). The organs were placed in triple sterile packaging. Thermal stability was monitored for 48 h by using four negative temperature coefficient (NTC) thermistors (three intrapulmonary and one extrapulmonary) with data acquisition at a 0.1-s interval. Another datalogger (Hantek® HTM208B) was used for control and validation. Thermal distribution was further characterized by infrared thermography (FLIR C3-X).

RESULTS: In Experiment 1 (P1), intrapulmonary temperatures reached the predefined target range ($\leq 12^\circ\text{C}$) approximately 1.4 h after lung extraction, and the mean temperature recorded in the container was 8.01°C (standard deviation, 2.88°C). In Experiment 2 (P2), a mean temperature reduction of 6.6°C was observed, bringing the intrapulmonary temperature to the target range ($\leq 12^\circ\text{C}$) within approximately 2 h post-extraction.

CONCLUSION: The EMAIS-SR device effectively maintained lung-preservation temperatures within the target 8°C – 12°C range over 48 h. These results demonstrate the system's technical feasibility and its capacity to provide consistent thermal stability across intrapulmonary compartments.

INTRODUCTION

In the early twentieth century, Alexis Carrel demonstrated that reducing temperature to approximately 2°C , with the aim of reducing cellular metabolism and delaying ischemic injury, was a central strategy for preserving solid organs.¹ Pulmonary preservation historically evolved toward static cold storage at approximately 4°C (ice).²⁻³ However, experimental studies have suggested that intermediate temperatures, particularly around 10°C , could offer biological advantages mainly related to mitochondrial function, although technical limitations have maintained the clinical use of the 4°C standard for decades.⁴

The Toronto Lung Transplant Group revisited the investigation of lung preservation at 10°C , demonstrating that this temperature is optimal for preservation since it allows extension of preservation time, experimental viability, and, more recently, clinical application in human lung transplantation, with the potential to further expand preservation time.⁵ Despite these advances, the existing platforms for controlled preservation at intermediate temperatures, such as the LUNGguard®, are expensive and single-use, limiting their applicability in low- and middle-income countries.

In this context, the EMAIS-SR system has emerged as a national, reusable alternative with lower operational complexity. Originally developed for operation at 4°C , the device was modified for operation at 10°C , necessitating preclinical validation before its application in human studies.

OBJECTIVE

This study aimed to validate the thermal stability of the EMAIS-SR system at 10°C in a swine model by evaluating the organ-cooling curve and the time spent within the target temperature range.

METHODS

The study attempted to validate the thermal performance of the EMAIS-SR device in a swine model on the basis of experimental strategies previously adopted in animal models for organ preservation.⁶ Two independent experiments (P1 and P2) were performed with assessment of pulmonary thermal stability. All national regulations for the use of animals in research were followed, and the study was approved by the Institutional Animal Care and Use Committee (CEUA; protocol no. 2399/2026) of the Faculdade de Medicina of the Universidade de São Paulo (FMUSP).

Two female Landrace pigs, weighing approximately 30 kg, were used. The animals were obtained from a production unit affiliated with the Universidade de São Paulo, and all procedures were performed under veterinary supervision. The animals received pre-medication with intramuscular ketamine (15 mg/kg) and midazolam (0.2 mg/kg), which was followed by intravenous induction of anesthesia with propofol (7 mg/kg) and maintenance with isoflurane (1.5%–2%). After surgical access, the lung block was procured, and cold ischemia was initiated.

After procurement, the lungs were perfused with 3 L of isotonic saline solution (0.9% NaCl) through the pulmonary artery under continuous flow at 4°C. The double lung block was removed with the lung inflated to 30 cmH₂O, and stapling was performed with the Panther Linear Cutter Stapler®.

The organ was placed in triple sterile packaging in accordance with Brazilian regulations: the first bag contained the lung and cold saline solution; the second bag contained saline solution; and the third outer bag was maintained dry.⁷ The packaged organ was subsequently placed in the EMAIS-SR device, which was adjusted for operation at 10°C.⁸

Immediately after perfusion and at the end of the preservation period, thermal images of the lung surface were obtained using a handheld infrared camera FLIR C3-X. Thermal imaging was used as a complementary qualitative method to document the distribution of the surface temperature.⁹

Temperatures of the lung and the preservation environment were monitored using four negative temperature coefficient (NTC) sensors, of which three were positioned within the organ (right pulmonary artery, inferior vein of the mediastinal lobe, and parenchyma of the left upper lobe) and one was placed in the container. Measurements were acquired at 0.1-s intervals with a 10-point moving average and recorded using a dedicated datalogger. In parallel, an independent datalogger (Hantek® HTM208B) was placed

inside the preservation container to provide external temperature monitoring for comparison and validation. Data were analyzed over a 48-h period, focusing on thermal stability and the time spent within the target range of 8°C–12°C.^{10,11}

RESULTS

Thermal data analysis of the packaging temperature and the temperatures of the intrapulmonary compartments over 48 h of preservation was performed separately for the two independent experiments (P1 and P2).

In Experiment 1 (P1), intrapulmonary temperatures reached the predefined target range ($\leq 12^\circ\text{C}$) approximately 1.4 h after lung extraction. The mean temperature reduction required to achieve this range was 5.2°C, which was calculated across vascular and parenchymal compartments. Initial temperatures immediately after perfusion ranged from 14.7°C to 17.6°C, and they decreased to values between 10.7°C and 11.6°C at the time of target range entry (**Figure 1A**).

The mean temperature recorded in the container was 8.01°C (standard deviation [SD], 2.88°C). In the right pulmonary artery, the mean temperature was 8.77°C (SD, 2.06°C) and remained within the target 8°C–12°C range for 30 h. In the inferior vein of the mediastinal lobe, the mean temperature was 9.83°C (SD, 1.84°C), and it remained within the target range for 42 h. In the parenchyma of the left upper lobe, the mean temperature was 8.6°C (SD, 2.16°C), remaining within the target range for 26 hours (**Tables 1 and 2**).

Temperature measurements obtained using the independent control datalogger are presented in **Figure 1B**. According to this control logger, the mean internal control temperature for the container (BOX) was 8.35°C (SD, 1.84°C); the mean temperature in the right pulmonary artery was 9.17°C (SD, 1.83°C); and mean temperatures in the inferior vein and left upper lobe parenchyma were 9.58°C (SD, 1.63°C) and 8.89°C (SD, 1.76°C), respectively (**Figure 1B**).

Qualitative thermal imaging scans obtained immediately after lung perfusion demonstrated heterogeneous surface temperature distribution, with higher temperatures (18°C–21°C) predominantly observed in the central and hilar regions (**Figure 2A**). At the end of the preservation period, thermal images acquired immediately after opening the device demonstrated a more homogeneous surface temperature distribution across the lung parenchyma, showing values consistent with the temperature range recorded by intraparenchymal sensors, within 7°C–12°C (**Figure 2B**).

In Experiment 2 (P2), intrapulmonary temperatures reached the predefined target range ($\leq 12^\circ\text{C}$) within approximately 2 h after lung extraction. An average temperature decrease of 6.6°C was necessary to reach this range. Initial post-perfusion temperatures ranged from 10.9°C to 19.6°C, decreasing to values between 7.8°C and 10.7°C at the time of entry into the target range (**Figure 3A**).

The mean packaging temperature was 8.44°C (SD, 1.42°C) and remained within the target 8°C–12°C range for 39 h. In the right pulmonary artery, the mean temperature was 9.05°C (SD, 1.51°C) and remained within the target range for 41 h. In the inferior vein of the mediastinal lobe, the mean temperature was 10.9°C (SD,

2.39°C), and it remained within the target range for 39 h. In the left upper lobe, the mean temperature was 8.62°C (SD, 1.08°C), and it remained within the 8°C–12°C range for 33 h (Tables 3 and 4).

Temperature measurements obtained using the independent control datalogger are registered in Figure 3B. According to this

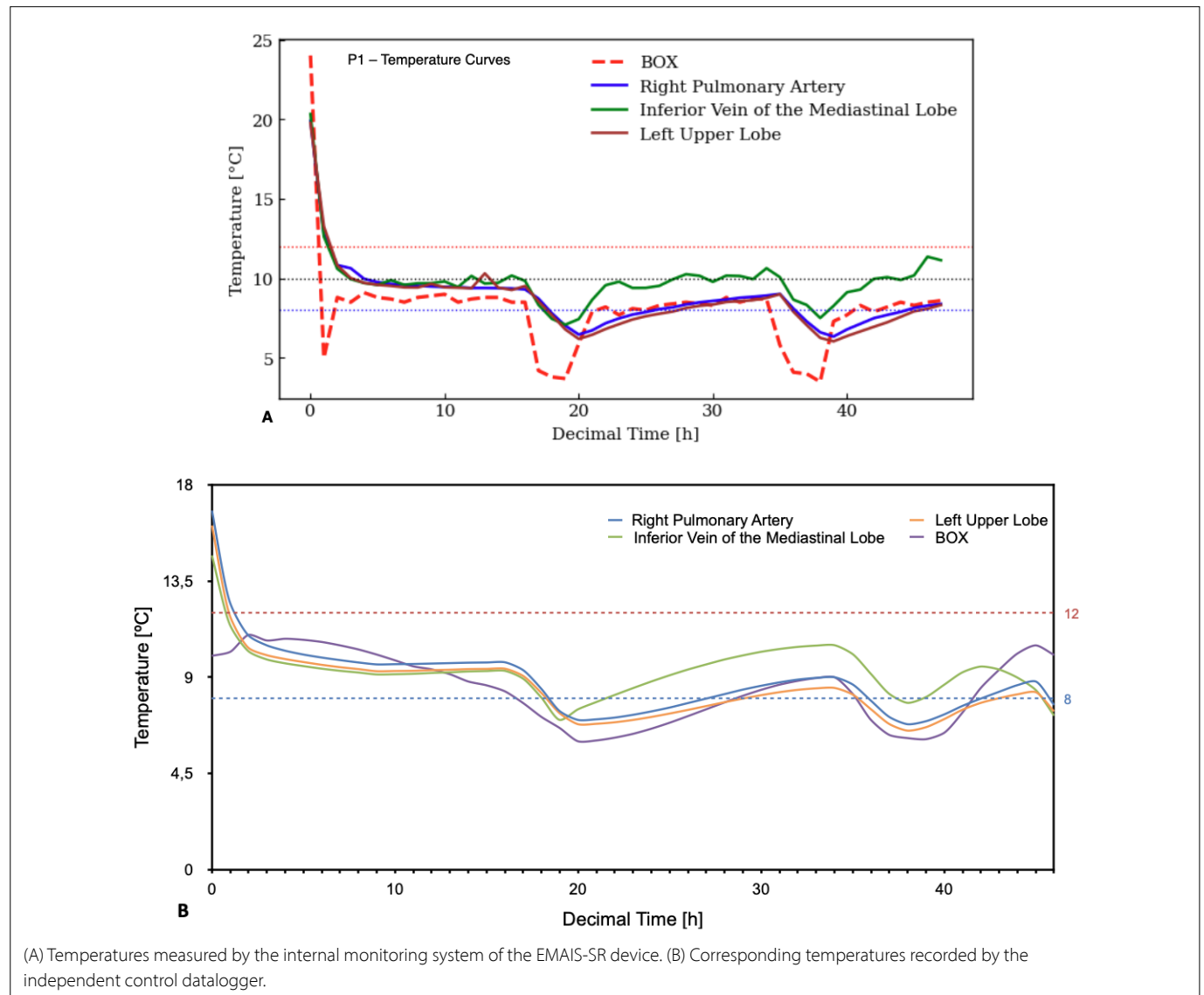


Figure 1. Temperature curves for the preservation container (BOX) and the intrapulmonary compartments during Experiment 1 (P1).

Table 1. Descriptive temperature statistics recorded in the preservation container and in different lung regions over 48 h of preservation during Experiment 1 (P1)

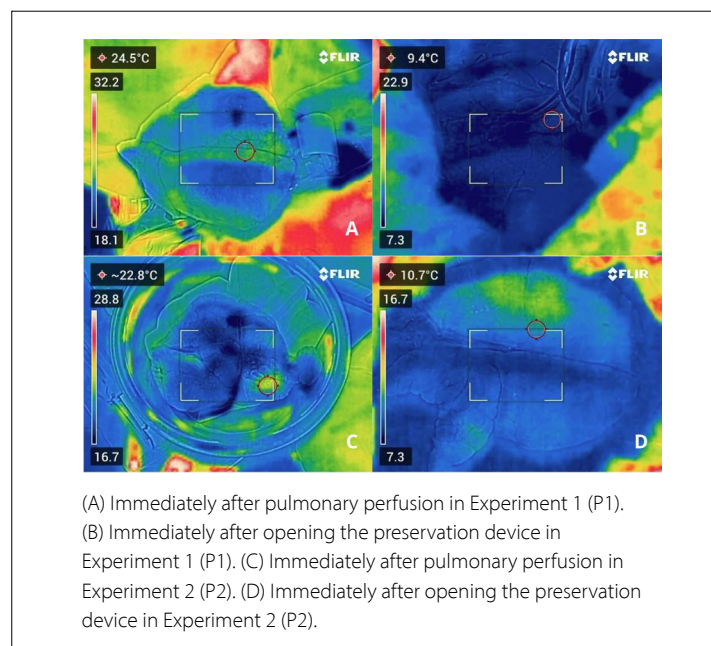
Sensor	Mean (°C)	SD (°C)	Min (°C)	Max (°C)
BOX	8.01	2.88	3.5	24
Right pulmonary artery	8.77	2.06	6.34	19.89
Inferior vein of the mediastinal lobe	9.83	1.84	7.09	20.32
Left upper lobe	8.6	2.16	6.04	19.75

BOX, preservation container; Max, maximum temperature; Min, minimum temperature; SD, standard deviation.

Table 2. Cumulative time during which the temperature remained within the 8°C–12°C range in the preservation container and in different lung regions during Experiment 1 (P1)

Sensor	Time within 8°C–12°C (h)
BOX	33
Right pulmonary artery	30
Inferior vein of the mediastinal lobe	42
Left upper lobe	26

BOX, preservation container.

**Figure 2.** Thermal images of the lung surface.

device, the mean internal temperature for the container (BOX) was 10.66°C (SD, 0.82°C), and the mean temperature in the right pulmonary artery was 9.77°C (SD, 1.68°C). The mean temperatures in the inferior vein of the mediastinal lobe and the left upper lobe were 9.01°C (SD, 1.05°C) and 8.84°C (SD, 0.61°C), respectively (**Figure 3B**).

For Experiment 2 (P2), thermal imaging performed immediately after pulmonary perfusion showed elevated surface temperatures mainly distributed over the central lung regions, with values ranging from 20°C to 23°C, while the peripheral areas exhibited lower temperatures (**Figure 2C**). After completion of the preservation period, thermal images obtained showed values predominantly within the 7°C–12°C range, in agreement with intraparenchymal temperature recordings (**Figure 2D**).

DISCUSSION

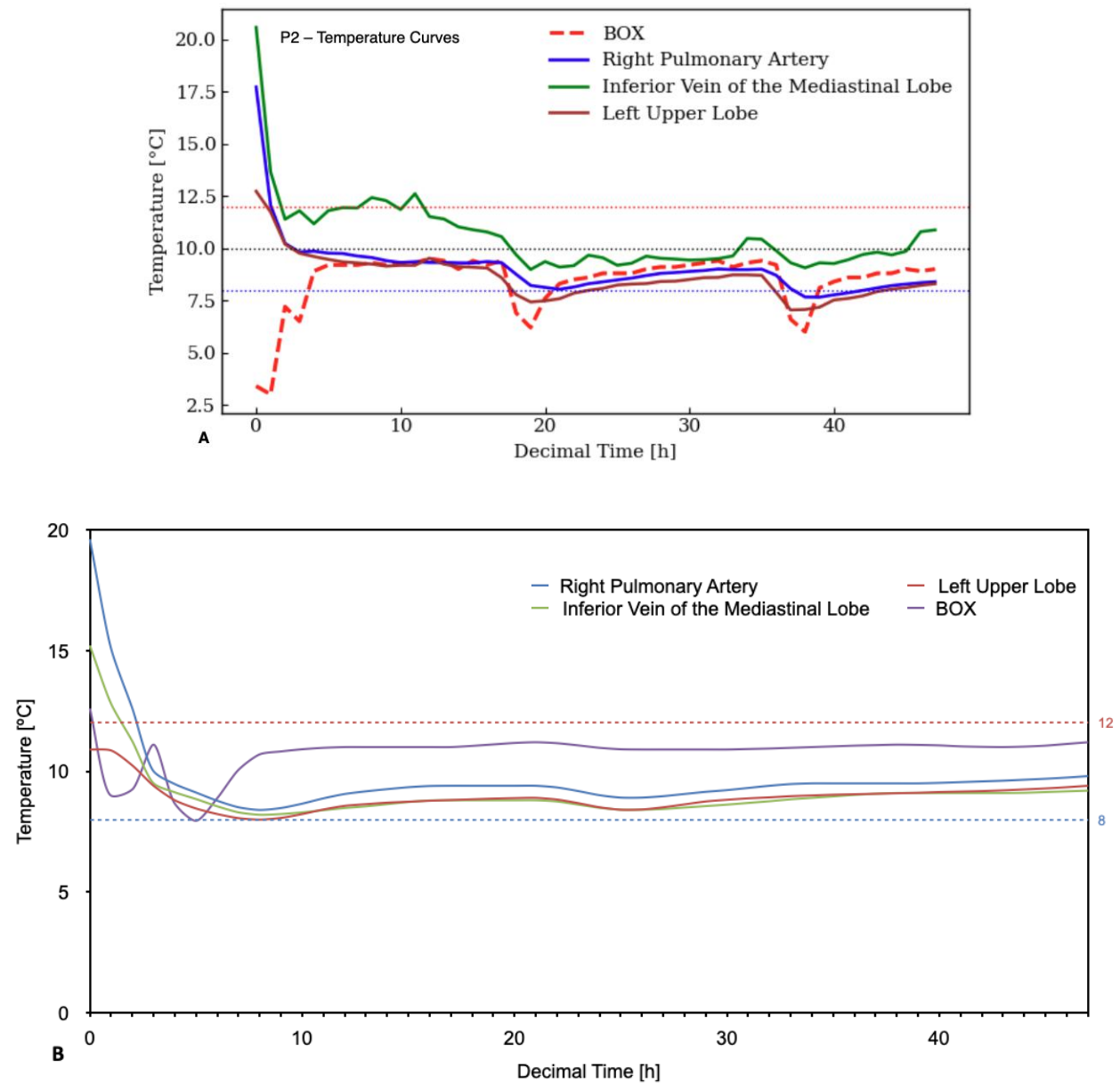
This pilot study demonstrated that the EMAIS-SR device, adjusted to operate at 10°C, was able to maintain lung-preservation temperatures within the predefined range of 8°C–12°C during a 48 h preservation period in a swine model. Temperature

measurements obtained from the preservation container and from intrapulmonary vascular and parenchymal sites showed concordant behavior over time, without sustained deviations outside the target range. These findings demonstrate the thermal stability of the EMAIS-SR system for a biological model and support its use in subsequent studies involving human lungs.^{9,11,12}

An additional relevant finding was the time required for the organ to reach the target temperature range after procurement. In the present study, the EMAIS-SR device achieved temperatures below 12°C within 1.7 h (range, 1.4–2 h) after lung procurement. Rapid lung cooling has not been suggested to be associated with structural or functional injury. In contrast, abrupt rewarming during transplantation can trigger pro-inflammatory cascades, metabolic dysregulation, and oxidative stress, thereby limiting the effective cold ischemia time of the lung.¹³ In this context, the EMAIS-SR system was designed to prevent unintended temperature elevations through active thermal control mechanisms.

Controlled hypothermic-preservation devices have been increasingly shown to outperform conventional ice-based storage in lung transplantation.^{9,11,12} Comparative studies have demonstrated that devices with active temperature control allow superior maintenance of mitochondrial integrity and cellular viability than ice-filled containers.^{5,14,15} Within this scenario, the EMAIS-SR system has emerged as a reusable alternative for lung transport. Importantly, previous studies have demonstrated effective microbiological decontamination of the device, supporting its safe reuse and addressing a key limitation of the existing disposable systems.⁷ This aspect is particularly relevant in low- and middle-income settings, where the high cost of single-use devices limits their widespread adoption and perpetuates reliance on conventional ice-based preservation.

The cooling dynamics of the EMAIS-SR system involve intermittent and controlled temperature drops to maintain the 10°C set-point. These fluctuations are visible as “valleys” in the NTC sensor graphs, demonstrating that the lung parenchyma tracks ambient thermal variations more slowly due to its mass and insulating properties. Despite these oscillations, thermal variation remained within the 8°C–12°C stability range specified in the experimental protocol. The literature still lacks standardized and universally accepted methods for real-time temperature verification.¹¹ The difficulty in obtaining absolute measurements of parenchymal temperature is evidenced by the differences observed between the internal monitoring system (NTC) and the independent control datalogger (Hantek® HTM208B). In Experiment 1 (P1), modest variations were observed, such as in the right pulmonary artery (8.77°C with the NTC versus 9.17°C with the control datalogger) and in the left upper lobe parenchyma (8.6°C versus 8.89°C). In Experiment 2 (P2), these disparities were more pronounced, with the internal system recording a container temperature of 8.44°C and the control datalogger recording a container temperature of 10.66°C, and inferior vein temperatures of



(A) Temperatures measured by the internal monitoring system of the EMAIS-SR device. (B) Corresponding temperatures recorded by the independent control datalogger.

Figure 3. Temperature curves for the preservation container (BOX) and the intrapulmonary compartments during Experiment 2 (P2).

Table 3. Descriptive temperature statistics recorded in the preservation container and in different lung regions over 48 h of preservation during Experiment 2 (P2)

Sensor	Mean (°C)	SD (°C)	Min (°C)	Max (°C)
BOX	8.44	1.42	3	9.5
Right pulmonary artery	9.05	1.51	7.66	17.72
Inferior vein of the mediastinal lobe	10.9	2.39	8.98	20.58
Left upper lobe	8.62	1.08	7.04	12.73

BOX, preservation container; Max, maximum temperature; Min, minimum temperature; SD, standard deviation.

Table 4. Cumulative time during which the temperature remained within the 8°C–12°C range in the preservation container and in different lung regions during Experiment 2 (P2)

Sensor	Time within 8°C–12°C (h)
BOX	39
Right pulmonary artery	41
Inferior vein of the mediastinal lobe	39
Left upper lobe	33

BOX, preservation container.

10.9°C versus 9.01°C. Anticipating this difficulty, the present study adopted multiple measurement methods (container sensors and external control), allowing for cross-validation that more closely approximates the organ's true thermal reality.

Preservation at temperatures around 10°C, in comparison with the current gold standard of 4°C (ice), has been consistently supported by experimental studies. These studies have demonstrated that prolonged graft preservation at 10°C ensures superior oxygenation and lung compliance while reducing airway pressures and edema, which is characterized by organ weight gain during ischemia. Furthermore, microscopic analyses have shown enhanced preservation at 10°C, evidenced by higher accumulation of the cytoprotective metabolites itaconate, glutamine, and N-acetylglutamine, which activate the innate mitochondrial anti-oxidative system that is essential for maintaining organelle health.¹⁵ In addition to experimental data, clinical studies have supported the adoption of preservation at 10°C, demonstrating increased preservation time and a consequent extension of the cold ischemia period. Clinically, this approach indicates a reduced incidence of primary graft dysfunction (PGD), the leading cause of early mortality following lung transplantation.⁵

Thus, the capacity of the EMAIS-SR system to maintain thermal stability within the 10°C range, combined with its reusability, represents a promising advancement for lung transplant logistics. This system can replace the current gold standard of ice-based transport, which has been shown to be inferior due to its inability to maintain the organ within the ideal range for viability, furthering the development of lung transplantation techniques.^{16–18}

Despite its promising results, this study had some limitations inherent to its experimental and pilot design. Preservation was performed using isotonic saline rather than a preservation solution, and the number of animals was limited for financial and ethical reasons. Furthermore, the observed differences between the measurement methods used in this study reinforce the need to develop more effective and precise thermal-monitoring tools and methods in lung preservation. Moreover, no histological or functional analyses were performed, because the primary objective of the study was to characterize the thermal behavior of the system.

These aspects will be addressed in subsequent studies, including investigations using human lungs declined for transplantation, wherein thermal stability can be correlated with morphological, metabolic, and functional parameters.

CONCLUSIONS

In this preclinical swine study, the EMAIS-SR device demonstrated the capacity to maintain lung-preservation temperatures predominantly within the target range of 8°C–12°C over a 48 h period. Thermal stability was observed in the preservation container and reflected across intrapulmonary compartments on the basis of continuous temperature monitoring. These findings support the technical feasibility of the system for lung preservation at intermediate temperature and support its progression to further translational evaluation.

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Authors' contributions: Reis FP: conceptualization, writing – review and editing, final approval; Guimarães PHA: investigation, methodology, final approval; Fantato MW: data curation, methodology, writing –

review and editing, final approval; Silva SF: project administration, resources, validation, writing – review and editing, final approval; Ruiz LM: formal analysis, writing – review and editing, final approval; Nepomuceno NA: conceptualization, supervision, final approval; Kitani EC: conceptualization, methodology, writing – original draft, final approval; Roza BA: formal analysis, validation, final approval; Pêgo Fernandes PM: conceptualization, writing – review and editing. All authors reviewed and approved the final version of the manuscript for publication.

Acknowledgments: The authors would like to thank Panther Healthcare for donating the surgical staplers used in this study. We also thank nurse Mariangela Belmonte Ribeiro for her invaluable assistance and technical expertise in the sterilization of the cannulas.

Sources of funding: None.

Conflicts of interest: Paulo Manuel Pêgo Fernandes, MD, PhD, is Editor-in-Chief of the *São Paulo Medical Journal*. The double-blinded peer review process was carried out by independent reviewers, and the authors declare no further conflicts of interest.

Data availability statement: Data supporting the findings of this study are available upon request from the corresponding author, Flavio Pola dos Reis.

Declaration of generative AI in scientific writing: During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to improve its readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

Date of first submission: February 16, 2026

Last received: February 19, 2026

Accepted: February 27, 2026

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Editor responsible for the evaluation process:

Marianne Yumi Nakai, MD, PhD (AE)



Potentially inappropriate medication use among older outpatients—prevalence and contributing factors: a retrospective study

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KEYWORDS (MeSH terms):

Potentially Inappropriate Medication List.
STOPP START Criteria.
Aged.

AUTHOR'S KEYWORDS:

Potentially Inappropriate Medication Use.
STOPP/START 3.
Older Adults.

ABSTRACT

BACKGROUND: Adverse outcomes associated with potentially inappropriate medication (PIM) use are prevalent among older adults.

OBJECTIVES: This study aimed to determine the frequency of PIM use older outpatients and assess its association with chronic comorbidities and geriatric syndromes using the screening tool of older persons' prescriptions (STOPP) and screening tool to alert to right treatment (START) version 3 criteria.

DESIGN AND SETTING: This retrospective study included patients aged 65 years or older who attended geriatric outpatient clinics between January and June 2024.

METHODS: PIM use was evaluated using the STOPP/START version 3 criteria. Polypharmacy was defined as the use of five or more medications.

RESULTS: Among the 107 patients (median age, 77 years; range, 65–91), 71% (n = 76) used PIM, with a median of two PIMs per patient (range, 0–6). PIM use was significantly more prevalent among patients with polypharmacy (odds ratio [OR] = 3.986; 95% CI: 1.493–10.639; p = 0.006). Analyses based on the STOPP criteria also revealed significant associations of PIM use with dementia (OR = 13.240; 95% CI: 1.504–116.529; p = 0.02), polypharmacy (OR = 4.352; 95% CI: 1.476–12.827; p = 0.008), and a history of falls (OR = 3.131; 95% CI: 1.017–9.634; p = 0.047).

CONCLUSION: Polypharmacy was significantly associated with greater odds of PIM use among older adults. Based on the STOPP version 3 criteria, dementia and a history of falls were also associated with increased PIM use in this population.

INTRODUCTION

The prevalence of potentially inappropriate medication (PIM) use among older adults ranges from 36.7% to 57.5%, depending on the guidelines applied, with even higher rates typically observed in low-income countries.^{1–3} Over the past two decades, the prevalence of PIM use has doubled.¹ In older populations, PIM use is associated with adverse outcomes such as falls, hip fractures, increased outpatient visits, and emergency department admissions.^{1–3}

The global population is aging rapidly.³ Older adults are particularly susceptible to PIM use because of the presence of multiple chronic conditions.⁴ PIM use in this demographic increases the risk of hospital admissions resulting from functional decline.⁵ To mitigate these risks, various guidelines have been established to minimize adverse events associated with PIM use and promote appropriate therapy.^{4–6} These guidelines focus on identifying medications that should be discontinued, detecting inadequate dosing, and recognizing omissions in necessary therapy.^{4–6} The screening tool of older persons' prescriptions (STOPP) and screening tool to alert to right treatment (START) criteria are the most widely used, and the third version has been recently introduced.⁴ This update incorporates new therapies and serves as an essential reference for optimizing medication safety in older adults. Ongoing evaluation of these guidelines will facilitate further refinements.

Therefore, the present study aimed to examine the prevalence of PIM use among older outpatients and evaluate its associations with chronic diseases and geriatric syndromes using the STOPP/START version 3 criteria.

MATERIALS AND METHODS

This retrospective study included patients aged ≥ 65 years who attended geriatric outpatient clinics between January and June 2024. The exclusion criteria comprised inability to complete

the assessments because of substantial health deterioration (e.g., advanced dementia, organ failure, ongoing cancer treatment, or ischemic sequelae), conditions that prevented the measurement of handgrip strength (e.g., arthritis, neuropathy, plegia, or spasticity), and incomplete data on comorbidities, the numbers of diseases and medications, or geriatric syndromes. The study adhered to the STROBE guidelines.⁷ Ethical approval was obtained from the local university hospital's ethics committee (approval no. 2024/798).

PIM use was assessed using the STOPP/START version 3 criteria.⁴ The vaccination section was excluded from the assessment. Polypharmacy was defined as the use of five or more medications.⁸

As part of the geriatric assessment, height and weight were measured, and body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).⁹ The recorded variables included age, sex, marital status, smoking status, comorbidities (including diabetes, hypertension, chronic obstructive pulmonary disease [COPD], ischemic heart disease, heart failure, cerebrovascular disease, Parkinson's disease, kidney disease, dementia, and depression), the numbers of diseases and medications, and geriatric syndromes. The assessed geriatric syndromes included a history of falls, probable sarcopenia, urinary or fecal incontinence, and constipation. Handgrip strength was measured using a dynamometer; and values below 27 kg for men or 16 kg for women were considered indicative of probable sarcopenia.¹⁰

Statistical analysis

Categorical data are presented as frequencies and percentages, and continuous data as means $\pm$ standard deviations or medians (minimum–maximum) according to their distribution. The Kolmogorov–Smirnov test was used to assess the normality of the data. For univariate analyses, the chi-square test, Mann–Whitney U test, or Student's t-test was used, as appropriate. Variables that were significant in the univariate analyses were subsequently evaluated using Pearson's or Spearman's correlation analysis. Because of the strong correlations among polypharmacy, the number of chronic diseases, and the number of medications, only polypharmacy was included in the multivariable analysis. Multivariable logistic regression analysis was performed, and the results were reported with 95% CI. Statistical significance was set at $p < 0.05$.

RESULTS

The median age of the 107 participants was 77 years (range: 65–91 years). The prevalence of PIM use was 71% ($n = 76$), and the median number of PIMs per patient was 2 (range: 0–6) (Table 1). Table 1 also summarizes the distribution of chronic diseases, including diabetes, hypertension, COPD, congestive heart failure, Parkinson's disease, chronic renal failure, dementia,

and depression, as well as the prevalence of geriatric syndromes such as polypharmacy, falls, probable sarcopenia, urinary or fecal incontinence, and constipation.

According to the START criteria, the prevalence of PIM use was 29% ($n = 31$). The medication groups most involved included oral bisphosphonates, acetylcholinesterase inhibitors, and memantine, along with the underdosing of antiplatelet and anticoagulant agents.

Using the STOPP criteria, the prevalence of PIM use was 63.6% ($n = 68$). The medications or medication classes most frequently implicated were nootropics used to treat dementia (e.g., ginkgo biloba and piracetam), nonsteroidal anti-inflammatory drugs, muscle relaxants, antiplatelet agents, anticoagulants, and proton pump inhibitors.

Univariate analysis showed significant associations between PIM use and age ($p = 0.02$), depression ($p = 0.04$), polypharmacy ($p < 0.001$), number of chronic diseases ($p = 0.02$), and number of chronic medications ($p < 0.001$) (Table 1). In the multivariable analysis, only polypharmacy (odds ratio [OR] = 3.986; 95% CI: 1.493–10.639; $p = 0.006$) remained statistically significant; the associations with age and depression were not significant (Table 2).

Univariate analysis based on the STOPP criteria showed significant associations between PIM use and age ($p = 0.005$), dementia ($p = 0.002$), depression ($p = 0.045$), polypharmacy ($p < 0.001$), the number of chronic diseases ($p = 0.004$), the number of chronic medications ($p < 0.001$), and a history of falls ($p = 0.019$). No significant associations were found with sex ($p = 0.895$), weight ($p = 0.067$), height ($p = 0.719$), BMI ($p = 0.105$), smoking status ($p = 0.28$), marital status ($p = 0.407$), diabetes ($p = 0.452$), hypertension ($p = 0.356$), COPD ($p = 0.447$), ischemic heart disease ($p = 0.098$), heart failure ($p = 0.208$), cerebrovascular disease ($p = 0.909$), Parkinson's disease ($p = 0.628$), chronic kidney disease ($p = 0.909$), probable sarcopenia ($p = 0.406$), urinary incontinence ($p = 0.597$), fecal incontinence ($p = 0.208$), or constipation ($p = 0.758$).

In the multivariable analysis based on the STOPP criteria, PIM use remained significantly associated with dementia (OR = 13.240; 95% CI: 1.504–116.529; $p = 0.02$), polypharmacy (OR = 4.352; 95% CI: 1.476–12.827; $p = 0.008$), and a history of falls (OR = 3.131; 95% CI: 1.017–9.634; $p = 0.047$) (Table 3).

DISCUSSION

In this study, the prevalence of PIM use among older outpatients was 71%. PIM use was significantly associated with polypharmacy. In the analysis based on the STOPP criteria, PIM use was significantly associated with dementia and a history of falls.

A meta-analysis by Tian et al.,¹ which included 94 studies comprising 371.2 million individuals aged ≥ 65 years across 17 regions, reported a PIM prevalence of 36.7%. The studies in the meta-analysis applied various assessment criteria, including multiple versions

Table 1. Baseline characteristics of the study population according to the presence of potentially inappropriate medication (PIM) use

	Presence of PIM (n = 76) 71%	Appropriate prescribing (n = 31) 29 %	Total Population (n = 107) 100%	p value
Age [□]	78 (65–91)	74 (65–87)	77 (65–91)	0.02*
Sex (n, %)				
Male	21 (27.6%)	10 (32.3%)	31 (29%)	0.632
Female	55 (72.4%)	21 (67.7%)	76 (71%)	
Weight (kg) [□]	72 ± 15.3	75.1 ± 14.5	72.9 ± 15.1	0.342
Height (cm) [□]	155 (140–183)	155 (143–178)	155 (140–183)	0.823
Body Mass Index (kg/m ²)	29.8 ± 6.2	31 ± 6.9	30.1 ± 6.4	0.361
Smoking Status (n, %)				
Smoker	4 (5.3%)	4 (12.9%)	8 (7.5%)	0.394
Quit	13 (17.1%)	5 (16.1%)	18 (16.8%)	
Never	59 (77.6%)	22 (71%)	81 (75.7%)	
Marital Status (n, %)				
Married	41 (53.9%)	19 (61.3%)	60 (56.1%)	0.198
Divorced	0 (0%)	1 (3.2%)	1 (0.9%)	
Widowed	35 (46.1%)	11 (35.5%)	46 (43%)	
Chronic disease (n, %)				
Diabetes mellitus	27 (35.5%)	8 (25.8%)	35 (32.7%)	0.331
Hypertension	56 (73.7%)	21 (67.7%)	77 (72%)	0.535
COPD	1 (1.3%)	0 (0%)	1 (0.9%)	0.521
Ischemic heart disease	20 (26.3%)	3 (9.7%)	23 (21.5%)	0.057
Congestive heart failure	7 (9.2%)	0 (0%)	7 (6.5%)	0.08
Cerebrovascular disease	3 (3.9%)	0 (0%)	3 (2.8%)	0.262
Parkinson's disease	4 (5.3%)	0 (0%)	4 (3.7%)	0.193
Chronic kidney disease	2 (2.6%)	1 (3.2%)	3 (2.8%)	0.866
Dementia	17 (22.4%)	2 (6.5%)	19 (17.8%)	0.051
Depression	14 (18.4%)	1 (3.2%)	15 (14%)	0.04*
Polypharmacy (n, %)	64 (84.2%)	15 (48.4%)	79 (73.8%)	< 0.001*
Number of chronic diseases [□]	3 (0–13)	2 (0–5)	3 (0–13)	0.002*
Number of chronic drugs [□]	7 (0–16)	3 (0–10)	6 (0–16)	< 0.001*
Geriatric syndromes (n, %)				
Falls	26 (34.2%)	5 (16.1%)	31 (29%)	0.061
Probable sarcopenia (HGS/27–16 kg)	33 (43.4%)	11 (35.5%)	44 (41.1%)	0.449
Urinary incontinence	42 (55.3%)	11 (35.5%)	53 (49.5%)	0.063
Faecal incontinence	2 (2.6%)	0 (0%)	2 (1.9%)	0.362
Constipation	10 (13.2%)	5 (16.1%)	15 (14%)	0.688

BMI: body mass index; COPD: chronic obstructive pulmonary disease; HGS: handgrip strength; PIM: potentially inappropriate medication use. Data are presented as the mean ± standard deviation, median (interquartile range), or number (percentage) as applicable. [□] Data as presented as the median (interquartile range).

* Significant p values.

of the Beers and the STOPP/START criteria, but did not apply the recently introduced STOPP/START version 3 criteria or the 2023 Beers criteria.¹ The higher prevalence observed in the present study (71%) may be attributable to the use of the updated STOPP/START version 3 criteria.

A cohort study by Ortonobes et al.¹¹ including 674 inpatients aged ≥65 years assessed PIM use during hospitalization using the STOPP/START version 2 criteria and found a prevalence of 73.1%. Although Ortonobes et al. used an earlier version of the criteria and a pharmacologist's perspective, their findings were comparable

to those of the present study, likely due to the substantial overlap between the two versions. In contrast, Andrade et al. applied the local European Union (7)-PIM criteria to 96 institutionalized older adults and reported a higher PIM prevalence of 86%. This difference may be attributable to differences in both the screening criteria and the patient populations.¹²

Multiple previous studies have consistently reported an association between PIM use and polypharmacy, regardless of the screening tool used.^{13–16} For example, Kojima et al.¹³ used the STOPP for Japanese (STOPP-J) criteria to screen 67,169 individuals aged ≥65

Table 2. Multivariable analysis of factors associated with PIM use

	p	OR	95% CI	
			Lower	Upper
Age	0.09	1.063	0.99	1.141
Depression	0.188	4.193	0.497	35.354
Polypharmacy	0.006*	3.986	1.493	10.639

CI: confidence interval; OR: Odds ratio; PIM: potentially inappropriate medication use. * Significant p values.

Table 3. Multivariable analysis of factors associated with PIM use based on the STOPP criteria

	p	OR	95% CI	
			Lower	Upper
Age	0.167	1.050	0.980	1.126
Dementia	0.020*	13.240	1.504	116.529
Depression	0.151	3.351	0.644	17.439
Polypharmacy	0.008*	4.352	1.476	12.827
Falls	0.047*	3.131	1.017	9.634

CI: confidence interval, OR: odds ratio, PIM: potentially inappropriate medication use. * Significant p values.

years and identified an association between polypharmacy and PIM use. Similarly, Hagiwara et al.¹⁴ conducted a cross-sectional study of 67,531 older adults receiving long-term care and reported a comparable association using the STOPP-J criteria. Kelleci et al.¹⁵ also observed this association among 700 older outpatients assessed using the Beers criteria.

Assessments using the STOPP criteria identify PIMs more frequently than those using the START criteria, regardless of the version used. The results of the present study are consistent with these findings.^{15–18}

Consistent with the present findings, previous studies have identified associations between PIM use assessed using the STOPP criteria and a history of falls, regardless of the STOPP/START version used.^{19–21}

The present findings are also consistent with those of previous studies that demonstrated an association between PIM use, as determined using the STOPP criteria, and dementia.^{22–24}

A key strength of this study is the application of the recently introduced STOPP/START version 3 criteria and the performance of assessments by geriatric specialists. However, limitations include its retrospective design and the lack of data on vaccination status.

CONCLUSION

Polypharmacy was significantly associated with PIM use in older adults. Medication regimens should be regularly reviewed to identify PIMs and determine whether they can be safely discontinued, particularly among patients with polypharmacy, a history of falls, or dementia.

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Authors' contributions: Bozkurt ME and Ozturk E: conceptualization (equal), writing – review and editing (equal), investigation (equal), methodology (equal), data curation (equal), project administration (equal), resources (equal), validation (equal), formal analysis (equal), supervision (equal), writing – original draft (equal). All authors reviewed and approved the final version of the manuscript for publication.

Funding: None.

Conflicts of interest: None.

Data availability statement: The data used in this study contains sensitive or private information and cannot be shared publicly in accordance with the Ethics Committee of Mersin University. Data are available from the authors upon approval by the relevant committee.

Declaration of generative AI in scientific writing: During the preparation of this work, the authors used DeepSeek to check grammar, spelling, and references. After using this tool, the authors reviewed and edited the content as needed and accepted full responsibility for the final publication.

Date of first submission: February 26, 2026

Last received: May 8, 2026

Accepted: June 8, 2026

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Editor responsible for the evaluation process:

Marianne Yumi Nakai, MD, PhD (AE)
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Standard versus miniaturized percutaneous nephrolithotomy in pediatric patients: a systematic review and meta-analysis

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KEYWORDS (MeSH terms):

Urology.
Pediatrics.
Urolithiasis.
Nephrolithotomy, Percutaneous.

AUTHOR'S KEYWORDS:

Pediatric Endourology.
Miniaturized Surgery.
Percutaneous Renal Access.
Perioperative Complications.
Clavien-Dindo Classification.
Renal Stone Surgery.

ABSTRACT

BACKGROUND: Although miniaturized percutaneous nephrolithotomy (mini-PCNL) is a less invasive alternative to standard percutaneous nephrolithotomy (PCNL), its comparative outcomes in pediatric patients remain unclear.

OBJECTIVES: To compare the efficacy and safety of mini-PCNL versus standard PCNL in children with nephrolithiasis.

DESIGN AND SETTING: Systematic review and meta-analysis conducted according to PRISMA guidelines, including randomized controlled trials (RCTs) and retrospective cohort studies of pediatric patients undergoing mini-PCNL or standard PCNL.

METHODS: Major databases (Cochrane Library, PubMed/MEDLINE, and Embase) were searched through August 2024. The primary endpoint was the stone-free rate. Secondary endpoints included operative time, postoperative fever, and complications classified by the Clavien-Dindo system.

RESULTS: Five studies (two RCTs and three retrospective cohort studies) involving 490 patients (46% underwent mini-PCNL) were included. Stone-free rates were comparable between the groups (relative risk [RR], 0.98; 95% confidence interval [CI], 0.929–1.041; $p = 0.56$; $I^2 = 0\%$). Mini-PCNL was associated with longer operative times (mean difference [MD], 7.5 min; 95% CI, 0.2–14.9; $p = 0.04$; $I^2 = 78\%$) but a lower risk of Clavien-Dindo grade I complications (RR 0.464; 95% CI 0.442–0.944; $p < 0.05$; $I^2 = 0\%$). No significant differences were observed in postoperative fever or Clavien-Dindo grade II–IV complications.

CONCLUSION: Mini-PCNL and standard PCNL achieve comparable stone-free rates in pediatric patients. Mini-PCNL is associated with fewer minor complications, whereas standard PCNL is associated with shorter operative times. Both techniques demonstrate similar safety profiles with respect to major complications.

CLINICAL TRIAL OR SYSTEMATIC REVIEW REGISTRATION:

PROSPERO: CRD42024618399; available at: <https://www.crd.york.ac.uk/PROSPERO/view/CRD42024567890>.

INTRODUCTION

Standard percutaneous nephrolithotripsy (PCNL) is the procedure of choice for treating complex and large kidney stones in both adults and children.¹ However, bleeding requiring transfusion has been reported in up to 10% of cases and is associated with stone burden, operative time, the number of access tracts, and sheath size.^{2–5}

Recent innovations in standard PCNL have focused on reducing the size of access sheaths to minimize perioperative complications without compromising stone removal efficiency. Standard PCNL typically utilizes access sheaths ranging from 24F to 30F, whereas mini-PCNL employs smaller sheaths ranging from 14F to 20F, providing a less invasive alternative.^{6,7}

Meta-analyses comparing mini-PCNL (12–22F) with standard PCNL (> 22F) in adults have shown that both techniques achieve similar stone-free rates (SFRs). However, mini-PCNL is associated with less blood loss, lower transfusion rates, and shorter hospital stays. No significant differences have been observed between the two approaches in overall complication rates.^{6–8}

Evidence regarding the pediatric population remains limited, although recent studies have begun to address this gap. Therefore, this meta-analysis aimed to assess the efficacy and safety of mini-PCNL compared with standard PCNL for the management of kidney stones in children.

OBJECTIVE

To compare the efficacy and safety of mini-PCNL versus standard PCNL in children with nephrolithiasis.

METHODS

Literature search

This systematic review and meta-analysis was conducted in accordance with the Cochrane Collaboration and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines. It was prospectively registered in the International Prospective Register of Systematic Reviews under registration number CRD42024618399.

The research question was developed using the Patient, Intervention, Comparator, Outcome, and Study framework: Which surgical approach, standard PCNL or mini-PCNL, yields a higher stone-free rate and better perioperative outcomes.⁹

Eligibility criteria

Randomized controlled trials (RCTs) and prospective or retrospective observational studies comparing standard PCNL and mini-PCNL in patients aged < 18 years with renal calculi were included if they reported at least one outcome of interest. Studies were excluded if they lacked a control group, included overlapping patient populations, enrolled patients without renal calculi, or evaluated a combination of other interventions or control groups.

Search strategy and data extraction

PubMed, Embase, and the Cochrane Central Register of Controlled Trials were systematically searched through August 2024 using the following search strategy: (“children” OR “pediatric” OR “adolescents” OR “patients under 18 years” OR “infants” OR “young patients” OR “youth” OR “neonates”) AND (“renal calculi” OR “kidney stones” OR “urolithiasis” OR “nephrolithiasis” OR “urinary calculi” OR “urinary stones”) AND (“mini PCNL” OR “mini percutaneous nephrolithotomy” OR “pediatric mini percutaneous” OR “pediatric mini percutaneous nephrolithotomy” OR “minimally invasive PCNL” OR “mini nephrolithotomy” OR “micro PCNL” OR “super-mini percutaneous nephrolithotomy”) AND (“standard PCNL” OR “standard percutaneous nephrolithotomy” OR “conventional PCNL” OR “traditional PCNL” OR “regular PCNL”).

Two authors (C.F. and F.S.) independently extracted the data according to predefined eligibility criteria and performed the quality assessment. Disagreements were resolved by consensus.

Data extraction and endpoints

All variables were entered into an Excel spreadsheet for analysis and cross-checked by another author (J.M. and F.S.). The mean

and standard deviation (SD) were recorded for continuous variables whenever reported in the included studies. For variables reported as the median and interquartile range, the original data were converted to the mean and SD.

The recorded variables included the medium follow-up duration, number of patients, age, sex, study design, year of publication, stone size, stone-free rate, operative time, number of punctures, length of hospital stay, blood loss, transfusion rate, and complications stratified according to the Clavien–Dindo classification.¹⁰ Minor complications were defined as Clavien–Dindo grades I and II, whereas major complications were defined as Clavien–Dindo grades III–V.

Quality assessment

The quality of the RCTs was assessed using the Cochrane Risk of Bias 2 tool for randomized trials, which evaluates studies across five domains: selection bias, performance bias, detection bias, attrition bias, and reporting bias. Each study was classified as having a low, high, or unclear risk of bias. For non-randomized studies, the Risk of Bias in Non-randomized Studies of Interventions tool was utilized. Two authors (C.F. and F.S.) independently assessed the risk of bias, and any disagreements were resolved by consensus.

Statistical analysis

Treatment effects for continuous outcomes were estimated using the mean difference (MD) with 95% confidence intervals (CIs). Binary outcomes were analyzed using risk ratios (RR) with 95% CIs. For studies reporting continuous outcomes as medians and interquartile ranges, these values were converted to means and SD using established methods.¹¹ Given the anticipated heterogeneity across studies, a random-effects model using the Mantel–Haenszel method was applied for all outcomes. Heterogeneity was assessed using the I^2 statistic, with values of 0–25% indicating low heterogeneity, 25–50% indicating moderate heterogeneity, and > 50% indicating substantial heterogeneity.¹² Statistical analyses were performed using R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study selection

A total of 148 records were identified through the electronic database search through September 30, 2024 (**Figure 1**), including 22 from PubMed, 123 from Embase, and three from the Cochrane Central Register of Controlled Trials. After removing 32 duplicates, 92 records were excluded based on their title and abstract because they were not relevant to the research question. Twelve articles were assessed for full-text eligibility. Of these, five

were excluded because they did not involve the population of interest, one for not reporting the outcome of interest, and six for other reasons. Finally, two RCTs^{13,14} and three retrospective cohort studies comprising 490 patients^{15–17} were included in the systematic review and meta-analysis.

BASELINE CHARACTERISTICS OF THE INCLUDED STUDIES

Table 1 summarizes the characteristics of the included studies. All studies were published in English between 2015 and 2024. The study populations were predominantly male, with participant ages ranging from 7 months to 11 years.

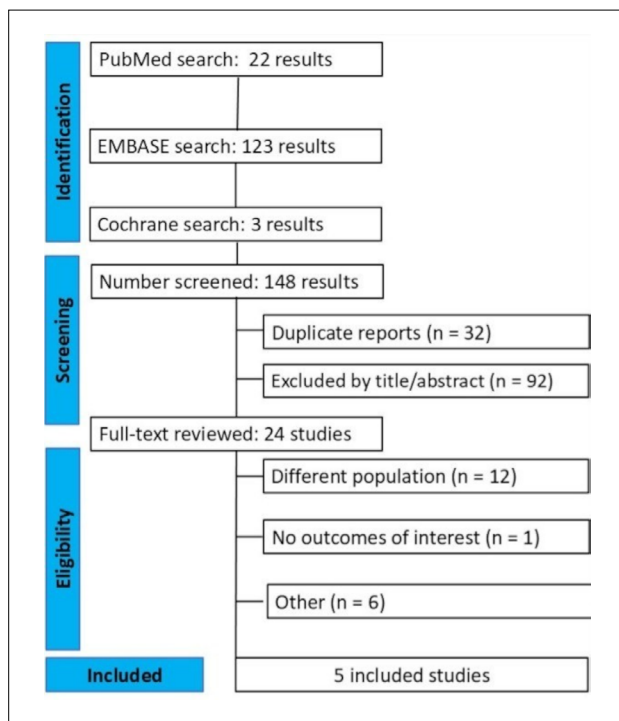


Figure 1. PRISMA flow diagram of the study selection process.

Pooled analysis of all studies: perioperative outcomes—operative time

Table 2 summarizes the perioperative outcomes comparing mini-PCNL and standard PCNL. Operative time was reported in all included studies. Kumar et al.¹⁴ reported the longest operative times in both the mini-PCNL and standard PCNL groups. The mean operative time was 90 min in the mini-PCNL group and 70 min in the standard PCNL group. This 20-min difference was statistically significant ($p < 0.05$), indicating a longer operative time with mini-PCNL. Conversely, Mahmood et al.¹⁷ reported the shortest operative times. The mean operative time was 41.53 min in the mini-PCNL group and 37.52 min in the standard PCNL group. This approximately 4-min difference was not statistically significant ($p = 0.073$), suggesting comparable operative times between the two techniques. The pooled analysis demonstrated that mini-PCNL was associated with a significantly longer operative time compared with standard PCNL (MD, 7.5 min; 95% CI, 0.2–14.9; $p = 0.04$; $I^2 = 78\%$). However, subgroup analysis showed that this difference was mainly driven by non-randomized studies, whereas randomized controlled trials did not demonstrate a statistically significant difference. Sensitivity analysis did not identify any source of heterogeneity (**Figure 2**).

Complications according to the Clavien–Dindo classification

The Clavien–Dindo classification of complications was assessed in four studies. Unlike the other studies, which used the Clavien–Dindo classification, Song et al.¹³ did not report complications using this system. Minor complications (Clavien–Dindo grade I–II) occurred more frequently in the standard PCNL groups, with incidences ranging from 17.7%–72%, compared with 11%–44% in the mini-PCNL groups. Among these complications, fever was the most common, affecting 15.5%–19.5% of patients undergoing standard PCNL compared with 5.7%–15.6%

Table 1. Baseline characteristics of the included studies

Study	Year	Country	Study design	No of patients (PCNL/MPCNL)	Age, MPCNL/PCNL	Male, n (%) MPCNL/PCNL	Stone size, MPCNL/PCNL
Akdogan	2024	Türkiye	Retrospective cohort	73/90	17.3 (7–24)/17.3 (8–24)**	36 (49.3)/50 (55.6)	165.4 (50–600)/151.6 (50–800) mm ²
Karkin	2024	Türkiye	Retrospective cohort	32/41	9.3 (4.1)/10.1 (5.4)*	19 (59.3)/29 (70.7)	2.1 (1.2)/2.3 (1.4) mm ²
Kumar	2023	India	RCT	25/25	9.4 (2.6)/10.4 (2.26)*	18 (72)/16 (64)	18.6 (2.56)/20.2 (3.58) mm ²
Mahmood	2019	Iraq	Retrospective cohort	59/75	6.91 (4.982)/6.20 (4.138)*	31 (52.5)/51 (68)	1.9 (1.162)/2.2 (1.424) cm
Song	2015	China	RCT	35/35	20.3 (6.35)/20.10 (7.22)*	–	2.32 (1.07)/2.4 (1.39) cm

* Age reported as median (minimum–maximum). * Age reported in months. * Age reported in years. MPCNL, miniaturized percutaneous nephrolithotomy; PCNL, percutaneous nephrolithotomy; RCT, randomized controlled trial.

of those undergoing mini-PCNL. Hematuria occurred in 1.7%–5.3% of patients in the standard PCNL groups and 1.4%–3.1% of those in the mini-PCNL groups. This difference reached statistical significance for grade I complications ($p = 0.047$), supporting a more favorable safety profile for the mini-PCNL approach. Regarding major complications (Clavien–Dindo grade $\geq$ III), standard PCNL was associated with a higher frequency of serious adverse events, with rates ranging from 1.1%–5.5%, whereas mini-PCNL showed incidences ranging from 0%–4.1%. Among the most severe complications, ureteral avulsion occurred in

1.1% of patients undergoing standard PCNL, and nephropleural fistula was reported in 4% of procedures. Although rare, these complications occurred exclusively or predominantly in the standard PCNL groups, suggesting that mini-PCNL may reduce the risk of serious surgical complications.

The pooled analysis demonstrated a significant difference between the groups for grade I complications (**Figure 3**: RR, 0.464; 95% CI, 0.442–0.944; $p < 0.05$; $I^2 = 0\%$). However, no significant differences were observed between the groups for grades II, III, and IV ($p > 0.05$ for all comparisons).

Table 2. Perioperative outcomes of mini-PCNL vs. standard PCNL

Study (Year)	Group	SFR (%)	Operative time (min)	Hemoglobin/Hematocrit decrease (g/dL)	Transfusion rate (%)	Minor complications (Clavien–Dindo grades I–II)	Major complications (Clavien–Dindo grades $\geq$ III)
2024	MPCNL	89	74.8*	–1.7% (Hct)*	1.4	19.2%	4.1%
	PCNL	90.8	62.8	–1.7% (Hct)	3.3	17.7%	5.5%
2024	MPCNL	81.2	85.4*	0.9*	3.1	21.9%	0
	PCNL	85.4	71.7	1.6	4.9	29.3%	0
2023	MPCNL	88	90*	1.1*	4	44%	0
	PCNL	92	70	1.7	8	72%	4%
2019	MPCNL	89.5	41.53	0.35	1.7	18%	0
	PCNL	94.7	37.52	0.57*	5.3	44%	0
2015	MPCNL	97.8	51.97	0.9	0	0	0
	PCNL	94.3	55	0.8	0	0	0

* Indicates a statistically significant difference ($p < 0.05$) between the mini-PCNL and standard PCNL groups for the corresponding outcome in the original study. Hct, hematocrit; MPCNL, miniaturized percutaneous nephrolithotomy; PCNL, percutaneous nephrolithotomy; SFR, stone-free rate.

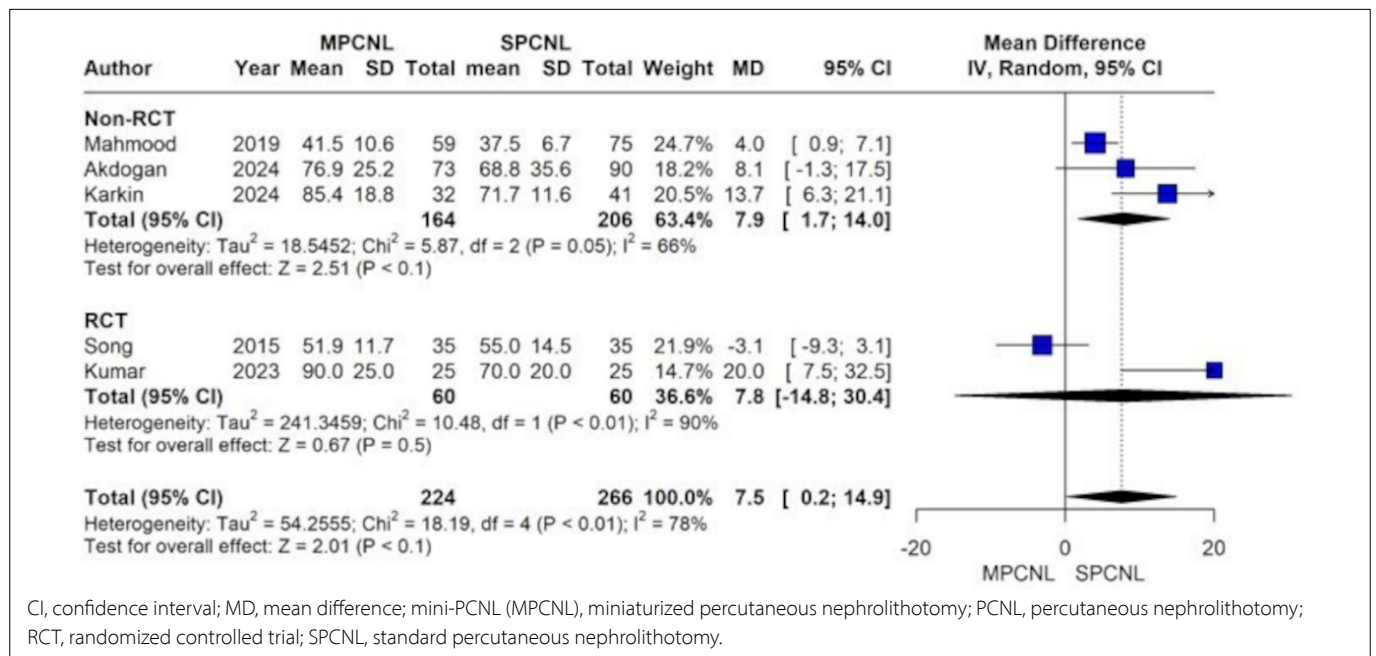


Figure 2. Forest plot of operative time comparing mini-PCNL and standard PCNL.

Postoperative fever

Postoperative fever was assessed in four studies. These studies included a total of 224 patients in the mini-PCNL group and 266 in the standard PCNL group. The pooled analysis showed no significant differences between the groups (Figure 4: RR, 0.772; 95% CI, 0.495–1.202; $p = 0.252$; $I^2 = 0\%$).

Stone-free rate

SFR was assessed in all included studies, although its definition varied. Most studies defined SFR as the absence of residual stones or the presence of clinically insignificant fragments. Mahmood et al.¹⁷ evaluated treatment success without specifying the allowable residual fragment size, focusing instead on complete stone removal. Kumar et al.¹⁴ and Karkin et al.¹⁶ defined SFR as residual fragments measuring <3 mm, whereas Akdogan et al.¹⁵ used a stricter cutoff of <2 mm. Song et al.¹³ considered residual fragments of up to 4 mm as stone-free. These varying definitions reflect differences in the assessment of SFR across studies.

Song et al.¹³ reported the highest SFR, with the rates of 97.14% in the mini-PCNL group and 94.29% in the standard PCNL group. Karkin et al.¹⁶ reported the lowest SFR, with rates of 81.25% and 85.37%, respectively. The pooled analysis showed no significant difference between the groups (Figure 5: RR, 0.98; 95% CI, 0.929–1.041; $p = 0.56$; $I^2 = 0\%$).

Quality assessment

The quality assessment indicated a low risk of bias across all assessed domains (Supplementary Figures 1 and 2).

The analysis of the studies included in this meta-analysis identified several methodological limitations that warrant consideration, although they do not undermine the overall validity of the findings. The main limitation was incomplete reporting in some studies, particularly regarding follow-up losses (D3) and outcome selection criteria (D5), resulting in an unclear risk of bias classifications for these domains.

The retrospective observational studies exhibited methodological characteristics requiring consideration, including challenges

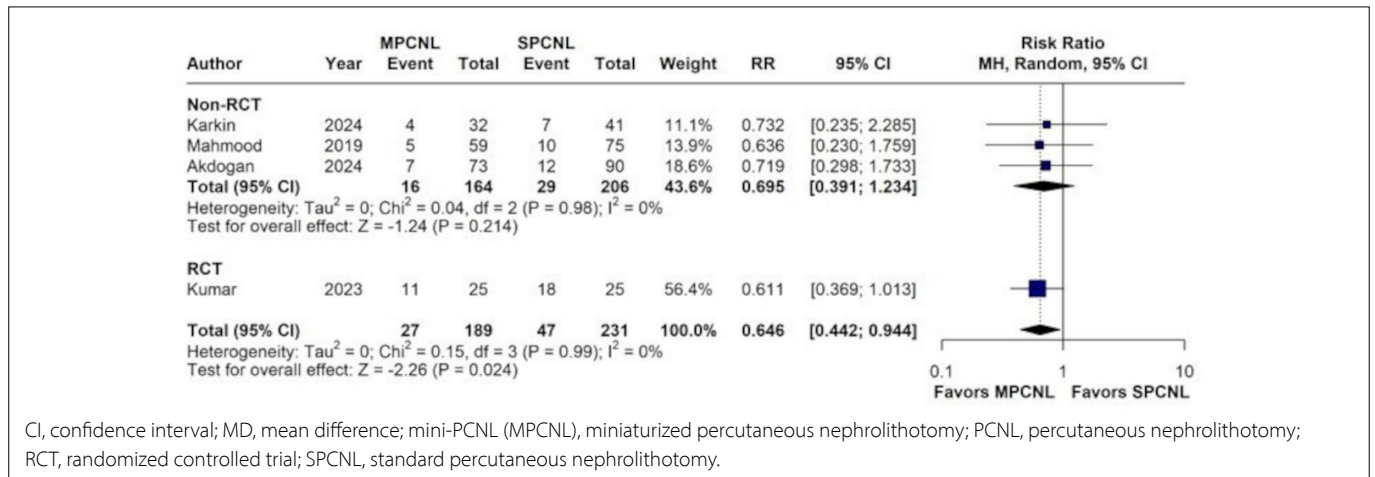


Figure 3. Forest plot of Clavien–Dindo grade I complications comparing mini-PCNL and standard PCNL.

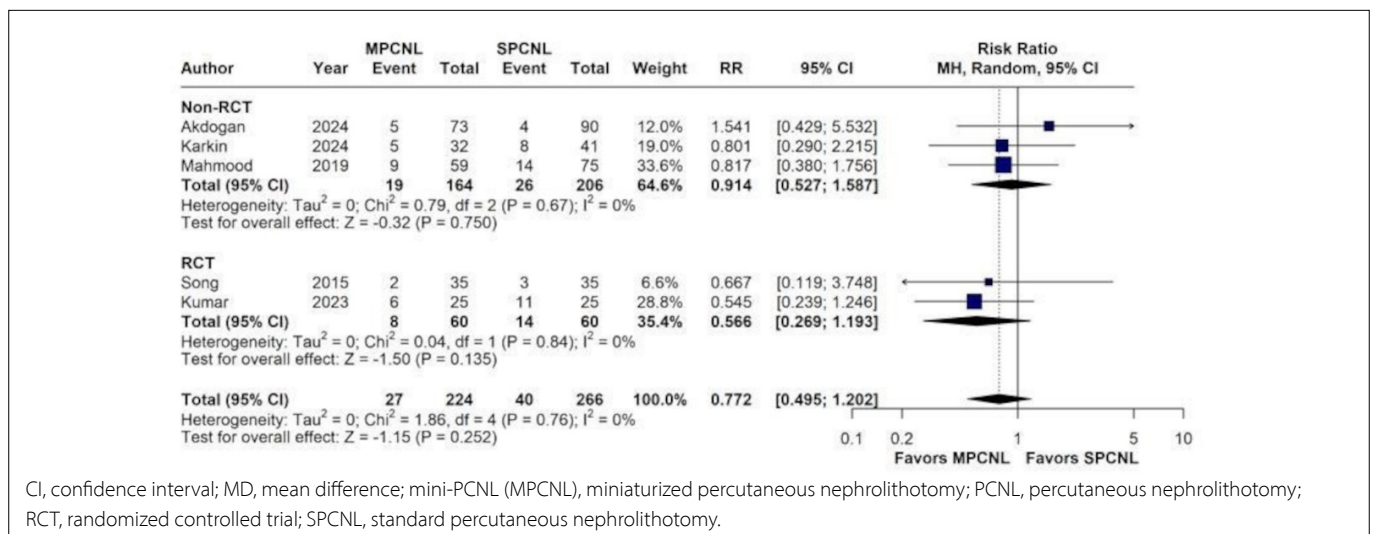
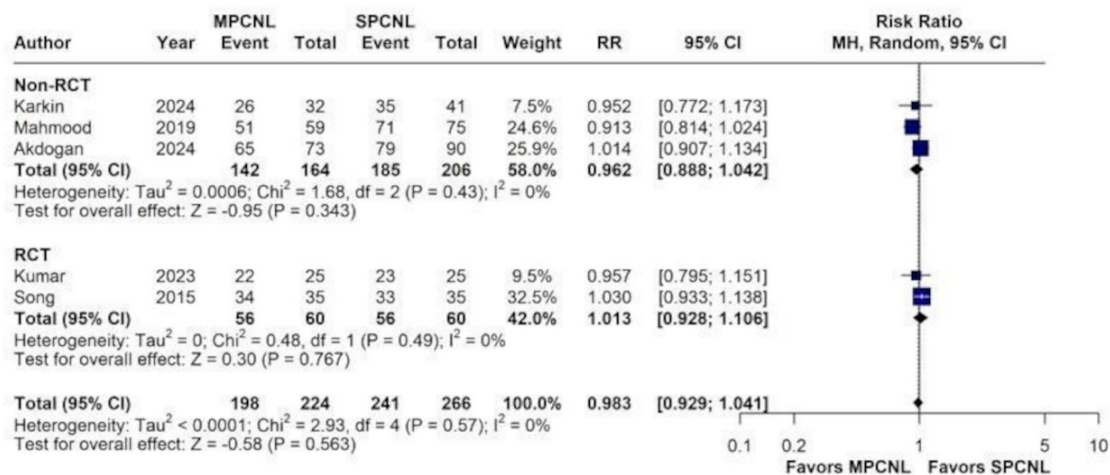


Figure 4. Forest plot of postoperative fever comparing mini-PCNL and standard PCNL.



CI, confidence interval; MD, mean difference; mini-PCNL (MPCNL), miniaturized percutaneous nephrolithotomy; PCNL, percutaneous nephrolithotomy; RCT, randomized controlled trial; SPCNL, standard percutaneous nephrolithotomy.

Figure 5. Forest plot of stone-free rate comparing mini-PCNL and standard PCNL.

in controlling for confounding variables and potential selection bias, particularly in the study of Mahmood et al.¹⁷ The study by Karkin et al.¹⁶ was notable for its 20% loss to follow-up. Variability was also observed among the studies in outcome measurement methods and the reporting of statistical procedures.

DISCUSSION

The main findings of this study can be grouped into three key areas: efficacy, safety, and operative aspects.

The most relevant finding was the absence of a significant difference in SFR between the two techniques analyzed (RR, 0.98; 95% CI, 0.929–1.041; $p = 0.56$). This finding is consistent with that of Qin et al.,¹⁸ who also reported no significant difference in SFR between mini-PCNL and standard PCNL (RR, 1.01; 95% CI, 0.98–1.04; $p = 0.57$).

Mini-PCNL demonstrated a more favorable safety profile, with a lower incidence of Clavien–Dindo grade I complications,¹⁰ such as postoperative pain and transient fever (RR, 0.464; 95% CI, 0.442–0.944; $p < 0.05$), possibly because of the reduced trauma associated with the use of smaller-caliber instruments. In contrast, no significant difference was observed in major complications (grades II–IV), including significant bleeding or the need for reoperation. Standard PCNL was associated with a significantly shorter operative time (MD, 7.5 min; $p = 0.04$), which may be related to greater familiarity with conventional instruments. However, this advantage was accompanied by a greater need for multiple punctures (RR, 2.946; 95% CI, 1.175–7.389; $p < 0.05$), a factor that may increase the risk of bleeding and renal parenchymal injury.

Although mini-PCNL required a slightly longer operative time, it was less invasive, requiring fewer additional access tracts.

The analysis of postoperative fever revealed no significant difference between the two techniques (RR, 0.772; 95% CI, 0.495–1.202;

$p = 0.252$; $I^2 = 0\%$), a finding that is consistent with that reported by Qin et al.,¹⁸ who also found no significant difference (RR, 1.22; 95% CI, 0.97–1.51; $p = 0.08$).

Additionally, no clinically relevant differences were observed in secondary outcomes, including hemoglobin loss, length of hospital stay, and the need for double-J catheter usage. Thus, although mini-PCNL was associated with a lower rate of minor complications, both techniques demonstrated comparable outcomes with respect to postoperative fever, hemorrhagic outcomes, and immediate postoperative recovery. Therefore, the choice between the two approaches should consider the patient's clinical profile, the desired operative time, and the goal of minimizing less clinically significant interventions.

Despite the promising results, some limitations should be considered.

The inclusion of studies with different designs, two RCTs^{13,14} and three retrospective cohorts,^{15–17} limits the direct comparability of the data. While the RCTs^{13,14} provide better control of potential bias, retrospective studies^{15–17} relied on medical records, which may be incomplete or inconsistent. Specifically, Mahmood et al.¹⁷ did not clearly describe the participant selection criteria, and Karkin et al.¹⁶ reported a high loss to follow-up rate (20%), which may have compromised the internal validity of the findings.

Five studies, including a total of 490 patients, were included, with an unequal distribution between the mini-PCNL (46%) and standard PCNL groups. The limited number of RCTs reduces the statistical power and generalizability of the findings. Several methodological limitations hindered the standardization of the results, including the lack of clearly defined exclusion criteria, unclear outcome assessment protocols, and inconsistent application of Clavien–Dindo classification (not reported in Song et al.¹³). Technical differences, such as instrument diameter (14F–20F in mini-PCNL

vs. 24F–30F in standard PCNL) and the use of fluoroscopic versus ultrasonographic guidance, were also not controlled and may have influenced outcomes such as bleeding and operative time.

Important clinical variables, such as patient age and stone burden, were not explored despite their potential influence on the outcomes. Furthermore, most studies were conducted in countries with distinct epidemiological characteristics and surgical practices (Türkiye, India, Iraq, and China), which may limit the generalizability of the findings to other populations.

Despite these limitations, mini-PCNL and standard PCNL demonstrated comparable efficacy for the treatment of pediatric kidney stones.

An important observation is that none of the included studies were conducted in high-income countries. This may reflect regional differences in case volume, the adoption of mini-PCNL techniques, the availability of specialized pediatric stone centers, and publication patterns. Countries with a higher burden of pediatric urolithiasis and greater experience with PCNL may therefore be disproportionately represented in the available literature. Consequently, although the pooled results suggest comparable efficacy and safety between mini-PCNL and standard PCNL, their generalizability to high-income healthcare settings remains uncertain and warrants further investigation.

Mini-PCNL was associated with a 53% reduction in mild complications, whereas standard PCNL was associated with a significantly shorter operative time, albeit with a greater need for multiple punctures. In contrast, no significant differences were observed in major complications, postoperative fever, or other secondary outcomes. Therefore, the choice of technique should be individualized, taking into account the patient's clinical characteristics, stone characteristics, the institutional context, and the therapeutic goal.

CONCLUSION

Mini-PCNL appears to achieve stone-free and complication rates comparable to those of standard PCNL in pediatric patients while potentially offering advantages in terms of blood loss and length of hospital stay. However, these findings should be interpreted with caution because of the limited number of available studies, the inclusion of retrospective data, and heterogeneity in study quality. Further well-designed prospective randomized studies are needed to confirm these findings.

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Date of first submission: November 3, 2025

Last received: June 4, 2026

Accepted: June 29, 2026

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Authors' contributions: Anunciação FS: methodology (equal), data curation (equal), formal analysis (equal), investigation (equal), software (equal), visualization (equal), writing – original draft (equal); Moreira JLML: data curation (equal), formal analysis (equal), investigation (equal), software (equal), visualization (equal), writing – original draft (equal); Fraga CCF: formal analysis (equal), visualization (equal), writing – original draft (equal); Suartz CV: conceptualization (equal), project administration (equal), supervision (equal), validation (equal), writing – review and editing (equal); Tiraboschi RB: supervision (equal), validation (equal), writing – review and editing (equal); Bessa J Jr: project administration (equal), supervision (equal), validation (equal), writing – review and editing (equal); Lopes RI: conceptualization (equal), project administration (equal), supervision (equal), validation (equal), writing – review and editing (equal). All authors reviewed and approved the final version of the manuscript for publication.

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: The data supporting the findings of this systematic review and meta-analysis, including the extracted dataset and statistical analysis files, are available upon reasonable request from the corresponding author, José Bessa Júnior.

Declaration of generative AI in scientific writing: The authors used GPT-5 (OpenAI) to assist in language editing and formatting of this manuscript. After using this tool, the authors reviewed and verified the content as accurate and take full responsibility for the final version.



Gender disparities among Brazil's top 2% of influential scientists for career-long impact

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Dear Editor,

The ranking of the world's top 2% most influential scientists, published by Stanford University, has gained recognition for its comprehensive methodology in evaluating scientific impact.¹ Developed by Professor John P.A. Ioannidis and his team, this ranking employs a composite metric based on data from the Scopus database to analyze scientists' contributions within their respective fields.

Researchers are classified both for their career-long impact and recent performance, which are determined by a score composed of six citation indicators. These include total impact (number of citations, Hirsch index), coauthorship adjustment (Schreiber's Hm index), and authorship order metrics (total citations in papers as single author; single or first author; single, first, or last author).² Although these metrics have limitations, Stanford University's ranking serves as a reference for assessing scientific output and impact, and identifying disparities that can guide policies that promote equity and scientific advancement.

In recent decades, Brazil has emerged as a significant contributor to global scientific production, despite historical challenges in science funding and regional inequalities. Female Brazilian researchers face barriers in opportunities, recognition, and career advancement, leading to their underrepresentation in prominent positions and in scientific rankings such as that of Stanford. This bias undermines gender equity and may limit innovation and diversity in Brazilian research.

Based on the ranking published on September 16, 2024, we extracted career-long data for researchers affiliated with Brazilian institutions, categorizing them by gender and geographic region (Central-West, Northeast, North, Southeast, and South).¹ Using descriptive analysis, we calculated the frequency of male and female scientists per region and displayed this distribution on a map.

Out of 217,093 scientists listed, 1,077 were affiliated with Brazilian institutions. Four entries were excluded due to duplication or inconsistencies, resulting in 1,073 (0.5%) scientists classified as the most influential for career-long impact. Among these, 892 (83.1%) were male and 181 (16.9%) were female scientists. This discrepancy was observed across all regions, with the proportion of female scientists ranging from 18.3% in the South to 10% in the North (**Figure 1**).

Gender disparity in Brazil stems from historical, cultural, social, and institutional factors that collectively create barriers to equity in science. Women face challenges regarding funding, collaboration networks, and access to leadership positions.³ The predominance of men in decision-making roles at universities and funding agencies limits opportunities for women's advancement. Furthermore, segregation in certain fields, inequalities in authorship roles, and reduced recognition in peer review may affect knowledge production, acknowledgment of women's contributions, and visibility in citation-based rankings.⁴

Although the proportion of female scientists in prominent positions is low nationwide, discrepancies are greater in less affluent regions such as the North and Northeast, where funding disparities exacerbate regional inequalities. In 2023, of the R\$ 5 billion (US\$ 0.9 billion) allocated by the National Fund for Scientific and Technological Development (FNDCT), the main science funding body in Brazil, only 4% was directed to institutions in the North and Northeast, whereas 93% was concentrated in the South and Southeast.⁵ This imbalance makes it more difficult for

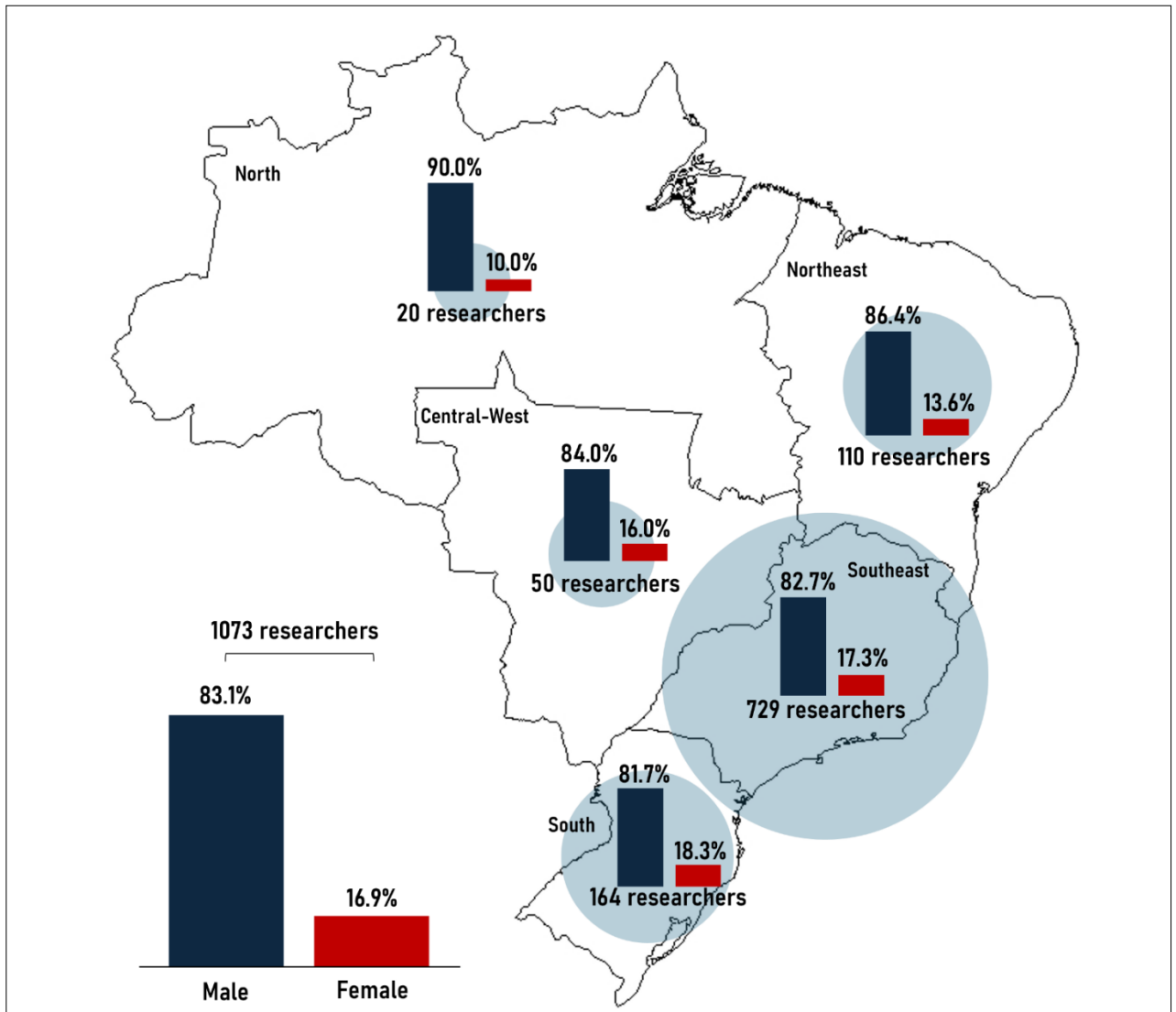


Figure 1. Distribution of male and female scientists in Brazil's top 2% of the most influential researchers by region.

women in underfunded regions to advance. Female researchers in Brazil have fewer research grants, published articles, and international collaborations,⁶ especially in resource-scarce regions. The coronavirus disease 2019 pandemic further accentuated these disparities, with countries such as Brazil showing a larger decline in women's publication rates.⁷

Despite advances in recent decades, gender inequality in academia remains a problem that requires effective policies and practices. Differences in career progression are impacted by factors such as maternity interruptions, discrimination, and family responsibilities. These factors create unfair competition between genders, and cultural change can take generations to materialize, leading to cumulative effects and losses in scientific development. To address this disparity, it is essential for universities, research institutes, and funding agencies to implement gender

equity policies, with clear goals to increase female participation at all academic levels.

Educational programs that inspire young girls to pursue science careers, challenging gender stereotypes, are crucial. Funding initiatives for women-led projects, especially in less favored regions, can help mitigate disparities. Ensuring transparency and recognition of contributions in publications can reduce biases in authorship attribution. Raising awareness about unconscious biases and stereotypes in academia, providing parental leave, offering flexible working hours, and making childcare services available in universities and research institutes are also essential. Regular disclosure of gender-disaggregated data on funding, publications, promotions, and other metrics can support monitoring and accountability efforts.

Addressing the identified gender disparities requires that evidence-based policies and institutional reforms be implemented

to promote gender equity at all academic levels. Such measures are essential not only for fairness but also to enhance the quality, innovation, and global competitiveness of scientific research.

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Authors' contributions: Quintans JSS: conceptualization, methodology, writing – review and editing; Guimarães AG: conceptualization, methodology, writing – review and editing; Quintans-Júnior L: conceptualization, methodology, investigation, formal analysis, writing – original draft, writing – review and editing; Araújo AAS: conceptualization, methodology, writing – review and editing; Santos VS: conceptualization, methodology, writing – review and editing; Martins-Filho PR: conceptualization, methodology, investigation, formal analysis, writing – original draft, writing – review and editing. All authors reviewed and approved the final version of the letter for publication.

Acknowledgments: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Sources of funding: None.

Conflicts of interest: None.

Data availability statement: Data supporting the findings of this study are available upon request from the corresponding author, Paulo Ricardo Martins-Filho.

Declaration of generative AI in scientific writing: During the preparation of this study, the authors did not use generative AI or AI-assisted technologies.

Date of first submission: April 28, 2025

Last received: May 20, 2026

Accepted: February 3, 2026

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INSTRUCTIONS FOR AUTHORS

Scope and indexing

São Paulo Medical Journal (formerly Revista Paulista de Medicina) was founded in 1932 and is published bimonthly by Associação Paulista de Medicina, a regional medical association in Brazil.

The Journal accepts articles in English in the fields of evidence-based health, including internal medicine, epidemiology and public health, specialized medicine (gynecology & obstetrics, mental health, surgery, pediatrics, urology, neurology and many others), and also physical therapy, speech therapy, psychology, nursing and healthcare management/administration.

São Paulo Medical Journal's articles are indexed in MEDLINE, LILACS, SciELO, Science Citation Index Expanded, Journal Citation Reports/Science Edition (ISI) and EBSCO Publishing.

Editorial policy

Papers with a commercial objective will not be accepted: please review the Journal's conflicts of interest policy below.

São Paulo Medical Journal accepts manuscripts previously deposited in a trusted preprint server.

São Paulo Medical Journal supports Open Science practices. It invites reviewers to join Open Peer Review practices through acceptance that their identities can be revealed to the authors of articles. However, this is purely an invitation: reviewers may also continue to provide their input anonymously.

São Paulo Medical Journal is an open-access publication. This means that it publishes full texts online with free access for readers.

São Paulo Medical Journal applies a publication fee in the form of an article processing charge (APC) for all studies conducted outside of Brazil. This rate will be charged to the corresponding author when the study has been accepted on the grounds of its scientific merit. This fee is US\$ 500.00 and is independent of the length of the text. The corresponding author should wait to receive the journal's invoice before making the payment. The article will only be published after presentation of the proof of payment. Submission is free for all. Associação Paulista de Medicina provides financial support for the Journal.

Articles accepted for publication become the Journal's property for copyright purposes, in accordance with Creative Commons attribution type BY.

Transparency and integrity: guidelines for writing

The Journal recommends that all articles submitted should comply with the editorial quality standards established in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals,¹ as updated in the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals. These standards were created and published by the International

Committee of Medical Journal Editors (ICMJE) as a step towards integrity and transparency in science reporting and they were updated in December 2018.¹

All studies published in *São Paulo Medical Journal* must be described in accordance with the specific guidelines for papers reporting on clinical trials (CONSORT),² systematic reviews and meta-analyses (PRISMA),^{3,4} observational studies (STROBE),^{5,6} case reports (CARE)⁷ and accuracy studies on diagnostic tests (STARD).^{8,9} These guidelines ensure that all methodological procedures have been described, and that no result has been omitted. If none of the above reporting guidelines are adequate for the study design, authors are encouraged to visit the EQUATOR Network website (<http://www.equator-network.org/>) to search for appropriate tools.

Conflicts of interest

Authors are required to describe any conflicts of interest that may exist regarding the research or the publication of the article. Failure to disclose any conflicts of interest is a form of misconduct.

Conflicts of interest may be financial or non-financial. The Journal recommends that the item "Conflicts of interest" at <http://www.icmje.org> should be read to obtain clarifications regarding what may or may not be considered to be a conflict of interest. The existence and declaration of conflicts of interest is not an impediment to publication at all.

Acknowledgements and funding

Grants, bursaries and any other financial support for studies must be mentioned separately, after the references, in a section named "Acknowledgements." Any financial support should be acknowledged, always with the funding agency name, and with the protocol number whenever possible. Donation of materials used in the research can and should be acknowledged too.

This section should also be used to acknowledge any other contributions from individuals or professionals who have helped in producing or reviewing the study, and whose contributions to the publication do not constitute authorship.

Authorship

The Journal supports the position taken by the ICMJE (<http://www.icmje.org>) regarding authorship. All authors should read ICMJE's recommendations to obtain clarifications regarding the criteria for authorship and to verify whether all of them have made enough contributions to be considered authors.¹⁰

All authors of articles published in *São Paulo Medical Journal* need to have contributed actively to the discussion of the study results and should review and approve the final version that is to be released. If one author has not contributed enough or has not approved the final version of the manuscript, he/she must be transferred to the Acknowledgement section.

The corresponding author is the primary guarantor of all ethical issues relating to the manuscript, before, during and after its publication. However, *São Paulo Medical Journal* and ICMJE consider that all authors are held fully responsible for the study, regarding the accuracy or integrity of data and data interpretation in the text. Contributions such as data collection only do not constitute authorship.

The addition or deletion of authors' names in the manuscript byline is possible only if the corresponding author provides the reason for the rearrangement and a written signed agreement from all authors. Modifications to the order of the authors are possible, but also need to be justified. Authors whose names are removed or inserted must agree with this in writing. Publication of the article cannot proceed without a declaration of authorship contributions signed by all authors.

São Paulo Medical Journal supports the ORCID initiative. All authors should create an ORCID identification (ID) record (in www.orcid.org) before submitting their article and should link the submission to their existing ORCID ID in the electronic submission system. ORCID identifications help to distinguish researchers with similar names, give credit to contributors and link authors to their professional affiliations. In addition, this may increase the ability of search engines to retrieve articles.

São Paulo Medical Journal supports Open Science practices. Authors must therefore complete an open science compliance form, which is available from: https://wp.scielo.org/wp-content/uploads/Open-Science-Compliance-Form_en.docx.

Redundant or duplicate publication

São Paulo Medical Journal will avoid publishing redundant or duplicate articles. The Journal agrees with the ICMJE definition of redundant publication,¹¹ i.e. an attempt to report or publish the same results from a study twice. This includes but is not limited to publication of patient cohort data that has already been published, without clear reference to the previous publication. In situations in which authors are making a secondary analysis on data that has already published elsewhere, they must state this clearly. Moreover, the outcomes assessed in each analysis should be clearly differentiated.

The Journal's peer review policy and procedures

After receipt of the article through the electronic submission system, it will be read by the editorial team, who will check whether the text complies with the Journal's Instructions for Authors regarding format. The Journal has adopted the *CrossRef Similarity Check* system for identifying plagiarism and any text that has been plagiarized, in whole or in part, will be promptly rejected. Self-plagiarism will also be monitored.

When the general format of the manuscript is deemed acceptable and fully compliant with these Instructions for Authors, and only then, the editorial team will submit the article to the Editor-in-Chief, who will firstly evaluate its scope. If the editor finds that the topic is of interest for publication, he will assign at least two reviewers/referees

with expertise in the theme, to evaluate the quality of the study. After a period varying from one to several weeks, the authors will then receive the reviewers' evaluations and will be required to provide all further information requested and the corrections that may be necessary for publication. These reviewers, as well as the Editorial Team and the Editor-in-Chief, may also deem the article to be unsuitable for publication by *São Paulo Medical Journal* at this point.

At the time of manuscript submission, the authors will be asked to indicate the names of three to five referees. All of them should be from outside the institution where the authors work and at least two should preferably be from outside Brazil. The Editor-in-Chief is free to choose them to review the paper or to rely on the *São Paulo Medical Journal's* Editorial Board alone.

Articles will be rejected without peer review if:

- they do not present Ethics Committee approval (or a justification for the absence of this);
- they fail to adhere to the format for text and figures described here.

After peer review

Peer reviewers, associated editors and the Editor-in-Chief may ask for clarifications or changes to be made to the manuscript. The authors should then send their article back to the Journal, with the modifications made as requested. Changes to the text should be highlighted (in a different color or using a text editor tool to track changes). Failure to show the changes clearly might result in the paper being returned to the authors.

The modified article must be accompanied by a letter answering the referees' comments, point by point. The modified article and the response letter are presented to the editorial team and reviewers, who will verify whether the problems have been resolved adequately. The text and the reviewers' final evaluations, along with the response letter, will then be sent to the Editor-in-Chief for a decision.

Manuscripts that are found to be suitable for publication through their scientific merit will be considered "provisionally accepted". However, all articles will subsequently be scrutinized to check for any problems regarding the reporting, i.e. sentence construction, spelling, grammar, numerical/statistical problems, bibliographical references and other matters that may arise, especially in the Methods section. The adherence to reporting guidelines will be checked at this point, and the staff will point out any information regarding methodology or results that the authors should provide. This is done in order to ensure transparency and integrity of publication, and to allow reproducibility.

The editorial team will then provide page proofs for the authors to review and approve. No article is published without this final author approval. All authors should review the proof, although the Journal asks the corresponding author to give final approval.

Submission

Articles should be submitted only after they have been formatted as described below. Texts must be submitted exclusively through the Internet, using the Journal's electronic submission system, which

is available at <http://mc04.manuscriptcentral.com/spmj-scielo>. Submissions sent by e-mail or through the post will not be accepted.

The manuscript should be divided into two files. The first of these, the main document (“blinded”), should contain the article title, article type, keywords and abstract, article text, references and tables, but must omit all information about the authors. The second of these, the “title page”, should contain all the information about the authors.

To format these documents, use Times New Roman font, font size 12, line spacing 1.5, justified text and numbered pages.

The corresponding author is responsible for the submission. However, all authors should approve the final version of the manuscript that is to be submitted and should be aware of and approve any changes that might be made after peer review.

Covering letter

All manuscripts must be submitted with a covering letter signed at least by the corresponding author. The letter must contain the following five essential items relating to the manuscript:

1. a declaration that the manuscript is original and that the text is not under consideration by any other journal;
2. a statement that the manuscript has been approved by all authors, who agree to cede the copyrights to the Journal, disclose all sources of funding and declare all potential conflicts of interest;
3. a statement that the study protocol was endorsed by an Internal Review Board (Ethics Committee), including the date and number of the approval (in the case of original articles). This is required for absolutely all studies involving human subjects or patient data (such as medical records), in accordance with the Committee on Publication Ethics (COPE) guidelines, and even for case reports. A copy of the approval document must be submitted to the Journal;
4. each author should indicate a valid, up-to-date email address for contact;
5. a list of a minimum of five potential referees outside of the authors' institutions, who could be invited, at the Editor-in-Chief's discretion, to evaluate the manuscript.

General guidelines for original articles

The following are considered to be full-text original articles: clinical trials; cohort, case-control, prevalence, incidence, accuracy and cost-effectiveness studies; case series (i.e. case reports on more than three patients analyzed together); and systematic reviews with or without meta-analysis. These types of article should be written with a maximum of 3,500 words (from the introduction to the end of the conclusion).

Typical main headings in the text include Introduction, Methods, Results, Discussion and Conclusion. The authors can and should use short subheadings too, especially those concerning the reporting guideline items.

Trial and systematic review registration policy

São Paulo Medical Journal supports the clinical trial registration policies of the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE) and recognizes the importance of these initiatives for registration and international dissemination of information on randomized clinical trials, with open access. Thus, since 2008, manuscripts on clinical trials are accepted for publication if they have received an identification number from one of the public clinical trial registration database (such as ClinicalTrials.gov and/or REBEC and/or the World Health Organization; the options are stated at <http://www.icmje.org>). The identification number should be declared at the end of the abstract. Articles describing systematic reviews must provide the protocol registration number from a reliable database, such as PROSPERO, Open Science Framework, Cochrane, Joanna Briggs and others. Articles presenting clinical trials or systematic reviews without registration protocols will be promptly rejected without peer review.

Results from cases with DNA sequences must be deposited in appropriate public databases. The protocol number or URL can be requested at any time during the editorial review. Publication of other research data in public repositories is also recommended, since it contributes towards replicability of research, increases article visibility and possibly improves access to health information.

Sample size

All studies published in SPMJ must present a description of how the sample size was arrived at. If it was a convenience or purposive sample, the authors must declare so and explain the characteristics of this sample and recruitment method. For clinical trials, for instance, it is mandatory to inform each of the three main values used to calculate sample size:

- power (usually 80% or more);
- level of significance (usually 0.05 or lower);
- clinically meaningful difference (effect size targeted), according to the main outcome measurement.

Regardless of study results (if “positive” or “negative”), the journal will probably reject articles of trials using underpowered samples, when sample size has not been properly calculated or the calculation has not been fully described as indicated above.

Abbreviations, acronyms and products

Abbreviations and acronyms must not be used, even those in everyday use, unless they are defined when first used in the text. However, authors should avoid them for clarity whenever possible. Drugs or medications must be referred to using their generic names (without capital letters), with avoidance of casual mention of commercial or brand names.

Interventions

All drugs, including anesthetics, should be followed by the dosage and posology used.

Any product cited in the Methods section, such as diagnostic or therapeutic equipment, tests, reagents, instruments, utensils, prostheses, orthoses and intraoperative devices, must be described together with the manufacturer's name and place (city and country) of manufacture in parentheses. The version of the software used should be mentioned.

Any other interventions, such as exercises, psychological assessments or educational sessions, should be described in enough details to allow reproducibility. The Journal recommends that the TIDieR reporting guidelines should be used to describe interventions, both in clinical trials and in observational studies.¹³

Supplementary material

Because supplementary material comprises documents that do not form part of the text of the manuscript, *São Paulo Medical Journal* will not publish it. The authors should cite an access link that allows readers to view the supplementary material.

Short communications

Short communications are reports on the results from ongoing studies or studies that have recently been concluded for which urgent publication is important. They should be structured in the same way as original articles. The authors of this kind of communication should explain, in the covering letter, why they believe that publication is urgent. Short communications and case reports must be limited to 1,000 words (from the introduction to the end of the conclusion).

Case reports, case series, narrative reviews and letters to the editor

Starting in June 2018, only individual case reports dealing with situations of public health emergencies will be accepted by *São Paulo Medical Journal*. Case reports that had already been accepted for publication up to May 2018 will still be published in a timely manner.

After initial evaluation of scope by the editor-in-chief, case reports, case series and narrative reviews will be considered for peer-review evaluation only when accompanied by a systematic search of the literature, in which relevant studies found (based on their level of evidence) are presented and discussed.¹² The search strategy for each database and the number of articles obtained from each database should be shown in a table. This is mandatory for all case reports, case series and narrative reviews submitted for publication. Failure to provide the search description will lead to rejection before peer review.

The access route to the electronic databases used should be stated (for example, PubMed, OVID, Elsevier or Bireme). For the search strategies, MeSH terms must be used for Medline, LILACS, and Cochrane Library. DeCS terms must be used for LILACS.

EMTREE terms must be used for Embase. Also, for LILACS, the search strategy must be conducted using English (MeSH), Spanish (DeCS) and Portuguese (DeCS) terms concomitantly. The search strategies must be presented exactly as they were used during the search, including parentheses, quotation marks and Boolean operators (AND, OR, and NOT). The search dates should be indicated in the text or in the table.

Patients have the right to privacy. Submission of case reports and case series must contain a declaration that all patients gave their consent to have their cases reported (even for patients cared for in public institutions), in text and images (photographs or imaging examination reproductions). The Journal will take care to cover any anatomical part or examination section that might allow patient identification. For deceased patients whose relatives cannot be contacted, the authors should consult the Editor-in-Chief. All case reports and case series must be evaluated and approved by an ethics committee.

Case reports should be reported in accordance with the CARE Statement,⁷ including a timeline of interventions. They should be structured in the same way as original articles.

Case reports must not be submitted as letters. Letters to the editor address articles that have been published in the *São Paulo Medical Journal* or may deal with health issues of interest. In the category of letters to the editor, the text has a free format, but must not exceed 500 words and five references.

FORMAT: FOR ALL TYPES OF ARTICLES

Title page

The title page must contain the following items:

1. Type of paper (original article, review or updating article, short communication or letter to the editor);
2. Title of the paper in English, which should be brief but informative, and should mention the study design.¹⁴ Clinical trial, cohort, cross-sectional or case-control study, and systematic review are the most common study designs. Note: the study design declared in the title should be the same in the methods and in the abstract;
3. Full name of each author. The editorial policy of the *São Paulo Medical Journal* is that abbreviations of authors' names must not be used; therefore, we ask that names be stated in full, without using abbreviations;
4. Place or institution where the work was developed, city and country;
5. Each author should indicate the way his/her name should be used in indexing. For example: for "João Costa Andrade", the indexed name could be "Costa-Andrade J." or "Andrade JC", as preferred;
6. The author's professional background (Physician, Pharmacist, Nurse, Dietitian or another professional description, or Undergraduate Student); and his/her position currently held (for

- example, Master's or Doctoral Student, Assistant Professor, Associate Professor or Professor), in the department and institution where he/she works, and the city and country (affiliations);
7. Each author should present his/her ORCID identification number (as obtained from HYPERLINK "<http://www.orcid.org/>" www.orcid.org/);
 8. Each author must inform his contribution, preferably following the CRediT system (see above in Authorship);
 9. Date and venue of the event at which the paper was presented, if applicable, such as congresses, seminars or dissertation or thesis presentations.
 10. Sources of financial support for the study, bursaries or funding for purchasing or donation of equipment or drugs. The protocol number for the funding must be presented with the name of the issuing institution. For Brazilian authors, all grants that can be considered to be related to production of the study must be declared, such as fellowships for undergraduate, master's and doctoral students; along with possible support for post-graduate programs (such as CAPES) and for the authors individually, such as awards for established investigators (productivity; CNPq), accompanied by the respective grant numbers.
 11. Description of any conflicts of interest held by the authors (see above).
 12. Complete postal address, e-mail address and telephone number of the author to be contacted about the publication process in the Journal (the "corresponding author"). This author should also indicate a postal address, e-mail address and telephone number that can be published together with the article. *São Paulo Medical Journal* recommends that an office address (rather than a residential address) should be informed for publication.

Second page: abstract and keywords

The second page must include the title and a structured abstract in English with a maximum of 250 words. References must not be cited in the abstract.

The following headings must be used in the structured abstract:

- Background – Describe the context and rationale for the study;
- Objectives - Describe the study aims. These aims need to be concordant with the study objectives in the main text of the article, and with the conclusions;
- Design and setting – Declare the study design correctly, and the setting (type of institution or center and geographical location);
- Methods – Describe the methods briefly. It is not necessary to give all the details on statistics in the abstract;
- Results – Report the primary results;
- Conclusions – Make a succinct statement about data interpretation, answering the research question presented previously. Check that this is concordant with the conclusions in the main text of the article;
- Clinical Trial or Systematic Review Registration – Mandatory for clinical trials and systematic reviews; optional for observational

studies. List the URL, as well as the Unique Identifier, on the publicly accessible website on which the trial is registered.

- MeSH Terms - Three to five keywords in English must be chosen from the Medical Subject Headings (MeSH) list of Index Medicus, which is available at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=mesh>. These terms will help librarians to quickly index the article.
- Author keywords - The authors should also add three to six "author keywords" that they think express the main article themes. These keywords should be different from the MeSH terms and preferably different from words already used in the title and abstract, so as to improve the discoverability of the article by readers doing a search in PubMed. They provide an additional chance for the article to be retrieved, read and cited. Combinations of words and variations (different wording or plurals, for example) are encouraged.

References

For any manuscript, all statements in the text that do not result from the study presented for publication in the *São Paulo Medical Journal* but from other studies must be accompanied by a quotation of the source of the data. All statements regarding health statistics and epidemiological data should generally be followed by references to the sources that generated this information, even if the data are only available electronically.

São Paulo Medical Journal uses the reference style known as the "Vancouver style," as recommended by the International Committee of Medical Journal Editors (ICMJE). Follow the instructions and examples at www.icmje.org, item "References", for the format.

In the text, the references must be numbered in the order of citation. The citation numbers must be inserted after periods/full stops or commas in sentences, and in superscript (without parentheses or square brackets). References cited in the legends of tables and figures must maintain sequence with the references mentioned in the text.

In the list of references, all the authors must be listed if there are up to and including five authors; if there are six or more, the first three should be cited, followed by the expression "et al." For books, the city of publication and the name of the publishing house are mandatory. For texts published on the internet, the complete uniform resource locator (URL) or address is necessary (not only the main home page of a website or link), so that by copying the complete address into a computer internet browser, the Journal's readers will be taken to the exact document cited, and not to a general website.

At the end of each reference, please insert the "PMID" number (for papers indexed in PubMed) and the link to the "DOI" number if available.

Authors are responsible for providing a complete and accurate list of references. All references cited in the text must appear in the reference list, and every item in the reference list must be cited in the text. Also, citations must be in the correct sequence.

Manuscripts that do not follow these guidelines for references will be returned to the authors for adjustments.

The reference list should be inserted after the conclusions and before the tables and figures.

Figures and tables

Images must be submitted at a minimum size that is reproducible in the printed edition. Figures should be sent at a resolution of 300 DPI and minimum size of 2,500 pixels (width) and be recorded in ".jpg" or ".tif" format. Images submitted in inadequate formats will not be accepted.

Images must not be embedded inside Microsoft PowerPoint or Microsoft Word documents, because this reduces the image size. Authors must send the images separately, outside of .doc or .ppt documents. Failure to send the original images at appropriate sizes leads to paper rejection before peer review.

Flowcharts are an exception: these must be drawn in an editable document (such as Microsoft Word or PowerPoint), and should not be sent as an image that can't be changed.

Figures such as bars of line graphs should be accompanied by the tables of data from which they have been generated (for example, sending them in the Microsoft Excel spreadsheets, and not as image files). This allows the Journal to correct legends and titles if necessary, and to format the graphs according to the Journal's style. Graphs generated from software such as SPSS or RevMan must be generated at the appropriate size, so that they can be printed (see above). Authors must provide internal legends/captions in correct English.

All the figures and tables should be cited in the text. All figures and tables must contain legends or titles that precisely describe their content and the context or sample from which the information was obtained (i.e. what the results presented are and what the kind of sample or setting was). The reader should be able to understand the content of the figures and tables simply by reading the titles (without the need to consult the text), i.e. titles should be complete. Acronyms or abbreviations in figure and table titles are not acceptable. If it is necessary to use acronyms or abbreviations inside a table or figure (for better formatting), they must be spelled out in a legend below the table or figure.

For figures relating to microscopic findings (i.e. histopathological results), a scale must be embedded in the image to indicate the magnification used (just like in a map scale). The staining agents (in histology or immunohistochemistry evaluations) should be specified in the figure legend.

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Realize uma pesquisa única e obtenha resultados das principais bases de dados de saúde.



Suporte Personalizado:

Encontre assistência especializada, agendando atendimentos para dúvidas ou pesquisas específicas.



Acesso às Informações Mais Recentes:

Mantenha-se atualizado com as últimas pesquisas para uma prática médica ainda mais assertiva.

Escaneie para
acessar agora



10% de desconto¹ no plano SulAmérica Saúde para médicos associados à APM.

130 ANOS
SulAmérica



Benefícios



Cuidado 360°: oferta promocional com Saúde + Odonto e Vida sem acréscimo na mensalidade²



Opções de planos com reembolso⁴



Faça uma cotação



(11) 3188-4200

¹ Condição válida apenas para novos clientes, com CNPJ de 3 a 29 vidas. O desconto é aplicado automaticamente na contratação do plano de saúde da operadora SulAmérica.

² Condição promocional válida apenas por 12 ou 24 meses de vigência, conforme vigência do contrato do plano de saúde, podendo ser prorrogado a critério da SulAmérica. Apenas novos proponentes serão elegíveis a esta promoção. O proponente poderá optar por aderir apenas ao Seguro Saúde, a seu exclusivo critério. Este material contém informações resumidas que poderão sofrer alterações sem prévio aviso. As coberturas e a rede referenciada variam de acordo com o Plano contratado. O valor do prêmio do Seguro Odontológico será informado no momento da contratação, e este valor será subsidiado pela SulAmérica, durante o período da campanha. Os Seguros de Vida da SulAmérica estão devidamente registrados na SUSEP. As coberturas do Seguro de Vida, que são válidas durante o período de vigência da apólice, possuem os seguintes números de processos na SUSEP: Cobertura de Morte – 15414.003475/2006-67 e Invalidez Permanente Total ou Parcial por Acidente – 15414.003485/2006-01. Os registros na SUSEP não implicam, por parte da autarquia, incentivo ou recomendação à sua comercialização. Os serviços de assistências não constituem coberturas de seguro e, portanto, não garantem indenização ou reembolso de quaisquer despesas efetuadas. Os Seguros de Vida da SulAmérica, farão parte do contrato de Seguro Saúde, como um benefício adicional. O Seguro Saúde, com um benefício adicional de Seguro de Vida, e o Seguro Odontológico, serão oferecidos pela SulAmérica Companhia de Seguro Saúde, CNPJ/MF nº 01.685.053/0001-56. SulAmérica Serviços de Saúde S.A., CNPJ/MF nº 02.866.602/0001-51 - Registro ANS 416428. O Seguro Saúde, com um benefício adicional de Seguro de Vida, obedece às Condições Gerais, que devem ser lidas previamente à sua contratação, conforme link: <https://portal.sulamericaseguros.com.br/para-empresa/saude/>. O Seguro Odontológico obedece às Condições Gerais, que devem ser lidas previamente à sua contratação, conforme link: <https://portal.sulamericaseguros.com.br/para-empresa/odonto/>. A apólice do Seguro de Vida será emitida pela SULAMÉRICA SEGUROS DE PESSOAS E PREVIDÊNCIA S.A., CNPJ: 01.704.513/0001-46 com registro na SUSEP nº 6220, e obedece suas Condições Gerais, que devem ser lidas previamente à sua contratação, conforme link: <https://portal.sulamericaseguros.com.br/para-voce/vida/segundo-menu-servicos-gerais/consulta-de-condicoes-gerais/>. SAC 0800 722 0504 e Ouvidoria 0800-725-3374, horário atendimento: segunda a sexta, das 8h30 às 17h30.

³ Verifique com o consultor as opções de planos com reembolso. Planos de saúde empresarial, conforme as regras da ANS. Informações resumidas.

A comercialização dos planos respeita a área de abrangência dos produtos e das respectivas operadoras. A disponibilidade dos produtos pode variar de acordo com a região e a entidade de classe com a qual os proponentes mantêm o vínculo.

Os planos podem ser coparticipados. Para conhecer a tabela completa de procedimentos e valores de coparticipação, consulte a operadora.

Todas as informações referentes aos planos, incluindo condições, preços, rede de prestadores e sua abrangência geográfica, são de responsabilidade exclusiva das respectivas operadoras de saúde e podem estar sujeitas a alterações por parte delas, mesmo após a contratação do plano, respeitadas as disposições contratuais e legais (Lei nº 9.656/98). Janeiro.