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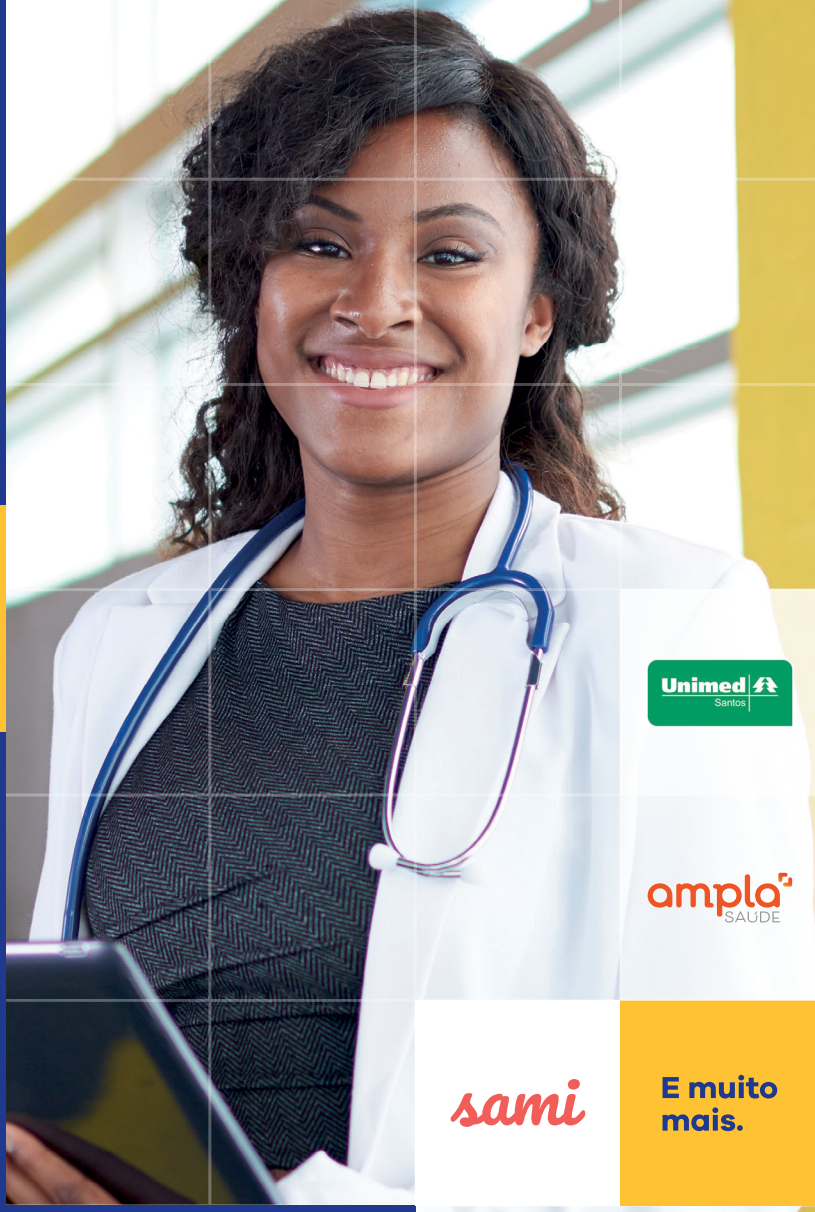

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Medical Residency – where we are and future challenges

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The concept of medical residency has its roots in ancient medicinal practices, such as in Greece, where teachings are transmitted by experienced professionals through practical observation. This informal system persisted until the emergence of universities in the Middle Ages, when practical and theoretical training was introduced.[1,2] Notably, the University of Bologna in 1088 and the University of Paris in 1150.

In the mid-19th century, Johns Hopkins Hospital became the cornerstone medical residency program. Notable pioneers in this program, such as William Halsted (1852–1922) and William Osler (1849–1919), emphasized a humanistic approach with direct patient observation and mentorship. In this innovative teaching model, residents began living in the hospital, hence the term "residency." The model evolved with emphasis on competency-based education, interdisciplinary collaboration, and technological advances.[3]

Medical residency represents a fundamental stage of a physician's education, consolidating the theoretical learning acquired in medical schools and promoting the development of practical, ethical, and human competencies essential to medical practice. It is an intensive educational process based on the principle of in-service education, in which the resident participates in real-world clinical and hospital environments, with qualified supervision. Confronted with real challenges in healthcare, this experience not only provides technical depth in the specialties, but also sharpens clinical reasoning, responsible decision-making, and appreciation of teamwork.

Residents are an essential part of many healthcare teams. In teaching hospitals, they participate in strategic roles, especially in areas of high demand, human resource shortages, and regions of greater social vulnerability. Therefore, investing in well-structured residency programs is an important health indicator.

Initial Challenges for the Dissemination of Medical Residency

In Brazil, medical residency was first institutionalized at the Hospital das Clínicas of the Faculty of Medicine of the Universidade de São Paulo in 1944 under the leadership of Professor Alfredo Balabram. The initial medical residency provided structured practical postgraduate training programs for newly graduated doctors. This initiative expanded into other institutions, such as the Hospital dos Servidores do Estado of Rio de Janeiro in 1948.[4]

However, the official recognition of medical residency in Brazil occurred only in 1977, with the creation of the National Medical Residency Commission (CNRM), through Decree nº 80.281.[4] Since then, medical residency has been the formal training pathway for medical specialization, governed by technical and ethical criteria initially established by the Ministry of Education and later by the Ministry of Health.[5]

This model, although strongly influenced by the North American experience, has progressively incorporated the needs of the Unified Health System (SUS – *Sistema Único de Saúde*), prioritizing the training of professionals committed to our social reality. Medical residency is a consolidated model of specialized training. However, its implementation faced several obstacles, locally and internationally. The first challenge was the perceived need for medical training beyond graduation, which required cultural paradigm shifts, and institutional and legal changes. In the United States, the adoption of this teaching model was initially seen as an unnecessary

extension of professional training. The consolidation of residency only gained strength with the growing need for specialist doctors after the war and with the creation of the Accreditation Council for Graduate Medical Education (ACGME) in 1981, which brought in quality assurance criteria and supervision requirements. This body is currently responsible for accrediting all medical training programs in the United States.

In Europe, the scenario was more heterogeneous as each country developed a different model according to its own healthcare system. A common challenge is the integration of residency into already-established hospital systems, which were often geared toward theoretical education. In the United Kingdom, the implementation of the residency model encountered substantial barriers due to the rigidity of the university system and the need to reconcile practical training with the National Health Service. Even after the creation of the European Union, diversity in medical residency models persisted.

The challenges in Brazil were no different. Among the initial obstacles were the scarcity of hospitals with adequate training capacity, the lack of qualified supervisors, and the need to align with the SUS. The advancement of medical residency required recognition of the complexity of modern medicine, which necessitated specialized training and supervised environments. The challenges pointed to a constant need for articulation between public policies, training institutions, and professionals committed to quality healthcare.

Currently, in Brazil, contrasting challenges are arising with the uncontrolled growth of medical schools, mostly private schools that lack the capacity to create and maintain their own teaching hospitals. Public hospitals often welcome teaching practices as they represent a way to sustain organizational structures and expand healthcare services. Private institutions, on the other hand, often aim to avoid the necessary infrastructural investment, which may initially seem advantageous. However, this lack soon revealed the fragility of these institutions due to a lack of long-term commitment. It is common to find students from different institutions training within the same hospital, compromising the creation of an organized teaching system.

It is essential that the state exercises a more effective regulatory role through the Ministry of Education and the National Medical Residency Commission. The accreditation of new programs should be conditioned on the existence of their own teaching infrastructures, with hospitals or health centers properly equipped, a minimum number of preceptors, and articulation with the local health network. Finally, it is essential that public universities, university hospitals, and medical entities take a clear and unified stance on this. Medical education is a complex process that requires time, structural resources, and institutional commitment to teaching, research, and care.

Adoption of Affirmative Actions

The adoption of affirmative action based on racial and socioeconomic quotas in higher education aims to correct historical inequalities in access to professional training. Discussions on the topic are controversial and require multifaceted political action. The term 'affirmative action' emerged in the United States in the 1960s, aiming to reduce disparities between Whites and Blacks through government policies. The "National Resident Matching Program" encourages institutions to adopt holistic criteria for evaluating candidates, going beyond grades and scientific publications. However, in 2023, the U.S. Supreme Court banned the use of racial criteria for university admissions in favor of meritocracy, although many institutions remained committed to diversity as a strategy to reduce health disparities.[6]

In Europe, the scenario is more conservative, and most countries prioritize academic and meritocratic criteria. However, countries such as the United Kingdom have invested in inclusion and support programs for students from vulnerable backgrounds, focusing on the equity of opportunities rather than quota reservations. The discussion of racial quotas still faces resistance, partly because of different historical and demographic contexts.

In Brazil, the application of racial and social quotas for undergraduate admissions has advanced, with different public institutions adopting this policy to reduce social disparities. The introduction of this policy in medical residency is much more complex and has sparked a greater debate. Opponents point out that this strategy has already been implemented at the undergraduate level, and duplication is not justified. Some public institutions have begun implementing policies to reserve a percentage of residency places for Black, Brown, and Indigenous individuals and those from public schools. The State sees these policies as positively impacting program diversity. However, the challengesincluding peer acceptance and the isolated adoption of quotas have not shown the expected effects, making the implementation of robust public policies from early educational stages necessary.

Challenges in the Selection Process for Medical Residents

In the United States, the "National Resident Matching Program" is a highly competitive selection process, with a demand greater than the number of available positions. In Europe, processes vary between countries, and the lack of standardization is a challenge for professional mobility among EU member states.

In Brazil, the selection process is decentralized, with health institutions and universities applying their own theoretical and practical examinations, leading to multiple applications and travel demands on the candidates. Major institutions are highly competitive because of the disproportion between the available places and demand. Among other factors, the demand for positions in large urban centers discourages the placement of doctors in underserved

areas, requiring additional measures. The growing demand for medical specialists requires that selection processes be not only efficient, but also fair and aligned with health system needs.

The assessment process must examine not only technical knowledge but also ethical decision-making and emotional behavior in high-complexity environments. However, one of the great challenges is identifying whether the resident has the right profile for the specialty, including resilience and empathy with patients, which are traits that are not always measurable through objective evaluations. During residency, programs should combine continuous and multimodal assessment methods, including self-assessment, peer evaluation, and structured direct observations. Strengthening the feedback culture and training preceptors for formative assessments are also essential strategies for addressing these challenges and ensuring excellent medical training.

Among various assessment tools, the Mini-Clinical Evaluation Exercise (Mini-CEX) stands out because it allows for structured evaluations of clinical performance in real time, followed by immediate feedback. This instrument is effective for assessing different aspects by observation, including patient interaction, communication skills, physical examination skills, and diagnostic formulations.

Future Challenges of Medical Residency

According to the 2023 Medical Demography Report in Brazil, 4,951 accredited residency programs are offered by 789 institutions accredited by the Ministry of Education, covering 55 medical specialties and 59 areas of practice recognized by the Joint Specialties Commission.[7]

In recent years, the number of medical residency programs has increased in an attempt to keep pace with the unregulated expansion of medical schools. Of note, approximately 60% of these programs are funded by federal, state, and municipal governments.

One of the main federal government initiatives is the National Program to Support the Training of Specialist Doctors in Strategic Areas (Pro-Residency), which supports the training of specialists in areas recognized as priority regions by the Unified Health System.

In the coming years, great challenges await. Considering that medical residency represents the most decisive stage in the training of specialist doctors. The expansion of training centers, appreciation of preceptorship, and alignment with the real needs of the Unified Health System represent important determinants for guiding future goals.

Another relevant challenge is the incorporation of new competencies required in today's context. For example, the management of chronic diseases, palliative care, mental health, technology in medicine, and effective communication with patients and teams. These skills remain underexplored in medical education research.

Given these challenges, it is necessary to reaffirm the commitment to medical residency that trains specialists who are technically competent, ethically committed, and whose responsibilities align with real-world needs. The quality of today's medical education defines the quality of tomorrow's healthcare.

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Association between soft drink consumption and cardiovascular disease risk among Brazilian adults: a cross-sectional study

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Cardiovascular risk estimation.

ABSTRACT

BACKGROUND: Inadequate diet is considered a major risk factor for chronic noncommunicable diseases and mortality. Among the ultra-processed foods, sweetened soft drinks are significant contributors to high-calorie diets and are associated with adverse health outcomes.

OBJECTIVE: To estimate the association between soft drink consumption and the risk of cardiovascular events.

DESIGN AND SETTING: A cross-sectional study was conducted using data of adults aged ≥ 18 years from the 2013 National Health Survey.

METHODS: The explanatory variable was the daily consumption of sugar-sweetened soft drinks. Cardiovascular risk (CVR) was calculated using the Framingham score. Multinomial logistic regression was used for the analyses. Two models were used: one adjusted for age and body mass index and the other for age and waist circumference. Both models were applied to the general population and stratified by race and educational attainment.

RESULTS: The study sample consisted of 8,391 participants. Individuals with sugary soda consumption ≥ 0.4 cups/day were associated with a higher CVR, which escalated with increasing consumption of soft drinks.

CONCLUSION: CVR was observed across all consumption categories and difference in risk was based on the intake quantity.

INTRODUCTION

The concept of diet quality reflects an evaluation of various food types, nutrients, and dietary constituents in relation to established dietary recommendations and/or health outcomes, which can be verified through diet quality indices.[1] Inadequate diet is considered a major risk factor for chronic noncommunicable diseases and mortality.[2] Among foods that undergo a high degree of industrial processing, ultra-processed foods are considered the most harmful to health and their consumption is associated with an increased incidence of diseases.[3]

Sweetened soft drinks, classified as ultra-processed foods, account for a large proportion of high-calorie diets, which are associated with excessive weight gain.[4]

These beverages are consumed by a considerable proportion of the Brazilian population.[5] Consumption patterns differ among sociodemographic, regional, and economic variables of the population.[5,6]

Evidence that increased soft drink consumption is associated with adverse health outcomes is not recent.[7] These beverages are typically sweetened with corn syrup or sucrose; a component of these sugars is the monosaccharide fructose which, when consumed frequently, results in increased visceral and intramuscular fat deposition,[8] elevated levels of uric acid,[9] insulin resistance, and dyslipidemia.[10]

Ultra-processed and sugar-sweetened beverages such as soft drinks increase the risk of obesity, type 2 diabetes, hypertension, and all-cause mortality.[11] They are also associated with a higher incidence of metabolic syndrome and its components.[12] Results from a cohort of Mexicans showed that participants in the highest quintile of refined food consumption, which included soft drink consumption, had a 10% higher cardiovascular risk (CVR) over 10 years than those in the lowest quintile of consumption.[13] A meta-analysis of prospective studies revealed that soft drink intake

was associated with a dose-dependent increase in the risk of acute myocardial infarction and stroke.[14]

Previous analyses have been reported an association between soft drink consumption and CVR factors, such as hypertension and obesity, in the Brazilian population.[12,15,16] However, to our knowledge, no previous studies have analyzed the association between the consumption of these beverages and the risk of cardiovascular events in adults using a representative sample of the Brazilian population. This knowledge is essential for the strengthening prevention and health promotion measures, encouraging healthy eating patterns, and guiding public policies aimed at controlling soft drink consumption, with a focus on reducing future cardiovascular events in the Brazilian population.

OBJECTIVE

This study aimed to estimate the association between soft drink consumption and the risk of cardiovascular events. We conducted a national survey of a representative sample of the Brazilian population. Thus, we tested the hypothesis of an association between an increased consumption of sugar-sweetened beverages and an elevated cardiovascular disease risk.

METHODS

Design and study population

This cross-sectional study was based on data from the 2013 National Health Survey (NHS), with adults aged ≥ 18 years. Data from laboratory tests collected in 2014 and 2015 were used and the two databases were correlated. The survey refers to the first laboratory edition of the NHS conducted in 2014 and 2015, which is the only Brazilian survey to collect laboratory tests from a representative sample of the national population, resulting in greater accuracy of the estimates.[17] The methodology of the NHS sampling process and the sub-sample for laboratory data collection is detailed in previous studies.[17-19]

The exclusion criteria for this study were the same as those adopted for the construction of the CVR estimates proposed by D'Agostino et al. These criteria excluded individuals aged < 30 years and > 74 years as well as individuals who reported being diagnosed with heart disease or stroke.[20]

After applying the exclusion criteria, we obtained a sample of 8,391 individuals.

Laboratory data collection

The collection of biological material was performed at the homes of the participants after they received an explanation of the procedure and signed the consent form for collection.[17] After blood collection, the samples were centrifuged and the resulting serum and plasma were stored in refrigerators at 4°C; the entire process was performed using calibrated equipment.[21]

The laboratory tests performed on the blood samples in this study included glycated hemoglobin, total cholesterol, and low- and high-density lipoprotein (LDL and HDL, respectively) levels.

Study variables

The dependent variable, CVR at 10 years, was constructed according to the Framingham score criteria.[20] The variables HDL, total cholesterol, treated-, and untreated- systolic blood pressure were considered, in numerical categories. Diabetes and smoking status were self-reported (yes/no). All the variables were adjusted for sex. Further details and cutoff points for each variable were specified as described by D'Agostino et al.[20]

The 10-year CVR categories were low CVR < 5%, medium CVR 5–20%, and high CVR ≥ 20%, based on the guidelines of the Brazilian Society of Cardiology.[22]

The explanatory variable in this study was the daily consumption of a standard portion (1 cup) of sugary drinks based on the following question: "*How many days a week do you usually drink soda (or artificial juice)*? The responses were categorized as follows: < 0.1 cup/day, 0.1–0.39 cups/day, 0.4–0.99 cups/day, and ≥ 1.0 cups/day.[9]

The sociodemographic variables used were sex (male, female), age (30–74 years), race/skin color grouped into either White or Other (black, yellow, brown, and indigenous), and educational level categorized as low (individuals without formal education to those who did not complete middle school) and high (individuals who completed middle school, did not complete high school, and completed high school).

Physical activity levels were recorded based on the following questions: *"How many days a week do you usually practice physical exercises or sports?"*; "*In general, on the day you usually practice exercise or sports, how many hours does this activity last?*"; and "*In general, on the day you usually practice exercise or sports, how many minutes does this activity last*?" Physical activity was defined as at least 150 min. of moderate activity per week or 75 min. of vigorous activity per week, during leisure time.[23]

The variable smoking (non-smoker, ex-smoker, and smoker) was created from the following questions: "*Do you currently smoke any tobacco products?*" and " *In the past, have you smoked any tobacco products daily?*"[18,24]

The variable alcohol consumption (no, light/moderate, and heavy drinking) was constructed based on the following questions: "*How often do you usually consume alcoholic beverages?*" and "*In the past 30 days, have you consumed 5 or more drinks on a single occasion?*[23] Habitual drinking (drinking in the past 30 days, regardless of dose) and heavy drinking (drinking five or more drinks for men and four or more drinks for women on a single occasion in the past 30 days) were considered.[18]

Regarding anthropometric variables, body mass index (BMI) was categorized as underweight (< 18.5 kg/m^2), eutrophic (18.5–25 kg/m^2), overweight (25–30 kg/m^2), and obese (≥ 30 kg/m^2). Altered waist circumference (WC) constituted "no" (< 88 cm in women and < 102 cm in men) and "yes" (≥ 88 cm in women and ≥ 102 cm in men).[24]

Hypertension was defined with a systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg or use of antihypertensive medications,[25,26] obtained in response to the question, "*In the past two weeks, have you taken medication for hypertension (high blood pressure)?*"[23] In all, three measurements were obtained at 2-min intervals; subsequently, the mean of the three readings was recorded as the definitive value for data analysis.[21]

Finally, laboratory variables included altered HDL cholesterol (≤ 40 mg/dL in men and ≤ 50 mg/dL in women, altered total cholesterol (≥ 200 mg/dL), altered LDL cholesterol (≥ 130 mg/dL),[24] and the diagnosis of diabetes when individuals presented HbA1c levels ≥ 6.5%.[27,28]

Data analysis

Initially, descriptive analysis was performed using the relative frequency of the data and the chi-square test for comparison of proportions according to sociodemographic variables, lifestyle and anthropometric and laboratory measurements.

Next, multivariate regression was performed using multinomial logistic regression to verify the association between the explanatory variables and the 10-year CVR variables in the three categories (low, medium, and high CVR). The association between sugar-sweetened beverage consumption and CVR was estimated using odds ratios (OR) and 95% confidence interval (95%CI). The reference category of the exposure variable used for comparison between analyses was soft drink consumption < 0.1 cup/day.

Two models were used: one adjusted for age and BMI, and the other for age and WC. The adjustment variables were selected based on the literature,[11,29-32] specifically BMI and WC, because the other variables suggested in the literature were already part of the proposed CVR score. The models were run for the general population and stratified by skin color/race (White and Other) and education (low/high) owing to differences in racial admixture and socioeconomic factors presented in the study population that differed from the population originally used for the Framingham score.

Data analyses were performed using the Stata 14.0 software (Stata Corp., College Station, Texas, United States) in the *survey* module, which included complex sample structure data for population estimates.

Ethical aspects

The NHS was approved by the National Research Ethics Committee of the Brazilian Ministry of Health in July 2013. Participation in the research was voluntary and information confidentiality was guaranteed. The research participants signed an informed consent form and authorized the collection of laboratory test results.[17,18,21]

RESULTS

Table 1 presents the distribution of clinical and sociodemographic profile variables according to soft drink consumption categories. The category with the highest consumption consisted predominantly of men (52.99%), individuals aged 30–39 years (40.11%), those with low educational levels (51.80%), and consumers of nonalcoholic beverage (70.83%). Additionally, in the high consumption category, there was a higher prevalence of nonsmokers (64.21%), individuals with normal weight (43.74%), normal WC (65.23%), normal lipid profile (total cholesterol, 71.43%; LDL, 84.16%), nondiabetics (94.00%), non-hypertensive individuals, and those with low CVR (65.39%).

Figure 1 shows the OR estimates for medium/high CVR. Soft drink consumption between 0.4–0.99 servings/day was associated with higher odds of high cardiovascular risk (CVR) scores in both the model adjusted for BMI (OR = 1.78; 95%CI = 1.27–2.50) and the model adjusted for WC (OR = 1.66; 95%CI = 1.18–2.34). Consumption of ≥ 1.0 serving/day was associated with higher odds of both medium (OR = 1.41; 95%CI = 1.07–1.86) and high CVR scores (OR = 1.97; 95%CI = 1.39–2.80) in the BMI-adjusted model. In the WC-adjusted model, this level of consumption was associated only with higher odds of high CVR scores (OR = 1.76; 95%CI = 1.23–2.52).

Table 2 presents the models stratified by skin color, race, and education. Overall, the significant association between soft drink consumption and CVR scores remained consistent in the 'Other' race/skin color category, as well as in both high- and low-education strata. These models were adjusted for age BMI and WC.

Figure 2 presents the conditional probabilities of low, medium, and high CVR scores according to the level of consumption of soft drink servings. As consumption increased, the probability of a low CVR score decreased, while the probability of a high CVR score increased.

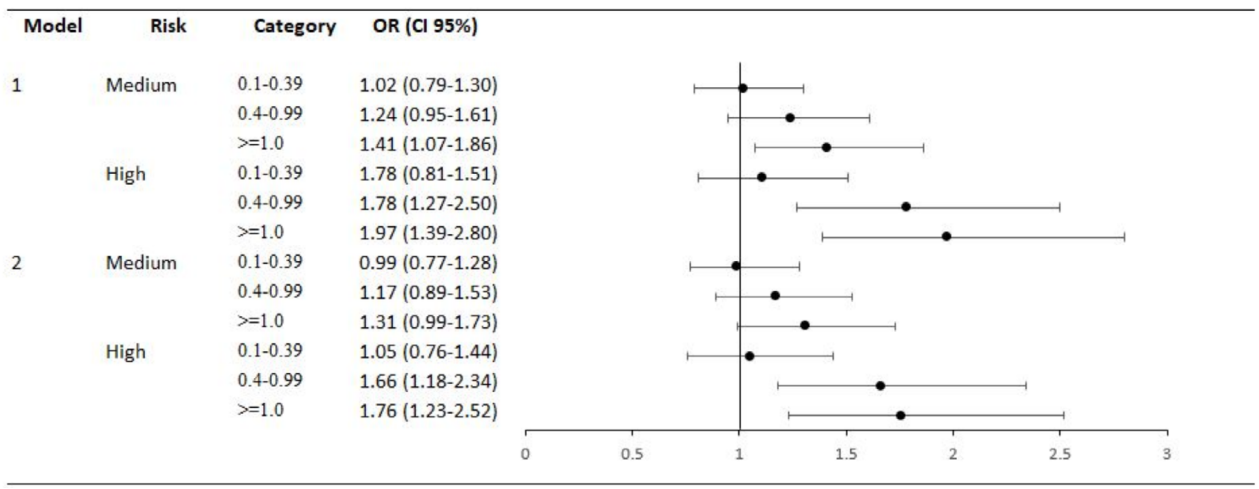
DISCUSSION

Our data showed that consumption of sugar-sweetened soft drinks may increase the CVR scores in 10 years, with variations according to the quantity consumed, adjusted by age and anthropometric measures (BMI and WC), which are measures of adiposity highly associated with metabolic outcomes and chronic inflammation.[29] The analyses also showed significance when stratified by race/color and educational level. The results presented here indicate that the daily consumption of one or more standard servings of soft drinks per day was independently and

Table 1. Prevalences of the population by clinical and sociodemographic characteristics of the Brazilian adults in relation to consumption of sweetened soft drinks. National Health Survey, 2013.

Variables	Soft drink consumption (cups/day)				P
	< 0.1	0.1–0.39	0.4–0.99	≥ 1.0	
	% (95% CI)	% (95%CI)	% (95%CI)	% (95%CI)	
Sex					
Male	38.44 (36.03–40.91)	43.52 (40.77–46.31)	58.56 (55.45–61.59)	52.99 (49.88–56.09)	< 0.001
Female	61.56 (59.09–63.97)	56.48 (53.69–59.23)	41.44 (38.41–44.55)	47.01 (43.91–50.12)	
Age (years)					
30–39	19.11 (17.04–21.37)	29.10 (26.03–32.06)	35.43 (31.94–39.07)	40.11 (36.45–43.89)	< 0.001
40–49	26.27 (23–87–28.82)	26.86 (24.23–29.65)	28.32 (25.15–31.73)	29.12 (25.86–32.60)	
50–59	26.18 (23.87–28.63)	23.78(21.31–26.44)	23.27 (20.14–26.72)	19.81 (17.00–22.96)	
60–74	28.44 (26.16–30.83)	20.26 (17.98–22.75)	12.98 (10.82–15.50)	10.96 (9.03–13.23)	
Color					
White	50.42 (47.96–52.88)	47.19 (44.43–49.98)	45.72 (42.44–49.03)	48.55 (45.41–51.70)	0.2709
Black	8.40 (07.15–09.85)	9.50 (8.01–11.23)	10.57 (08.69–12.80)	9.26 (7.52–11.35)	
Yellow	0.98 (0.510–1.88)	0.38 (0.21–0.69)	0.66 (0.39–01.12)	0.49 (0.23–1.01)	
Brown	39.89 (37.59–42.24)	42.58 (39.94–45.27)	42.78 (39.68–45.93)	41.38 (38.41–44.41)	
Indigenous	0.30 (0.17–0.54)	0.34 (0.15–0.79)	0.27 (0.13–0.56)	0.33 (0.18–0.61)	
Level of education					
Low	60.34 (57.82–62.82)	54.27 (51.47–57.04)	48.93 (45.70–52.17)	51.80 (48.66–54.93)	< 0.001
High	39.66 (37.18–42.18)	45.73 (42.96–48.53)	51.07 (47.83–54.30)	48.20 (45.07–51.34)	
Physical activity					
Yes	21.14 (19.12–23.32)	22.03 (19.80–24.43)	22.94 (20.15–26.00)	21.83 (19.26–24.65)	0.7973
No	78.86 (76.68–80.88)	77.97 (75.57–80.20)	77.06 (74.00–79.85)	78.17 (75.35–80.74)	
Consumption of alcoholic beverages					
Non-drinker	80.40 (78.31–82.34)	79.36 (76.90–81.61)	68.12 (64.88–71.20)	70.83 (67.87–73.63)	< 0.001
Light/Moderate	14.49 (12.76–16.41)	16.91 (14.82–19.23)	23.93 (21.13–26.97)	19.24 (16.89–21.83)	
Abusive	5.10 (4.15–6.26)	3.74 (2.80-4.98)	7.95 (6.25–10.06)	9.93 (8.12–12.08)	
Smoking					
Non-smoker	65.15 (62.81–67.42)	70.12 (67.54–72.57)	70.13 (67.09–73.01)	64.21 (61.15–67.16)	0.0005
Ex-smoker	19.58 (17.77–21.51)	17.93 (15.94–20.10)	15.45 (13.40–17.74)	17.93 (15.77–20.31)	
Smoker	15.27 (13.60–17.11)	11.96 (10.25–13.90)	14.42 (12.16–17.02)	17.86 (15.48–20.53)	
BMI					
Low weight	2.60 (1.94–3.49)	2.31 (1.59–3.32)	1.74 (1.08–2.80)	3.40 (2.27–5.06)	0.0041
Eutrophic	36.65 (34.29–39.08)	38.44 (35.75–41.19)	42.21 (38.99–45.49)	43.74 (40.65–46.89)	
Overweight	36.60 (34.28–38.99)	37.22 (34.63–39.88)	35.42 (32.42–38.55)	32.63 (29.83–35.57)	
Obesity	24.14 (22.09–26.32)	22.04 (19.85–24.39)	20.63 (18.17–23.33)	20.22 (17.82–22.86)	
Altered waist circumference					
Yes	46.45 (44.01–48.90)	41.28 (38.62–43.99)	32.77 (29.91–35.76)	34.77 (31.90–37.75)	< 0.001
No	53.55 (51.10–55.99)	58.72 (56.01–61.38)	67.23 (64.24–70.09)	65.23 (62.25–68.10)	
Altered HDL cholesterol					
Yes	54.10 (51.63–56.55)	51.55 (48.78–54.31)	50.28 (47.02–53.53)	54.35 (51.20–57.47)	0.1600
No	45.90 (43.45–48.37)	48.45 (45.69–51.22)	49.72 (46.47–52.98)	45.65 (42.53–48.80)	
Altered total cholesterol					
Yes	36.95 (34.62–39.33)	33.50 (31.02–36.07)	30.94 (28.11–33.92)	28.57 (25.91–31.38)	< 0.001
No	63.05 (60.67–65.38)	66.50 (63.93–68.98)	69.06 (66.08–71.89)	71.43 (68.62–74.09)	
Altered LDL cholesterol					
Yes	21.50 (19.57–23.57)	18.47 (16.65–20.56)	17.39 (15.20–19.83)	15.84 (13.80–18.12)	0.0013
No	78.50 (76.43–80.43)	81.53 (79.44–83.45)	82.61 (80.17–84.80)	84.16 (81.88–86.20)	
Diabetes (HbA1c > 6.5 or medication)					
Yes	12.62 (11.13–14.27)	8.41 (7.06–9.98)	6.44 (5.11–8.09)	6.00 (4.75–7.56)	< 0.001
No	87.38 (85.73–88.87)	91.59 (90.02–92.94)	93.56 (91.91–94.89)	94.00 (92.44–95.25)	
Hypertension					
Yes	28.35 (26.16–30.64)	24.80 (22.49–27.25)	19.96 (17.61–22.55)	21.80 (19.28–24.54)	< 0.001
No	71.65 (69.36–73.84)	75.20 (72.75–77.51)	80.04 (77.45–82.39)	78.20 (75.46–80.72)	
Cardiovascular risk					
Low	39.68 (37.25–42.17)	52.47 (49.72–55.20)	61.60 (58.47–64.65)	65.39 (62.42–68.24)	< 0.001
Medium	37.09 (34.76–39.47)	31.53 (29.08–34.09)	25.64 (22.99–28.49)	24.51 (21.94–27.27)	
High	23.23 (21.30–25.28)	15.99 (14.17–18.00)	12.75 (10.87–14.90)	10.10 (8.55–11.90)	

*Pearson's chi-square test; CI = confidence interval; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein.



*Model 1 adjusted for age and body mass index; Model 2 adjusted for age and waist circumference. OR = odds ratio; CI = confidence interval.

Figure 1. Odds ratio and 95% confidence interval for medium/high cardiovascular risk according to the consumption of daily servings of sweetened soft drinks in the Brazilian population. National Health Survey, 2013.

Table 2. Odds ratio and 95% confidence interval (95% CI) for medium/high 10-year risk score according to consumption of sweetened soft drinks in the adult Brazilian population stratified by race and education. National Health Survey, 2013.

	Medium risk				High risk			
	OR (95%CI)*	P*	OR (95%CI)**	P**	OR (95%CI)*	P*	OR (95%CI)**	P**
Soft drink consumption (cups/day)								
White								
< 0.1	Ref.		Ref.		Ref.		Ref.	
0.1–0.39	1.04 (0.71–1.51)	0.843	0.99 (0.68-1.46)	0.982	0.99 (0.61–1.58)	0.955	0.89 (0.55–1.45)	0.651
0.4–0.99	1.16 (0.77–1.75)	0.467	1.07 (0.70-1.62)	0.760	1.67 **(1.01–2.74)**	0.044	1.48 (0.88–2.47)	0.136
≥ 1.0	1.25 (0.82–1.91)	0.296	1.13 (0.74-1.72)	0.573	1.64 (0.97–2.78)	0.065	1.42 (0.82–2.45)	0.205
Other								
< 0.1	Ref.		Ref.		Ref.		Ref.	
0.1–0.39	0.97 (0.71–1.33)	0.861	0.97 (0.71-1.33)	0.857	1.21 (0.80–1.81)	0.365	1.19 (0.78–1.78)	0.016
0.4–0.99	1.26 (0.90–1.76)	0.179	1.22 (0.86–1.71)	0.259	1.83 (1.16–2.89)	0.009	**1.76 (1.11–2.79)**	0.016
≥ 1.0	1.60 (1.13–2.26)	0.018	1.52 **(1.07–2.14)**	0.018	2.45 **(1.55–3.87)**	<0.0001	**2.24 (1.41–3.57)**	0.001
Low educational level								
< 0.1	Ref.		Ref.		Ref.		Ref.	
0.1–0.39	0.89 (0.65–1.22)	0.468	0.89 (0.65–1.22)	0.466	0.94 (0.65–1.37)	0.766	0.92 (0.63–1.33)	0.646
0.4–0.99	1.05 (0.74–1.49)	0.761	1.01 (0.71–1.44)	0.955	1.44 (0.95–2.18)	0.083	1.35 (0.88–2.05)	0.164
≥ 1.0	1.16 (0.80–1.67)	0.428	1.11 (0.77–1.60)	0.576	**1.83 (1.19–2.83)**	0.006	**1.71 (1.10–2.65)**	0.016
High educational level								
< 0.1	Ref.		Ref.		Ref.		Ref.	
0.1–0.39	1.21 (0.79–1.83)	0.373	1.15 (0.70–1.75)	0.528	1.36 (0.73–2.50)	0.330	1.27 (0.68–2.37)	0.446
0.4–0.99	**1.61 (1.07–2.42)**	0.022	**1.53 (1.00–2.32)**	0.046	**2.72 (1.48–5.00)**	0.001	**2.61 (1.38–4.94)**	0.003
≥ 1.0	**1.80 (1.19–2.73)**	0.005	**1.63 (1.08–2.48)**	0.0021	**1.98 (1.04–3.79)**	0.038	1.70 (0.86–3.36)	0.127

*Age-adjusted model of body mass index.

**Age adjusted model waist circumference.

OR = odds ratio; CI = confidence interval.

significantly associated with a greater probability of increased CVR scores. Similar results were observed in the subgroup analyses stratified by race/color and anthropometric measures.

The database used in this study was a representative subset of the Brazilian population, which mitigated the selection bias. The NHS was conducted between 2013 and 2014. Biological materials were collected from a subsample in 2014–2015 to perform laboratory tests on blood and urine samples. A survey in the Brazilian population with clinical data and biological fluid tests has never been conducted previously, which justifies its use despite being conducted

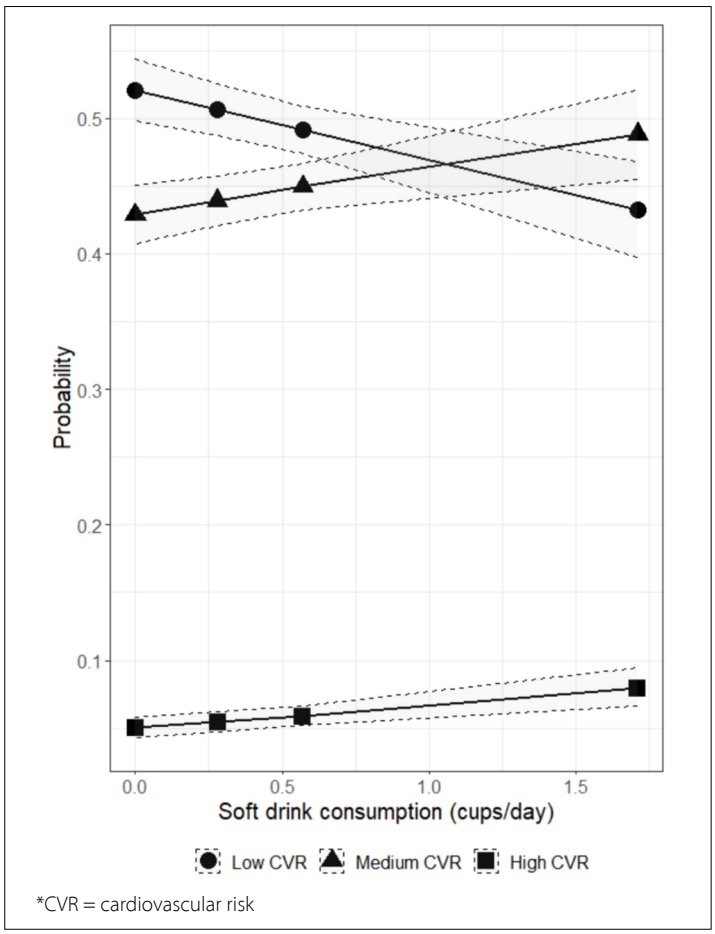


Figure 2. Conditional probability of low, medium, and high cardiovascular risk scores according to soft drink consumption levels, adjusted for body mass index and age. National Health Survey, 2013.

in 2014. Thus, the estimate of CVR score in this study is considered representative of the Brazilian population.[17] CVR estimates in other national studies were from restricted samples of clinical trials and specific populations.[33,34] Measurement of CVR score has been used secularly in several previous and recent studies, which have demonstrated its validity.[20,21,35]

In this study, soft drink consumption was measured through direct interviews. Participants were questioned about their usual consumption of sweetened soft drinks. Food consumption surveys are always susceptible to measurement bias, particularly memory bias. However, in most similar studies, the consumption of sweetened beverages was measured using questionnaires on the frequency of usual consumption, dietary history, or 24-hour recall. Beverage consumption levels were categorized by percentile distribution in standard serving units, using the no-consumption category as a reference. In this study, we adopted the methodology of a similar study on standard serving units.[16]

Most studies in the literature are from cross-sectional, longitudinal, and meta-analysis studies, adding up to approximately 310,000 participants predominantly in White or Black populations in the United States, Finland, and China.[29] Based on these studies, consumers who are in the highest quartile had a 20% and 26% excess risk of metabolic syndrome and diabetes, respectively, when compared to the lowest quartile of soft drink consumption.[29] In a cross-sectional study not representative of the Brazilian population, the association between high consumption of soft drinks and the probability of metabolic syndrome was 95%.[16] In a recent cohort study with a sample of 12,048 adults, consumption of 5–20 g/day of sugar (in this study a standard serving contains approximately 18.5 g of sugar) was associated with a risk of metabolic syndrome, particularly in women.[36]

Two longitudinal studies have shown an increased risk of coronary heart disease with the consumption of soft drinks. In the cohort of 51,529 male health professionals aged 40–75 years, participants with consumption in the highest quartile compared with those in the lowest quartile had a 20% increased risk of coronary heart disease; these results were adjusted for appropriate confounding factors.[31] Similarly, in another study, women from the *Nurse's Health Study* showed a dose-response relationship between soft drink consumption and coronary heart disease; a median consumption of 1.2–2 standard servings/day, increased the risk of coronary heart disease by 23–25%, respectively.[32] In the present study 27% and 21% of male and female participants, respectively, consumed ≥ 1 standard servings/day. Other studies have also suggested that high consumption of soft drinks is associated with weight gain, increased fat mass, hypertension, and a risky lipid profile,[11,29,37] which in turn are also associated with increased CVR.

Typically, carbonated beverages in Brazil are sweetened with sucrose, a carbohydrate composed of glucose and fructose, causing high glycemic load that can potentiate the risk of metabolic diseases and increase the risk of metabolic deterioration.[9] This metabolic impairment has been widely studied and associated with the development of cardiovascular involvement and type 2 diabetes.[38]

The present study has some limitations. These designs considered the possibility of reverse causality, which could contradict the association between soft drink consumption and CVR. Additionally, insulin resistance indicators can be considered mediators of high sugar consumption and CVR. However, the magnitude of the association did not change when participants with glycated hemoglobin levels ≥ 6.5 or those receiving oral hypoglycemic agents or insulin were excluded. Another limitation is that national surveys are not designed to test specific hypotheses of association and are therefore susceptible to residual confounding. However, the greatest methodological advantage of this study lies in its ability to estimate the 10-year CVR scores representative of the Brazilian population— a population parameter rarely obtained by health surveys—which indicates the novel character of the results.

The results of this study suggest associative relationships, and not necessarily causal relationships, which are limited by the cross-sectional design of this investigation. However, the abundant literature in this area suggests a significant role of sugar consumption through beverages with added sugar, which may be a major source of calorie intake in the Brazilian population, with the aggravating factor of altering metabolic homeostasis. Thus, the findings of this study may help support public policies that aim to reduce the consumption of high-calorie processed foods.

CONCLUSION

In this study, the association between soft drink consumption and 10-year CVR scores was shown to be independent of age and adiposity measures such as BMI and WC. Future studies using more robust methodologies, such as longitudinal studies and larger population samples, are required to better understand the underlying mechanisms, strengthen the scientific evidence, and inform effective public health policies.

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Effect of planned visual education on university students' attitudes and beliefs regarding skin cancer: a cluster-randomized controlled trial

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ABSTRACT

BACKGROUND: The incidence of skin cancer is increasing globally. However, it is largely preventable through early detection. Therefore, raising public awareness through education is essential.

OBJECTIVE: This study aimed to evaluate the effect of a planned visual education program—based on the Health Belief Model—on university students' attitudes and beliefs regarding skin cancer.

DESIGN AND SETTING: This cluster-randomized controlled trial was conducted in two departments at a university in Manisa, Türkiye.

METHODS: The study was conducted with 116 university students, divided equally into an intervention group (n = 58) and a control group (n = 58). Data were collected using the Student Information Form and the Health Belief Model Scale in Skin Cancer.

RESULTS: Following the visual education program based on the Health Belief Model, significant differences were observed between the intervention and control groups in perceived severity, perceived susceptibility, perceived barriers, perceived benefits, and self-efficacy scores. While the intervention group showed significant improvements across these domains, no significant difference was found in perceived severity scores.

CONCLUSION: The findings indicate that planned visual education based on the Health Belief Model positively influenced students' attitudes and beliefs regarding skin cancer.

CLINICAL TRIAL REGISTRATION: The research was recorded at https://clinicaltrials.gov/study/NCT05788939.

INTRODUCTION

The incidence rate of skin cancer is steadily increasing both worldwide and in Türkiye.[1,2] According to 2014 data, the incidence was 28.3 per 100,000 in men and 18 per 100,000 in women.[3] Skin cancer is the most common type of cancer among individuals aged 25–29 years and the second most common type in those aged 15–29 years.[4,5] Melanoma—the most fatal form of skin cancer—is particularly prevalent among adolescents and young adults.[6] Therefore, primary prevention strategies for skin cancer should specifically target this population.[7,8]

The most significant risk factor for skin cancer is exposure to ultraviolet (UV) radiation.[9,10] Youth and childhood are the most vulnerable periods for skin damage and UV exposure. The amount of UV radiation absorbed during these years substantially contributes to skin cancer risk in adulthood. Additionally, approximately 25–50% of an individual's lifetime sun and UV exposure occurs between the ages of 18 and 21.[10,11] Young people frequently engage in outdoor social activities that further increase their exposure to sunlight.[12]

Adolescence is a developmental stage marked by substantial physical, psychological, and social change during which lifelong health behaviors are established.[13] Young individuals may struggle to adapt to these changes. Although skin cancer is highly preventable through proper sun protection measures, studies indicate that young people often do not adhere to recommended protective behaviors.[9–11] Consequently, it is essential to implement educational initiatives aimed at skin cancer prevention among young people.[14]

To promote healthy behaviors and improve sun protection and skin cancer prevention practices among young individuals, visual education campaigns incorporating videos, brochures, and posters that reinforce learning are recommended.[15] In European countries, such campaigns are

implemented to raise awareness and knowledge about skin cancer and the risks associated with sun exposure.[16–18] However, few studies have focused on university students,[18–22] and limited research has explored the use of visual education programs incorporating digital applications to increase skin cancer awareness in Türkiye.[23,24]

Developing preventive behaviors for skin cancer requires individuals to reflect on their attitudes and beliefs regarding the disease.[5,9] The Health Belief Model (HBM)—a widely used framework in health promotion—proposes that health behaviors are influenced by individuals' beliefs and perceptions. Although the HBM is frequently applied to explain and guide preventive health behaviors,[25] the number of studies investigating its use in the context of skin cancer[26–29] is limited and largely descriptive.[30] To our knowledge, no studies have examined the impact of a planned visual education program based on the HBM on university students' attitudes and beliefs about skin cancer. Therefore, this study was conducted to assess the effects of such an intervention.

OBJECTIVE

The aim of this study was to assess the effects of a planned visual education program—based on the Health Belief Model—on university students' attitudes and beliefs about skin cancer.

METHODS

This study was conducted as a cluster-randomized controlled trial. The sample comprised 116 second-year students at Manisa Celal Bayar University (MCBU). Participants were enrolled in the Faculty of Economics and Administrative Sciences and the Faculty of Technology. The study was conducted between February and September 2019. Simple random sampling was used to determine the sample. A draw was conducted among the faculties at MCBU. The Faculty of Economics and Administrative Sciences was assigned as the intervention group (IG), and the Faculty of Technology was assigned as the control group (CG). Among the seven departments within the Faculty of Economics and Administrative Sciences and the four departments within the Faculty of Technology, lots were drawn again using simple random sampling. The Department of Econometrics was assigned as the IG, and the Department of Mechatronics Engineering was assigned as the CG. The research was conducted as a single-blind study in which participants were blinded; they did not know whether they belonged to the IG or CG.

A power analysis was conducted to determine an adequate sample size that would yield reliable results and allow for statistical analysis. Based on repeated-measures analysis of variance using the G*Power 3.1 software (Heinrich Heine University, Düsseldorf), the minimum required sample size was calculated to be 51 participants per group, assuming 80% power, a significance level of 0.05, and a medium effect size (0.5) with two repeated measurements. A total of 58 students were recruited for each group. The post hoc power of the study was computed as 0.95 at a significance level of $\alpha = 0.05$.

The inclusion criteria were as follows: being 18 years of age or older, actively enrolled in formal education and attending classes, and voluntarily agreeing to participate in the study.

Ethical standards

Approval was obtained from the Ethics Committee of the Faculty of Medicine, Manisa Celal Bayar University Health Sciences (dated 27/06/2018; protocol no. 20.478.486), along with institutional permissions, prior to conducting the study. This research was carried out in accordance with the principles of the Declaration of Helsinki. The aim and procedure of the study were explained to the students, and informed consent forms were signed. Upon completion of the study, the CG received the same educational content, including slides and videos, and project-related keychains were distributed.

HYPOTHESES

- Hypothesis 1 (H1-1): There is a difference between the IG and CG in perceived susceptibility to skin cancer as a significant health issue.
- Hypothesis 2 (H1-2): There is a difference between the IG and CG in their beliefs about developing skin cancer and its consequences.
- Hypothesis 3 (H1-3): There is a difference between the IG and CG in their beliefs that recommendations for preventing skin cancer are useful.
- Hypothesis 4 (H1-4): There is a difference between the IG and CG in perceived barriers to preventing skin cancer.
- Hypothesis 5 (H1-5): There is a difference between the IG and CG in their confidence in taking preventive measures against skin cancer.
- Hypothesis 6 (H1-6): There is a time-based difference within the IG in perceived susceptibility to skin cancer.
- Hypothesis 7 (H1-7): There is a time-based difference within the IG in beliefs about developing skin cancer and its consequences.
- Hypothesis 8 (H1-8): There is a time-based difference within the IG in beliefs that recommendations for preventing skin cancer are useful.
- Hypothesis 9 (H1-9): There is a time-based difference within the IG in perceived barriers to preventing skin cancer.
- Hypothesis 10 (H1-10): There is a time-based difference within the IG in confidence regarding taking preventive measures against skin cancer.

Instruments

The study data were collected using the Student Information Form and the HBM Scale in Skin Cancer.

The Student Information Form

This form was developed by the researchers based on a review of the relevant literature.[20,21,31] It consists of 24 questions concerning participants' sociodemographic characteristics.

The Health Belief Model Scale in Skin Cancer (HBMSSC)

The scale was developed by Dogan and Caydam.[32] The HBMSSC includes five sub-dimensions: perceived benefit, perceived susceptibility, perceived barriers, perceived severity, and self-efficacy. It comprises 26 items and is a Likert-type scale. Each item is rated on a scale from 1 ("strongly disagree") to 5 ("strongly agree"). The total Cronbach's alpha coefficient for the scale is 0.87. The Cronbach's alpha coefficients for the five sub-dimensions are 0.79, 0.89, 0.65, 0.77, and 0.86, respectively. The "perceived barriers" sub-dimension is reverse-coded. The HBMSSC does not calculate a total score; instead, scores are derived independently for each sub-dimension. Higher scores in the "perceived severity," "perceived benefit," "perceived susceptibility," and "self-efficacy" sub-dimensions indicate stronger perceptions in each respective domain.[32] In this study, the Cronbach's alpha coefficient for the total HBMSSC was 0.89 in the intervention group. The sub-dimension coefficients were 0.86, 0.94, 0.81, 0.71, and 0.92, respectively. In the control group, the total Cronbach's alpha was 0.78, and the sub-dimension values were 0.86, 0.81, 0.89, 0.75, and 0.90, respectively.

Data collection

Data were collected twice from the CG: before the training (pre-test) and seven months after the training (post-test). For the IG, data were collected four times: before the planned visual education program (pre-test), at the first and third months during the follow-up period, and at seven months post-intervention (post-test) (**Figure 1**).

Planned visual education program

The educational materials included a PowerPoint presentation, two videos titled Mr. Sun and Dear 16-Year-Old Me, a skin cancer model, three posters, and a brochure. Professional support was obtained from a media company to produce subtitled versions of the Mr. Sun video. The researcher presented the content of the presentation slides, brochure, and posters.

Pre-test: The forms were distributed to students, who were asked to complete them. The presentation and skin cancer model were shown, and brochures were handed out.

1st Month: Students completed the HBMSSC. The videos were shown, and bandanas—intended to be worn as bracelets—were distributed. An Instagram page (@derikanseri), created for the campaign, was introduced. Students were informed that the first 20 people who followed the page, took a photo wearing the bandana, and tagged the page would receive gift items (sunscreen for the first 10 participants and beach towels for the next 10). Posters were displayed throughout the faculty building. A WhatsApp group was created to send periodic reminder messages to participants.

3rd Month: Students again completed the HBMSSC. Participants who followed the Instagram page received their designated gifts. Students were reminded to stay engaged during the summer and were informed that new surprises would be announced in

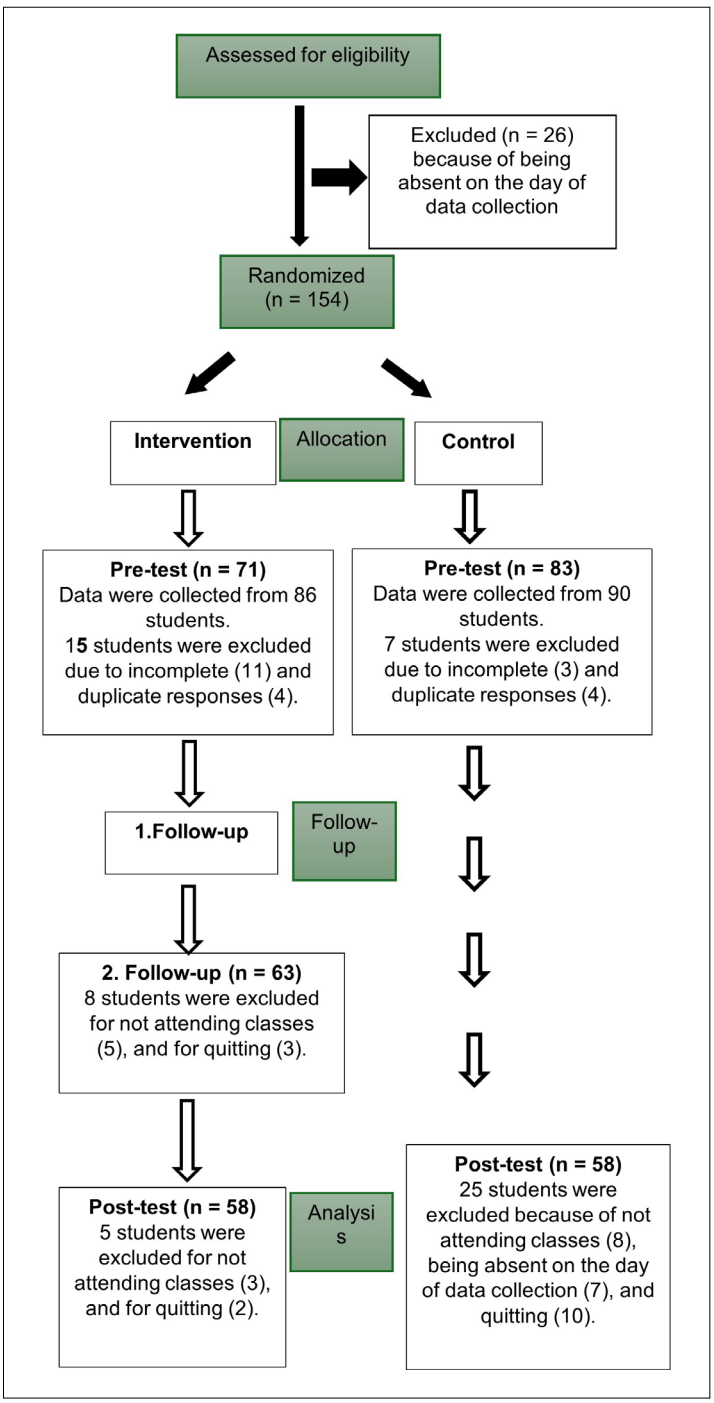

Figure 1. Flow diagram of the study.

September. Skin cancer awareness messages were sent monthly via Instagram, WhatsApp, and text message applications in June, July, and August.

Post-test: Students completed the final HBMSSC. They were informed of the study's conclusion, and all participants received a gift—a keychain designed by the researchers featuring sun protection imagery.

Statistical analysis

SPSS version 21.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. Descriptive statistics were presented as mean ± standard deviation and frequency. To determine the suitability of the data for normal distribution, the decision was based on the "mean ± 2 standard deviations" rule, where 95.44% of the data were considered to fall within this range, indicating a normal distribution. As the data met the assumption of normality, parametric tests were applied. Between-group differences were analyzed using the independent samples t-test, while within-group differences were evaluated using the paired samples t-test. Repeated-measures analysis of variance was used when comparing more than two time points. Categorical variables were assessed using the chi-square test. Statistical significance was set at $p < 0.05$, with a 5% margin of error. A priori power analysis was conducted using G*Power version 3.1 (Heinrich Heine University, Düsseldorf) to determine the minimum required sample size.[33] Statistical consultation was obtained from a professional data analysis company.

RESULTS

No significant differences were observed between the groups in terms of sociodemographic characteristics or skin type ($p > 0.05$) (**Table 1**).

While there were no significant differences in pre-test scores between the groups for perceived severity, perceived barriers, perceived susceptibility, perceived benefit, and self-efficacy ($p > 0.05$), significant differences were found in post-test scores ($p < 0.01$; $p < 0.001$; $p < 0.001$; $p < 0.0001$; and $p < 0.0001$, respectively). No significant differences were observed between the pre-test and post-test scores for any of the five dimensions in the control group ($p > 0.05$) (**Table 2**).

Although the difference in perceived severity scores between the pre-test and post-test in the IG was not statistically significant ($p > 0.05$), the mean post-test score was higher than the pre-test score. Significant differences were observed between pre-test and post-test mean scores in the IG for perceived benefit ($p < 0.001$), perceived susceptibility ($p < 0.05$), perceived barriers ($p < 0.05$), and self-efficacy ($p < 0.001$) (**Table 2**).

Significant differences were found across follow-up measurements in the IG for perceived barriers ($p < 0.01$), perceived susceptibility ($p < 0.05$), perceived benefit ($p < 0.001$), and self-efficacy ($p < 0.05$). However, no significant difference was observed in perceived severity scores across follow-ups ($p > 0.05$) (**Table 3**).

DISCUSSION

Perceived susceptibility refers to the extent to which individuals believe they are at risk of a disease or condition, with higher perceived risk typically associated with healthier behaviors.[25,34] A significant difference was found in perceived susceptibility scores between the groups in the post-test (confirming H1-1) and across follow-ups within the IG (confirming H6-1). Similarly, studies on skin cancer in farmers[31] and osteoporosis in women reported[35] differences in perceived susceptibility during follow-ups after training. In contrast to our findings, a previous study[36] on breast self-examination among university students found no significant difference in perceived susceptibility scores during follow-up. This discrepancy may be explained by the fact that the sample in the referenced study consisted of nursing students.

A significant difference in perceived susceptibility scores was also observed between the first and third months. In this context, incorporating video content into educational programs may help promote behavioral changes to prevent skin cancer. Existing literature supports this approach.[15,37,38]

Perceived benefit is defined as an individual's belief that a particular behavior will effectively prevent a disease.[25,34] A significant difference was observed in perceived benefit scores between groups in the post-test (confirming H3-1) and across follow-ups within the IG (confirming H8-1). Similar to our findings, one study reported differences in perceived benefit scores among farmers across follow-ups.[28] In our study, the difference in scores appeared primarily between the first and third months, following delivery of the intervention via slides, brochures, and videos. These results suggest that visual education based on the HBM strengthened students' beliefs in the usefulness of recommendations for skin cancer prevention. Supporting evidence in the literature indicates that visual education increases young people's knowledge and awareness of skin cancer,[15] improves sun protection and prevention behaviors,[15] and enhances skin self-examination practices.[21]

Perceived severity refers to the extent to which an individual considers the consequences of a disease to be serious, with stronger perceptions generally associated with increased health-protective behaviors.[25,34] A significant difference was observed in post-test perceived severity scores between the groups in this study (confirming H2-1). However, no significant change was found in perceived severity scores within the IG across time (rejecting H7-1). In contrast to these findings, a previous study reported significant changes in farmers' perceived severity scores during follow-up,[29] which may be attributed to the younger age of participants in our sample. Another study also found that visual education based

Table 1. Sociodemographic characteristics and skin type profiles of students in the intervention and control groups (n = 116)

	Intervention Group (n = 58)		Control Group (n = 58)		Significance
	$\bar{x} \pm SD$	Min-Max	$\bar{x} \pm SD$	Min-Max	
Age (years)	20.84 ± 1.08	19-24	20.91 ± 1.18	19-24	t = –0.326 p = 0.745
Gender	**n**	**%**	**n**	**%**	
Female	35	60.3	28	51.7	$x^2 = 1.702$ p = 0.192
Male	23	39.7	30	48.3	
Place of long-term residence					
Village/province	22	37.9	30	51.7	$x^2 = 2.231$ p = 0.135
City	36	62.1	28	47.3	
Sunbelt of residence					
First belt	47	81.1	39	67.2	$x^2 = 2.878$ p = 0.090
Second, third, and fourth belts	11	18.9	19	32.8	
Paternal education level					
Primary school	14	24.1	15	25.9	$x^2 = 1.761$ p = 0.623
Secondary school	15	25.9	11	18.9	
High school	19	32.8	17	29.3	
University	10	17.2	15	25.9	
Paternal occupation					
Civil servant	11	19.0	22	37.9	$x^2 = 6.701$ p = 0.082
Retired	12	20.7	7	12.1	
Self-employed	18	31.0	11	19.0	
Worker	17	29.3	18	31.0	
Maternal education level					
Primary school	22	37.9	20	34.5	$x^2 = 0.459$ p = 0.928
Secondary school	15	25.9	15	25.9	
High school	15	25.9	18	31.0	
University	6	10.3	5	8.6	
Maternal occupation					
Civil servant	5	8.6	5	8.6	$x^2 = 0.136$ p = 0.987
Retired	6	10.4	7	12.1	
Worker	9	15.5	8	13.8	
Housewife	38	65.5	38	65.5	
Perceived income					
Income ≤ expenses	46	79.3	47	81.0	$x^2 = 0.054$ p = 0.816
Income > expenses	12	20.7	11	19.0	
Hair color					
Fair	5	8.6	7	12.1	$x^2 = 0.402$ p = 0.818
Brown	30	51.7	28	48.3	
Black	23	39.7	23	39.6	
Eye color					
Blue/Green/Hazel	15	25.9	13	22.5	$x^2 = 0.195$ p = 0.907
Brown	37	63.8	39	67.2	
Black	6	10.3	6	10.3	
Skin color					
Fair	20	34.5	19	32.7	$x^2 = 0.143$ p = 0.931
Auburn/Light brown	26	44.8	28	48.3	
Brown/Brunette	12	20.7	11	19.0	
Skin type					
Burns easily, does not tan or tans very little	21	36.2	12	20.7	$x^2 = 5.417$ p = 0.114
Burns, tans over time	12	20.7	20	34.5	
Burns very little, tans quickly	12	20.7	16	27.6	
Does not burn, tans quickly	13	22.4	10	17.2	
Birthmark status on the skin					
Yes	22	37.9	18	31.0	$x^2 = 0.611$ p = 0.415
No	36	62.1	40	69.0	
History of sunburn in the last 12 months					
No	23	39.7	26	44.9	$x^2 = 0.323$ p = 0.851
Once	20	34.5	18	31.0	
Twice or more	15	25.8	14	24.1	
	$\bar{x} \pm SD$	Min-Max	$\bar{x} \pm SD$	Min-Max	
Time in the sun between 10:00 a.m. and 4:00 p.m. in summer (hours)	2.51 ± 1.21	0.5-6	2.78 ± 1.62	0-6	t = –1.004 p = 0.371
Number of nevus on the skin	16.53 ± 22.61	0-100	19.73 ± 24.63	0-150	t = –0.742 p = 0.46

Max = maximum; Min = minimum; SD = standard deviation; t = independent samples t-test; $\bar{x}$ = Mean; x^2 = chi-square test; [a] $p < 0.05$.

Table 2. Comparison of mean scores on the health belief model scale in skin cancer between intervention and control groups (n = 116)

Sub-dimension	Group	Pre-test (1st Month)		Post-test (7th Month)		Between-Group Significance	
		$\bar{x} \pm SD$		$\bar{x} \pm SD$		t	p
Perceived Susceptibility	Intervention	24.86 ± 6.02		27.01 ± 2.76		**−2.567**	**0.013[a]**
	Control	24.94 ± 5.18		24.05 ± 5.52		0.901	0.370
	Significance	t	P	**t**	**P**		
		0.083	0.934	**3.654**	**0.000[c]**		
Perceived Benefit	Intervention	20.56 ± 5.49		24.81 ± 3.27		**−5.364**	**0.000[c]**
	Control	20.50 ± 4.40		20.53 ± 4.04		−0.044	0.965
	Significance	t	P	**t**	**P**		
		0.075	0.941	**6.106**	**0.000[c]**		
Perceived Severity	Intervention	16.22 ± 3.19		17.12 ± 2.36		−1.914	0.061
	Control	15.18 ± 3.77		15.39 ± 3.95		−0.288	0.774
	Significance	t	P	**t**	**P**		
		1.544	0.114	**2.850**	**0.005[b]**		
Perceived Barriers	Intervention	13.67 ± 3.93		16.15 ± 2.61		**−4.335**	**0.000[c]**
	Control	14.48 ± 3.50		13.67 ± 3.15		1.849	0.070
	Significance	t	P	**t**	**P**		
		−1.239	0.218	**4.616**	**0.000[c]**		
Self-efficacy	Intervention	23.53 ± 5.47		25.15 ± 3.51		**−2.063**	**0.044[a]**
	Control	22.72 ± 5.35		21.84 ± 4.84		1.440	0.155
	Significance	t	P	**t**	**P**		
		0.980	0.422	**0.211**	**0.000[c]**		

SD = standard deviation; t = independent samples t-test; $\bar{x}$ = Mean; [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$

Table 3. Within-group comparison of mean scores on the Health Belief Model Scale in Skin Cancer at pre-test, 1st, 3rd, and 7th months in the intervention group (n = 58)

Sub-dimension	Follow ups	$\bar{x} \pm SD$	Pairwise Comparisons					
			I		t	p	F	p
Perceived Susceptibility	Pre-test (I1)	24.86 ± 6.02	I 1-2	I1 < I2	−1.561	0.124		
	1st month (I2)	26.20 ± 2.97	I 1-3	I1 < I3	**−2.292**	**0.026**	**3.298**	**0.027[a]**
	3rd month (I3)	26.91 ± 2.62	I 1-4	I1 < I4	**−2.567**	**0.013**		
	7th month (I4)	27.01 ± 2.76	I 2-3	I2 < I3	**−2.362**	**0.022**	**E.S.**	
			I 2-4	I2 < I4	−1.281	0.205	0.152	
			I 3-4	I3 < I4	−0.171	0.864		
Perceived Benefit	Pre-test (I1)	20.56 ± 5.49	I 1-2	I1 < I2	**−3.576**	**0.001**		
	1st month (I2)	23.68 ± 4.36	I 1-3	I1 < I3	**−7.686**	**0.000**	**12.345**	**0.000[c]**
	3rd month (I3)	24.89 ± 3.30	I 1-4	I1 < I4	**−5.364**	**0.000**		
	7th month (I4)	24.81 ± 3.27	I 2-3	I2 < I3	**−2.820**	**0.007**	**E.S.**	
			I 2-4	I2 < I4	−1.480	0.144	0.402	
			I 3-4	I3 > I4	0.136	0.892		
Perceived Severity	Pre-test (I1)	16.22 ± 3.19	I 1-2	I1 < I2	−0.422	0.674		
	1st month (I2)	16.41 ± 2.69	I 1-3	I1 < I3	−1.955	0.056	2.724	0.053
	3rd month (I3)	17.08 ± 2.52	I 1-4	I1 < I4	−1.914	0.061		
	7th month (I4)	17.12 ± 2ç36	I 2-3	I2 < I3	**−2.413**	**0.019**	**E.S.**	
			I 2-4	I2 < I4	−1.769	0.082	0.129	
			I 3-4	I3 < I4	−0.100	0.920		
Perceived Barriers	Pre-test (I1)	13.67 ± 3.93	I 1-2	I1 < I2	−1.852	0.069		
	1st month (I2)	14.82 ± 2.90	I 1-3	I1 < I3	**−3.178**	**0.002**	**6.637**	**0.001[b]**
	3rd month (I3)	15.62 ± 2.28	I 1-4	I1 < I4	**−4.335**	**0.000**		
	7th month (I4)	16.15 ± 2.61	I 2-3	I2 < I3	**−2.165**	**0.035**	**E.S.**	
			I 2-4	I2 < I4	**−2.463**	**0.017**	0.266	
			I 3-4	I3 < I4	−1.209	0.232		
Self-efficacy	Pre-test (I1)	23.53 ± 5.47	I 1-2	I1 < I2	−0.468	0.642		
	1st month (I2)	23.91 ± 3.38	I 1-3	I1 < I3	−0.463	0.149	3.420	**0.023[a]**
	3rd month (I3)	24.67 ± 3.23	I 1-4	I1 < I4	**−2.063**	**0.044**		
	7th month (I4)	25.15 ± 3.51	I 2-3	I2 < I3	**−2.297**	**0.025**	**E.S.**	
			I 2-4	I2 < I4	**−2.233**	**0.023**	0.157	
			I 3-4	I3 < I4	−0.969	0.337		

E.S. = effect size; F = Pillai's Trace test; I = intervention; SD = standard deviation; t = independent samples t-test; $\bar{x}$ = Mean; [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$.

on the HBM did not significantly affect perceived severity scores related to breast cancer prevention among university students.[36]

Perceived barriers refer to an individual's beliefs about obstacles that may prevent them from adopting health behaviors.[25,34] A significant difference was found in post-test perceived barriers scores between groups (confirming H4-1) and across follow-ups within the IG (confirming H9-1). Our findings are consistent with previous studies.[29,35] These results suggest that HBM-based training reduced perceived barriers to skin cancer prevention. Notably, perceived barrier scores decreased following the video-based component of the training. Armstrong et al.[15] found that video-based education was a more effective teaching tool than written materials for promoting sun protection among young people. Therefore, incorporating video-based educational materials into behavior change programs for skin cancer prevention appears to be beneficial.

Self-efficacy refers to an individual's confidence in their ability to take action.[25,34] A significant difference was observed in post-test self-efficacy scores between the groups (confirming H5-1) and across follow-ups within the IG (confirming H10-1). These findings are consistent with previous studies on skin cancer in farmers[29] and osteoporosis in women.[35] In another study involving workers, a positive change in knowledge and attitudes toward skin cancer was observed following a planned visual education program.[39] Accordingly, it can be concluded that planned visual training increased individuals' confidence and knowledge regarding the ability to engage in preventive behaviors for skin cancer.

The difference in mean self-efficacy scores across follow-up periods appears to be associated with the activities implemented between the pre-test and post-test. Specifically, the improvement was observed following the delivery of video-based training to the IG. Prior research has shown that video-based education is more effective than written materials and enhances both sunscreen use and related knowledge.[36] Other studies have recommended combining visual educational materials.[18,19] A study on young people's knowledge and awareness of skin cancer found that training programs incorporating video, brochures, and PowerPoint presentations improved behaviors related to skin self-examination.[23] Based on these findings, video training can be considered a valuable component of educational interventions aimed at strengthening individuals' belief in their ability to adopt skin cancer prevention practices.

This study had several limitations. First, as participation was voluntary, not all students in the targeted classes took part in the study. Second, the sample consisted of students from a single university, which limits the generalizability of the findings to the broader population of university students. Third, to ensure accurate comparison across follow-up periods, only data from participants who completed all follow-ups were included in the analysis. Fourth, the results were based on self-reported data. Nonetheless, the authors believe that the data collection tool was effective in evaluating the impact of the planned visual education program based on the HBM.

CONCLUSION

In conclusion, the findings indicate that planned visual education based on HBM had a positive effect on perceived benefit, perceived severity, perceived susceptibility, perceived barriers, and self-efficacy. Based on these findings, it is recommended that health professionals implement skin cancer education and screening programs for young people, incorporating visual education tools grounded in the HBM—particularly those that emphasize video-based content—to promote preventive behaviors related to skin cancer.

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Selenium intake, food sources, and associated factors in Brazilian Longitudinal Study of Adult Health (ELSA-Brasil): a cross-sectional study

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ABSTRACT

BACKGROUND: Selenium is essential to human health. There are few reports on the analysis of selenium intake in the Brazilian population; however, data have shown that Brazilian are in the deficient range of consumption.

OBJECTIVES: This study aimed to identify the major foods that contribute to dietary selenium and determine the socioeconomic and lifestyle factors associated with selenium intake.

DESIGN AND SETTING: This cross-sectional study used baseline data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil).

METHODS: Selenium consumption was evaluated using a food frequency questionnaire (FFQ) developed and validated for the ELSA-Brasil. To determine the contribution of selenium consumption, each food item was divided by the total selenium intake of the population. Associations between selenium intake (mg/day, dependent variable) and sociodemographic and lifestyle factors (predictors) were tested using multiple linear regression analyses.

RESULTS: The sample comprised 14,780 participants, most of whom were adults (78.5%). Individuals with income ≥ 3 minimum wages were mostly concentrated in the 5th quintile of selenium consumption; positive and significant correlations were found between selenium intake and female sex, age ≥ 60 years, income ≥ 3 minimum wages, higher or postgraduate education, alcohol consumption, and moderate physical activity level. Nuts and fish contributed the most to dietary selenium.

CONCLUSION: Nuts, meat, and fish contributed the most to the dietary intake of selenium, and selenium intake was associated with sociodemographic and lifestyle factors among the evaluated individuals.

INTRODUCTION

Selenium (Se) is an essential mineral for human health; after iodine, it is the second most important nutrient for proper functioning of the thyroid.[1] It acts as a cofactor of more than 25 selenoproteins and selenoenzymes, which are involved in diverse physiological processes.[2] Unlike other minerals, selenium is incorporated into proteins by means of a co-translational mechanism as part of the amino acid selenocysteine (SeCys), the 21st proteinogenic amino acid in humans.[3]

Daily intake of selenium is necessary to maintain natural metabolism and homeostasis in the human body. The known biological functions of selenium in the human body include defense against oxidative stress, regulation of thyroid hormone, protection against oxidative damage, reducing the risk of chronic non-communicable diseases, and increasing resistance of the immune system in the form of selenoproteins.[4]

The Recommended Dietary Allowance (RDA) of selenium for both men and women is 55 µg/day. The Tolerable Upper Intake Level (UL) of selenium for adults is set at 400 µg/day to prevent selenosis caused by high intake of selenium.[5] Selenium consumption is mostly dependent on the food content and dietary supplements.[6] Fruits and vegetables, in general, have a low content of this micronutrient.[7–9]

The concentration of selenium in food depends on the content of selenium present in the soil in which the plants is grown; thus, the concentration of selenium in food varies worldwide. There are few reports on the analyses of selenium in Brazilian soils; however, data have shown that many soils are in the deficient range.[10–13]

Few food items such as Brazilian nuts and kidney beef are considered the best sources of selenium.[14] In addition to these, selenium is present in beef, chicken, fish, dairy products,

and eggs.[7,8,15,16] In regions with soil containing adequate selenium, wheat represents a good source, and thus, consumption of breads and cereals can contribute to the nutrient intake.[6,17–19]

Many studies show that personal factors, such as sex, age, physical activity level, and socioeconomic factors based on income and education, may affect the food consumption of a person or population.[20,21] People from lower socioeconomic background have a higher incidence of premature death, heart diseases, and cancer than those from socioeconomically advantaged groups.[22–24] In Brazil, the consumption of milk, fish, lean meat, fruits, vegetables, and whole grains is positively related to family income,[25] which may influence selenium intake in the Brazilian population.

OBJECTIVE

Thus, this study aimed to identify the main foods that contribute to the dietary consumption of selenium and to determine the socioeconomic and lifestyle factors associated with selenium intake among participants of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil).

METHODS

Study design and population

This cross-sectional study used baseline data from the ELSA-Brasil, which is a multicenter cohort study focused on the incidence of cardiovascular diseases and diabetes in the Brazilian population.[26,27] This cohort involved 15,105 participants of both sexes, aged 35–74 years at baseline, recruited between August 2008 and December 2010.[26,27] Detailed methodological information and baseline data have been published elsewhere.[26,27] We excluded participants without food consumption information (n = 24) and individuals below the 1st percentile and above the 99th percentile of the total energy intake estimates (n = 301) to exclude possibly invalid food intake data. The final sample comprised 14,780 participants. The ELSA-Brasil was approved by the Research Ethics Committee of each institution where the project was conducted.

Food consumption assessment

To evaluate the habitual consumption of participants in the last 12 months, a food frequency questionnaire (FFQ) was developed and validated for the ELSA-Brasil.[28] This semi quantitative FFQ designed by the ELSA-Brasil had 114 food items and was answered by interview. The FFQ used the Nutrition Data System for Research (NDSR) software (University of Minnesota, Minneapolis, 2010) for data analysis. Participants were asked at the end of the FFQ if they had changed their dietary intake in the past six months, and if the answer was "yes," the participant would inform reason for changes in their dietary habits. Individuals who did not have any dietary data or who did not have a plausible daily caloric intake (< 500 or > 6,000 kcal) were excluded.

The proportion of selenium in diet was calculated based on the consumption of all foods containing this nutrient. Selenium intake was adjusted for the total energy intake using the residue method[29] to reduce errors associated with food consumption measurements, and the total selenium intake was divided into quintiles (1st quintile = lowest intake; 5th quintile = highest intake). Energy-adjusted values were used for stratification of quantiles and linear regression analyses.

Socioeconomic and lifestyle factors

At baseline, data was collected from interviews, anthropometric measurements, and clinical examinations.[26] Socioeconomic and lifestyle variables were selected based on previous studies that addressed these determinants of food intake in the Brazilian adult population.[21,30] Accordingly, we selected sex, age, schooling, income, self-reported skin color, smoking and alcohol habits, nutritional status, and physical activity level as variables in this study.[31]

The participants were classified according to sex as male and female and according to age as adult (34–59 years) and older (≥ 60 years). We categorized schooling as "complete elementary school," "complete high school," and "higher education or postgraduate." To classify the family income *per capita,* we calculated it as equivalent to the average minimum wage between 2008 and 2010 (R$ 463.33) and then stratified into < 3 or ≥ 3 minimum wages. Self-reported skin color was categorized as "white" and "not white".[32]

Alcohol consumption was measured in grams of ethanol per week as informed in the FFQ, and participants were classified into two categories: current and past alcohol intake. We evaluated smoking status using a semi-structured questionnaire on smoking habits at the time of interview and at a predetermined time in the past. Based on these questions, participants were categorized as "non-smokers," "former smokers," or "current smokers."

To assess the nutritional status of participants, we considered the body mass index (BMI) calculated as weight (kg) divided by height squared (kg/m²) and classified according to the World Health Organization criteria defined as: low weight (< 18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25–29.9 kg/m²), and obesity (≥ 30 kg/m²).[33] Physical activity level was evaluated based on the International Physical Activity Questionnaire (IPAQ),[34,35] and categorized as light, moderate, and vigorous physical activity level at leisure time.[36] In this study, we considered physical activity level during leisure time only because it was better related to sociodemographic variables, such as schooling, income, sex, and age,[37] used in epidemiological studies.[38]

Statistical analysis

Energy-adjusted selenium consumption was stratified into quintiles to better represent dietary selenium ranking. Sociodemographic and lifestyle factors were described as frequencies and percentages according to the lowest (1st quintile) and highest (5th quintile) levels of selenium intake and sex of the participants. Pearson's chi-square test was used to verify differences between groups.

To measure the proportion of food contributing to selenium intake, we used the methodology proposed by Block et al.[39] Aiming to obtain the contribution of food in selenium consumption, each food item was divided by the total population selenium intake. Food items were listed as major contributors according to their contribution rankings.[40]

Associations between energy-adjusted selenium intake (mg/day, dependent variable) and sociodemographic and lifestyle factors (predictors) were tested by multiple linear regression analyses using the stepwise backward method.

The energy-adjusted selenium intake variable approaches normality based on the results of the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots, thus meeting this assumption for multiple linear regression. The multivariable model was further adjusted for self-reported changes in dietary habits over the past six months (yes or no) and supplement use (no, regular, or not regular). Since we did not have information about the presence of selenium in supplements, we adjusted the multivariable model for the use of supplements. Since information regarding the use of supplements was self-reported, we cannot exclude the possibility of memory bias. All analyses were performed using the Stata® (version 14) software, assuming a statistical significance level of 5%.

RESULTS

Our sample consisted of 14,780 participants, mostly adults (78.5%), females (54.5%), non-smokers (57.1%), self-reported as white (52.5%), and with higher education status (53%). Regarding the nutritional status of the participants, 35.9% were classified as normal weight, 40.3% were overweight, and 22.9% were obese. The distribution of sociodemographic and lifestyle characteristics according to the selenium intake of all individuals is presented in **Table 1** and those stratified by sex are presented in **Table 2**. Individuals with income ≥ 3 minimum wages as well as men and women with higher education were mostly concentrated in the 5th quintile of selenium consumption (**Table 2**), whereas those who self-reported as white, who practice light physical activity, who were non-smokers and alcohol consumption had selenium intake levels in the last quintile (**Table 2**).

The top 10 foods contributing to selenium intake for all participants are described in **Table 3**. Nuts were the largest contributor (28.9%), followed by cooked fish (8.6%), boneless meat (7.7%), French bread (6.9%), white rice (5.7%), fried fish (3.9%), spaghetti (3.5%), whole-grain bread (2.5%), light bread (2.2%), and whole milk (0.2%) (**Table 3**).

Regarding the contributing foods stratified by sex, there was a difference only from the sixth-ranked foods (**Table 4**). Except nutritional status, all sociodemographic and lifestyle variables investigated were dependent on selenium intake (**Table 5**). As shown in **Table 4**, positive and significant correlations were found between intake of selenium and the following factors: female sex, age ≥ 60 years, income ≥ 3 minimum wages, complete high school and higher or postgraduate education levels, alcohol consumption, and moderate and vigorous physical activity levels. In contrast, smoking and "not white" skin color were negatively associated with selenium intake (**Table 5**).

Table 1. Distribution of sociodemographic and lifestyle characteristics of the study population according to the selenium intake quintiles. ELSA-Brasil, 2008–2010.

Characteristic	N	%	P
Age, years			
Adult	11.598	78.47	< 0.001
Elderly	3.182	21.53	
Body mass index, kg/m²			
Less than 18.5	139	0.94	< 0.001
18.5 to 24.9	5.309	35.93	
25 to 30	5.946	40.25	
More than 30	3.386	22.88	
Self-reported race			
White	7.665	52.46	< 0.001
Not White	6.947	47.54	
Education level			
Complete primary education	1.852	12.53	< 0.001
Complete high school	5.094	34.47	
Higher education or postgraduate	7.834	53	
Income			
< 3SM	7.370	49,86	< 0.001
≥ 3SM	7.410	50,14	
Smoking			
Non-smoker	8.434	57.07	< 0.001
Former smoker	4.429	29.97	
Current smoker	1.916	12.96	
Alcohol intake			
Past	2.969	22.5	< 0.001
Current	10.224	77.5	
Leisure-time physical activity			
Light	11.437	77.04	< 0.001
Moderate	2.029	13.93	
Vigorous	1.314	09.02	

Selenium intake quintiles range from 32.44 mcg/day (minimum) to 1099.34 mcg/day (maximum). Values are presented as absolute numbers (n) or percentages (%). Differences between groups were tested using the Pearson's chi-square test. Statistical significance was set at P < 0.05.

Table 2. Sociodemographic and lifestyle data according to selenium intake stratified by sex. ELSA-Brasil, 2008–2010.

Characteristic	Selenium intake (quintiles)									
	Male					Female				
	1º Q		5º Q		P	1º Q		5º Q		P
Selenium quintiles (mcg/day, min-max)	35.77–105.04		223.06–1054.91			32.44–105.05		223–1099.34		
Age (n, %)										
Adult	1.368	80.7	934	71.03	< 0.001	1.059	81.68	1.203	73.31	< 0.001
Elderly	291	17.54	381	28.97		238	18.35	438	26.69	
BMI (n, %)										
Less than 18.5	23	1.39	8	0.61		10	0.77	18	1.1	
18.5 to 24.9	566	34.14	472	35.92	0.003	467	36.3	719	48.81	< 0.001
25 to 30	719	43.37	608	46.27		463	35.73	555	38.82	
More than 30	350	21.11	226	17.2		356	27.47	349	21.27	
Self-reported race (n, %)										
White	693	42.33	879	68.14	< 0.001	497	38.53	997	61.35	< 0.001
Not White	944	57.67	411	31.86		793	61.47	628	38.65	
Education level (n, %)										
Complete primary education	430	25.92	80	06.08	< 0.001	219	16.89	68	4.14	< 0.001
Complete high school	753	45.39	230	17.49		634	48.88	359	21.88	
Higher education or postgraduate	479	28.69	1.005	76.43		444	34.23	1.214	73.98	
Income (n, %)										
< 3SM	1.160	69,92	359	27.3	< 0.001	901	69.47	476	29.01	< 0.001
≥ 3SM	499	30,08	956	72.7		396	30.53	1.165	70.99	
Smoking (n, %)										
Non-smoker	777	46.84	702	53,38	< 0.001	778	59.98	983	59.9	< 0.001
Former smoker	559	33.69	475	36.12		307	23.67	504	30.71	
Current smoker	323	19.47	138	10.49		212	16.35	154	9.38	
Alcohol intake (n, %)										
Past	374	23.94	190	15	< 0.001	303	29.94	294	20.01	< 0.001
Current	1.188	76.06	1.077	85		709	70.06	1.175	79.99	
Leisure-time physical activity (n, %)										
Light	1.289	79.08	831	63.92	< 0.001	1.117	87.61	1.192	73.76	< 0.001
Moderate	207	12.7	243	18.69		110	8.63	265	16.4	
Vigorous	134	8.22	226	17.38		48	3.76	159	9.84	

A chi-square test was conducted considering all quintiles of selenium intake; however, for better interpretation and data presentation, only the extremes (1st and 5th quintiles) are included in this table. The P values refer to the analysis considering all quintiles. Statistical significance was set at $P < 0.05$.

Table 3. Main food sources of selenium intake. ELSA-Brasil, 2008–2010.

Rank	Food	PC	SD	95% confidence interval
1st	Nuts	28.95	0.82	27.35; 30.56
2nd	Cooked fish	8.63	0.1	8.43; 8.83
3rd	Boneless meat	7.66	0.08	7.52; 7.81
4th	French bread	6.97	0.06	6.85; 7.1
5th	White rice	5.72	0.04	5.65; 5.8
6th	Fried fish	3.91	0.06	3.79; 4.03
7th	Spaghetti	3.53	0.04	3.46; 3.6
8th	Whole-grain bread	2.54	0.04	2.50; 2.62
9th	Light bread	2.2	0.03	2.15; 2.59
10th	Whole milk	02.03	0.03	1.98; 2.08

Nuts include Brazilian nuts, peanuts, cashew nuts, walnuts, and almonds.
PC, percentage contribution; SD, standard deviation.

DISCUSSION

In this study, we identified food sources that were major contributors to the total selenium intake, which included nuts, cooked fish, boneless meat, French bread, and white rice among ELSA-Brasil participants (2008–2010). The nuts consumed included Brazilian nuts (52.6%), peanuts (18.8%), cashew nuts (15%), walnuts (6.8%), and almonds (6.8%). Brazilian nuts represent the largest fraction of nut consumption, which justifies their high selenium concentration and consumption. These foods are part of regular food consumption of Brazilian families, except nuts, owing to their high cost, and meat in individuals from lower socioeconomic classes. In addition, nuts consumption is not common in most regions of Brazil being not a part of the Brazilian eating habits, representing only 0.2% of the total calories consumed per day.[41] A cross-sectional population-based study aimed to identify foods contributing to mineral intake among residents in urban areas of São Paulo revealed that white rice, meat, and bread were the main foods contributing to selenium intake.[16]

Table 4. Main food sources of selenium intake by stratified by sex. ELSA-Brasil 2008–2010

Rank	Food	PC	SD	95% confidence interval
		Men		
1st	Nuts	26.88	1.43	24.07; 29.69
2nd	Cooked fish	8.5	0.15	8.21; 8.78
3rd	Boneless meat	8.12	0.12	7.89; 8.35
4th	French bread	7.74	0.09	7.55; 7.93
5th	White rice	6.79	0.06	6.67; 6.9
6th	Fried fish	4.31	0.09	4.13; 4.5
7th	Spaghetti	3.88	0.06	3.76; 4
8th	Bean	2.44	0.03	2.38; 2.49
9th	Whole milk	2.08	0.04	2; 2.16
10th	Whole-grain bread	1.97	0.05	1.86; 2.07
		Women		
1st	Nuts	30.98	0.81	29.39; 32.57
2nd	Cooked fish	8.76	0.14	8.49; 9.02
3rd	Boneless meat	7.22	0.09	7.03; 7.4
4th	French bread	6.22	0.08	6.06; 6.38
5th	White rice	4.68	0.04	4.60; 4.75
6th	Spaghetti	3.18	0.04	3.10; 3.25
7th	Whole-grain bread	3.11	0.06	2.99; 3.22
8th	Light bread	2.79	0.04	2.71; 2.88
9th	Oat	2.12	0.04	2.04; 2.19
10th	Cooked chicken	2.05	0.04	1.98; 2.12

Nuts include Brazilian nuts, peanuts, cashew nuts, walnuts, and almonds.
PC, percentage contribution; SD, standard deviation.

Many people rarely eat foods that are good sources of selenium, such as Brazilian nuts, the richest dietary source of selenium.[7,8,13,15] However, bread and cereals are commonly consumed and make a substantial contribution to selenium intake, followed by animal products. Ferreira et al.[42] evaluated the selenium content of various foods consumed in the country from different regions and found that the staple foods in the Brazilian diet, such as white rice, beans, wheat flour, corn flour, and cassava flour, contained low levels of this mineral. Significant amount of selenium was present only in meat (both beef and fish). These findings indicate that the Brazilian population is susceptible to selenium deficiency, which is further aggravated among lower-income individuals as they have restricted access to animal foods.

Socioeconomic characteristics, such as sex, age, race/ethnicity, education level, and income, can influence selenium intake among the ELSA-Brasil participants. Dietary selenium intake is higher in older people than in adults, and there is a positive association between age and micronutrient consumption. This observation may reflect healthier diet consumed by older people to minimize age-related disorders, as described by Andrade.[43]

In our analysis, women had a high selenium intake, which might be due to better care of diet quality by women than men. A multi-national study including participants from 23 countries revealed that women focus more on healthy eating habits than do men, and that health beliefs explain a large proportion of dietary behavior.[44] Several other studies conducted in Brazil and other

Table 5. Multiple linear regression model between selenium intake and sociodemographic and lifestyle factors. ELSA-Brasil, 2008–2010.

Predictors	β	95% confidence interval	P
Sex (reference: male)			
Female	6.01	2.51; 9.5	< 0.001
Age group (reference: adults)			
Older people	12.85	8.54; 17.16	< 0.001
Self-reported skin color (reference: white)			
Non-white	(−8.66)	(−12.31; −5.01)	< 0.001
Education level (reference: complete primary school)			
Complete high school	7.22	1.28; 13.16	0.017
Higher education or postgraduate	33.34	26.96; 39.71	< 0.001
Income per capita (reference: < 3 minimum salaries)			
≥ 3 minimum wages	23.95	19.75; 28.14	< 0.001
Smoking (reference: non-smoker)			
Former smoker	6.29	2.43; 10.16	0.001
Current smoker	(−7,77)	(−13.02; −2.53)	0.004
Alcohol (reference: non-consumer)			
Current consumption	4.38	0.19; 8.56	0.040
Nutritional status (reference: normal weight)			
Low weight	(−9.47)	(−27.67; 8.73)	0.308
Overweight	(−11.84)	(−30.04; 6.36)	0.202
Obesity	(−14.7)	(−33.07; 3.68)	0.117
Physical activity level (reference: light)			
Moderate	10.03	5.02; 15.04	< 0.001
Vigorous	15.52	9.57; 21.48	< 0.001

countries also found a better dietary profile in women.[44–47] In addition, women with normal weight (BMI: 18.5–24.9 kg/m²) had a high intake of dietary selenium, reflecting better diet profile adopted by women and their concern about diet quality. A similar study with the same sample from the ELSA-Brasil found a greater consumption of antioxidants, such as vitamins A and E and selenium, by females.[48]

Regarding ethnicity-based dietary differences, individuals who self-reported as "white" (52.5%) had a high intake of dietary selenium. According to our findings, selenium intake, based on the NHANES III 24-h dietary recall, was slightly higher among Whites than Blacks.[49] Racial variations in selenium status has been reported in previous studies as well.[49–52] Non-white population experiences significant disparities in selenium intake, which may be linked to both socioeconomic factors and lack of nutritional knowledge. Previous studies suggest that lower consumption of selenium-rich foods is prevalent among Blacks than among Whites.[49,51,54] Selenium deficiency in this population is concerning as selenium plays a crucial role in antioxidant defense and cardiovascular health.[3,6]

Limited nutritional education and access to resources have contributed to these disparities. Many individuals in underserved communities lack awareness of their specific dietary needs, including the importance of micronutrients, such as selenium, in preventing chronic diseases and supporting mental health.[3] This knowledge gap leads to poor dietary choices and exacerbates health risks.[24]

High school education level is associated with a higher consumption of nutrients such as selenium. In our study, individuals with higher education levels or postgraduates were mostly concentrated in the 5th quintile of selenium consumption for men and women. Consistent with our observations, a previous study by Cardoso et al.[53] with the same ELSA-Brasil sample found that the consumption of a "healthy" diet characterized by fruit, vegetables, whole grains, and low sugar/low fat patterns were present in strikingly higher proportions among individuals with higher education levels.

Socioeconomic status has an important impact on the lifestyle of individuals by determining access to services, goods, and products, including food. Medina et al.[54] verified a better profile of food consumption in high-income and educated social groups. In our study, individuals with higher incomes were mostly concentrated in the 5th quintile of selenium consumption for both men and women, and there was a positive association between high income and consumption of this micronutrient. Although the consumption of selenium was higher in women, not all individuals who self-reported as "white" with high education and higher income had adequate consumption of selenium because the choice of foods in the diet may be determined by other reasons, such as family or personal preferences, availability of foods near home or place of work, seasonal foods, or knowledge about healthy and unhealthy foods.

Individuals who were former smokers, moderate and vigorous physical activity, and alcohol consumption were positively associated with selenium intake. The dietary habits of smokers are usually characterized by a lower intake of antioxidants such as selenium and fiber.[55,56] This suggests that these differences may contribute to the deleterious effects of tobacco smoke components on the risk of cancer and coronary heart disease.[56]

In the present study, alcohol consumption was significantly higher among men and women in the 5th quintile of selenium consumption, and there was a positive correlation between current alcohol consumption and selenium intake. Alcohol consumption is usually accompanied by the consumption of protein snacks, such as meat and nuts, which contribute to the intake of foods rich in selenium. Isobe et al.[57] in a cohort study of 1,183 subjects observed that alcohol intake was associated with relatively selenium-rich foods such as seafood. The results from the NHANES 2003–2008 showed that men consumed more energy from meat and fish on drinking days than on non-drinking days.[58]

A physically active lifestyle can modify eating habits. It has been suggested that physical activity could be a possible gateway for healthier eating and better food choices.[59,60] In our study, there was a positive correlation between light physical activity and selenium intake.

In this study, foods that contributed mostly to dietary selenium intake were nuts, meat, and fish among the individuals evaluated, and low intake of selenium was associated with the following conditions: male sex, low education, low income, non-white skin color, and younger age, indicating that the Brazilian population may be susceptible to selenium deficiency; public policies focused on the increased intake of foods rich in selenium must be directed specifically to groups with specific characteristics of low selenium intake.

CONCLUSION

Nuts, meat, and fish contributed the most to selenium intake in the diet. In addition, selenium intake was associated with sociodemographic and lifestyle factors, such as sex, age group, education, income, ethnicity, smoking, and physical activity among the evaluated individuals.

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Subclinical hypothyroidism, focusing on carpal tunnel syndrome and peroneal neuropathy at the fibular head: a prospective case-control study

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ABSTRACT

BACKGROUND: Peripheral nerves may be affected in subclinical hypothyroidism (SH).

OBJECTIVES: This study aimed to investigate the presence of common entrapment mononeuropathies in patients with SH.

DESIGN AND SETTING: A prospective case-control study conducted between September 2022 and November 2023 at Adana City Training and Research Hospital, Adana, Türkiye.

METHODS: SH patients without neurological complaints and healthy individuals over the age of 18 were included. Serum levels of free T3, free T4, thyroid-stimulating hormone (TSH), thyroid peroxidase (TPO) antibody, and creatine kinase (CK) were measured. All participants underwent nerve conduction studies of the upper and lower extremities.

RESULTS: Thirty patients with SH and 40 healthy individuals were included in the study. The percentage reduction in compound muscle action potential (CMAP) amplitude across the knee segment was 2.8 ± 3.5% in healthy individuals and 6.7 ± 6.6% in SH patients (P = 0.017). Apart from this significant difference, other nerve conduction study findings did not differ between the two groups. A positive correlation was observed between CK levels and the percentage reduction in peroneal nerve CMAP amplitude across the knee segment (P = 0.021, r = 0.421). Additionally, there was a positive correlation between TPO antibody levels and F-wave latency in both the median and ulnar nerves (P = 0.028 r = 0.400/P = 0.005 r = 0.501). Electrodiagnostic evaluations revealed carpal tunnel syndrome (CTS) in four patients, peroneal neuropathy at the fibular head (PNFH) in four patients, and ulnar neuropathy at the elbow (UNE) in one patient.

CONCLUSION: This study suggests that patients with SH may develop subclinical CTS and PNFH, but not UNE. Accordingly, it highlights the importance of avoiding risk factors that may contribute to the development of CTS and PNFH. Serum CK and TPO antibody levels may be useful in monitoring subclinical neuropathy in SH.

INTRODUCTION

Peripheral nerves can become compressed while passing through narrow structures, leading to entrapment mononeuropathies, which manifest clinically as symptoms ranging from paresthesias to weakness.[1–3] Carpal tunnel syndrome (CTS) and ulnar neuropathy at the elbow (UNE) are common forms of upper extremity entrapment mononeuropathies. Meanwhile, peroneal neuropathy at the fibular head (PNFH) is a prevalent lower extremity entrapment neuropathy.[2] Many factors, including certain occupations and chronic diseases like diabetes mellitus and thyroid disease, are associated with these neuropathies.[1–4] Neuropathy linked to thyroid conditions can lead to reduced deep tendon reflexes, muscle weakness, neuropathic pain, and paresthesias, significantly affecting daily activities. Although the precise pathophysiology of neuropathy in thyroid diseases is not fully understood, reductions in adenosine triphosphate activity and metabolic alterations are believed to play a role. These changes may cause damage to the nerve cell body, myelin sheath, or axons.[6,7] While it is well-established that thyroid diseases are risk factors for neuropathies such as CTS and polyneuropathy, their relationship with UNE or PNFH remains less understood.[4–7]

OBJECTIVE

Electrophysiological tests are crucial in diagnosing entrapment mononeuropathies, providing essential information for both diagnosis and prognosis.[8,9] In motor and sensory nerve

conduction studies, common electrophysiological abnormalities linked to thyroid diseases include reduced potential amplitudes and moderately slowed conduction velocities.[7] Notably, these abnormalities have been observed in patients with subclinical hypothyroidism (SH) who do not have neuropathic symptoms, suggesting that thyroid diseases might exert a subclinical impact on peripheral nerves.[10] Research has been conducted to determine whether there is an increased predisposition to CTS, UNE and PNFH in patients with SH who do not present neuropathic complaints.

METHODS

Subjects and study design

Patients with SH displaying normal thyroid function and healthy individuals who presented to the Internal Medicine Department of the University of Health Sciences Adana City Training and Research Hospital (ACTRH) between September 2022 and November 2023 were included in this study. Ethics committee approval for this prospective case-control study was obtained from the ACTRH Clinical Research Ethics Committee (approval number 1934/105/2022). Written consent was secured from all participants. Neurological examinations were conducted on both SH patients and healthy individuals, and the levels of serum free T3 (T3), free T4 (T4), thyroid stimulating hormone (TSH), thyroid peroxidase (TPO) antibody, and creatine kinase (CK) were assessed. SH was considered in patients exhibiting the following characteristics:[11–13] 1) positivity for thyroglobulin antibody and/or thyroid peroxidase antibody; 2) thyroid ultrasonography results consistent with SH. Patients were required to have been diagnosed with SH for at least one month. Both SH patients and healthy individuals were excluded from the study if they exhibited any of the following: 1) sensory abnormalities or weakness in the extremities; 2) abnormalities in neurological examinations; 3) any neurodegenerative disease; 4) conditions or diseases predisposing to neuropathy, such as diabetes mellitus; 5) abnormal levels of T3, T4, or TSH. Additionally, healthy individuals with abnormal levels of TPO antibody, thyroglobulin antibody, or CK were also excluded from the study. Neurophysiological tests were conducted on all participants at the University of Health Sciences ACTRH Neurophysiology Laboratory. These tests, along with the assessment of serum T3, T4, TSH, TPO antibody, and CK levels, were all performed on the same day.

Electrodiagnostic tests

Nerve conduction studies were performed on one upper and one lower extremity of each participant using the Cadwell Sierra Summit EMG unit (Cadwell Laboratories, Kennewick, Washington, USA). Electrodiagnostic tests were conducted only if the extremity temperature was above 32°C; otherwise, the limb was warmed prior to testing. For motor and sensory nerve conduction studies, the high- and low-pass filter settings were 20 Hz–10 kHz and 20 Hz–2 kHz, respectively. Surface electrodes were used for both stimulation and recording, and stimulation was applied supramaximally. Sweep speed and sensitivity were set to 5 ms/2 mV for motor studies and 1 ms/10 μV for sensory studies. Compound nerve action potential (CNAP) and compound muscle action potential (CMAP) amplitudes were calculated by measuring from peak to peak. Median and ulnar nerve CNAPs were recorded antidromically from the 2nd and 5th fingers, respectively. Sural and superficial peroneal nerve CNAPs were also obtained antidromically. Sensory nerve conduction velocity (NCV) was calculated using peak latency for the median, ulnar, and sural nerves, and onset latency for the superficial peroneal nerve. Stimulation sites for motor nerve conduction studies were as follows: median nerve—wrist to elbow; ulnar nerve—wrist, below elbow, and above elbow; peroneal nerve—ankle, below the fibular head, and popliteal fossa; posterior tibial nerve—ankle to popliteal fossa. Peroneal nerve CMAPs were recorded from both the extensor digitorum brevis and tibialis anterior muscles. Among the ten F-wave responses recorded, the one with the shortest latency was selected for analysis.

Identifying entrapment neuropathy

CTS was considered electrophysiologically if any of the following criteria were met:[14–16] 1) median sensory NCV across the 2nd finger–wrist segment < 39 m/s; 2) In addition to slowed median sensory NCV, median nerve CMAP distal latency > 3.7 ms; 3) If the median nerve CNAP could not be obtained, and the median nerve CMAP distal latency was > 3.7 ms.

UNE was diagnosed electrophysiologically if any of the following findings were present:[16–18] 1) Ulnar motor NCV across the elbow segment < 43 m/s; 2) CMAP amplitude reduction across the elbow segment > 20%; 3) A difference in ulnar motor NCV > 15 m/s between the forearm and elbow segments.

PNFH was diagnosed electrophysiologically if any of the following criteria were met:[17,19,20] 1) peroneal motor NCV < 40.1 m/s (recorded from the extensor digitorum brevis [EDB]) or < 41 m/s (recorded from the tibialis anterior [TA]) across the knee segment; 2) CMAP amplitude decrease > 25% across the knee segment; 3) peroneal motor NCV difference > 6 m/s between the leg segment and the knee segment.

Statistical analysis

Numerical data are presented with the mean ± standard deviation (SD). Frequency and percentage were used to define categorical variables. Pearson's chi-square test was used to compare categorical data between two groups. Numerical data were

compared between the two groups with the Mann-Whitney U test. Correlation analyses were performed using Spearman's correlation test. It was considered statistically significant if $P < 0.05$. SPSS 22.0 program was used for statistical analysis.

RESULTS

Thirty-three patients with SH were initially assessed; however, three were excluded due to the presence of diabetes mellitus and polyneuropathy, confirmed by nerve conduction studies. Two of these three patients also had electrophysiological findings consistent with CTS. Ultimately, 30 SH patients (4 male, 26 female) and 40 healthy individuals (7 male, 33 female) were included in the study ($P = 0.747$ for sex comparison). The mean ages of the SH patients and healthy individuals were 39.9 ± 12.6 years (range: 18–65) and 38.5 ± 11.4 years (range: 18–64), respectively ($P = 0.557$). The mean body mass index (BMI) was 30.3 ± 5.7 kg/m² (range: 21.5–42.9) for SH patients and 25.3 ± 3.8 kg/m² (range: 17.9–27.4) for healthy individuals ($P < 0.001$). The mean duration of SH was 7.2 ± 7.6 months (range: 1–30), with 21 patients (70%) having a disease duration of less than 6 months. All SH patients were receiving levothyroxine therapy. The mean values of laboratory parameters in SH patients were as follows: T3, 5.4 ± 7.9 ng/dL; T4, 0.9 ± 0.2 ng/dL; TSH, 3.9 ± 2.7 mIU/L; TPO antibody, 485.8 ± 544.7 IU/mL; and CK, 94.7 ± 46.3 U/L. T3, T4, and TSH levels were within the normal range in both SH patients and healthy individuals.

Comparisons of sensory and motor nerve conduction study findings between the groups are presented in **Tables 1** and **2**, respectively. Nerve conduction studies were performed on the right/left upper and lower extremities in 22/8 SH patients and 24/16 healthy individuals ($P = 0.245$). **Figure 1** illustrates the percentage reduction in peroneal nerve CMAP amplitude across the segment from below the fibular head to the popliteal fossa, comparing the two groups. Patients with electrodiagnostic findings suggestive of entrapment neuropathy are listed in **Table 3**. A negative correlation was observed between TPO antibody levels and both median nerve CNAP amplitude in the 2nd finger–wrist segment and ulnar motor NCV across the elbow segment ($P = 0.004$ $r = -0.509$/$P = 0.022$ $r = -0.416$) (**Figure 2**). A positive correlation was found between TPO antibody levels and F-wave latency in both the median and ulnar nerves ($P = 0.028$ $r = 0.400$/$P = 0.005$ $r = 0.501$). Additionally, a positive correlation was identified between T3 levels and ulnar motor NCV across the elbow segment, while a negative correlation was found between T3 levels and the NCV difference between the elbow and forearm segments ($P = 0.032$ $r = 0.392$, $P = 0.031$ $r = -0.394$). **Figure 3** displays the positive correlation between CK levels and the percentage reduction in peroneal nerve CMAP amplitude across the segment from below the fibular head to the popliteal fossa segment ($P = 0.021$, $r = 0.421$).

DISCUSSION

In the present study, unlike other nerve conduction study findings, the reduction in peroneal nerve CMAP amplitude across the knee segment was greater in SH patients than in healthy individuals. Although the patients were asymptomatic, electrodiagnostic findings were consistent with CTS in four patients, PN in four patients, and UNE in one patient. Additionally, associations were observed between TPO antibody, T3, and CK levels and certain nerve conduction study findings.

The association between neuropathy—particularly CTS—and SH is well established.[4] In the present study, subclinical CTS was also identified in some patients. However, the pathophysiology of CTS and other peripheral neuropathies in SH remains unclear. One proposed mechanism is mucinous infiltration of the tissues surrounding the nerve.[5,7,21] Another possible explanation is impaired Na^+/K^+ pump function due to reduced ATPase activity in SH, which may disrupt axonal transport and lead to peripheral neuropathy.[5,7] Although UNE is a common entrapment mononeuropathy, only one patient in this study showed electrophysiological findings consistent with subclinical UNE. This may suggest that UNE is a relatively rare manifestation in SH. Whether thyroid disease is a definitive risk factor for UNE remains unclear.[22]

The association between PNFH and factors such as weight loss, prolonged leg positioning (e.g., crossing the legs or squatting), and chronic diseases is well documented.[20,23,24] In cases of PNFH related to weight loss, the peroneal nerve becomes more susceptible

Table 1. Comparison of sensory nerve conduction study findings between healthy individuals and patients with SH

Sensory nerve conduction study	Healthy individuals	SH patients	P value
Median nerve CNAP amplitude (μV)	41.8 ± 21.3 (39.6) (14.2-110.4)	44.0 ± 16.8 (43.1) (19.7-79.5)	0.336
Median sensory NCV- 2nd digit-wrist (m/s)	47.2 ± 4 (46.5) (41.1-58)	46.2 ± 4.5 (47) (36-53.2)	0.517
Ulnar nerve CNAP amplitude (μV)	44.7 ± 17.6 (42.9) (13.3-96.3)	51.2 ± 21.8 (51.1) (9.5-85.7)	0.149
Ulnar sensory NCV- 5th digit-wrist (m/s)	45.0 ± 4.3 (44.5) (39.1-56)	45.3 ± 4.4 (44.8) (36.7-55.7)	0.771
Sural nerve CNAP amplitude (μV)	19.2 ± 7.4 (18.5) (8.3-35)	17.0 ± 8.3 (15.4) (5.2-44.1)	0.194
Sural sensory NCV (m/s)	43.5 ± 5.6 (41.9) (35-56)	44.5 ± 6.2 (42.5) (35-63)	0.458
Superficial peroneal nerve CNAP amplitude (μV)	12.2 ± 4.3 (11.4) (5.3-20.9)	12.8 ± 5.3 (12.1) (6-28)	0.981
Superficial peroneal sensory NCV (m/s)	49.6 ± 6.1 (49.2) (39-64)	50.6 ± 7.3 (52.5) (35-60)	0.204

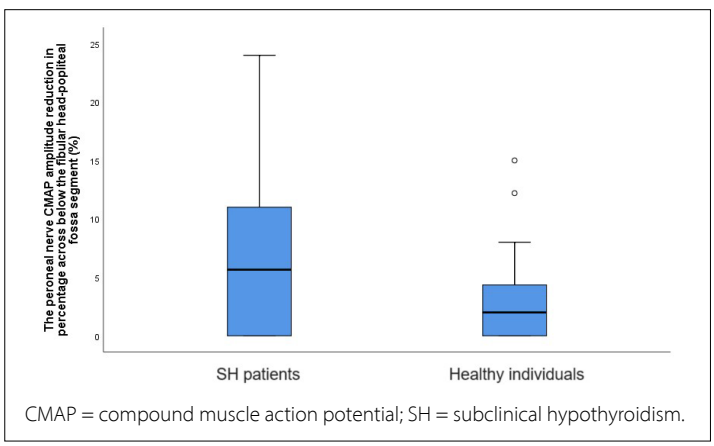
CNAP = compound nerve action potential; SH = subclinical hypothyroidism; NCV = nerve conduction study.

Table 2. Comparison of motor nerve conduction study findings between healthy individuals and patients with SH

Motor nerve conduction study	Healthy individuals Mean SD	SH patients Mean SD	P value
Median nerve			
Distal latency (ms)	2.9 ± 0.3 (2.9) (2.2-3.7)	2.9 ± 0.4 (2.9) (2.2-3.8)	0.664
CMAP amplitude (mV)	15.1 ± 5.1 (15.1) (5.1-26.7)	13.8 ± 4.7 (12.8) (7.6-24.2)	0.178
Motor NCV- wrist-elbow (m/s)	57.6 ± 4.9 (57) (50-67.3)	59.8 ± 4.6 (59.4) (52.1-71)	0.079
F-wave latency (ms)	25.9 ± 1.9 (26.1) (22.2-31.1)	26.1 ± 1.9 (25.8) (23-31)	0.905
Ulnar nerve			
Distal latency (ms)	2.2 ± 0.3 (2.2) (1.8-3)	2.2 ± 0.3 (2.2) (1.9-3.2)	0.633
CMAP amplitude (mV)	14.3 ± 3.1 (14.2) (8.8-21.6)	16.6 ± 7.7 (15.3) (11.2-56)	0.070
Motor NCV- wrist-below elbow (m/s)	63.2 ± 4.2 (63.4) (54.2-69)	64.5 ± 5.6 (65) (50-72.4)	0.121
Motor NCV- below elbow-above elbow (m/s)	59.1 ± 7.3 (59.5) (43-71)	60.0 ± 6.6 (60.7) (47-70)	0.605
CMAP amplitude reduction in percentage- below elbow-above elbow (%)	3.7 ± 4.3 (2.7) (0-14.9)	5.9 ± 5.8 (4.9) (0-17)	0.143
Motor NCV difference between elbow segment and forearm segment	4.1 ± 7.2 (5) (-11.9-16)	4.5 ± 6.1 (4.5) (-10.2-18.1)	0.934
F-wave latency (ms)	26.9 ± 3.7 (26.3) (23-45.8)	25.5 ± 2 (25.5) (22-29.6)	0.098
Peroneal nerve-EDB			
Distal latency (ms)	3.4 ± 0.5 (3.3) (2.7-4.5)	3.3 ± 0.5 (3.3) (2.3-4.5)	0.399
CMAP amplitude (mV)	9.4 ± 4.7 (8.8) (3.3-27.2)	8.2 ± 3.6 (7.7) (2.8-19.1)	0.262
Motor NCV- ankle-below fibular head (m/s)	50.3 ± 3.8 (48.5) (42.7-59)	52.1 ± 5.2 (51.4) (46.6-69.8)	0.137
Motor NCV- below fibular head-popliteal fossa (m/s)	52.2 ± 7.6 (50) (41.1-70)	52.8 ± 6.8 (53) (38.8-70)	0.304
CMAP amplitude reduction in percentage- below fibular head-popliteal fossa (%)	2.8 ± 3.5 (2) (0-15)	6.7 ± 6.6 (5.7) (0-24)	**0.017**
Motor NCV difference between knee segment and leg segment	-1.9 ± 8.6 (0) (-23.5-11)	-0.7 ± 4.7 (-1.7) (-8.5-13)	0.614
*F-wave latency (ms)**	46.3 ± 2.9 (45.8) (42-52.5)	45.6 ± 2 (45.6) (42-49)	0.496
Peroneal nerve-TA			
CMAP amplitude (mV)	10.2 ± 3 (10.1) 4.0-17.2)	9.1 ± 3 (9.1) (3.7-14)	0.146
Motor NCV- ankle-below fibular head (m/s)	55.5 ± 9.5 (55) (41-72)	58.5 ± 8.2 (58.7) (44-71)	0.208
CMAP amplitude reduction in percentage- below the fibular head-popliteal fossa (%)	3.5 ± 4.2 (1.6) (0-13.9)	4.7 ± 7.1 (3) (0-34.9)	0.577
Posterior tibial nerve			
Distal latency (ms)	4.1 ± 0.8 (4.1) (2.7-5.9)	3.8 ± 0.8 (3.6) (2.3-6)	0.062
CMAP amplitude (mV)	14.1 ± 4.9 (13.3) (5.8-29)	12.4 ± 6.2 (10.8) (4.5-31)	0.047
Motor NCV- ankle-popliteal fossa (m/s)	46.2 ± 4.6 (45.6) (36-60)	48.0 ± 6.1 (48.3) (35.5-58)	0.154
F-wave latency (ms)	48.2 ± 4.1 (48.2) (42-59)	46.7 ± 3.5 (47.1) (40-56.1)	0.194

CMAP = compound muscle action potential; SH = subclinical hypothyroidism; NCV = nerve conduction study.

* F-wave was obtained in 34 healthy individuals and 24 patients with SH.



CMAP = compound muscle action potential; SH = subclinical hypothyroidism.

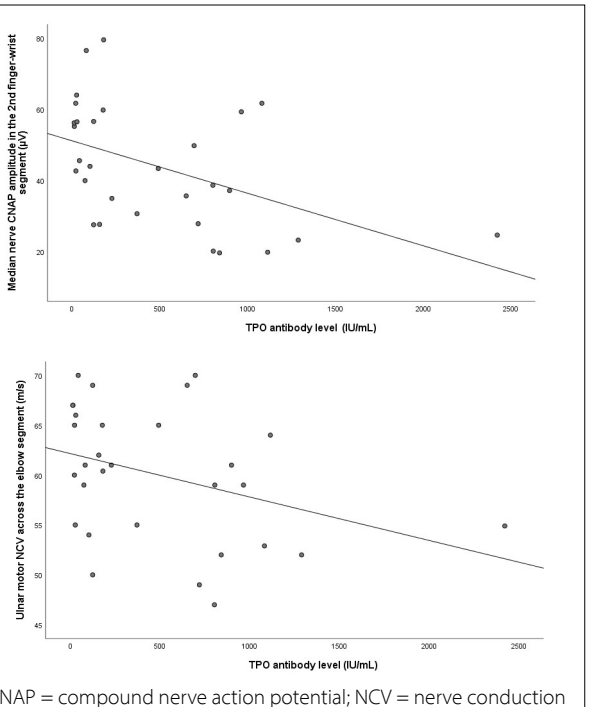
Figure 1. Comparison of peroneal nerve CMAP amplitude reduction in percentage across below the fibular head-popliteal fossa segment between healthy individuals and SH patients.

to entrapment due to the reduction of protective adipose tissue around the fibular head.[20,23,24] Patients with SH may experience fluctuations in weight, which can similarly reduce the protective tissue surrounding the peroneal nerve.[20,23–25] The observed positive correlation between serum CK levels and the reduction in peroneal nerve CMAP amplitude across the knee segment may suggest that this relationship is mediated by the loss of protective tissue in SH. However, the occurrence of PNFH in patients who have experienced weight loss but do not report prolonged leg positioning or trauma indicates that non-mechanical factors may also contribute to its development.[26,27] It has been proposed that metabolic changes, such as disruptions in lipid metabolism associated with weight loss, may play a role in the pathophysiology of PNFH.[26–28] Similarly, metabolic alterations in SH may lead to a deficiency of nutrients essential for nerve function, resulting in peripheral nerve damage.[7] The peroneal nerve conduction abnormalities observed in this study could also be attributed to autoimmune mechanisms involving cytokines.[12] Another possible explanation is mucinous infiltration of the tissues surrounding the nerve or impaired axonal transport caused by reduced Na^+/K^+ pump activity due to decreased

Table 3. Patients with electrodiagnostic findings suggestive of entrapment neuropathy according to neurophysiology tests

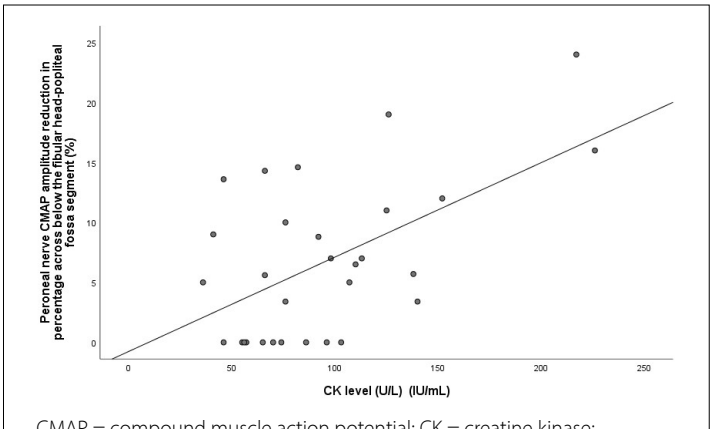
Patient	Age (years)/sex	Electrodiagnostic abnormality	Entrapment neuropathy according to neurophysiology tests
Patient 3	48/F	Median sensory NCV- 2nd digit-wrist (m/s)	CTS
Patient 5	42/F	CMAP amplitude reduction in percentage- below fibular head-popliteal fossa (%) – TA	PNFH
Patient 6	18/F	Peroneal Motor NCV difference between knee segment and leg segment -EDB	PNFH
Patient 11	36/M	Median sensory NCV- 2nd digit-wrist (m/s), Median nerve CMAP distal latency (ms)	CTS
Patient 13	64/M	Median sensory NCV- 2nd digit-wrist (m/s), Median nerve CMAP distal latency (ms), CMAP amplitude reduction in percentage- below fibular head-popliteal fossa (%) – EDB	CTS, PNFH
Patient 14	38/F	Peroneal Motor NCV difference between knee segment and leg segment -EDB	PNFH
Patient 27	43/F	Ulnar Motor NCV difference between elbow segment and forearm segment	UNE
Patient 30	49/F	Median sensory NCV- 2nd digit-wrist (m/s)	CTS

CMAP = compound muscle action potential; CNAP = compound nerve action potential; CTS = carpal tunnel syndrome; EDB = extensor digitorum brevis; NCV = nerve conduction velocity; PNFH = peroneal neuropathy at the fibular head; TA = tibialis anterior; UNE = ulnar neuropathy at the elbow.



CNAP = compound nerve action potential; NCV = nerve conduction velocity; SH = subclinical hypothyroidism; TPO thyroid peroxidase.

Figure 2. Correlation between TPO antibody level and median nerve CNAP amplitude in the 2nd finger-wrist segment/ulnar motor NCV across the elbow segment in SH patients.



CMAP = compound muscle action potential; CK = creatine kinase; SH = subclinical hypothyroidism; TPO = thyroid peroxidase.

Figure 3. Correlation between CK level and peroneal nerve CMAP amplitude reduction in percentage across below the fibular head-popliteal fossa segment in SH patients.

thyroid hormone levels, as has been suggested in the pathogenesis of CTS in hypothyroid conditions.[5,7,21]

Reduction in peroneal nerve CMAP amplitude at the fibular head is a characteristic electrophysiological feature of PNFH. Most patients with PNFH who do not have chronic diseases and whose condition is associated with prolonged leg postures or weight loss typically show improvement.[20,23,24] These features suggest that although partial axonal degeneration may occur in PNFH, demyelinating processes are more prominent. Both axonal degeneration and segmental demyelination have been implicated in the pathophysiology of subclinical hypothyroidism.[4,7,10,29,30] The reduction in peroneal nerve CMAP amplitude across the knee segment observed in this study suggests that neuropathies associated with subclinical hypothyroidism may also exhibit prominent demyelinating features. Furthermore, the association of demyelinating neuropathies—such as acute inflammatory demyelinating polyradiculopathy—with subclinical hypothyroidism supports the findings of the present study.[29,30]

Although some studies have not identified electrodiagnostic abnormalities in patients with hypothyroidism, the majority suggest that both subclinical and clinical nerve involvement may occur.[4,7,10,31] We believe that these discrepancies—or the observation that some studies report abnormalities in certain nerves while others do not—may be attributed to methodological differences. Factors such as disease duration, age, and sex could have influenced the results in both previous studies and the current one. Age and female sex have been reported as risk factors for hypothyroidism.[6]

In the present study, associations were found between nerve conduction study findings and TPO antibody, T3, and CK levels in SH patients. These results suggest that thyroid function tests, along with TPO antibody and CK levels, may be helpful in monitoring subclinical neuropathy. It has also been reported that as basal TSH levels increase, abnormalities in nerve conduction studies become more pronounced.[10]

This study has several limitations. The duration of SH varied between 1 and 30 months; however, it is important to note that the disease duration was less than 6 months in the majority of patients. BMI differed between healthy individuals and SH patients, which, while consistent with findings from previous studies may have influenced the results and thus represents a potential limitation. [4,6,7] Another limitation is that all SH patients were receiving treatment for hypothyroidism. We believe that comparative studies involving treated and untreated patients could offer valuable insights into the relationship between hypothyroidism and neuropathy, particularly regarding peroneal nerve conduction. Additionally, individuals with conditions known to predispose to polyneuropathy or entrapment mononeuropathy—such as diabetes mellitus—were not included as a separate group in this study. Including such individuals, both with and without subclinical hypothyroidism, in future research could help generate more comprehensive findings.

CONCLUSION

This study demonstrated that subclinical CTS and PNFH may occur in SH, whereas UNE was not observed. It is recommended to avoid risk factors such as excessive use of the upper extremities—which may contribute to CTS—as well as weight loss and prolonged maintenance of the same leg posture, which may increase the risk of PNFH. Additionally, T3, TPO antibody, and CK levels may serve as useful markers for monitoring the subclinical effects of SH on peripheral nerves. However, further studies are needed to confirm the reliability and clinical significance of these findings.

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Epidemiology of severe heart disease among Unified Health System (SUS) users in Rio Grande do Norte: a cross-sectional study

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Clinical profile.

ABSTRACT

BACKGROUND: Severe heart disease has high prevalence, morbidity, and mortality rates. Heart stimulation is important in the final stages of heart disease. The concentration of procedures in a service allows for the epidemiological analysis of our population.

OBJECTIVE: To analyze the epidemiological profile of severe heart disease in Rio Grande do Norte by registering all patients undergoing artificial cardiac stimulation (ACS) in a Unified Health System reference service in Rio Grande do Norte.

DESIGN AND SETTING: This cross-sectional study included all patients who underwent ACS procedures at the Hospital Onofre Lopes, Universidade Federal do Rio Grande do Norte (UFRN), from 2006 to 2021. Sociodemographic characteristics, procedures, and health conditions were examined. Additionally, a spatial analysis of casuistry was performed according to the municipality of origin.

METHODS: This cross-sectional study analyzed data derived from patients treated at Hospital Onofre Lopes, UFRN, from 2006 to 2021, including sociodemographic characteristics, procedures, and health conditions.

RESULTS: A total of 894 patients (male, 59.8%; mean age: 65.5 years) were included. Third-degree atrioventricular block was indicated in 191 patients, an ischemic etiology was found in 269 patients, whereas dyspnea was reported by 398 patients. Furthermore, 69.5%, 24.4%, and 31.7% of patients had hypertension, diabetes, and dyslipidemia, respectively. Spatial analysis showed no significant differences in the formation of clusters.

CONCLUSIONS: The characteristics of the service contributed to possible differences in the literature. The spatial distribution of severe heart disease was random in the state, indicating an adequate distribution of reference services even in the absence of a defined flowchart for such services.

INTRODUCTION

Severe heart disease has a high prevalence and incidence, with a substantial impact on morbidity and mortality in general population. Low investment in health, inadequate access to care, and insufficient follow-up services at the primary or tertiary level are potential risk factors.[1] In addition to medications, patients require support in the final stages of heart disease, and artificial cardiac stimulation (ACS) is necessary for treating heart failure (HF).[2,3]

In the Unified Health System (SUS), procedures are performed in public or private referral centers. Complex procedures performed for severe heart disease are restricted to some centers, usually public, according to a specific ordinance by the Ministry of Health.[4] In Rio Grande do Norte, complex procedures are performed in this period exclusively at the Hospital Universitário Onofre Lopes (HUOL), Universidade Federal do Rio Grande do Norte (UFRN). Thus, the HUOL focuses on all SUS patients undergoing procedures, specifically those with severe heart disease. Therefore, this study represented practically all SUS patients in Rio Grande do Norte who underwent complex procedures related to severe heart disease. Data on this population in our country are scarce. In Brazil, few centers portray their populations based on epidemiological patterns.

Universal access to health services is not only a constitutional guarantee and a pillar of the SUS but also an expression of the right to citizenship. Citizenship has a supervisory and action role with the potential for concrete results, change, and improvement in care. Access to health services is a transformative tool of reality.[5]

Spatial analysis can describe the characteristics and patterns existing in geographical spaces for a given factor and establish relationships among different variables quantitatively.[6] It is a

fundamental instrument in public health that enables the development of technologies for data analysis in a geographical space. A detailed study on the health situation and its trends allows for the identification of variables that reveal the social, economic, and environmental structure of the environment.[7]

Access to specialized public health service by the entire population, irrespective of their social condition or place of origin, is a premise and obligation of the SUS. This has not been previously evaluated in relation to ACS in Rio Grande do Norte. Given the group of highly complex procedures and the absence of well-defined flowcharts for access to the reference service, spatial analysis can aggregate information for health managers and the scientific community. Additionally, the analysis of this sample establishes an epidemiological profile that characterizes an SUS referral service and provides an estimate of the prevalence of severe heart disease in a state in Northeastern Brazil.

OBJECTIVE

The current study primarily aimed to evaluate the epidemiological profile of SUS patients in a referral service for ACS in Rio Grande do Norte from 2006 to 2021. The specific objectives were (i) to analyze the characteristics of the population served in relation to demographic and epidemiological peculiarities, use of medications, and particularities of procedures and (ii) to descriptively evaluate the spatial distribution of patients served based on the municipality of origin.

METHODS

This quantitative, analytical, cross-sectional retrospective study was conducted at the HUOL, a reference center for ACS by the SUS in Rio Grande do Norte. Data were collected from primary sources, and medical records were analyzed. All patients undergoing ACS procedures in this service from 2006 to 2021 were included, totaling 894 patients.

Data were obtained by reviewing the medical records based on the institution's list of procedures. The analysis was performed using physical records requested from a specific sector. In cases of nonlocation, electronic medical records were searched. Because the sample comprised all procedures performed during that period, there were no exclusion criteria.

For the sociodemographic profiles, data related to age and sex were evaluated. The variables assessed in the epidemiological profile were divided into four groups—namely, (i) epidemiological characteristics (residence, underlying disease, symptomatology); (ii) comorbidities (systemic arterial hypertension, diabetes, kidney failure, dyslipidemia, HF, smoking, catheterization changes, valvular heart disease); (iii) use of medications (amiodarone, angiotensin-converting enzyme [ACE] inhibitors, digoxin, diuretic, acetylsalicylic acid [ASA], spironolactone, beta-blockers); and (iv) characteristics of the procedures (electrocardiographic alteration, procedure, need for temporary pacemaker, implanted device, stimulation mode, and complications).

Data were stored in Microsoft Excel spreadsheets and were analyzed via Stata, a statistical software for data science, to establish the epidemiological profile. Categorical variables were expressed as absolute values and relative frequencies, whereas continuous variables were presented as measures of central tendencies.

For the spatial analysis, the rate was established based on the number of procedures per 100,000 inhabitants in the municipality of origin. Thematic maps were prepared using QGIS software, and univariate spatial analysis was performed with Global Moran's I index using GeoDa software to verify the level of spatial interdependence between the analysis units (municipalities).

A reporting guide for observational studies (STROBE Statement) was used. Data collection was initiated after the approval of the project by the Research Ethics Committee of UFRN (opinion number: 4.880.641) on August 3, 2021.

RESULTS

From 2006 to 2021, 894 ACS procedures were performed at the HUOL-UFRN. Among the patients treated, 533 were male (59.8%). The patients' mean age was 63.8 years for males (median, 65 years) and 68.1 years for females (median, 69 years), with age extremes ranging from 13 to 100 years.

Regarding the patients' region of origin, more than half came from the metropolitan region of Natal, Rio Grande do Norte. The predominant underlying etiology was ischemia, which was detected in 269 (30.6%) patients. Chagas disease, valvular heart disease, and other etiologies were identified in 98 (11.2%), 98 (11.2%), and 28 (3.2%) patients, respectively. A causal etiology was not established in 421 (47.9%) patients.

Presyncope or syncope was the main complaint in 462 patients (52.7%), among whom 38% and 14.7% had syncope and presyncope, respectively. Dyspnea was reported in 398 (45.4%) patients. Only 7 (0.8%) patients presented with a cardiorespiratory arrest event, and symptomatology was not reported in 9 (1%) patients (**Figure 1**).

With respect to related comorbidities, systemic arterial hypertension occurred in 612 patients (69.5%), congestive HF in 557 (63.2%), dyslipidemia in 280 (31.7%), diabetes mellitus in 215 (24.4%), renal failure in 122 (14.4%), and valvular heart disease on Doppler echocardiography in 75 (9.3%). Regarding smoking, 462 (52.3%) patients denied current or previous smoking, 58 (6.6%) were smokers, and 363 (41.4%) reported having stopped smoking for at least 5 years. Obstructive lesions in the coronary arteries were verified at catheterization in 260 patients (29.6%), including 213 who did not undergo the examination (**Figure 2**).

The most directly used medications with cardiovascular effects were ACE inhibitors or angiotensin receptor blockers in 657 patients (74.6%), beta-blockers in 577 (65.5%), spironolactone in

478 (54.3%), diuretics in 466 (52.9%), ASA in 379 (43.1%), digoxin in 252 (28.7%), and amiodarone in 218 (24.7%).

Electrocardiographic alterations indicative of the procedure were left bundle branch block in 245 patients with HF (27.4% of the sample), followed by third-degree atrioventricular (AV) block in 191 patients (21.4%). A total of 163 (18.3%) patients underwent generator replacement. Tachycardia events determined the need for the procedure in 121 (13.5%) patients.

The most commonly performed procedure was device implantation (n = 610, 68.6%), followed by generator replacement due to battery exhaustion and/or electrode dysfunction (n = 218, 24.5%) and other types of approaches (n = 61, 6.9%).

According to the type of device used, 346 (39.1%) patients required a conventional pacemaker implant, 292 (33%) patients required atrio-biventricular pacemaker (cardiac resynchronization therapy [CRT]) implants, and 154 (17.4%) patients required a double-chamber implantable cardioverter-defibrillator (ICD). ICD with CRT, which was the most complex prosthesis, was used in 94 (10.6%) patients.

Dual-chamber stimulation, stimulating the atrium and ventricle(s), corresponded to the mode in 810 (90.8%) patients, with only ventricular stimulation in 76 (8.5%) patients and only atrial stimulation in three (0.3%) patients. In the three procedures, the system was completely removed without device replacement (0.3%).

The complication rate was 7.6% (**Figure 3**). The mean left ventricular ejection fraction (LVEF) was 33.6% (median, 30%). The mean creatinine level was 1.04 mg/dL (median,

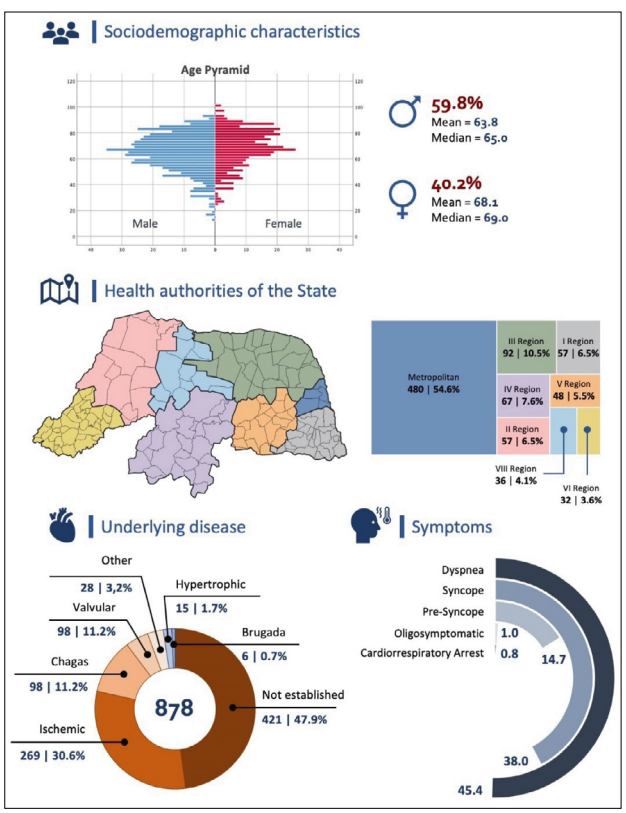

Figure 1. Infographic shows the demographic and epidemiological characteristics of the sample. Rio Grande do Norte, 2021.

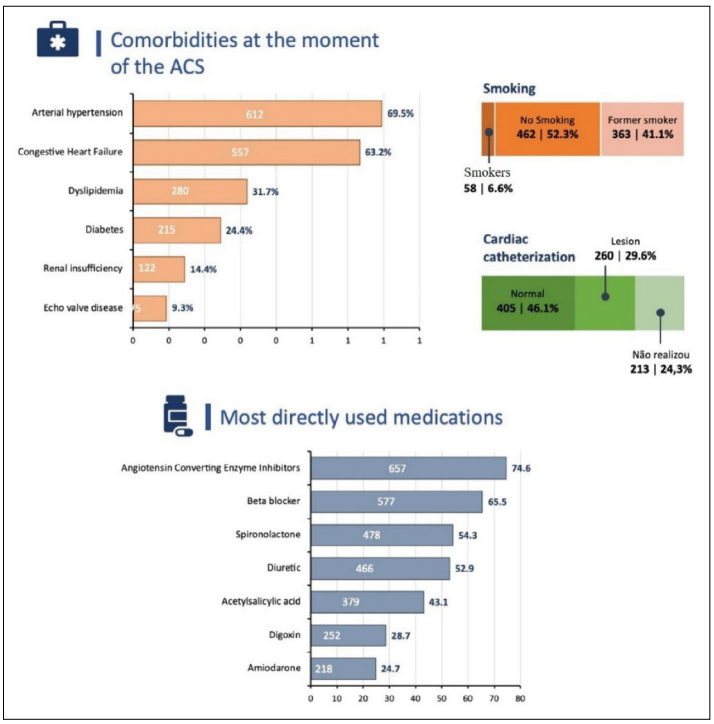

Figure 2. Infographic illustrates the clinical characteristics, medications, and risk factors. Rio Grande do Norte, 2021.

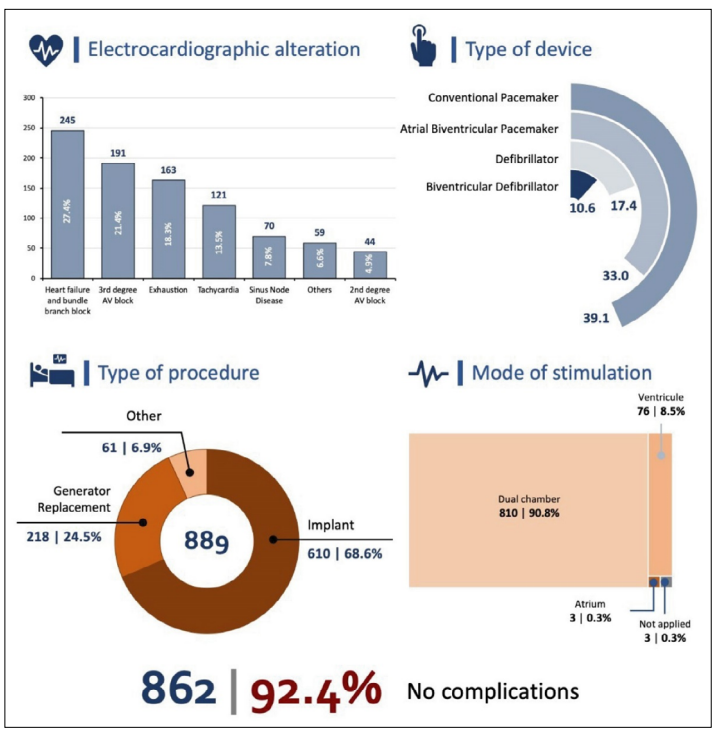

Figure 3. Infographic shows the technical characteristics related to ACS procedures. Rio Grande do Norte, 2021.

1 mg/dL). The mean serum potassium level was 4.19 mg/dL (median, 4.6 mg/dL).

Regarding the patients' origin, the majority ($n = 384$) of patients originated from Natal, whereas 47 and 38 patients were from Parnamirim and Mossoró, respectively. Of the 168 municipalities, 110 referred patients during the study period.

Based on the population of municipalities in 2014, the rate of procedures per 100,000 inhabitants was evaluated (**Figure 4**) to estimate the prevalence of severe heart disease.[7] Natal (the capital city) appeared in position 22, with a rate of 44.55, whereas Mossoró (the second largest city in the state) was only in position 91, with a rate of 13.37.[7]

As for spatial influence, the Global Moran's I index value obtained was 0.046, without statistical significance, indicating no spatial pattern in the distribution of procedures per 100,000 inhabitants. The distribution of socioeconomic variables (Human Development Index and *per capita* income) was also quite different from the rate of procedures per inhabitant (Figure 4), suggesting the absence of socioeconomic determination.

DISCUSSION

Severe heart disease is associated with complex medical procedures. Despite potentially fatal situations, medical technology can support and improve the patients' quality of life. The most complex ACS procedures encompass this universe almost entirely. As the HUOL is the only service in the state dedicated to complex procedures, we consider this record of patients undergoing ACS to be a portrait of severe heart disease within the SUS in Rio Grande do Norte.

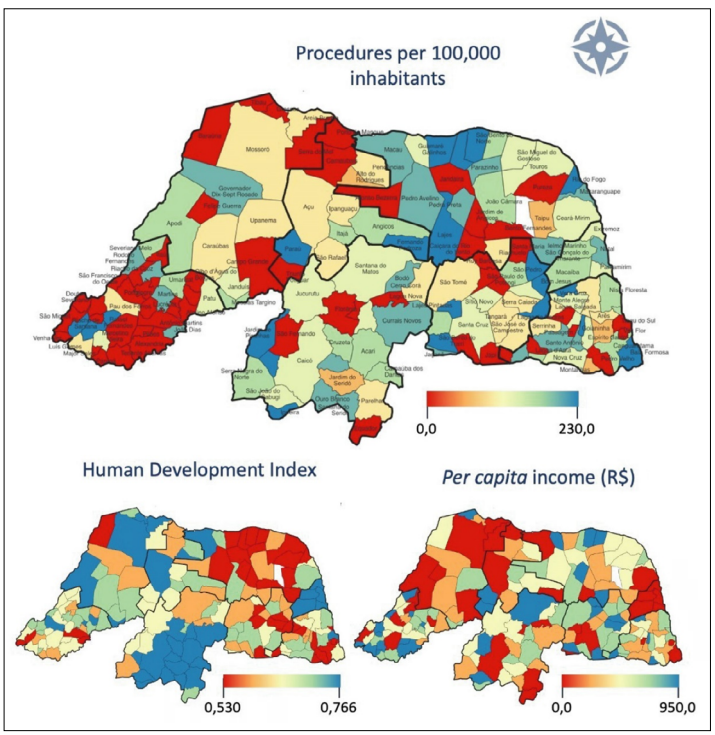

Figure 4. Infographic of thematic maps for the distribution of procedures, Human Development Index, and per capita income. Rio Grande do Norte, 2021.

The Brazilian Register of Cardiac Pacemakers, Defibrillators, and Resynchronizers (BRCP) is a national database established to collect data on cardiac pacemakers, defibrillators, and resynchronizers; such an obligation stems from ordinance number 41 of March 1994 by the Ministry of Health.[8] While its completion depends on the initiative of the team, the obtained data allow for the establishment of a procedural profile in Brazil. On August 1, 2008, the Ministry of Health, by ordinance number 1,559, instituted the National Policy for Regulation of the SUS, which determines the competences of federative entities. Among other obligations of the municipality are to guarantee access to the referenced population according to agreed and integrated programming and to act in an integrated manner at the State High Complexity Regulation Center (CERAC).[9]

Comparing our data with the results of analysis conducted by Pachón et al.,[10] based on the BRCP, we observed a higher percentage of ischemic etiology, dyspnea as a complaint, lower degree of sinus node disease, and second-degree AV block. These differences can be explained by the higher profiles of patients undergoing CRT in our sample. The lower profile of ischemic patients in the aforementioned study might have been underestimated by the use of the Registro Brasileiro de Marcapassos (RBM) as a database. RBM data were analyzed from 2000 to 2014, with a focus on the number of procedures and their distribution by region in the country.[11]

Analysis with an active search of data from 2018 and 2019 in a cardiological reference center did not include patients undergoing complex procedures.[12] In this study, females were slightly predominant (51.8%), with a mean age of 72.9 years. These data contrast with our sample as well as with the etiological findings of Chagas disease and ischemia. The sensitivity and existence of less invasive serological investigations may explain these differences.

In the international context, Spain has published annual reports on ACS procedures since 1997. The analysis was performed with a focus on technical aspects,[13] the most recent of which coincides with our data regarding male predominance, syncope as a causal factor, and the percentage of first implants and generator changes. Differences were observed in the occurrence of ischemic etiology, HF indicative of the procedure, and the percentage of biventricular stimulation or resynchronizers.

The Italian Association of Arrhythmology published data for 2018.[14] With more than 400 centers, the population of this registry presents a higher age group, with the data being close to our findings. In 2011, we had data records of the holders of ACS devices in France,[15] with data similar to ours in relation to the type of device used. Other centers such as those in Denmark present periodic epidemiological records with a greater focus on implanted devices and procedural complications.[16] Recently, the Danish registry demonstrated similar results with regard to epidemiological data.[17]

The Biopace registry selected patients from 2003 to 2007 in Europe and Australia.[18] Compared to our data, this population had a higher mean age and proportion of males. We observed the same proportion of patients with ischemic disease, as well as a high proportion of AV block and tachycardia. The characteristics of our service, including the selection of patients for CRT and the absence of emergency care, may explain these differences.

National data with a clinical focus are reported by analyzing specific comorbidities, such as hypertrophic heart disease[19] and Chagas disease.[20,21] Other centers report clinical analyses but with a lower volume of procedures.[22–24] A Nigerian study showed single-center data, with a smaller volume of patients.[22] Casola Crespo et al.[24] present data from a Cuban center, with a higher number of procedures, but without data on CRT and ICD. They reported a higher prevalence of ventricular pacemakers than double-chamber pacemakers.

Dubernet et al.[25] show data from Chile, focusing on the procedure with a predominance of conventional pacemakers. Aktoz et al.[26] evaluated the influence of gender and demographic data on the type of device used between 2006 and 2016. Equality was observed in relation to sex, with a mean age higher than that in our study. The proportion of ICD use in relation to pacemaker use is consistent with our findings. The high prevalence of ventricular devices (single chamber) in this study differs from general findings and our sample.

Khanal et al.[27] conducted a study on the influence of gender on device type. The percentage of single-chamber systems was higher than that reported in the general literature and in our population. The data from our study in relation to the percentage of ventricular pacemaker implants agreed with the national sample, with the majority of patients receiving double-chamber stimulation.

Symptoms associated with resynchronizer implants were prevalent in our study. These points are explained by the service being referenced without a gateway to emergencies, which require the most non-complex procedures that are carried out mostly in the private network.

The representativeness of older individuals was similar to that reported in the literature, with advanced age being a predictive factor for the need for ACS. The slight predominance in male individuals is corroborated by the literature, which shows that sex is not a relevant factor in the emergence of these pathologies. We observed a high incidence of ischemic etiology, probably because the study was limited to a tertiary school hospital with access to invasive diagnostic technologies. The lower proportion of emergency procedures also contributed to a greater etiological investigation. Dyspnoea was highlighted by a higher proportion of complex procedures related to resynchronization.

Clinical conditions such as hypertension and diabetes were predominant in our study population, reinforcing the role of deterioration of the conduction system and cardiac function associated with these risk factors. High use of medications such as ACE inhibitors, spironolactone, and beta-blockers is associated with the prevalence of HF.

Among the basic electrocardiographic changes, our profile explains the Left Branch Block as the main change, because it is required for resynchronization.

The HUOL at UFRN is a tertiary referral hospital for SUS patients in Rio Grande do Norte. It serves the entire state with a varied profile of patients with different complexities and severities. The characteristics of the service may explain the differences between our sample and those of other centers and services. The hospital does not provide emergency care, with all demands for ACS procedures being referenced from other centers. The availability of pacemaker procedures in affiliated hospitals associated with a lack of emergency care explains the lower proportion of less complex procedures. As procedures of greater complexity are offered only in the HUOL, their proportion in relation to conventional pacemakers increases. We then obtained a picture of almost the entire population of the state subjected to resynchronizer and defibrillator implantations using the SUS.

Spatial evaluation was considered to have no impact when analyzing the distribution of patients by municipality of origin. We were unable to establish clear clusters of greater access to services by merely analyzing geographical aspects (proximity to the service), regions with greater purchasing power, or regions where access to medical services is easier. Geographical differences, social inequalities, and socioeconomic differences are not reflected in greater accessibility. This result indicates homogeneous accessibility despite the absence of an established flowchart.

In the 1988 Constitution, health was guaranteed as a universal right of the state for all citizens. Despite these advances, we live with an unequal and exclusionary reality. Legal guarantees were an important stage in the construction of the SUS; however, in practice, this is still a daily struggle for health professionals. To realize the right to health, we need a social model based on "human solidarity and social equality."[28] Unfortunately, a "selective, focused and exclusionary" access is observed. The challenge of upholding the constitution continues. The implementation of equitable access requires different actions, depending on the social segment and clinical situation.

The social situation excludes a part of the population, which is not always perceived by the public. Often, when these demands are perceived, managers lack data for an in-depth analysis; to formulate public policies to mitigate the problem.[29] The challenging context of the economic crisis that has set in the last decade is not only local, but also shows a series of counterpoints, such as the existence of barriers to users, such as queues for appointment and service.[30,31]

The absence of a flowchart may be a factor responsible for this absence of long waiting for the procedure. Although the eligible population was restricted to patients with more severe heart disease, the lack of identification by health professionals in their gateway services may be a limiting factor for our demand. This did not prevent patients from accessing the most varied locations. We observed that the patients had access to other care flows, often through direct medical contact with our services.

The apparent equity detected in access did not seem comfortable. We consider it necessary and urgent that these data be made available to managers to demonstrate the importance of this population, the service itself, and the need to develop defined flows to better identify these patients, allow access, and avoid the loss of patients who could benefit from these important tools.

Ultimately, this group of patients corresponded to the profile of patients in our state with more severe heart disease, which is representative of the prevalence of severe heart disease in Rio Grande do Norte. Analysis of the data provides useful information for developing better health policies for patients with serious heart disease.

CONCLUSION

This population corresponded to the profile of patients with advanced heart disease, which is representative of the prevalence of severe heart disease in Rio Grande do Norte. Evaluation of the clinical and epidemiological profiles of this sample showed a pattern compatible with the literature, with a higher proportion of patients with HF and a high number of complex procedures. We believe that the characteristics of the service contributed to the differences in our population in relation to the findings in the literature.

Despite the absence of a flowchart, the spatial distribution was not statistically significant with respect to the municipality of origin. The results should be shared with the public health managers of the state to collaborate in the elaboration of better health policies, focusing on providing patients with universal access to this important tool for heart disease treatment.

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HFE mutations in patients with iron overload in Santa Catarina: a cross-sectional study

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S65C mutation.
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ABSTRACT

BACKGROUND: Investigating the frequency and characteristics of iron overload cases with *HFE* gene mutation is crucial, given the population-level risks associated with excessive iron.

OBJECTIVE: To determine the frequency of *HFE* mutations in patients with iron overload in Santa Catarina, Brazil.

DESIGN AND SETTING: A cross-sectional study of patients with iron overload at the Ambulatory Department of the Centro de Hematologia e Hemoterapia de Santa Catarina (Hemorrede-HEMOSC) in Santa Catarina.

METHODS: ***HFE*** genotype frequencies were determined, and a division were made between carriers of ***HFE***-C282Y/C282Y mutations and carriers of other *HFE*-non-C282Y/C282Y mutations, according to each region of Santa Catarina. Binary logistic regression was used for association between sex and age with genetic mutation trait.

RESULTS: Among the 1,022 patients, 10.4% had secondary hemochromatosis, and 89.6% were evaluated for iron overload due to hereditary hemochromatosis (HH). Of these, 367 underwent genetic testing, which revealed *HFE* mutations in 77.3%. Most patients with *HFE* mutations had non-C282Y/C282Y-hemochromatosis, especially H63D/WT (> 39%), regardless of the Santa Catarina region. The frequency of C282Y/C282Y was higher in the West (20.9%) and North (28.3%) regions. Adjusted association analysis showed that men have an increased chance of hemochromatosis when involving "non-C282Y/C282Y" mutations (OR: 2.77; 95% CI: 1.60–6.608).

CONCLUSIONS: The data show the magnitude and characteristics of iron overload cases with *HFE* mutations in Santa Catarina. As most patients referred for treatment have H63D mutation, we suggest further studies to assess whether other factors, including dietary habits and mandatory iron fortification policies, contribute to iron overload or HH manifestation.

INTRODUCTION

Hereditary hemochromatosis (HH) is an autosomal recessive disease, characterized by an excessive increase in absorption of dietary iron due to alterations in the hepcidin hormone expression, which reduces its concentration and consequently increases the amount of iron accumulation in the human body.[1,2] Under normal physiological conditions, hepcidin is a negative iron metabolism regulator, i.e., its physical expression is reduced only when there is an increase in the need for iron in the human body.[1,3–5] Since the human body does not have specific mechanisms to regulate iron excretion, excess iron can be absorbed and cause toxicity. This progressive accumulation of iron leads to the formation of free radicals causing oxidative damage in organs and tissues, especially in the liver, heart and pancreas.[5,6] HH related to the *HFE* gene, defined as type 1, represents more than 90% of the known cases of this disease and appears as mutant alleles C282Y, H63D, and S65C. Penetrance into HH of other *HFE* genotypes could also occur, but would depend on greater exposure to non-genetic factors.[1,4,8] The homozygous C282Y mutation (C282Y/C282Y) is the most precociuous and most severe iron overload-related subtype among *HFE* mutations.[1,4,5,7]

Diagnosis of HH is based on the detection of iron overload with elevated serum ferritin level and elevated transferrin saturation level in conjunction with genetic mutations. Early diagnosis and prompt treatment can prevent serious clinical manifestations such as heart disease, arthritis, liver disease, and diabetes.[1,9,10]

Regular therapeutic phlebotomy (therapeutic bloodletting) is the most effective treatment for HH, which aims to move the accumulated iron in tissues into the blood by inducing a

negative balance in serum iron.[2,7] Less exposure to environmental risk factors can attenuate phenotypic expression and facilitate treatment by allowing longer intervals between phlebotomies. Examples of environmental factors include habitual consumption of iron-enriched foods, excessive vitamin C intake, and excessive alcohol consumption.[1,5,8,9,11–14]

According to data on populations of Caucasian origin, the estimated prevalence of HH type 1 is one case for every 200 individuals.[15,16] Studies on HH prevalence in Brazil are scarce; however, some studies suggest that, in certain regions of the country, the allele frequencies of *HFE* mutations can be comparable to those in countries where HH is considered a public health problem.[17–19] This is due to Brazil's genetic heterogeneity, where *HFE* mutations and the phenotypic HH expression could present variations and different frequencies, depending on the colonizing ethnic groups of various regions and the influence of environmental factors.[7,8,12,16,20–23]

As data on HH in Santa Catarina is lacking, and to describe the prevalence of type 1 HH cases in a Brazilian population of predominantly European descent, we investigated the frequency of *HFE* mutations in patients from Santa Catarina assisted by the Sistema Único de Saúde (Unified Health System, Brazil's public healthcare system) through the Centro de Hematologia e Hemoterapia de Santa Catarina (Hemorrede-HEMOSC, the Hematology and Hemotherapy Center of Santa Catarina).

METHODS

A cross-sectional study was conducted using secondary data to describe the frequency of *HFE* mutations in patients with iron overload in Santa Catarina who were referred for investigation and treatment at Hemorrede-HEMOSC. The study was conducted using data from January 1 to December 31, 2016. Cases were selected from medical records by searching for the International Classification of Diseases – 10 (ICD-10) codes E83.1 (iron metabolism diseases) and T4.4 (intoxication by iron and its compounds). For the sampling procedure, all medical records of patients assisted at Hemorrede due to iron overload (ICD-10 and ICD-T4.4) were analyzed. Analyses were conducted between December 2017 and December 2018.

To describe the frequency of *HFE* mutations, the exclusion criteria were absence of genetic testing for HH, medical records of patients diagnosed with *HFE*-HH without indicating the mutation type, and patients diagnosed with secondary hemochromatosis (hemoglobinopathies, sideroblastic anemia, polycythemia vera, hereditary spherocytosis, porphyria cutanea tarda, viral hepatitis, or alcoholic liver disease).

The data were grouped according to the state's regions, namely Greater Florianópolis, Itajaí River Valley, North, South, West, and the Serrano Highland.[24] The mutations frequency observed for the Serrano Highland region is not shown, as no genetic testing data are available for that region. All regions of the state have populations predominantly of European descent, mainly Italians, Germans, and Portuguese; thus, they carry a higher risk of having *HFE* mutations.[25,26] Information was obtained regarding age, sex, skin color, genotyping test results, as well as the frequency and distribution of *HFE* mutations. Skin color was self-declared by the public healthcare user, within the standards used by the Instituto Brasileiro de Geografia e Estatística (IBGE, the Brazilian Institute of Geography and Statistics). In the public healthcare information systems, these are categorized as white, black, yellow, brown, dark-skinned, or indigenous.[27] Genotyping for *HFE* mutations is performed by polymerase chain reaction analysis[28] in laboratories outsourced to HEMOSC. The frequency and distribution of *HFE* gene mutations in patients from Santa Catarina were noted for each region of the state. The age variable was categorized between patients aged ≤ 40 years versus > 40 years, which is the cut-off point referring to the age group for most type 1 HH diagnoses.[2] For the skin color variable, Santa Catarina's low prevalence of black, yellow, brown, dark-skinned, and indigenous population was not considered; therefore, only two categories were established, namely White and Non-white.[29] For the *HFE* genotype frequency analysis, genotypes were divided into six categories, namely, C282Y/C282Y mutation carriers (homozygous C282Y) and carriers of other non-C282Y/C282Y *HFE* genotypes; C282Y/WT (C282Y heterozygous); C282Y/H63D (compound heterozygote C282Y/H63D); H63D/H63D (homozygous H63D); H63D/WT (heterozygous H63D); and all the S65C types as one single group (S65C-grouped). The S65C category included both homozygous and heterozygous patients with this mutation (S65C/S65C, S65C/WT, and C282Y/S65C). Wild-type (WT) was used to signal a non-mutant allele.

The collected data were recorded and organized into electronic spreadsheets (Excel), and data files were generated. After the files were checked, they were transferred to statistical analysis software. The data were verified through consensus between two researchers not involved in initial data collection and by reviewing the data the researcher responsible for statistical analysis. The characterization data were described using absolute and relative frequencies, and genotype frequencies (95% CI) were also presented. Binary logistic regression was used to determine the association between sex and age (adjusted analysis) for carriers of the genetic traits (C282Y/C282Y mutation versus other non-C282Y/C282Y *HFE* genotypes). The cut-off for significance was set at 5%, and the data were analyzed using STATA 15 software (Stata Inc., College Station, TX, USA). The research project was approved by the Ethics Committee of the Federal University of Santa Catarina (UFSC) (CAAE: 64252017.2.0000.0121) and the Ethics Committee of the Center for Hematology and Hemotherapy of Santa Catarina (HEMOSC) (CAAE: 64252017.2.3001.0110). This study followed the ethical principles established in Resolutions No. 466/2012 and No. 580/2018 of the National Health Council in Brazil.

RESULTS

During the study period, 1,022 outpatients were assessed for iron overload at Hemorrede-HEMOSC. Of these, 206 (10.37%) had secondary hemochromatosis. The remaining 806 patients (89.63%) were assessed for HH. Of these 806 patients, 367 (35.2%) underwent genetic testing to identify HH, of whom 77.3% tested positive for *HFE* mutations, as shown in **Table 1**. Of the total number of patients who underwent genetic testing for *HFE* mutations, 89.6% were aged over 40 years, 91.0% were male, and all but one had white skin**.**

HFE genotype frequencies in patients undergoing treatment showed the relevance of the number of iron overload cases in *HFE* mutations in Santa Catarina. **Table 2** shows which mutations are involved in these cases, while **Figure 1** shows the distribution thereof among the regions. The percentage of patients with homozygous C282Y/C282Y in Santa Catarina was 17%, and the most frequent mutation among patients in this state was heterozygous H63D (48.2%), which was repeated in all regions of the state. The compound heterozygous mutation C282Y/H63D was the third most frequent mutation in the state (12.4%) and the highest in the Greater Florianopolis region (23.8%).

The non-C282Y/C282Y *HFE* mutation was detected in 83% of the patients and the adjusted analysis association between the sex and age of genetic trait carriers (C282Y/C282Y and other non-C282Y/C282Y *HFE* genotypes) showed that men, have higher chances of having hemochromatosis with non-C282Y/C282Y mutations (OR: 2.77; 95% CI: 1.60–6.608) compared to women.

Table 1. Distribution of patients according to age, sex, skin color and presence of genetic alteration in the *HFE* gene in Santa Catarina, Brazil, 2016

Variable		N	%
Age (years)	≤ 40	38	10.4
	> 40	329	89.6
	Total	**367**	**100**
Sex	Male	334	91
	Female	33	9
	Total	**367**	**100**
Skin color	White	366	96.3
	Non-White**	1	3.7
	Total	**367**	**100**
***HFE* gene mutation**	Yes	282	77.3
	Not	83	22.7
	Total	**365*** **	**100**

* N: number of patients; ** Brown; *** Two subjects excluded from the sample (patients diagnosed with ***HFE***-HH, but without indicating the mutation type).

Table 2. Frequency of *HFE* genotypes in patients from Santa Catarina, Brazil, 2016

HFE genotype	N	%	95% CI
C282Y/C282Y	48	17	13–21.9
C282Y/WT	30	10.6	7.5–14.8
H63D/H63D	24	8.5	5.7–12.4
H63D/WT	136	48.2	42.4–54.1
C282Y/H63D	35	12.4	9–16.8
S65C-grouped**	9	3.2	1.7–6
Total	**282**	**100**	

* N: number of patients in each group; WT: wild type; ** All the S65C types as one single group (two patients S65C/S65C, two patients S65C/C282Y e five patients S65C/WT).

DISCUSSION

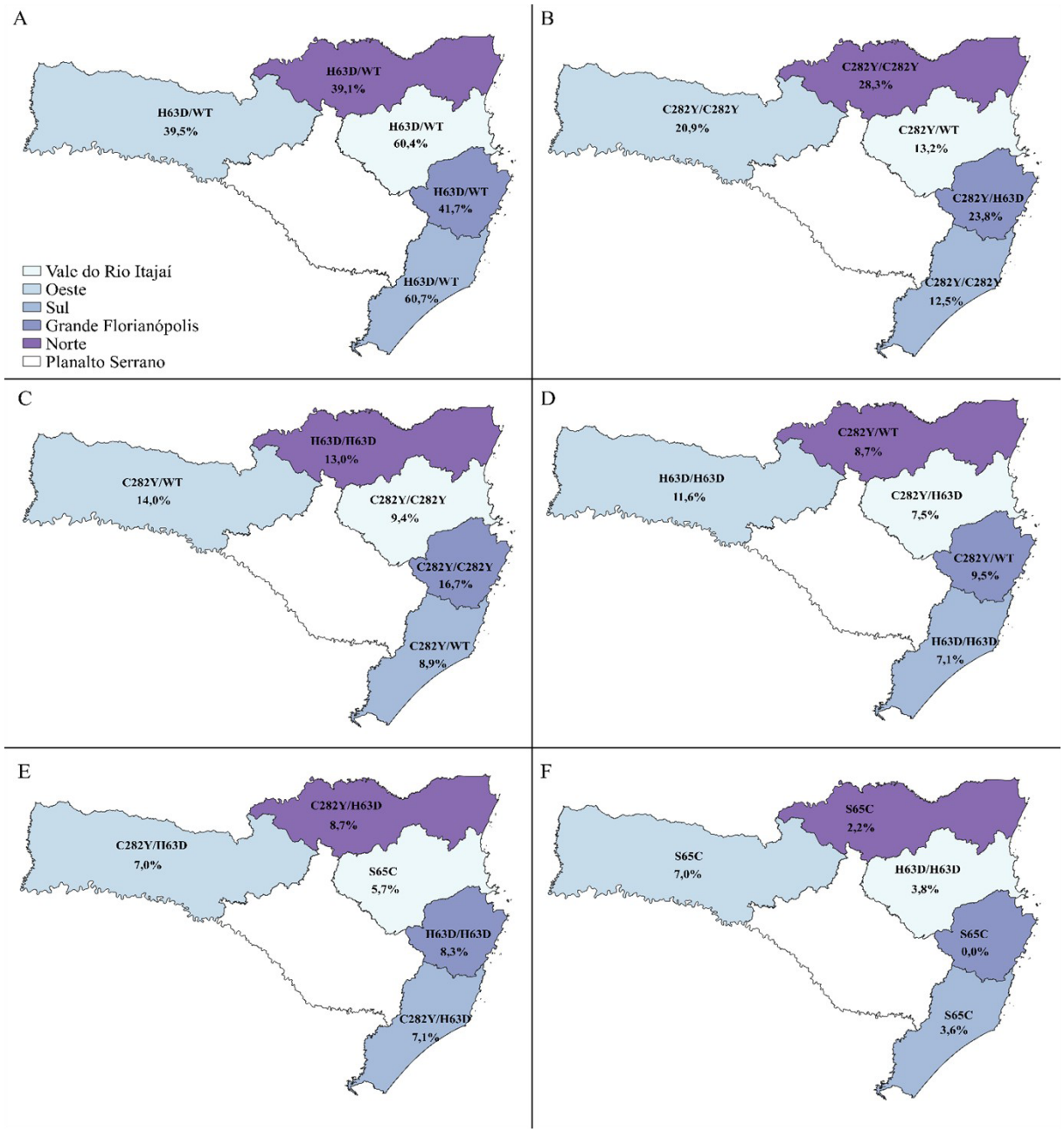
In the present study, cases of iron overload were found predominately in male, white-skinned patients over 40 years of age with mutations in the *HFE* gene, which agrees with reports on HH type 1.[1,2,8] Although clinical manifestations differ between patients, they tend to be related to the amount of iron accumulated in the body, and they generally begin after 40 years of age, which is the age after which most people are diagnosed with HH.[2,9] Since 22.7% of patients did not have an *HFE* mutation, some other genetic mutation for HH may be participating, such as genes encoding other proteins involved in iron metabolism, including hemojuvelin (*HJV*), hepcidin (*HAMP*), transferrin receptor (*TfR2*), or ferroportin (*SLC40A1*).[1,5,10] This proportion of non-*HFE* HH cases is similar to the value of 24% noted by Cançado et al.[22] in a study involving fifty patients with iron overload being treated in São Paulo.

The low S65C mutation frequency in patients from Santa Catarina is similar to the reported *HFE*-HH cases.[21,30,31] This low frequency is also found in healthy individuals without phenotypic disease expression.[18,32–34]

The C282Y/C282Y mutation was more common in patients in Santa Catarina than those in Rio Grande do Norte (2.67%) and Espírito Santo (5%), and was similar to that described for São Paulo (21.6%)[21,30,31]; furthermore, it is also similar to a value described in a study in the United States.[35] Of note, Gallego et al.[35] investigated 222 men over 59 years of age with the previously known mutation (*HFE* C282Y/C282Y or C282Y/H63D), of whom 24.4% had a clinical diagnosis compatible with HH-*HFE* C282Y/C282Y and 3.4% whose clinical diagnoses were compatible with C282Y/H63D mutation-related HH-*HFE*.

When considering the frequency among regions of Santa Catarina, the C282Y/C282Y mutation was least common in Vale do Itajaí and South (**Figure 1**), with values close to those (10%) reported in another study involving only patients from the South region of Santa Catarina.[36] The present study shows the heterogeneity of *HFE* mutations, being similar to that observed in southern European countries, such as Italy.[37]

The highest number of C282Y/H63D patients, compared to those with C282Y/C282Y within Santa Catarina, was noted in the Greater Florianopolis region, which is also where most patients



Panel A shows the most frequent genotypes for each region, Panel B shows the second most frequent, and so on. Geographic regions were according to Instituto Brasileiro de Geografia e Estatística (IBGE), 2017: Vale do Rio Itajaí (Itajaí River Valley); Oeste (West); Sul (South); Grande Florianópolis (Greater Florianópolis); Norte (North); and the Planalto Serrano (Serrano Highland). The West Region also includes the Midwest Region. No genetic testing data were available from the Serrano Highland region. WT, wild-type; S65C: S65C-grouped, with a total of two patients with S65C/S65C, two patients with C282/S65C, and five patients with S65C/WT.

Figure 1. Frequency of *HFE* genotypes in patients, according to each region of Santa Catarina, 2016.

had been screened for *HFE* mutations. In Espírito Santo, a notable association was found between compound heterozygous cases with the H63D mutation and HH when compared to people without phenotypic expression.[31] In another study conducted in Spain, which has a Mediterranean population, researchers noted greater phenotypic expression for the compound heterozygote mutation C282Y/H63D than other *HFE* mutations,[12] as seen in patients in the Greater Florianópolis region. The frequency of the C282Y/H63D mutation found (12.3%) was similar to that noted for patients in São Paulo (14–15%)[22,31] and higher than those in the Northeast

Region of Brazil (5.02%),[30] as well as in Italian (5.3%) and Swedish (7.1%) patients.[37,38]

The heterozygous H63D mutation showed a similar frequency to that noted in a study conducted in the South region of Santa Catarina, which profiled patients undergoing phlebotomy and being treated at a private hematology clinic, where 44% of patients with *HFE* mutations had heterozygous H63D.[36] As most patients referred for treatment at HEMOSC have the H63D mutation, regardless of region (**Figure 1**), additional factors could be contributing to iron overload and HH manifestation in this population.[10]

Bell et al.[11] conducted a study in Norway that showed that excessive consumption of iron for at least five years was significantly associated with the clinical hemochromatosis expression in patients with non-C282Y/C282Y *HFE* mutations. Since 2002, the Brazilian population has regularly eaten mandatorily iron-fortified foods (wheat flour, corn flour, and preparations that contain either or both).[39,40] This may also have contributed to iron overload in carriers of non-C282Y/C282Y *HFE* mutations.[11–13,41–46] Guidelines advise that patients with HH should avoid daily consumption of iron-fortified foods,[2,8,9] highlighting the importance of offering such people foods without iron fortification.

The present study shows that male sex can be a significant predictor of iron overload development in non-C282Y/C282Y*HFE*-HH cases, suggesting that further studies should assess whether men suffer greater environmental exposure to factors such as excessive iron-rich food consumption (e.g., meat or iron-fortified foods), alcohol, or vitamin C compared to women with the same condition. In addition, it is still unknown whether female physiological conditions could mitigate the effect of exposure to these environmental factors. Further, if this is indeed the case, it remains unknown whether there would be greater relevance for non-C282Y/C282Y *HFE* mutations , since women lose iron during menstruation and require more iron during pregnancy, as well as being affected by the antioxidant effects of estrogen and the naturally higher hepcidin concentrations in women when they are overweight.[46]

The present study has some limitations. First, the data are from a convenience sample, where patients with iron overload were referred to HEMOSC, although this is the main treatment center for these cases. Second, we lacked data for one of six regions of Santa Catarina. Third, environmental factors, comorbidities, and biochemical data were not investigated; thus, these were not considered in the analytical association model. Next, we could not make many comparisons with other results, since studies in this field are limited in Brazil. Finally, only laboratory tests for *HFE* were used, since they are easily accessible. Laboratories that perform genetic testing for mutations other than those of *HFE* are scarce,[10] thereby limiting investigations to determine whether mutations in other iron homeostasis-related genes could also be involved.

However, the present study describes *HFE* mutation frequency, which is the main HH-related gene, in patients with iron overload treated at a public healthcare center specializing in hematology and hemotherapy in Santa Catarina. Moreover, it can help map and plan public interventions aimed at patients with hemochromatosis and those in at-risk groups, including those with non-C282Y/C282Y mutations.

Among such interventions, education is particularly relevant. This could include developing courses and other educational materials to raise awareness about excess iron, its possible causes, and the associated health risks. It could also foster understanding of the importance of early diagnosis by quantifying serum ferritin and transferrin saturation indices in routine examinations among clinical managers. Individuals with laboratory findings suggestive of hemochromatosis or a family history of the condition would then undergo investigation for HH-related mutations. These actions, supported by case mapping, could be expanded to all states in Brazil, strengthening the involvement of different segments of society, including public and private organizations, health professionals, and teaching, extension, and research institutions. Furthermore, recognizing this as a public health issue, it is important to encourage the establishment of associations for patients with HH to seek improvements and share responsibilities for addressing this condition. Another opportunity lies in promoting better coordination between federal public bodies and industries, particularly the flour industry, to facilitate the production and accessibility of common foods without iron supplementation, which would be a crucial measure to mitigate excess iron levels in individuals with this condition.

CONCLUSION

The data obtained in this study showed that most patients with *HFE* mutations have non-C282Y/C282Y hemochromatosis regardless of their region in Santa Catarina, whereas patients with the homozygous C282Y/C282Y mutation were more common in the West and North regions of the state. Further studies are needed to investigate the association of these cases with environmental risk factors such as lifestyle and eating habits, including the consumption of iron-fortified foods. It is also suggested that further studies should confirm whether additional pathological conditions (such as ferroportin disease and metabolic syndrome that can be associated with iron overload) exist in cases of non-C282Y/C282Y *HFE*-hemochromatosis, as well as the presence of other mutations involved in iron regulation, in addition to the *HFE* gene.

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Percutaneous tracheostomy in COVID-19 patients: a retrospective cohort study

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ABSTRACT

BACKGROUND: The coronavirus disease 2019 (COVID-19) pandemic has placed unprecedented strain on healthcare systems, particularly on critically ill patients requiring prolonged mechanical ventilation (MV). Percutaneous tracheostomy (PT) has emerged as a potential strategy to facilitate weaning, reduce intensive care unit (ICU) stay, and optimize resource use. However, the timing, safety, and outcomes of PT in COVID-19 patients remain debatable.

OBJECTIVES: This study aimed to describe the technical aspects of the procedure and evaluate the early safety of our technique to healthcare professionals, as well as the short-term factors affecting survival in 103 consecutive patients after tracheostomy.

METHODS: We retrospectively analyzed patients with COVID-19 who underwent PT between March 2020 and June 2020 at Hospital Alemão Oswaldo Cruz, São Paulo. The factors considered for analysis included age, sex, timing of tracheostomy, proportion of affected lungs, comorbidities, fraction of inspired oxygen on MV, and availability of professional private equipment. Univariate analysis was performed for screening, and variables with $P < 0.20$ were included in the multivariate Cox proportional hazards regression model.

RESULTS: Most patients were male, with a median age of 68 years. The most common comorbidities were hypertension (n = 55/52%), diabetes (n = 37/36%), and heart disease (n = 24/21%). Patients over 60 years old had reduced survival (hazard ratio [HR] = 3.35; P = 0.003), and those who underwent high nasal flow catheter (HR = 0.49; P = 0.02) and PT earlier (< 10 days) had better survival (HR = 0.37; P = 0.04).

CONCLUSION: Early PT in selected patients may reduce the duration of MV and lead to shorter ICU stays. The health system is overloaded by the scarcity of ventilators and beds for critically ill patients.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a worldwide pandemic, with over 225 million cases diagnosed to date.[1] While most COVID-19 patients do not require supportive care, 10–15% of patients develop acute respiratory distress that requires invasive ventilatory support.[2] More than 190 countries registered cases that reached an outcome, and a total of 4.641.746 resulted in mortality.[1] In Brazil, there were 31.895.385 COVID-19 cases and 561.762 deaths, with a 2.8% lethality rate. In São Paulo, the epicenter of the disease in Brazil, there were 4.113.741 confirmed cases and 140.677 deaths, with a lethality rate of 3.9%.[3]

Mechanical ventilation (MV) for patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is associated with prolonged airway intubation and a high worldwide mortality of at least 50–67%.[4] Based on previous experience with severe acute respiratory syndrome (SARS) in 2003, aerosol-generating procedures such as tracheal intubation or tracheostomy were considered high-risk procedures for the transmission of SARS-CoV-2 to healthcare professionals (HCP).[4]

Tracheostomy is a common procedure in critically ill patients who require an extended period of MV. Use of tracheostomy can facilitate weaning from MV and potentially increase the availability of intensive care unit (ICU) beds.[4] When the COVID-19 pandemic spread to Italy and Spain, ICUs experienced a massive influx of patients who were critically ill, many becoming candidates for tracheostomy. However, tracheostomy is an aerosol-generating procedure; therefore, HCP are at risk of infection during insertion and subsequent care, even when appropriate personal protective equipment (PPE) is used.[4]

The American Academy of Otolaryngology and the ENT academy in the United Kingdom have stated that providers should "avoid tracheotomy in COVID-19 positive or suspected patients"

because of the risks to HCP. These guidelines, based on limited evidence, recommend that tracheostomies should not be performed before 2–3 weeks after intubation, preferably after negative COVID-19 testing, and recommend open tracheostomy (OT) in these circumstances as opposed to percutaneous tracheostomy (PT).[4,5]

The indications and ideal timing of tracheostomy in patients with COVID-19 remain controversial. Although several studies have been published with recommendations on proper and safe tracheostomy in these patients, the indications and timing of the procedure remain unclear and are mostly based on previous experience gained from the 2003 SARS-CoV-1 epidemic.[6,7]

A recently published report has discussed the timing of tracheostomy in COVID-19 patients. The authors emphasize that tracheostomy reduces ICU stay and, in the context of prolonged MV, should be suggested within 7–14 days in order to avoid potential tracheal damage.[7] Performing an early tracheostomy in a critical patient (before 10 days of MV) seems to reduce the risk of mortality and increase the probability of discharge from the ICU.[8] Although it is a probability, some studies show promising results.[3,9] In addition, according to our own experience, tracheostomy reduces mortality and sometimes influences discharge time (**Figure 3**).

In this manuscript, we describe the technical aspects of the procedure, evaluate the early safety of our technique for HCP, and identify short-term factors affecting survival in 103 consecutive patients after tracheostomy.

METHODS

We retrospectively analyzed 103 consecutive patients with COVID-19 who underwent PT between March 10, 2020 and June 30, 2020, at Hospital Alemão Oswaldo Cruz, São Paulo. All the patients agreed to participate in this study.

This study included all mechanically ventilated patients who were both COVID-19 positive and received a tracheostomy consultation. Each patient was evaluated individually by the multidisciplinary team, and the appropriateness of tracheostomy was assessed by considering patient prognosis and goals of care, potential benefit of the procedure, and tolerability of the procedure. The main criteria for patient selection for tracheostomy were possibility of long MV time, comorbidities, positive end-expiratory pressure (PEEP) < 12 mmHg, and fraction of inspired oxygen (FiO_2) < 70%.[3]

The patients had SARS-CoV-2 infection documented by a nasopharyngeal swab for reverse transcriptase polymerase chain reaction (PCR) assay and developed severe respiratory failure requiring MV. Data were collected following a medical record review of each patient's chart. This study was approved by the institutional review board (4.849.515).

Patients with the following characteristics were considered eligible for the analysis: (1) SARS-CoV-2 confirmed by testing, (2) MV due to COVID-19-related respiratory failure , and (3) underwent PT.

Several COVID-19-related factors were considered, including age, sex, timing of tracheostomy, type of technique, proportion of lungs affected, comorbidities, duration of and FiO_2 fraction on MV, availability of PPE, and current patient status. All phases of routine tracheostomy care were considered in the review: the perioperative step was preferable in the ICU, appropriate PPE was used, and ventilation was maintained during tracheotomy until cuff inflation and circuit reconnection; the postoperative step was closed, inline suctioning, closed circuit with high-efficiency particulate arrestance (HEPA) filter if on mechanical ventilatory support, and heat and moisture exchange (HME) when off ventilatory support.[3]

The concurrent goals of these modifications were to mitigate the risk to the HCP while preserving the risk-benefit profile for patients and the feasibility and safety of PT. In addition, we collected variables such as O_2 saturation when entering the ICU, FiO_2 at admission to the ICU, partial pressure of oxygen (PaO_2)/FiO_2 relation, SAPS 3 score, use of noninvasive MV, use of high-flow nasal catheter (HFNC), and total time of orotracheal tube administration before PT.

All patients underwent chest CT prior to PT to evaluate the extent of lung lesions by SARS-CoV-2, which was divided into three degrees: 25%–50%, 50%–75%, and > 75%. MV settings recommended for the procedure were a PEEP ≤ 12 mmHg, FiO_2 ≤ 70%, respiratory rate ≤ 25 breaths per minute, and partial pressure of carbon dioxide ≤ 60 mmHg. Patients with multi-organ failure (MOF) were excluded.

All personnel involved in this procedure used full PPE, according to the following institutional policies: hair cover, N95 mask, surgical mask, face shield, gown, and two layers of gloves upon entering the room. Maximal oxygenation was performed before the procedure (FiO_2 = 100%).

PT was performed in the ICU according to the usual technique, with all patients under sedation and using muscle blockers to avoid coughing and aerosolizing the virus. With the ventilator in the standby mode, we deflated the endotracheal tube (ETT) cuff and retracted the ETT into the proximal trachea/subglottis. Before the airway opening, bronchoscopy was performed for secretion suction and tracheal puncture. The ETT cuff was reinflated, and the ventilator was restarted. The path was dilated with the "PORTEX KIT" dilator (PORTEX Medical). Before insertion of the tracheostomy cannula, the ventilator was again set to standby mode and the ETT cuff was deflated. A PT tube Portex #8.0 was inserted into the trachea for all for females and #9.0 for all males. The bronchoscope was advanced through the tracheostomy tube to confirm the position and correct size of the tracheostomy tube and remove any blood or secretions from the airway. The ventilator was then connected and restarted once the circuit was closed.[3]

Our tracheostomy team consists of:

- two thoracic surgeons (one for bronchoscopy);

- one intensive care physician and one nurse;
- one physiotherapist.

The endpoints for this study were the safety and feasibility of our bedside PT in mechanically ventilated patients with COVID-19 in the ICU to bring to them a better prognosis and life expectancy; early patient outcomes as decreased complications, mortality, ICU stay, MV time, and early HCPs; time of tracheostomy; and factors affecting survival.

Statistical analyses

The SPSS 22.0® package for Windows (IBM Corp. Released 2020) was used. Patient characteristics were analyzed using frequencies and percentages for qualitative variables and medians and interquartile ranges for quantitative variables. Univariate analysis was performed for screening, and variables with $P < 0.20$ were included in the multivariate Cox proportional hazards regression model. The Cox model yielded hazard ratios (HRs) with 95% confidence intervals (95% CI), establishing the likelihood of death according to the number of tracheostomy days.

RESULTS

The patients included in this review (n = 103) underwent PT in the ICU between March 2020 and June 2021. All patients underwent surgery performed by the same team using the same surgical protocol and technique.

The patient characteristics, including demographics, lung injury, and respiratory support type and measures, are shown in **Table 1**. Most patients were male, and the most frequent comorbidity (**Table 2**) was hypertension (n = 55/52%). The most common comorbidities associated with death were hypertension (n = 27) and heart disease (n = 16) (**Table 2**).

When admitted to the ICU, 15 patients required an FiO_2 fraction of 100% on MV. The median relation PaO_2/FiO_2 in all patients was 141 (percentile range, 84–800), the median number of days of MV before tracheostomy was 16 (percentile range,

Table 1. Patient demographics

Variables	Total group n = 103	Early PT n = 14	Late PT n = 89
Sex (male); n (%)	76 (70)	8 (57)	68 (76)
Age (years); median [25%–75%]	68 [57–77]	71 [60–90]	69 [56–77]
CT scan (%) involvement; n (%)			
25%–50%	54 (52)	9 (64)	45 (51)
50%–75%	31 (30)	3 (22)	28 (31)
> 75%	14 (14)	1 (7)	13 (15)
No signs of COVID-19	4 (4)	1 (7)	3 (3)
Comorbidities n (%)			
Hypertension	55 (53)	6 (43)	49 (55)
Obesity	7 (7)	2 (14)	5 (6)
Diabetes	37 (36)	2 (14)	35 (39)
Dyslipidemia	14 (13)	0 (0)	14 (15)
Smoking history	7 (7)	0 (0)	7 (100)
Heart disease	24 (23)	3 (21)	21 (24)
Lung disease	9 (9)	0 (0)	9 (100)
Kidney disease	3 (3)	0 (0)	3 (100)
Thyroid disorder	17 (16)	1 (7)	16 (18)
Neoplasia	13 (13)	0 (0)	13 (100)
SAPS 3; median [25%–75%]	48 [41–55]	53 [37–63]	48 [43–55]
SpO_2 at admission in hospital; median [25%–75%]	93 [90–95]	93 [89–95]	94 [91–96]
FiO_2 at admission in ICU; median [25%–75%]	97 [65–100]	100 [40–100]	100 [65–100]
PaO_2/FiO_2 at admission in ICU; mediam [25%–75%]	141 [108–215]	187 [114–217]	141 [99–236]
Use of non-invasive MV; n (%)	60 (58)	7 (50)	53 (59)
Use of HFNC n (%)	35 (34)	1 (7)	34 (38)
Orotracheal cannula (days); median [25%–75%]	16 [13–19]	7 [6–10]	16 [14–19]
ICU before PT (days); median [25%–75%]	17 [14–21]	9 [7–10]	18 [15–21]
Days in hospital before PT; median [25%–75%]	20 [16–24]	10 [7–13]	21 [18–25]
Total time in MV; median [25%–75%]	33 [24–44]	22 [13–44]	34 [25–44]
Total days in hospital; median [25%–75%]	48 [37–73]	48 [37–69]	45 [25–92]

n = number of individuals; % = percentage of individuals; CT = computed tomography; SAPS = Simplified Acute Physiology Score; SpO_2 = oxygen saturation; Fi = inspiratory fraction; ICU = intensive care unit; Pa = partial pressure; MV = mechanical ventilation; PT = percutaneous tracheostomy; HFNC = high-flow nasal catheter. Data are expressed as median and percentile (25%–75%) or as the number and percentage of patients. No data were missing.

13–19 days), and 62% of patients underwent non-invasive MV. The median hospital stay before PT was 20 days (percentile range, 16–24 days), and the median hospital stay was 48 days (percentile range, 37–73 days).

The median time on MV prior to PT was 16 days (percentile range, 13–19 days) after initial intubation. The median total time of MV was 33 days (percentile range, 24–44 days). To date, none of the team members has developed any symptoms and/or tested positive for COVID-19.

Univariate analysis

Table 2 shows the results of the univariate Cox proportional hazards regression analysis of the factors associated with mortality. Survival time was defined as the total days of hospitalization.

Age, comorbidities such as heart disease, SAPS 3 prognostic score, use of HFNC, and number of days in the orotracheal cannula prior to tracheostomy were included in the multivariate analysis (P < 0.20). Older patients (> 60 years old) had a significantly decreased survival probability than younger individuals (HR = 3.38; P = 0.005) (**Figure 1**). In addition, it appears that patients who used oxygen in HFNC (HR = 0.59; P = 0.10; **Figure 2**) and those undergoing tracheostomy earlier (≤ 10 days) (HR = 0.54; P = 0.020; **Figure 3**) had a better survival probability than those who underwent tracheostomy later. However, these factors were not significant in univariate analysis (**Figure 2**, **3**).

Table 2. Univariate and multivariate analysis for death in tracheostomized patients in ICU

	Univariate		Multivariate	
	HR	P value	HR	P value
Sex	1.36	0.35		
Age (> 60 years/old)	**3.38**	**0.005**	**1.02**	**0.01**
Hypertension	1.13	0.66		
Diabetes	1.31	0.33		
Obesity	1.67	0.47		
Heart disease	**1.5**	**0.17**		
Lung disease	1.67	0.23		
CT findings	0.93	0.78		
SAPS 3	**1.02**	**0.13**		
SpO_2	1	0.98		
FiO_2	**1**	**0.2**		
PaO_2/FiO_2	0.99	0.39		
Non-invasive MV	**0.67**	**0.16**		
HFNC	**0.59**	**0.1**	**0.49**	**0.03**
Days in ICU before PT	1	0.74		
Days in hospital before PT	1	0.93		
PT < 10 days	**0.54**	**0.2**	**0.4**	**0.04**

HR = hazard ratio; CT = computed tomography; SAPS 3 = Simplified Acute Physiology Score 3; SpO_2 = oxygen saturation; Fi = inspiratory fraction; Pa = partial pressure; MV = mechanical ventilation; ICU = intensive care unit; PT = percutaneous tracheostomy; HFNC = high-flow nasal catheter. Dependent variable = days of hospitalization until outcome (hospital discharge or death).

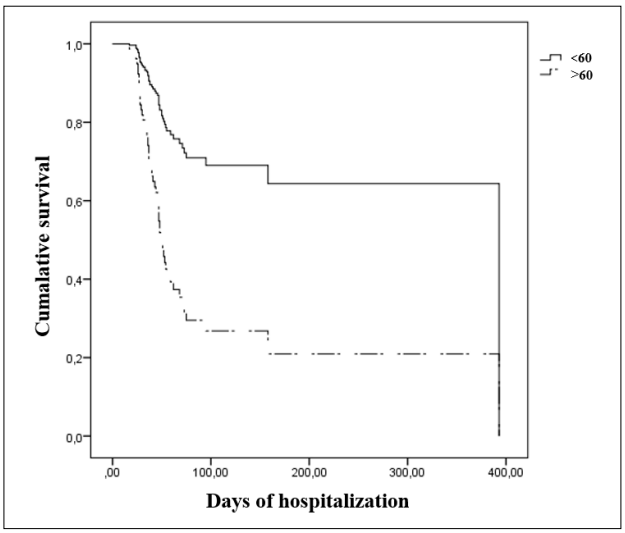

Figure 1. Cumulative survival according to age (HR = 3.38; P = 0.005).

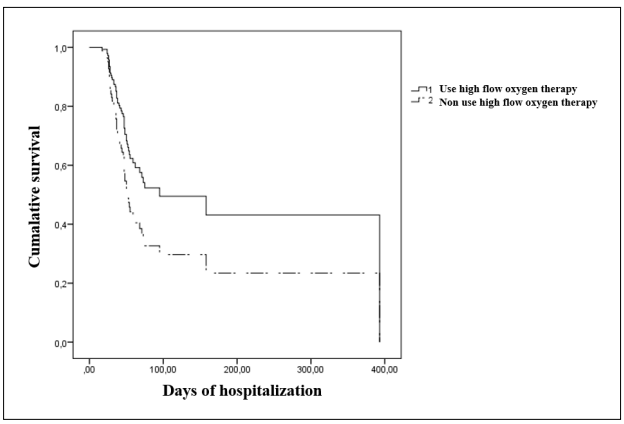

Figure 2. Cumulative survival according to the use of high-flow nasal therapy (HR = 0.59; P = 0.1).

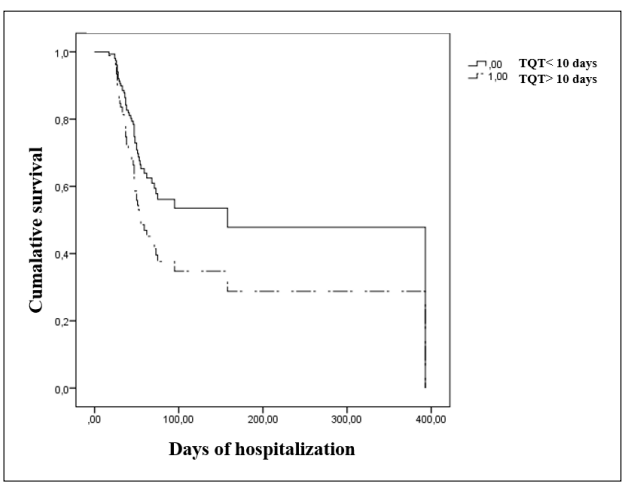

Figure 3. Cumulative survival in individuals who underwent tracheostomy before 10 days before orotracheal cannula (HR = 0.54; P = 0.2).

Multivariate analysis

In the multivariate analysis, patients over 60 years old had a reduced survival (HR = 3.35; P = 0.003; **Figure 4**). Patients who used HFNC prior to orotracheal intubation presented a better survival than those who did not (HR = 0.49; P = 0.02; **Table 2** and **Figure 4**). Patients who underwent PT earlier (< 10 days) also had better survival than those who underwent this procedure later (HR = 0.37; P = 0.04; **Figure 4**).

DISCUSSION

The COVID-19 pandemic has pushed healthcare systems globally to their limits, with the unprecedented task of managing large volumes of critically ill patients. In this context, tracheostomy has emerged as an imperative component of care with a heightened risk of viral transmission to HCP and requires careful attention.[9,10,11,12] Of primary concern in all countries is the relative scarcity of mechanical ventilators to support critically ill patients.[13,14] This resource scarcity could lead to a push to perform tracheostomies. However, whether this would allow for a more expeditious ventilator weaning process remains unclear.

To the best of our knowledge, this is the largest sample from a single center published to date. The optimal timing of tracheostomy varies by clinical context. Outside of the current pandemic, it is generally recommended to be performed within two weeks post-intubation, as prolonged intubation is associated with post-intubation laryngotracheal stenosis.[6] In our study, early tracheostomy patients had a better prognosis than late tracheostomy patients (**Table 1**). Currently, there is no evidence regarding the optimal timing of tracheostomy.

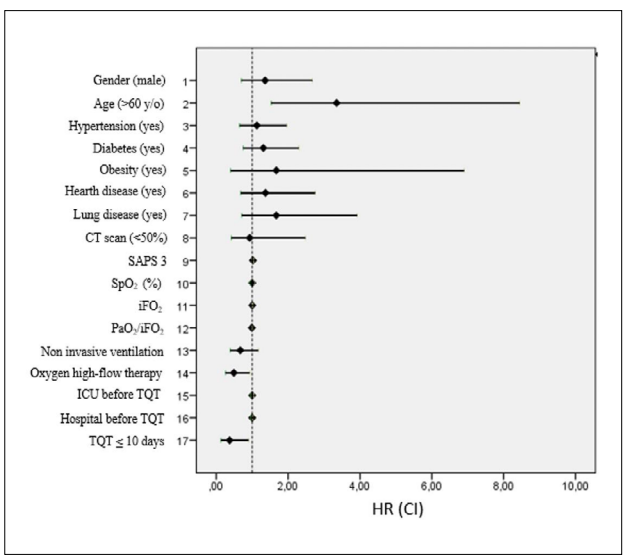

Figure 4. Hazard ratios and confidence intervals corresponding to multivariate analysis for age, use of oxygen high flow therapy, and days prior to tracheostomy; corresponding to univariate analysis for the other variables.

Two different tracheostomy techniques are currently available: OT and PT. The first is frequently performed in the operating room, although it can be performed at the bedside. No data are available to establish the superiority of one approach over the other in terms of infectious transmission or safety.[12,15]

The role of identifying PCR test status in COVID-19 patients ahead of tracheostomy is unclear.[16,17] Delaying tracheostomy to achieve negative test results is likely to prolong endotracheal ventilation and thus defer the potential benefits of tracheostomy while increasing the risk of complications related to endotracheal intubation.[17]

PT may be performed by an intensive care specialist in the ICU; however, the thoracic surgeon normally participates in the procedure. It consists of a percutaneous tracheal puncture guided by bronchoscopy and progressive dilatation before insertion of the tracheostomy cannula, as described previously. A few complications have been described, including unexpected decannulation, wound infection, and postoperative bleeding.[6,7,11]

Major complications of tracheostomy are rare. The risks of mortality, trachea-innominate fistula, and tracheoesophageal fistula from this procedure are all less than 1%. Early bleeding complications at the stoma are common (approximately 5%).[18]

Although it is not possible to define the optimal timing, we believe that tracheostomy in a stable or clinically improved COVID-19 patient should not be proposed before the 14th day after orotracheal intubation.[19,20]

Early tracheostomy accelerates weaning from the ventilator and may have a critical role in freeing up ventilators, ICU beds, and staff during surges.[21] This consideration is important, because resource scarcity may limit access to life-saving interventions for other patients.[22] As shown by other authors, we argue that tracheostomy before 14 days has a role in a select group of patients with COVID-19 respiratory failure requiring prolonged MV. **Figure 3** shows the cumulative survival in individuals who underwent tracheostomy before 10 days before orotracheal cannula.

The most important consideration regarding tracheostomy is not timing but whether the procedure is indicated. Critically ill patients requiring invasive ventilation have up to 50% mortality.[23] There are several factors favoring early tracheostomy, including the cumulative dose of sedation, pulmonary hygiene, physical rehabilitation, airway complications, ventilator-associated muscle atrophy, ventilator-associated pneumonia, tracheal injury, ICU capacity in surge, and physical rehabilitation. However, there are factors that favor delayed tracheostomy, such as MOF, need for prone positioning, inability to tolerate the procedure (MOF), and risks to HCP.[23]

Numerous randomized trials have demonstrated the benefits of early tracheostomy in appropriately selected patients. Tracheostomy

reduces the cumulative sedation dose and allows for earlier participation in physical therapy and rehabilitation; this improvement in early mobility lessens the risk of critical illness myopathy.[24] Early tracheostomy is also associated with earlier walking, talking, and eating.[25] Earlier extubation lowers the risk of airway complications arising from prolonged translaryngeal intubation, such as focal tracheomalacia and tracheal stenosis.

Published information from the 2003 SARS outbreak showed that OT was the preferred technique. Current guidelines for choosing OT or PT for COVID-19 patients differ between countries based on each one's preferences.[13]

The 103 patients included in this study underwent PT by the same team using the same surgical protocol and technique. All procedures were performed at the bedside and no HCP involved in this process were infected. We emphasize the importance of being used to the procedure, making it safer and faster and minimizing viral exposure.

In our experience, the mortality rate among ICU COVID-19 patients undergoing tracheostomy was 30%, similar to the previously reported overall mortality rate. Deceased patients had at least one comorbidity (73% with cardiovascular problems and 36% with diabetes mellitus) and presented with > 75% lung affection. They were ventilated under FiO_2 of 100% whenever entering the ICU, and had a mean total MV for 40 days.

Based on our data and those of previous publications, we could infer that tracheostomy did not affect the natural history of these patients. However, patients who underwent tracheostomy earlier (< 10 days) showed better survival. These findings may be related to fewer MV-related complications and early weaning from the ventilators, allowing these patients to leave the ICU before those who underwent the procedure later. In addition, these patients were in a more stable condition that allowed them to tolerate the procedure. These data were analyzed using multivariate analysis ($P < 0.04$).

Another interesting observation is the trend toward a significant association between the duration of intubation and overall survival; specifically, in our cohort, intubation longer than 20 days (median, 33 days) resulted in an increased risk of death. This result could support the choice to postpone OT in COVID-19 patients, as previously suggested for SARS-CoV-1 patients.[13] We noticed better survival in patients who underwent HFNC and delayed or avoided the need for MV, which had an impact on outcomes in both univariate ($P = 0.10$) and multivariate analyses ($P = 0.02$).

As shown by other authors, the survival rate of COVID-19 patients requiring MV is extremely poor (< 30%). Otherwise, this argues against early tracheostomy.[20,26]

Although it is not possible to define an optimal timing, we believe that tracheostomy in a stable or clinically improved COVID-19 patient should not be performed before the 7th day after orotracheal intubation.[20,27,28]

Currently, evidence supporting a specific technique for tracheostomy in terms of minimizing the risk of HCP is lacking.[5] There is no evidence to confirm whether the viral load of a patient at a specific time correlates with transmission risk to HCP. However, it has been shown that viral load does not correlate well with the severity of symptoms; therefore, not all critically ill patients will have high viral loads.[16] Still, there is a consensus that providing adequate PPE for HCP is mandatory to mitigate infection in aerosol-generating procedures.

Some guidelines strongly advise that patients should test negative for COVID-19 before proceeding with tracheostomy, although the negative test should not diminish the exposure risk.[6] Even in a recently published multi-society consensus statement, the writing panel could not find any evidence for recommending a specific tracheostomy timing in patients with COVID-19-related respiratory failure.[29,30]

Early post-tracheostomy care is fundamental to minimize the risk of aerosol generation in HCP and other patients. Early provinces include keeping the cuff inflated, in-line airway suction, and aversion of humidified oxygen whenever feasible.[15] The changing of the tracheostomy tube and progress on decannulation protocols should be judged case by case, since such procedures are also aerosol generators.[31]

In our view, an enhanced level of PPE represents the safest possible level of protection for HCP and should be compulsory when performing PT. In our institution, complete PPE is available, and those involved in this process should receive PPE usage training, as this can represent a fount of contamination if not used properly.[29,30]

Currently, the infection rate associated with tracheostomy is unknown. There is no evidence to support the use of a specific technique to minimize the risk of HCP exposure to airborne droplets. Although solid data on SARS-CoV-2 HCP infectivity are scarce, infection and death during HCP have been described.[31]

A major limitation of our study is that the patients were recruited and compared from a single center.

CONCLUSION

Our study demonstrated the feasibility and importance of early PT in COVID-19 patients. All 103 patients successfully underwent PT, and no major complications were reported. Tracheostomy may reduce the duration of MV and lead to shorter ICU stays. The health system is overloaded by the scarcity of ventilators and beds for critically ill patients, and the timing of PT should be taken into account. In conclusion, early PT in selected patients may reduce the duration of MV and lead to shorter ICUs stays.

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Availability of data	Sample statement
All data are incorporated into the article and its online supplementary material.	The data underlying this article are available in the article and in its online supplementary material.
Data available on request.	The data underlying this article will be shared on reasonable request to the corresponding author.

Self-perception of health and quality of life in patients on renal replacement therapy undergoing hemodialysis: a case-control study

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ABSTRACT

BACKGROUND: Chronic kidney disease (CKD) presents challenges to human health and quality of life, with care primarily focusing on renal function and comorbidity management. Several studies confirm the relationship between oral and systemic conditions of patients. Therefore, CKD and periodontal disease can be related because they are both inflammatory conditions that further increase the risk of other pathologies. The impact of CKD on oral health and overall quality of life is an area of interest.

OBJECTIVES: To evaluate patients with CKD undergoing renal replacement therapy regarding the levels of self-perception of oral health and quality of life.

DESIGN SETTING: This case-control study was conducted jointly at the Universidade de Passo Fundo and Hospital São Vicente de Paulo, Brazil.

METHODS: This study included two patient groups: 1) Case group with CKD (CGA) comprising 116 patients; 2) Control group without CKD (CGO) composed of 124 patients. We used a structured questionnaire and the Oral Health Impact Profile (OHIP-14). We used the Chi-square and Wilcoxon-Mann-Whitney tests as well as an analysis of variance measure ($P < 0.05$).

RESULTS: The systemic diseases most prevalent among our patient cohort included hypertension (16.9% CGO and 75.9% CGA) and diabetes mellitus (8.9% CGO and 38.8% CGA). The systemic health perception was good in 66.9% of the CGO group and average in 42.2% of CGA group members. Oral health perception was good in 46% of CGO and 50% of CGA group members. Results of the self-assessment for quality of life showed a statistically significant difference between the groups for physical domain, physical disability, and social disadvantage. A comparison between the control and case (CKD) groups, based on the OHIP-14 score, showed statistically significant differences in the functionality ($P < 0.006$), physical disability ($P < 0.042$), and social disadvantage ($P < 0.031$) domains for the CKD group.

CONCLUSION: Patients with CKD have lower rates of self-perception of oral health and quality of life than individuals without CKD.

INTRODUCTION

The prevalence of chronic kidney disease (CKD) is rapidly increasing worldwide, representing a significant financial burden for healthcare systems.[1] CKD is classified into the following five stages: renal disorders with a regular glomerular filtration rate; renal disorders with a slight reduction in the glomerular filtration rate;[2] moderate reduction in glomerular filtration rate;[3] severe reduction in the glomerular filtration rate;[4] and kidney failure.[5] The glomerular filtration rate value reveals the level of kidney dysfunction to the clinician.

It is estimated that CKD has a worldwide prevalence of 11–13% among adults.[2] However, CKD prevalence has increased both in the industrialized countries and worldwide, and this is important because of its high morbidity and mortality.[3–8] Between 30% and 50% of all cases of end-stage renal failure may be attributed to diabetes mellitus and arterial hypertension.[9] Nutritional factors and depression may impact the quality of life of individuals undergoing renal replacement therapy.[10,11]

Several studies have validated the relationship between the oral and systemic conditions of patients. Therefore, CKD and periodontal disease may be related because they are both inflammatory conditions that further increase the risk of other pathologies, such as diabetes, hypertension, atherosclerosis, liver diseases, and changes in the intestinal microbiota, thereby reducing the quality of life of individuals. Thus, the lack of oral health care in patients with CKD causes

more serious clinical problems, affecting the quality of life and risk of transplant, and may even lead to death.[8,9]

However, several studies have shown an association between oral diseases and CKD, and regular oral care and instruction can be a relevant strategy to reduce CKD.[12–16] Strategies for facing this global health problem include identifying risk factors associated with comorbidities and complications and adequately managing the pertinent issues.[13,14]

OBJECTIVE

This case-control study aimed to evaluate self-perception of the level of oral health and quality of life in patients with CKD undergoing renal replacement therapy by comparing the case group (having CKD) with the control group.

METHODS

The present study was approved by the Research Ethics Committee of the Universidade de Passo Fundo, Brazil, in March 2020 (Ethical Approval Protocol Number 3.929.915), and by the Research Ethics Committee of the Hospital São Vicente de Paulo (HSVP) (No. 3.929.915), in Passo Fundo, Rio Grande do Sul, in November 2019. The methodology of this study was based on the Strengthening of the Reporting of Observational Studies in Epidemiology guidelines.

Study design

This is a case-control observational study. The predictor variable is CKD, while the outcome variables are self-perception of oral health and quality of life. The participants were divided into two groups. The case group (Group 1; CGA; n = 116) included patients with CKD undergoing hemodialysis, and the control group (Group 2; CGO; n = 124) included patients without CKD. These patients were evaluated between September 2020 and June 2021.

Questionnaires

A questionnaire with closed questions about sociodemographic data and systemic and oral health was employed. Also, all patients answered the Oral Health Impact Profile (OHIP-14) questionnaire that assesses oral health-related quality of life.

The individuals who agreed to participate in the present study were requested to complete a self-assessment questionnaire structured by the authors according to the investigative objectives, containing questions about socioeconomic and demographic aspects, behavioral factors, general and oral health conditions, and items of the OHIP-14 quality of life index. The data were collected in a questionnaire-interview format, where evaluators asked the questions and patients undergoing renal replacement therapy responded according to the given alternatives. An informed consent form (ICF) was also provided, describing the significance of consent for participating in the present study.

Sample

We selected volunteers with CKD (CGA group) undergoing hemodialysis at the HSVP in Passo Fundo, Rio Grande do Sul (n = 116 individuals) to establish the case-cohort for this study. For comparative purposes, a CGO group was recruited with a similar profile regarding sex and age attributes and was comprised of patients of the Faculdade de Odontologia of the Universidade de Passo Fundo, Brazil (n = 124 individuals).

Inclusion criteria

This study included participants who met the following selection criteria:

- CGA group: Individuals who had been undergoing hemodialysis at HSVP, Brazil for at least 1 year, were ≥ 18 years of age, and consented to participate by signing the ICF.
- CGO group: Individuals receiving dental treatment at the Faculdade de Odontologia, Universidade de Passo Fundo, Brazil, who were ≥ 18 years old and agreed to participate by signing the ICF.

Exclusion criteria

This study excluded participants who did not meet the following criteria:

- Individuals under the age of 18 because obtaining informed consent from minors is not feasible. Additionally, those with special needs were excluded to ensure that all participants could fully understand and engage with the research requirements. Lastly, individuals who did not provide informed consent to participate were also excluded, in accordance with ethical considerations emphasizing the importance of voluntary participation.

Pilot study

A pilot study was performed at the Faculdade de Odontologia of the Universidade de Passo Fundo, Brazil, employing other patients who did participate in this study. The pilot study was necessary to adjust the data collection instruments and identify the most suitable approach method for the analyzed population.

The research team (two examiners) was trained and calibrated at the Universidade de Passo Fundo, Brazil, before starting the study, with a theoretical-practical module carried out in a classroom setting using image projections and organized by the coordinator of this research. Examiner agreement was based on the pilot study, obtaining the Kappa coefficient (0.92).

The data were tabulated in Microsoft Excel (Microsoft, Redmond, Washington) and the statistical analysis was carried

out using SPSS software, version 20.0, (IBM, Chicago). The Chi-square test (categorical variables) analyzed the quantitative data, and the non-parametric Wilcoxon-Mann-Whitney test analyzed the quantitative variables. The accepted statistical significance level between groups was $P < 0.05$.

The OHIP-14 analyses involved working with continuous variables, comparing the mean values of each domain and the overall score according to the group (control or case) by employing one-way analysis of variance, and using SPSS software. The statistical significance level established for all analyses was 5% ($P < 0.05$). A linear model was tested to verify the mean of the overall score according to the group.

RESULTS

The present study involved the participation of 240 individuals, comprising the CGO group (Control group; Faculdade de Odontologia, Universidade de Passo Fundo, Brazil; n = 124) and the CGA group (Case group; Hospital São Vicente de Paulo, Passo Fundo, Brazil; n = 116).

The participants were divided into five age groups. Among the CGO members, the age group of 20–35 years (31.5%) had the highest number of participants. Among the CGA members, the most prevalent age group was 35–50 years old (36.2%). Regarding sex, the CGO group had 59.7% men and 40.3% women, while the CGA group included 58.6% men and 41.4% women (Table 1).

As for skin color, white skin was the most frequent in both groups: 78.2% in the CGO and 60.3% in the CGA group. Regarding marital status, the CGO group had 42.7% single participants, and the CGA group had 51.7% married individuals. The responses to the employment query showed that 83.6% of the CGO group were working, while 65.3% of the CGA group had worked previously but were not currently employed. When evaluating the education of participants, the results indicated primarily that 50% of the CGO group had completed high school, while 38.8% of the CGA group had completed primary school.

As for the most prevalent systemic diseases in the study cohort, the CGO group had hypertension (16.9%), respiratory problems (12.9%), and diabetes mellitus (8.9%). The CGA group showed a higher prevalence of systemic conditions: hypertension (75.9%), anemia (50.9%), and diabetes mellitus (38.8%) (Tables 2, 3).

Table 4 shows that the CGA group members displayed the highest mean value in the functional, physical disability, and social disadvantage domains. A generalized linear model showed that the CGO group tended to have lower mean values in the overall OHIP-14 score compared with the CGA group ($\beta = -0.221$ (-0.32–0.12; $P < 0.001$).

DISCUSSION

The present study accepts the proposed hypothesis that patients with CKD undergoing hemodialysis have a lower self-perception of quality of life and oral health compared with control patients. Patients with CKD showed a higher prevalence of systemic diseases, such as arterial hypertension, diabetes mellitus, and anemia. These patients also showed the worse indicators of oral health-related quality of life in the functionality, physical disability, and social disadvantage domains. Applying the OHIP-14 questionnaire as well as a clinical and sociodemographic questionnaire to both groups (case and control) ensured the comparison and confirmation of results.

Table 1. Comparison of systemic diseases in the CGO and CGA groups (Wilcoxon Man-Whitney U test; P value)

Systemic disease	CGO, mean	CGA, mean	P value
Hypertension	86.36	157.03	0*
Chronic Kidney Disease	62.5	182.5	0*
Diabetes mellitus	103.15	139.05	0*
Respiratory problems	117.48	123.72	0.266
Depression	113.68	127.79	0.008*
Cancer	117.9	123.38	0.098
Anemia	91.94	151.03	0*
Nutritional problems	109.9	131.83	0*
Cardiovascular disease	111.68	129.93	0.001*
Lupus	118	123.17	0.2
Hepatitis C	120	121.03	0.301
Total	**124**	**116**	

* Indicates statistical significance between groups; CGO, control group; CGA, case group.

Table 2. Description of the self-perception of systemic, oral, and chewing health

Perception of systemic health	CGO, n (%)	CGA, n (%)
Very good	20 (16.1%)	5 (4.3%)
Good	83 (66.9%)	47 (40.58%)
Regular	18 (14.5%)	49 (42.2%)
Poor	2 (1.6%)	11 (9.5%)
Very poor	**1 (0.8%)**	**4 (3.4%)**
Total	**124 (100%)**	**116 (100%)**
Oral health perception		
Very good	19 (15.3%)	5 (4.3%)
Good	57 (46%)	58 (50%)
Regular	30 (24.2%)	30 (25.9%)
Poor	14 (11.3%)	18 (15.5%)
Very poor	**4 (3.2%)**	**5 (4.3%)**
Total	**124 (100%)**	**116 (100%)**
Chewing health perception		
Very good	26 (21%)	21 (18.1%)
Good	71 (57.3%)	54 (46.6%)
Regular	19 (15.3%)	32 (27.6%)
Poor	5 (4%)	7 (6%)
Very poor	**3 (2.4%)**	**2(1.7%)**
Total	**124 (100%)**	**116 (100%)**

CGO, control group; CGA, case group.

Table 3. Assessment of self-perception, oral condition, and dental care

Oral health conditions	CGO, n (%)	CGA, n (%)
Xerostomia		
No	103 (83.1%)	97 (78.2%)
Yes	21 (16.9%)	27 (21.8%)
Total	**124 (100%)**	**116 (100%)** **100 (100%)**
Last visit to the dentist		
Never	2 (1.6%)	39 (33.6%)
< 6 months	81 (65.3%)	21 (18.1%)
6 months–1 year	21 (16.9%)	18 (15.5%)
1–2 years	10 (8.1%)	17 (14.7%)
2–5 years	9 (7.3%)	9 (7.8%)
5–20 years	1 (0.8%)	12 (9.6%)
Total	**124 (100%)**	**116 (100%)**
Last service location		
Health Center	17 (13.7%)	51 (44%)
Private service	42 (33.9%)	57 (49.1%)
Other	1 (0.8%)	6 (5.3%)
University	63 (50.8%)	0 (0%)
Could not inform	1 (0.8%)	2 (1.8%)
Total	**124 (100%)**	**116 (100%)**
Reason for the last visit		
Routine	35 (28.2%)	22 (19%)
Pain	10 (8.1%)	18 (15.5%)
Bleeding	3 (2.4%)	1 (0.9%)
Caries/rest/fill	21 (16.9%)	15 (12.9%)
Root canal treatment	12 (9.7%)	4 (3.4%)
Extraction	13 (10.5%)	28 (24.1%)
Dentures	7 (5.6%)	18 (15.5%)
Other	23 (18.5%)	10 (8.6%)
Total	**124 (100%)**	**116 (100%)**
Pain when chewing		
No	99 (79.8%)	95 (81.9%)
Yes	25 (20.2%)	21 (18.1%)
Total	**124 (100%)**	**116 (100%)**
Difficulty chewing solid food		
No	88 (71%)	76 (65.5%)
Yes	36 (29%)	40 (34.5%)
Total	**124 (100%)**	**116 (100%)**
Difficulty swallowing		
No	118 (95.2%)	107 (92.2%)
Yes	6 (4.8%)	9 (7.8%)
Total	**124 (100%)**	**116 (100%)**
Pain in the mouth or teeth		
No	101 (81.5%)	92 (79.3%)
Yes	23 (18.5%)	24 (20.7%)
Total	**124 (100%)**	**116 (100%)**
Pain intensity		
Mild	15 (12.1%)	12 (10.3%)
Moderate	10 (8.1%)	8 (6.9%)
Severe	0 (0%)	5 (4.3%)
None	99 (79.8%)	91 (78.4%)
Total	**124 (100%)**	**116 (100%)**

CGO, control group; CGA, case group.

Our present findings revealed that men were more prevalent in the samples (CGO 59.7% and CGA 58.6%), corroborating previous studies that showed male prevalence rates of 58%,[17] 60.6%,[18] 56.5%,[19] and 67%.[20] Regarding age, our study found a prevalence of the 20-35 years age group in the CGO (31.5%) and of the 35–50 years age group (36.2%) in the CGA group.

The data on employment and source of income were compelling. Among the 116 patients with CKD, only 5.2% were currently employed, while 58.6% were retired or had a "household" occupation. These data can be explained on the basis that CKD requires permanent treatment, and adequate adherence to regimens and collaboration are conditions for disease control and therapy success.[21] People often spend years on hemodialysis and have to visit hospitals or specialized clinics up to three times a week for 2–4 hours per visit.[2,11,22]

As for the most prevalent systemic diseases in the analyzed CKD sample, arterial hypertension was the most prevalent (75.9%) followed by diabetes mellitus (38.8%). Another study on quality of life and CKD found similar results, where arterial hypertension was the most prevalent condition, followed by diabetes mellitus.[10,23] Epidemiological studies explain these results by claiming that blood pressure and the usual risk factors for cardiovascular diseases are directly related to target organ damage, including vascular stiffness and poor outcomes in hemodialysis patients.[24] Another study has reported that renal dysfunction related to arterial hypertension contributes to increasing intraluminal hydrostatic pressure.[25]

Regarding the relationship between diabetes mellitus and CKD in the Brazilian population, approximately 35.6% patients with CKD have systemic arterial hypertension and diabetes mellitus.[22] This number is much higher than our prevalence data. When linking renal dysfunction to diabetes, described in the literature as diabetic nephropathy, several factors work together by weakening the glomerular basement membrane, expanding the mesangial matrix, decreasing the number of podocytes, and promoting glomerulosclerosis and tubule-interstitial fibrosis.[25]

The second most prevalent disease in the CGA group was anemia, occurring in half the patients (50.9%). Anemia is defined as hemoglobin levels < 13 g/dL in men and < 12 g/dL in women. Anemia is a common CKD complication resulting from interferences with erythropoietin production.[26,27] These results can be explained by changes in renal physiology, considering that the kidneys perform multiple functions that can be didactically characterized as filtration, reabsorption, homeostasis, endocrinological and metabolic function.[16]

Some diseases showed a statistically significant difference when pairing the case (CGA) and control (CGO) datasets and comparing them for systemic health as follows: systemic arterial hypertension (P = 0), CKD (P = 0), diabetes mellitus (P = 0), depression

Table 4. Mean and standard deviation (SD) according to the overall score and OHIP-14 domains of the control and case groups of hemodialysis patients (n = 240)

Group		Variables							
		Overall score	Functional domain	Physical pain	Psychological discomfort	Physical disability	Psychological disability	Social disability	Social disadvantage
CGO	Mean	5.91	0.52	1.72	1.25	0.53	0.96	0.5	0.4
(n = 112)	SD	7.19	1.18	1.94	1.89	1.3	1.56	1.07	0.95
CGA	**Mean**	**6.9**	**0.86**	**1.75**	**1.43**	**0.75**	**1.04**	**0.49**	**0.55**
(n = 116)	**SD**	**8.24**	**1.55**	**1.98**	**1.98**	**1.49**	**1.77**	**0.99**	**1.3**
	P*	**0.324**	**0.006***	**0.536**	**0.352**	**0.042***	**0.165**	**0.592**	**0.031***

CGO, control group; CGA, case group; OHIP-14, Oral Health Impact Profile; SD, standard deviation.

(P = 0.008), anemia (P = 0), nutritional problems (P = 0), and cardiovascular disease (P = 0.001). These results demonstrate that the CGA group is more likely to develop or already have systemic diseases.

Oral cavity conditions also affect the quality of life. Thus, the impact of oral health has been studied extensively and has gained importance in the literature.[26–28] When left untreated and neglected by patients, worsening is frequently observed.[29] Besides local problems, oral conditions can also work as modifying factors for systemic conditions such as diabetes mellitus, respiratory diseases, and CKD.[30]

In this context, a comparison between the control and case (CGA; having CKD) groups based on the OHIP-14 score and its internal domains showed a statistically significant difference in the CGA group for functionality (P < 0.006), physical disability (P < 0.042), and social disadvantage (P < 0.031) domains. Overall, the patient group without CKD (CGO group) exhibited a better quality of life compared with those affected by CKD, particularly in the assessed domains.[31–33] A study performed in Iran with patients undergoing dialysis found a negative impact of oral conditions (assessed with the OHIP-14 questionnaire) on the quality of life of these patients.[34]

The present study has some limitations. The primary limitation concerns the non-feasibility of performing clinical examinations of the study participants due to the COVID-19 pandemic-related restrictions. Thus, important data such as the Decayed, Missing, and Filled Teeth index, gingival bleeding index, probing depth, radiographic examinations, and oral mucosa lesions could not be measured. Other limitations include the non-feasibility of standardizing the hemodialysis time for the CGA group members and the memory bias and subjectivity of questionnaire responses.

CONCLUSION

Patients with CKD (CGA group) exhibited lower rates of self-perception of oral health and quality of life than individuals in the CGO (control) group.

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Translation, cross-cultural adaptation and validation of the Weight Management Questionnaire in the Brazilian population: a cross-sectional study

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ABSTRACT

BACKGROUND: The prevalence of obesity has increased considerably worldwide, and it has become an important risk factor for chronic non-communicable diseases.

OBJECTIVES: To translate, cross-culturally adapt, and validate the Weight Management Questionnaire (WMQ) to evaluate weight control practices for health monitoring and intervention in Brazil.

DESIGN AND SETTING: This was a quantitative cross-sectional study.

METHODS: Obese and physically active lean individuals aged 18–59 years were included in this study. The tool used as an adaptation was the WMQ. The cross-cultural adaptation was conducted in five phases. For structural validity, confirmatory factor analysis (CFA) was used to verify the internal structure considering two domains: diet and physical activity. To determine construct validity, a comparison was performed between different groups (lean versus obese). Reliability was assessed using the intraclass correlation coefficient (ICC).

RESULTS: The short version of the WMQ after CFA presented eight items. Thus, the structure with two domains presented adequate fit indices: chi-square/degree of freedom = 1.66, comparative fit index = 0.996, Tucker-Lewis index = 0.994, root mean square error of approximation = 0.057, and standardized root mean square residual = 0.047. For construct validity, a significant difference was observed between the groups ($P < 0.05$) in both domains. We observed adequate reliability for both domains (ICC ≥ 0.854).

CONCLUSION: The WMQ can be used for the Brazilian population, as it is reliable and has adequate internal structure, supporting its use in future research.

INTRODUCTION

The World Health Organization (WHO) defines obesity as a chronic condition determined by excessive fat accumulation that leads to negative health.[1] The prevalence of obesity has increased considerably worldwide in the last decades, which has resulted in an increase in lifestyle-related diseases. Obesity is an important risk factor for non-communicable diseases (NCD), such as diabetes, hypertension, and cardiovascular diseases, among others.[2]

A sedentary lifestyle has become one of the most important risk factors for the development of NCD. Although there is sufficient scientific evidence to confirm the benefits of regular physical activity (PA), currently 31.1% of the global adult population does not meet the minimum PA recommendations (≥ 150 min of moderate or vigorous intensity activity per week).[3] Adherence to an unhealthy lifestyle may cause obesity and, in consequence, NCD. According to the WHO, lifestyle is defined as a set of behaviors and habits that are influenced by the process of socialization; they comprise the consumption of substances such as tobacco, alcohol, coffee, tea, dietary habits, and physical exercise.[4]

Epidemiologically, in developing countries, obesity mainly affects middle-aged adults, whereas in developed countries, it affects both sexes at the same proportion and people of all age ranges.[5] Overweight and obesity may reach levels of 89% and 85% in men and women, respectively, by 2030. This will cause an increase in the prevalence of obesity-related coronary cardiac disease by

97%, cancer by 61%, and type 2 diabetes by 21%. Therefore, health costs increase considerably, overloading the public health system.[5]

Currently, in Brazil, various instruments analyze obesity and physical activity in isolation in different populations. An example of this is the Lipedema Screening Questionnaire that assesses knowledge about this clinical condition in women.[6] Another tool committed to investigating the level of physical activity in Brazilians in the last 12 months is the Baecke Habitual Physical Activity Questionnaire (BHPAQ).[7]

Advice on lifestyle changes, such as nutritional education on calorie deficits and regular exercise, is the cornerstone of managing all lifestyle-related diseases. Understanding the determinants of non-adherence to advice on lifestyle changes may help physicians plan and develop interventions focused on assisting patients in achieving long-term healthy weight loss.[8]

In this context, the Weight Management Questionnaire (WMQ) was created and validated in India with 14 items in a Likert scale format and divided into two domains: items 1 to 12 are related to the diet domain, and items 13 and 14 are related to the physical activity domain. The questionnaire showed satisfactory structural validity and adequate internal consistency.[8] To the best of our knowledge, only the original version of the WMQ has been published,[8] thus our study is the first cross-cultural adaptation.

The rationale for translating and adapting the WMQ is the need for a valid, reliable, and culturally appropriate instrument to assess eating habits and physical activity of Brazilian individuals. The hypothesis of this study was that there would be significant differences in the WMQ domain scores between the groups. Additionally, we expect a reliable two-dimensional instrument with adequate internal consistency. Given the scenario of validated instruments in Brazil and the importance of this tool, the need arose to translate, cross-culturally adapt, and validate the WMQ for Brazilian Portuguese by assessing eating habits and physical activity using a single tool in a succinct and objective way. To date, this questionnaire has not been validated in any country, and this is the first validation study of this tool.

METHODS

Study design

This quantitative cross-sectional study was conducted in São Luís (the capital of Maranhão, Northeast Brazil). São Luís is located on the coast of Maranhão and is officially part of the Brazilian Legal Amazon.[9] All participants included in the study validated their participation by signing an informed consent form. This study was approved by the institutional ethics committee (report number: 2,853,570). Before validating the questionnaire in the Brazilian population, written consent was obtained from the author of the original manuscript (Dr. Piyush Ranjan).

Sample size

For sample size, we adopted the guidelines of the Consensus-based Standards for the Selection of Health Measurement Instruments (COSMIN), which consider a sample size seven times the number of items of the instrument as long as the minimal sample size is 100 participants.[10]

Obese individuals with a body mass index (BMI) > 30 kg/m² and physically active lean individuals (BMI < 24.9 k/m²) aged 18–59 years, were included in this study. We excluded individuals who were unable to read and write Brazilian Portuguese, with cognitive or neurological alterations, or with any impairment in their ability to reply to the questionnaire.

Instruments

The data were collected online (Google Forms, Mountain View, California). To characterize the sample, we evaluated sociodemographic and personal data using the BHPAQ, a self-applicable self-report instrument that evaluates physical activity over the last 12 months. The BHPAQ is composed of 16 items divided into three domains: occupational physical activity (items 1–8), free-time sports physical activity (items 9–12), and leisure non-sports physical activity (items 13–16). Each domain is considered separately to calculate the final score. The total score ranged from 1 to 5; the higher the score, the greater the habitual physical activity. The BHPAQ has been adapted and validated in the Brazilian Portuguese population.[7]

The target tool of this study was the WMQ, an instrument that evaluates adherence to diet and exercises for weight management.[8] The questionnaire has 14 items in two domains: 12 items on the "diet" domain and 2 items on the "physical activity" domain. The responses were based on a Likert scale ranging from 1 to 5 points. Originally, the values obtained were added to the scores for each domain.

TRANSLATION AND ADAPTATION

Translation and adaptation into Brazilian Portuguese were performed in accordance with the Guidelines for the Process of Cross-Cultural Adaptation of Self-Report Measures,[11] which are divided into five steps:

1) Translation: Two independent translators, with Brazilian Portuguese as their native language and fluency in English, translated the original version of the questionnaire into Brazilian Portuguese.
2) Synthesis of the translations: After discussions and reviews, both translators observed by one of the researchers synthesized the two translated versions of the questionnaire independently and produced a single version in a consensual way.
3) Back-translation: Two independent translators (with no technical background in the health area and blind to the original version of the questionnaire), both with English as their mother

tongue and fluency in Portuguese, translated the Portuguese version of the questionnaire back to English.

4) Expert committee analysis: Six experts in the fields of nutrition, physical exercise, and health and wellness reviewed all translated and back-translated versions and corrected any discrepancies, creating a pre-final version of the WMQ.
5) Pre-final version: To test the pre-final version, the sample comprised of 34 randomly selected participants. Next, to check the instrument's measurement properties, the final version of the transculturally adapted WMQ was administered to 203 individuals (101 obese and 102 physically active, lean individuals).

DATA ANALYSIS

In terms of descriptive analysis, quantitative variables are presented as means and standard deviations, and qualitative variables are presented as numbers and percentages. Comparisons between the lean and obese groups were made using an independent t-test or chi-square test, according to the normality of the data.

For test-retest reliability analysis, we used a subsample of 34 participants. The individuals answered the WMQ at two time points, with an interval of seven days between evaluations.[12] We used the intraclass correlation coefficient (ICC), and a value equal to or higher than 0.75 was considered as the acceptability cut-off.[13–15] In addition, the standard error of measurement (SEM) and minimal detectable difference (MDD) were calculated.[12]

For structural validity, confirmatory factor analysis (CFA) was performed using R Studio (Boston) with the packages lavaan and semPlot. The WMQ is scored on a Likert scale (ordinal data). Therefore, CFA was performed by implementing a polychoric matrix and the extraction method using robust diagonally weighted least squares. The model fit was assessed using the following indices: root mean square error of approximation (RMSEA) with a confidence interval (CI) of 90%, comparative fit index (CFI), Tucker-Lewis index (TLI), standardized root mean square residual (SRMR), and chi-square/degrees of freedom (DF).

Values higher than 0.90 were considered appropriate for CFI and TLI, and values lower than 0.08 were considered appropriate for RMSEA and SRMR. Values below 3 were considered appropriate for the interpretation of chi-square/DF.[13] In CFA, factor loadings equal to or higher than 0.40 were considered appropriate for the domain. For WMQ refinement, modification indices > 10 were used to identify redundant items and items with lower factor loadings in every paired analysis were excluded.[14]

To determine construct validity, a comparison between recognizably distinct groups (lean versus obese) was performed using a t-test for independent samples, with a significance level of 5%.[10] The effect size was calculated based on Cohen's d, according to the website https://www.psychometrica.de/effect_size.html, with the following interpretations of the d value: 0.2 (weak), 0.5 (moderate) and > 0.8 (large effect size). Descriptive analysis, construct validity, and reliability were analyzed using SPSS software (version 17.0, Chicago).

RESULTS

Translation and cross-cultural adaptation

After translating the WMQ, we adapted the food items mentioned in the questionnaire to the Brazilian context. These adaptations were performed for items 3, 4, 5, 6, and 9, as shown in **Table 1**. There was 100% understanding of the items in the pre-final version of the WMQ.

Structural validity

CFA was employed to check the internal structure, considering two domains according to the original proposal of the questionnaire (Model 1): the diet domain (items 1 to 12) and the physical activity domain (items 13 and 14). The proposed model showed an inappropriate internal structure. As the internal structure of the proposed model was considered inappropriate, we excluded items with factor loadings below 0.40, including items 1 (0.19), 2 (0.33), 6 (0.29), and 9 (0.26). Subsequently, we applied CFA again with the exclusion of these four items from the diet domain (Model 2), and the values of the fit indices remained inappropriate (except for CFI): chi-square/DF = 9.77, CFI = 0.903, TLI = 0.871, RMSEA (90% CI) = 0.208 (0.188 to 0.229), and SRMR = 0.153, as reported in **Table 2**.

Table 1. Adaptation of food items of the Weight Management Questionnaire (WMQ)

Translated version	Adapted version
Item 3: laddu, barfi, jalebi, kulfi, chocolate, halwa, arroz doce.	Item 3: brigadeiro, cocada, doce de leite, goiabada, paçoca, pudim.
Item 4: puri, parathas, kachori, tikki, bhature, pakoras, samosas.	Item 4: pastel, batata frita, torresmo, salgados, alimentos fritos.
Item 5: namkeen, bhujia, pickles, chutney, papad.	Item 5: fast food, macarrão instantâneo, pipoca industrializada, salgadinhos industrializados.
Item 6: coalhada, lassi.	Item 6: suco, achocolatado.
Item 9: gordura de carneiro.	Item 9: manteiga, queijo.

Table 2. Fit indices after confirmatory factor analysis of the Weight Management Questionnaire (WMQ)

Fit indices	Model 1	Model 2
Chi-square/DF	7.35	9.77
CFI	0.846	0.903*
TLI	0.815	0.871
RMSEA (90% CI)	0.177 (0.164 to 0.191)	0.208 (0.188 to 0.229)
SRMR	0.15	0.153

RMSEA, root mean square error of approximation; CFI, comparative fit index; TLI, Tucker-Lewis index; SRMR, standardized root mean square residual; DF, degrees of freedom. * Adequate fit index.

With Model 2, the issues of the model were investigated using modification indices, and high correlations were identified among the instrument items, as indicated in **Table 3**, which resulted in the exclusion of items 7 and 8 (lowest factor loading in the analysis paired with modification indices). Following the exclusion of items 7 and 8, CFA was employed again, considering items 3, 4, 5, 10, 11, and 12 for the diet domain, and items 13 and 14 for the physical activity domain (Model 3), producing appropriate fit indices: chi-square/DF = 1.66, CFI = 0.996, TLI = 0.994, RMSEA (90% CI) = 0.057 (0.016 to 0.092), and SRMR = 0.047.

Figure 1 shows the relationship between the WMQ domains and the items with appropriate factor loadings (> 0.4). The diet (D1) and physical activity (D2) domains were negatively correlated; that is, they were inversely proportional. Therefore, the scores of the short version of the WMQ should be determined by domain, with diet domain scores from 6 to 30, and physical activity domain scores from 2 to 10. For interpretation, the higher the score in the diet domain, the better the individual's eating habits; the lower the score in the physical activity domain, the more active the individual. The Brazilian version of the WMQ can be accessed through the website questionariosbrasil.blogspot.com.

Table 3. Modification items (MI) and items excluded from Weight Management Questionnaire (WMQ)

Item	Description	Factor loading	MI	Item excluded
Item 7	How often do you eat fruit and salad?	0.68	228.336	Item 8
Item 8	How often do you eat vegetables and greens?	0.63		
Item 7	How often do you eat fruit and salad?	0.68	22.432	Item 7
Item 13	How many times do you exercise in a week?	0.95		

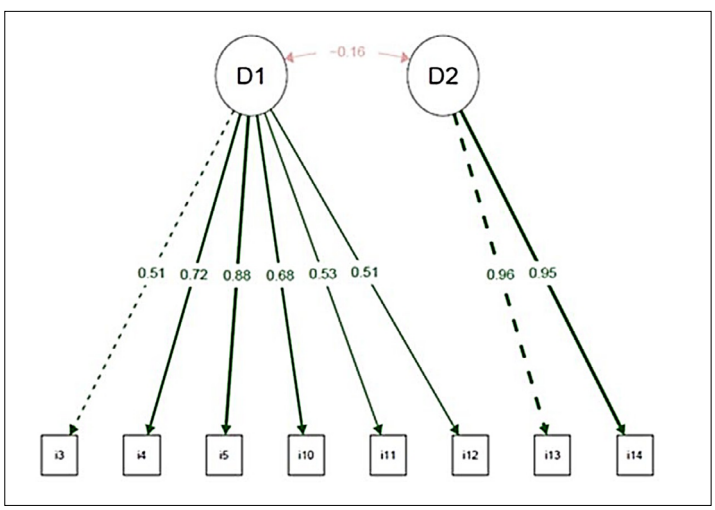

Figure 1. Path diagram showing the relationship between WMQ domains and items with their respective factor loadings.

Data descriptive analysis

Table 4 shows sample description, in addition to comparisons between the lean and obese groups. Significant differences were observed for weight, BMI, BHPAQ sports and leisure domain, and education level.

Construct validity

Construct validity was conducted by comparing two recognizably distinct groups: physically active lean individuals (BMI 18.5–24.9 kg/m²) versus obese individuals (BMI > 30 kg/m²). As **Table 5** shows, a significant difference was observed between the groups ($P < 0.05$), indicating that the WMQ construct was valid.

Test-retest reliability

In terms of the diet domain, appropriate test-retest reliability was observed in active lean (ICC = 0.977) and obese (CCI = 0.854) participants. Similarly, in the physical activity domain, satisfactory test-retest reliability was observed for the active lean (ICC = 0.940) and obese (ICC = 0.924) participants. Further details are presented in **Table 6**.

DISCUSSION

This study aimed to translate, transculturally adapt, and validate the WMQ in Brazilian Portuguese. After translation, synthesis of translations, back-translation, expert committee analysis, and a pre-final version test, the instrument was applied to a sample of 203 participants. The original questionnaire was created and validated for obese individuals with nonalcoholic fatty liver disease; however, it is worth mentioning that this Brazilian adaptation was made only for obese individuals because the questionnaire has generic characteristics.

For the short version of the WMQ for the Brazilian population, a two-dimensional tool was proposed with two domains: a diet domain with a score between 6 and 30, and a physical activity domain with a score between 2 and 10, presenting a valid internal structure with eight items after the execution of the structural validity. To interpret data from the short version, the higher the diet domain score, the better the eating habits, and the lower the physical activity domain score, the more active the individual, as the questionnaire domains are inversely proportional.

The questionnaire revealed a significant difference between the two distinct groups (obese versus active lean), thus presenting a valid construct. Regarding test-retest reliability, adequate reliability was observed in the active lean (ICC = 0.977) and obese (ICC = 0.854) participants. Similarly, for the physical activity domain, satisfactory test-retest reliability was observed for the active lean (ICC = 0.94) and obese (ICC = 0.924) participants.

It is important to highlight the similarities and differences between the original and our proposed versions of the WMQ. The original version used exploratory factor analysis, but did not

Table 4. Comparison among the variables of sample characterization of the active lean group and obese group

Quantitative variables	Active lean group (n = 102)		Obese group (n = 101)		P value
	Mean	Standard deviation	Mean	Standard deviation	
Age (years)	29.35	7.93	30.27	7.39	0.392
Weight (kg)	63.89	9.5	95.16	16.34	0.001[a]
Height (m)	1.68	0.09	1.68	0.1	0.929
BMI (kg/m^2)	22.45	1.64	33.56	3.77	0.001[a]
BHPAQ-O (score, 1–5)	2.56	0.64	2.56	0.61	0.992
BHPAQ-S (score, 1–5)	2.96	0.68	2.15	0.82	0.001[a]
BHPAQ-L (score, 1–5)	2.66	0.59	2.36	0.67	0.001[a]
Qualitative variables	**n**	**%**	**n**	**%**	**P value**
Sex	102		101		
Female	56	54.9	50	49.5	0.529
Male	46	45.06	51	40.59	
Education					
Incomplete secondary education	1	0.98	1	0.99	
Completed secondary education	11	10.78	25	24.75	
Incomplete higher education	23	22.54	13	12.87	
Completed higher education	38	37.25	34	33.66	0.038[b]
Incomplete post-graduation	12	11.76	5	4.95	
Completed post-graduation	17	16.66	23	22.77	
Marital status					
Single	84	82.35	72	71.28	
Married	13	12.74	24	23.76	0.242
Divorced	4	3.92	4	3.96	
Widower	1	0.98	1	0.99	

BHPAQ-O: occupational domain of the Baecke Habitual Physical Activity Questionnaire; BHPAQ-S: sports domain of the Baecke Habitual Physical Activity Questionnaire; BHPAQ-L: leisure domain of the Baecke Habitual Physical Activity Questionnaire. [a] Significant difference (independent t-test, P < 0.05); [b] Significant difference (chi-square test, P < 0.05).

Table 5. Comparison between the scores of the domains of the Weight Management Questionnaire (WMQ)

WMQ domains	Lean group	Obese group	p value	Cohen's d
Diet (6–30)	20.78 (4.9)	18.49 (5.44)	0.005*	0.442 (0.039, 0.923)
Physical activity (2–10)	3.37 (1.14)	7.27 (3.03)	< 0.001*	1.704 (1.149, 2.259)

* Significant difference (P < 0.05, independent t-test); Cohen's d interpretation: 0.2 (weak), 0.5 (moderate) and > 0.8 (large effect sizes).

Table 6. Reliability of the Weight Management Questionnaire (WMQ)

Parameters	Lean (n = 34) Values	Obese (n = 34) Values
Diet domain		
Test, mean (standard deviation)	21.32 (5.34)	17.76 (4.17)
Retest, mean (standard deviation)	20.88 (5.55)	18.02 (3.67)
ICC (95% CI)	0.977 (0.954, 0.988)	0.854 (0.707, 0.927)
SEM, score (%)	0.83 (3.91%)	1.50 (8.37%)
MDD, score (%)	2.29 (10.85%)	4.15 (23.31%)
Physical activity domain		
Test, mean (standard deviation)	3.32 (0.97)	7.61 (2.75)
Retest, mean (standard deviation)	3.41 (0.92)	6.73 (3.69)
ICC (95% CI)	0.94 (0.88, 0.97)	0.924 (0.847, 0.962)
SEM, score (%)	0.23 (6.88%)	0.89 (12.38%)
MDD, score (%)	0.64 (19.07%)	2.46 (34.32%)

ICC, intraclass correlation coefficient; CI, confidence interval; SEM, standard error of measurement; MDD, minimal detectable difference.

present the model fit indices for a more adequate understanding of the analysis. Also, the authors did not clearly describe the method used to identify the number of domains of the instrument, nor the factor loadings of the relationship between domain and items.[8] Our study used CFA considering the two-dimensional structure, however, some items were redundant. The shortened version proposed here presented adequate fit indices (chi-square/DF < 3, CFI and TLI > 0.9, RMSEA and SRMR < 0.08). In this sense, it is important that future studies in different cultures compare different structures to determine the most appropriate structure.

In addition to the factor analysis, the original version of the WMQ showed adequate internal consistency (Cronbach's alpha = 0.94). Our study did not use Cronbach's alpha because of the small number of items in the physical activity domain (only two items). However, the CFA we used was a much more robust analysis of the relationships between items and domains. In addition, our study identified adequate reliability in both domains; the original study did not evaluate reliability using ICC.[8]

Duarte et al.[16] reported the importance of translation, cultural adaptation, and validation of questionnaires in the health field, stressing that the adaptation of a questionnaire for use in a new culturally different population, when it is valid for the population studied, is the best and most appropriate way of guaranteeing a reliable and reproducible instrument, corroborating our results for the WMQ.

Concerning questionnaires similar to the constructs tested here, one study proposed the development of a new Portuguese version of the Sociocultural Attitudes Toward Appearance Questionnaire-4 (SATAQ-4),[17] culturally adapting it for use in different contexts of Brazilian Portuguese and evaluating its validity and reliability when applied to a sample of university students. The SATAQ-4 showed appropriate structural validity (CFI = 0.98, TLI = 0.98, RMSEA = 0.08), which was consistent with the WMQ values (CFI = 0.99, TLI = 0.99, RMSEA = 0.057).

The Exercise Adherence Rating Scale (EARS) is a tool designed to evaluate adherence to prescribed home physical activity, exclusively intended for individuals with chronic back pain.[18] The EARS-Br scale was translated, adapted, and validated to Brazilian Portuguese and presented acceptable internal consistency ($\alpha = 0.88$) and excellent reliability (ICC = 0.91).[19] The WMQ reliability was assessed based on a test-retest model, using the ICC, and it was considered appropriate and reliable, similarly to EARS-Br.

The consumption of ultra-processed food has grown considerably in Brazil; as a result, the prevalence of obesity has increased across all age ranges. For this reason, a study was conducted to test the validity and reliability of the Portuguese version of the Comprehensive Feeding Practices Questionnaire (CFPQ), which was translated, adapted, and applied to a sample of parents of preschool Brazilian children. The modified version of the CFPQ demonstrated significant internal reliability and appropriate validity in the population studied.[20]

Food literacy refers to the knowledge and competencies related to healthy food choices. Based on that, the process of transcultural adaptation and content validation of the Short Food Literacy Questionnaire (SFLQ) was performed for the Brazilian population.[21] The SFLQ-Br showed to be reliable, valid and stable, which assures that the instrument can be deemed useful to evaluate the food literacy of Brazilian adults.

The WMQ construct validity was satisfactory when comparing obese versus active lean individuals, as it produced a significant difference. The internal consistency indicated an appropriate value and the test-retest reliability was acceptable for the diet and physical activity domains.

The present study has several limitations. One of the main limitations of this study was that we did not perform an analysis of criterion validity because there was no pre-existing validated instrument with which to directly compare the WMQ. Although we performed CFA to assess structural validity, we did not conduct exploratory factor analysis, which could have provided further insight into the dimensionality of the WMQ. As our study had a cross-sectional design, it was not possible to assess responsiveness or identify the minimum clinically important differences. In addition, the database was established online and only in one region of Brazil. Therefore, emphasizing the importance of a country's cultural plurality, we recommend that further studies apply this tool to different regions of the country to corroborate the reproducibility of our study.

Regarding the clinical implications, this is the first study to translate, adapt, and validate the Indian version of the WMQ into Brazilian Portuguese, and we have shown it can be considered a reliable, valid, and useful tool in both clinical and scientific contexts. The direct use of the WMQ allows for the comprehensive assessment of eating habits and physical activity levels. The questionnaire consists of domains related to diet and exercise, enabling a detailed evaluation of lifestyle behaviors. In practice, the instrument can provide valuable data for healthcare professionals who, by using it, will be able to identify patterns and specific areas that will guide more personalized interventions, supporting more effective strategies for health promotion and control of diseases arising from sedentary lifestyles and improper eating habits.

CONCLUSION

The WMQ can be used in the Brazilian population as a reliable tool with an adequate internal structure and constructs, supporting its use in future research applications.

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The Brazilian version of the High-Activity Arthroplasty Score: psychometric property evaluation in hip replacement patients

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ABSTRACT

BACKGROUND: The High-Activity Arthroplasty Score (HAAS) is a reliable and valid self-administered questionnaire that was developed in British English and was designed to determine the level of physical activity in patients after lower limb arthroplasty (hip and/or knee). The Brazilian version (HAAS-Brazil) was developed after a cross-cultural adaptation in 2023.

OBJECTIVE: To evaluate the psychometric properties of HAAS-Brazil in patients after hip arthroplasty.

DESIGN AND SETTING: A cross-sectional quantitative and qualitative study was conducted in an orthopedic outpatient setting.

METHODS: Evidence for the validity of HAAS-Brazil was assessed via psychometric testing, which followed the COnsensus-based Standards for the selection of health status Measurement INstruments (COSMIN).

RESULTS: A total of 112 patients with a mean age of 56 years were included as participants; of these patients, 50.9% were female, with 44.6% being overweight and 85.7% being engaged in physical activity. HAAS-Brazil provided satisfactory evidence of content validity (CVC > 0.9), structural validity (AISP = 1; $H_i > 0.3$; $VI_{Mon} = 0$; $VI_{IIO} = 0$), construct validity ($\rho_{HOS\text{-}SP} = 0.696$; $\rho_{SF\text{-}12\,PSC} = 0.554$; $\rho_{SF\text{-}1\,MSC} = 0.338$), no ceiling or floor effect, acceptable internal consistency (Mokken $\rho = 0.707$; Cronbach $\alpha = 0.663$), and good reliability ($ICC_{(3,K)} = 0.840$; $P < 0.001$).

CONCLUSION: The HAAS-Brazil provided satisfactory validation evidence in patients who underwent hip arthroplasty.

INTRODUCTION

The functional outcomes of hip or knee arthroplasty can be assessed using health-related quality of life (HRQOL) instruments, including questionnaires and scales. The instruments available in the current literature evaluate pain as the principal symptom, which presents a limiting factor in the performance of low-demand activities of daily living (ADL).[1]

This emphasis on pain and ADL gives rise to a degree of difficulty when discerning individuals who demonstrate no pain limitations for low-demand activities such as ADL, but who endure limitations when participating in more demanding activities such as sports. Assessing important functional differences (e.g., walking on uneven ground, running, climbing stairs, and level of physical or sporting performance) is not possible with currently available instruments.[2]

In response, Talbot et al. developed and validated the High-Activity Arthroplasty Score (HAAS). This was designed to assess the physical function of patients after lower limb arthroplasty (hip and/or knee), incorporating a greater spectrum of physical and sporting activities, in addition to the customary emphasis on painful symptoms. It is a 4-item self-administered instrument in a scalogram format designed to assess physical function with items addressing (i) *Walking*, (ii) *Running*, (iii) *Stair Climbing*, and (iv) *Activity Level*. Each item assessed the highest capacity of the patient, reflecting a total score ranging from 0 to 18. Higher scores signify greater patient function.[2] The HAAS was developed in the British English language and then cross-culturally adapted into the Brazilian Portuguese language, that is, HAAS-Brazil. Psychometric properties were at the time, yet to be evaluated within the Brazilian population.[3]

OBJECTIVE

This study aimed to investigate the validity of the psychometric properties of the scores produced by the instrument in a sample of post-hip arthroplasty Brazilian patients. We hypothesized that HAAS-Brazil would present acceptable evidence of validity for this purpose.

METHODS

Ethical aspects

This study was approved by the Ethics Committee of Hospital Universitário Pedro Ernesto (number 50529321.3.0000.5259; August 30, 2021). All participants signed an informed consent statement through paper or online application in accordance with the Brazilian National Health Council 510th Resolution of April 7th, 2016.

Study design

This was a cross-sectional study of the quantitative-qualitative nature of the assessment of psychometric properties of scores produced by a self-administered instrument utilizing primary data collected between June 2023 and April 2024.

Construct definition and conceptual framework

The HAAS aims to measure the construct of physical activity, defined as any bodily movement produced by skeletal muscles that results in energy expenditure, referring to all movements, whether during moments of leisure, commuting, or as part of an individual's work, from the perspective of estimating the ability to perform certain activities through the subjectivity of self-report.[4-6] The items of the HAAS are related to the construct with respect to the conceptual framework of a reflective model.[7]

Instruments

HAAS is a self-administered instrument designed to evaluate physical function after lower limb arthroplasty (hip and/or knee), with a focus on higher-demand physical activities, such as sports participation.[2] HAAS contains four items organized as a scalogram.[7,8] Each item possesses its individual point system based within a hierarchical order which in sum, results in a possible range of scores from 0 to 18 points. Higher scores indicated better functional ability.[2,3,8]

The 12-Item Short-Form Health Survey (SF-12) is a short instrument for measuring general HRQOL and assessing both physical and mental health using two summary scores: a physical component summary (PCS-12) and a mental component summary (MCS-12). Higher scores indicated higher HRQOL.[9]

The Hip Outcome Score (HOS) is a 28-item instrument divided into two domains: ADL (19 items) and sports (9 items). It was developed to assess function in patients with hip disorders who are young and/or physically active but do not have severe degenerative abnormalities. Only the sports domain (HOS-Sp) was factored in for hypothesis testing in this study.[10]

Sample size, participants selection and data collection

Sample size was estimated according to the COnsensus-based Standards for the selection of health status Measurement INstruments (COSMIN) methodology of 7 times the number of items with at least 100 participants.[11,12] The selection considered a non-probabilistic convenience sampling of patients with at least 6 months after hip arthroplasty, American Society of Anesthesiologists (ASA) classification ≤ 2 by the time of elective surgery, and fluent Portuguese reading ability.[13] Arthroplasty surgery for a hip fracture was an exclusion criterion. Data were collected in a self-administered manner either by paper and pen on follow-up consultation or by digital collection through Google Forms.

Psychometric properties and statistical analyses

Validity Domain

Content validity

Content validity examines the extent to which the concepts of interest are comprehensively represented by the items in the questionnaire and is recognized as the most important property in a patient-related outcome measure (PROM).[11,12] Content validity was partially assessed on cross-cultural adaptation through expert panel opinion and the three-step test interview (TSTI) technique applied to volunteers evaluating the instrument.[3] This study aimed to assess relevance, comprehensiveness, and comprehensibleness in relation to the construct of interest. As such, the retest was administered using a 5-item Likert-scale per item for comprehension, an open-ended question, and three dichotomous questions about face validity, relevance, and comprehensibility in a subgroup of patients. The content validity coefficient (CVC) was employed to evaluate content validity evaluation.[14]

Structural validity

The internal structure refers to how different items in the PROM are related. Structural validity refers to the degree to which PROM scores adequately reflect the dimensionality of the construct to be measured. It was assessed by applying Mokken scale analysis (MSA), which addresses the assumptions inherent within a scalogram: 1) unidimensionality, 2) independence of item scoring, 3) monotonicity, and 4) invariant item ordering (IIO).[11,15-18] These assumptions were investigated using the automated item selection procedure (AISP), scalability coefficient analysis (H), and evaluation of item response function.[16,19,20]

Construct validity

Construct validity refers to the degree to which the scores of a PROM are consistent with hypotheses testing regarding its relationship with the scores of other instruments.[11,21] This study correlated HAAS scores with HOS-Sp, PSC-12, and MSC-12.

A positive correlation was expected between HAAS and HOS-Sp scores. In comparison with SF-12, a positive correlation was expected in PSC-12 while in MSC-12 this was not have been observed. Correlation was measured by Spearman test (ρ). Correlations with HOS-Sp and PSC-12 should be ≥ 0.50, with higher magnitude in HOS-Sp due to the similarity of the construct of measurement. Correlation with MSC-12 should be lower, i.e., 0.30 – 0.50, since it measures related, but dissimilar constructs.[11,15]

Ceiling and floor effect

Ceiling and floor effects are types of scale attenuation effects that are levels above or below variance, respectively, when an independent variable is no longer measurable. These effects are data gathering, which clusters responses, thereby compromising the ability to distinguish outcomes of patients on the highest and lowest levels of the instrument score.[22,23] Ceiling and floor effects contribute to measurement inaccuracy.[22,23] The presence of ceiling and floor effects was considered if there was clustering of > 15% of the maximum or minimum scores throughout the sample.[24]

Reliability Domain

Internal consistency

Internal consistency refers to the degree of interrelatedness among the items and will be assessed by Cronbach α and Mokken scale ρ coefficients.[25,26] Coefficient levels between 0.70 and 0.95 indicates a good internal consistency.[11]

Test-retest reliability

The test-retest reliability evaluates the extent to which scores for patients who exhibit no change in their clinical status and who, over time, remain the same for repeated measurement.[27] To evaluate the agreement between the test and a subsequent retest with a 48-hour to 3-week interval, the intraclass correlation coefficient (two-way mixed effects with average mean of measurements and absolute agreement $ICC_{(3,K)}$) was applied. By assuming no normality of scores, the Wilcoxon signed-rank test evaluates the null hypotheses of no difference between the mean scores of the two applications.[11,28]

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 28.0 (IBM, Armonk, New York, United States) with a level of significance of 5% and R programming with the Mokken package.[29,30]

RESULTS

Patients were invited to participate in the research either in person or via cell phone text messaging. Consent and adequate completion of the test were obtained from 112 participants, while 81 patients answered the retest. A subgroup of 26 patients completed a re-test for content validity evaluation. Patient characteristics are summarized in **Table 1**.

Content validity

Content validity was assessed on cross-cultural adaptation by means of an expert panel opinion and the TSTI technique applied to volunteers from a diverse socio-educational sample who evaluated the translated instrument.[3] To assess relevance, comprehensiveness, and comprehensibility among patients the CVC for each item was calculated (**Table 2**). Furthermore, all respondents answered affirmatively to question (1) "*Knowing that the aim of the questionnaire above is to evaluate the level of physical activity of patients after total hip replacement surgery, do you think it*

Table 1. Descriptive data of patients

Variables	Values
Sex, n (%)	
Male	55 (49.1%)
Female	57 (50.9%)
Age, years	55.92 ± 14.43
Weight, kg	77.43 ± 16.35
Height, m	1.68 ± 0.09
BMI*, kg/m^2	27.37 ± 4.85
Underweight, n (%)	2 (1.8%)
Eutrophic, n (%)	33 (29.5%)
Overweight, n (%)	50 (44.6%)
Obese, n (%)	27 (24.1%)
Follow up, months	33.19 ± 30.11
Minimum	6
Maximum	141
Physical activity, n (%)	
Yes	96 (85.7%)
No	15 (13.4%)
HAAS	
Test (n = 112)	9.58 ± 3.72
Retest (n = 81)	10.48 ± 3.47
HOS-Sp	74.41% ± 27.71%
SF-12	
PSC-12	49.49 ± 9.27
MSC-12	52.42 ± 9.66

BMI = body mass index; HAAS = High Activity Arthroplasty Score; HOS-Sp = Sports domain of Hip Outcome Score; SF-12 = 12-Item Short-Form Health Survey; PSC-12 = physical domain; MSC-12 = mental domain; *BMI categories according to *Ministério da Saúde* (Brazil).

accomplishes its purpose?" and (2) "*Do you think the questions of HAAS-Brazil are relevant to its purpose?*".

Structural validity

The AISP investigation attested to a 4-item single cluster, reflecting the unidimensionality of the scale. No violations of monotonicity or IIO were observed in accordance with the MSA assumptions (**Table 3**).

Construct validity

A positive correlation was observed between the HAAS and HOS-Sp scores and between the HAAS and PSC-12 scores. Meanwhile, MSC-12 scores showed a lower correlation with HAAS scores, as predicted by the initial hypothesis. These findings are summarized in **Table 4**.

Ceiling and floor effect

HAAS scores presented no ceiling or floor effect since the maximum (18) and minimum (0) scores accounted for less than 15% of the scores, as shown by the percentile analysis (P15 = 6.65; P85 = 13.35).

Table 2. Content validity coefficient results

Items	CVC
Walking	0.931
Running	0.977
Climbing stairs	0.969
Physical Activity	0.946
Total	0.956

CVC = content validity coefficient.

Table 3. Mokken Scale Analysis results

	Cluster	H_i (SE)	VI_{Mon}	P value	VI_{IIO}	P value
Walking	1	0.446 (0.077)	0	< 0.001	0	< 0.001
Running	1	0.509 (0.060)	0	< 0.001	0	< 0.001
Climbing stairs	1	0.370 (0.088)	0	< 0.001	0	< 0.001
Physical Activity	1	0.356 (0.085)	0	< 0.001	0	< 0.001

H_i = item scalability coefficient; VI_{Mon} = number of violations of monotonicity; VI_{IIO} = number of violations of invariant item ordering; SE = standard error.

Table 4. Spearman's test correlation (rhô) between scores

	PSC-12	MSC-12	HOS-Sp	HAAS
PSC-12	-			
MSC-12	-0.049	-		
HOS-Sp	0.607**	0.297	-	
HAAS	0.554**	0.338**	0.695**	-

PSC-12 = physical domain of short form 12; MSC-12 = mental domain of short form 12; HOS-Sp = Sports domain of Hip Outcome Score; HAAS = High Activity Arthroplasty Score; **P < 0.001.

Internal consistency

Internal consistency was assessed by Cronbach α coefficient (α = 0.663) favoring a questionable consistency while Mokken scale ρ (ρ = 0.707) was acceptable.

Test-retest reliability

The test-retest reliability was evaluated for 70 applications and deemed adequate ($ICC_{(3,K)}$ = 0.840 [F(69) = 6.225, P < 0.001]). The normality assumption of the sample favored a normal distribution for test scores (W = 0.988, P = 0.737), but was asymmetrical for retest scores (W = 0.952, P < 0.009). The Wilcoxon signed rank test was therefore performed to evaluate the difference between the paired applications, showing no significant difference (Z = -1.209, P = 0.230).

DISCUSSION

Validation evidence was investigated foremost from content validity, as recommended by COSMIN.[24] This investigation preceded evidence validation based on internal structure and, ultimately, evidence based on external measures.[24] Aiming to ensure that the items of the HAAS are relevant, comprehensive, and comprehensible with respect to the construct of interest. Content validity was assessed on the adaptation process by TSTI and CVC of respondents from various ages, socioeconomic, and scholarity levels.[3] This study enhanced the evaluation of content from a subgroup of patients, with a satisfactory performance of the instrument (CVC > 0.90) (**Table 2**).[14]

As HAAS-Brazil is a scalogram, its internal structure was evaluated by MSA, which is a model of non-parametric item response theory based on a non-parametric probabilistic version of scalogram.[16-19,30] MSA commands respect to four basic assumptions: 1) unidimensionality – items must measure only one latent trait (θ); 2) local independence of items' score – association between items is explained exclusively by θ; 3) monotonicity – higher θ levels imply greater probability of endorsement of higher difficulty items; and 4) IIO – the item response function to each item must not intercept.[16,17] AISP was therefore performed to evaluate if items cluster in one or more dimensions, which confirmed the unidimensionality of HAAS (**Table 3**).

As an adequate unidimensional instrument, the scalability coefficient (H) was used to investigate the model adjustment of the data and the quality of single items and each pair of items (H_i and H_{ij}, respectively). This analysis explored the efficacy of scalograms in mitigating Guttman errors. This demonstrates the instrument's ability to rank patients accurately based on their total scale scores. When evaluated in pairs, items should demonstrate positive covariance ($H_{ij} > 0$). Moreover, the scalability coefficient should be H > 0.30. The HAAS-Brazil produced by the response of post-hip arthroplasty patients attended to all assumptions of MSA.[16,20]

The internal consistency of instruments can be evaluated by Cronbach α coefficient, being the most widely employed estimator of reliability. However, there is increasing concern in the psychometric literature regarding its use. Because Cronbach α is negatively biased, especially in shorter scales such as HAAS, this study also evaluated internal consistency using the Mokken scale ρ (ρ = 0.707). HAAS-Brazil showed adequate internal consistency.[26,31]

After gathering satisfactory evidence of the internal structure validity of HAAS-Brazil, test-retest reliability was assessed using $ICC_{(3,K)}$. While the Shapiro-Wilk test favored normality in the test score distribution (W = 0.988, P = 0.737), we opted to employ non-parametric tests, such as the Wilcoxon signed-rank test. Physical activity is a construct that is empirically not expected to behave normally in the general population.[31] The retest was applied to 81 patients with an interval ranging from 5 to 122 days. Since the interval must provide an adequate timeline to prevent memory bias and avoid latent trait-level alteration due to clinical changes, a maximum cutoff of 21 days was adopted after expert deliberation.[11,14] The stability of test scores upon retesting, as undertaken by 71 patients, was satisfactorily attested. ($ICC_{(3,K)}$ = 0.840 [F(69) = 6.225, P < 0.001]; Z = -1.209, P = 0.230).

Construct validity, that is, evidence based on external measures, was evaluated by applying Spearman's test in accordance with the hypothesis of correlation between instruments. The strong correlation between the HAAS and HOS-Sp is best explained by the shared construct of interest, even though the HAAS centers on a broader definition of physical activity than the HOS-Sp, which focuses on sports. As a general HRQOL instrument, the SF-12 summary components are expected to possess lower correlation magnitudes with HAAS, particularly the MSC-12. The quality of the construct validity investigation resides in sound hypothesis testing, which was accomplished in this study.[15]

Responsiveness analysis was beyond the scope of this cross-sectional study and will be investigated in a subsequent prospective evaluation of post-hip arthroplasty patients. The assessment of psychometric properties in patients undergoing knee replacement is currently underway.

CONCLUSION

In this study, the psychometric properties of the Brazilian version of HAAS (i.e., HAAS-Brazil), which was adapted by Oliveira et al., were evaluated.[3] The most important finding was that the HAAS-Brazil of post-hip arthroplasty patients gathered sound evidence of validity and reliability. These results support the applicability of HAAS-Brazil in both clinical practice and research settings.

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Associations between selected genetic variants and lipid profile variability in response to statins in Alzheimer's disease: a prospective observational study

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ABSTRACT

BACKGROUND: Lipid profiles are largely determined by genetic variants, and lipid metabolism plays a crucial role in Alzheimer's disease.

OBJECTIVE: To investigate whether lipid profile variability in response to diverse statins could be affected by cholesterol metabolism-related genetic variants in Alzheimer's disease..

DESIGN AND SETTING: This prospective observational pharmacogenetic study was conducted at the Universidade Federal de São Paulo (Unifesp), Brazil.

METHODS: Consecutive outpatients were prospectively followed for lipid profile variations over one year, estimated by the associations between statin therapy and the following variants: rs2695121 (NR1H2), rs3846662 (HMGCR), rs11669576 (LDLR8), rs5930 (LDLR10), rs5882 and rs708272 (CETP), rs7412 and rs429358 (APOE), and ACE insertion/deletion polymorphism.

RESULTS: All polymorphisms in the 189 patients were in Hardy–Weinberg equilibrium. Statins resulted in lower total cholesterol and LDL cholesterol levels, whereas the effects on HDL cholesterol varied according to the statin used. Atorvastatin resulted in lower triglyceride level variations than simvastatin. APOE-ε4 carriers showed a better response to atorvastatin in elevating HDL-cholesterol than APOE-ε4 non-carriers. Carriers of the ACE insertion allele had cumulatively lower total cholesterol and LDL-cholesterol levels, regardless of statin therapy, but lower triglyceride levels when using atorvastatin. Carriers of rs11669576-G had lower total cholesterol and LDL-cholesterol levels when using simvastatin, and lower total cholesterol and triglycerides when using atorvastatin. Concerning CETP haplotypes, carriers of rs5882-A and rs708272-A benefitted the most from statins, which lowered total cholesterol and increased HDL-cholesterol levels, and from atorvastatin lowering triglycerides; however, the effects of atorvastatin lowering total cholesterol and LDL-cholesterol were more pronounced for carriers of rs5882-GG/rs708272-GG.

CONCLUSION: Lipid profile variations may be pharmacogenetically mediated in Alzheimer's disease, thus, confirming their high heritability.

INTRODUCTION

Cerebrovascular risk factors may be more important in the pathogenesis of Alzheimer's disease compared to other primary neurodegenerative diseases;[1] however, an intimate relationship exists between lipid metabolism and Alzheimer's disease. Midlife hypercholesterolemia in combination with other vascular risk factors causes earlier onset of late-onset Alzheimer's disease dementia.[2] However, such combinations may be protective against cognitive decline in patients with established dementia, possibly due to enhanced late life cerebral perfusion.[3] For instance, carriers of the ε4 allele of the apolipoprotein E (*APOE*) gene with a high 10-year coronary heart disease risk (dependent upon vascular risk factors such as higher total cholesterol and lower HDL-cholesterol), may have slower cognitive decline in the mild dementia stage.[4]

Genetic variants are responsible for approximately 30% of the variability in fasting blood cholesterol concentrations.[5] For instance, *APOE* in chromosome 19 is the most important gene that affects the incidence and the age at onset of late-onset Alzheimer's disease dementia,[2] as it encodes

a key extracellular lipid transport protein produced mostly by hepatocytes, which is responsible for clearance of triglyceride-rich lipoproteins.[6] The *Alu* repeat insertion/deletion polymorphism, within intron 16 of the angiotensin-converting enzyme gene (*ACE*) in chromosome 17, is associated with obesity[7] and higher blood pressure when boosting serum levels of the angiotensin-converting enzyme in the presence of the deletion allele.[8] This polymorphism could potentially affect white matter myelin loss in Alzheimer's disease as well as behavioral features;[9] however, limited studies have shown associations between this polymorphism and the dementia syndrome.[10] The 3-hydroxy-3-methylglutaryl CoA reductase gene (*HMGCR*) in chromosome 5 is the mechanistic gene that encodes the statin-binding domain of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase, which is involved in the regulation of the intracellular biosynthesis of cholesterol.[11] Furthermore, the intronic A allele of rs3846662 (*HMGCR*) is associated with lower LDL-cholesterol levels and reduced response to statin therapy.[12] Single nucleotide polymorphisms rs11669576 (*LDLR8*) and rs5930 (*LDLR10*) are among the prime genetic variants of the epidermal growth factor precursor homology domain of *LDLR* (the low-density lipoprotein cholesterol receptor gene) in chromosome 19 to be associated with dysfunctional cholesterol metabolism.[13] *NR1H2* in chromosome 19 encodes the liver X receptor β (LXR-β) isoform. When underexpressed, *NR1H2* increases cellular cholesterol levels and amyloidogenesis by downregulating the apolipoprotein E.[14] Specifically, the T allele of rs2695121 (*NR1H2*) may affect the risk of Alzheimer's disease and produce higher behavioral burden.[15] The cholesteryl ester transfer protein gene (*CETP)* in chromosome 16 encodes the hydrophobic cholesteryl ester transfer protein,[11] which is involved in reverse cholesterol transport (from peripheral tissues to the liver) and promotes the uptake of cholesterol in the liver by mediating the transfer of cholesteryl esters from high-density lipoproteins to apolipoprotein B-containing lipoproteins in exchange for triglycerides.[6] The protective *CETP* variants of rs5882 and rs708272 lead to lower serum levels of the cholesteryl ester transfer protein and, consequently, healthier lipid profiles.[15]

Statins are the most commonly prescribed lipid-lowering agents,[6] although they can be underused in older people because of their side effects.[16] They lower plasma cholesterol concentrations by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, providing benefits to patients with Alzheimer's disease because of their lipid-lowering effects by blocking cholesterol biosynthesis and upregulating the low-density lipoprotein receptor, and because of their antioxidant, antithrombotic, antiamyloidogenic, and vasodilatory properties.[17]

OBJECTIVE

In this longitudinal pharmacogenetic study, we aimed to determine whether variability in lipid profiles in response to diverse statins could be affected by selected genetic variants related to cholesterol metabolism in a cohort of outpatients with Alzheimer's disease dementia.

METHODS

Participants and clinical assessment

This prospective observational study recruited a convenience sample of consecutive outpatients with Alzheimer's disease dementia, based on the National Institute on Aging—Alzheimer's Association criteria[18], from the Behavioral Neurology Section of our university hospital between October 2010 and May 2014. After confirmation of the neurological diagnosis, all patients from this uncontrolled cohort were followed up for one year and had at least three consultations in which they were assessed for proxy reports regarding age, alcohol use, smoking, and lipid-lowering therapy with a statin; sex and body mass index were also assessed. Participation of each patient was concluded when the one year follow-up was completed. Only the first and last evaluations were considered for statistical analysis.

Diagnoses of dyslipidemias[19] and diabetes mellitus[20] were based on blood tests, whereas that of arterial hypertension was based on the JNC 7[21] report. Statin therapy was monitored yearlong. Dyslipidemia was managed according to specific guidelines.[19] Essentially, total cholesterol (desirable < 200 mg/dl), LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, and triglycerides target levels were determined based on the presence or absence of symptomatic ischemic heart disease, coronary heart disease risk equivalents (diabetes mellitus and clinical manifestations of non-coronary forms of atherosclerotic disease), and cardiovascular risk factors. Statins were introduced only if lifestyle recommendations were unsuccessful after three months, or unless the 10-year estimated coronary heart disease risk was > 10%. Lifestyle recommendations, including body weight control, regular physical activity, dietary therapy, and smoking cessation, were employed for all patients.[22] Statin therapy was discontinued in case of side effects. All efforts were directed towards reducing cholesterol and triglyceride levels to their target levels.

Genotyping

Genomic DNA was extracted from whole blood using a standard salting-out procedure. Subsequently, the following genotypes were determined by real-time Polymerase Chain Reactions: rs3846662 (*HMGCR*), rs2695121 (*NR1H2*), rs11669576 (*LDLR8*), rs5930 (*LDLR10*), rs5882 and rs708272 (*CETP*), and rs7412 and rs429358 (*APOE*). The use of TaqMan® SNP Genotyping Assays on the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, California, United States) was in accordance with standard protocols. In addition, the *ACE Alu* insertion/

deletion (I/D) polymorphism was determined using conventional polymerase chain reactions.[23] Genotyping procedures were performed after clinical data were collected from all patients.

Outcome measures

The primary outcome measure was variation in the levels of triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol according to the following independent variables: *APOE* haplotypes and genotypes of *ACE* I/D, *HMGCR*, *NR1H2*, *LDLR* or *CETP*, and lipid-lowering therapy with a statin. The secondary outcome was variation in the levels of triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol according to different statins. Haplotypes of *LDLR* and *CETP* were also considered in the analyses.

Statistical analyses

The Hardy–Weinberg equilibrium was estimated using the chi-square test. Lipid profile variables are expressed as absolute values and treated as continuous measures. A paired Student's t-test was used to assess variations in body mass index and lipid profile variables (considering baseline and final values after one year). Variations in triglycerides, total cholesterol, LDL cholesterol, and HDL-cholesterol at one year were then logarithmically transformed to meet the normality assumptions for a general linear model that was employed to evaluate associations between variations in these lipid profile variables with every genetic variant and statin therapy and adjusted for the following covariates: sex, baseline age, and body mass index variations in one year. Levels of significance from the general linear model were corrected for false discovery rates according to the Benjamini–Hochberg procedure to minimize the occurrence of type I errors. However, due to the preliminary nature of this study, the final results were presented with and without correction. Univariate analyses revealed the effects of statin therapy on lipid profile variations regardless of genetic variants, while multivariate analyses showed the results of interactions between genetic variants and the use or non-use of a statin on lipid profile variations. Concerning *APOE* haplotypes, the number of *APOE*-ε4 alleles was considered for the analysis rather than genotypes. Statistical significance was set at $P < 0.05$.

The dataset that was used for all statistics of this article is freely available for download at Mendeley Data (https://doi.org/10.17632/6n7z6hytrj.1).

Ethical considerations

All procedures in this study 1067/10 (CAAE 0540.0.174.000-10) were approved by the Ethics Committee of our university hospital on August 20, 2010 and were performed in accordance with the recommendations of the Declaration of Helsinki on Biomedical Research involving Human Participants. All invited patients and their legal representatives provided consent via a signed Informed Consent Form before assessment.

RESULTS

A total of 217 consecutive outpatients with late-onset Alzheimer's disease dementia were included in this study; 14 (6.5%) died, 7 (3.2%) were lost during follow-up, and 7 (3.2%) were excluded because of incomplete clinical data, resulting in a final cohort of 189 patients.

Table 1 presents the demographic, anthropometric, and clinical characteristics of the study population. Almost two-thirds of the patients were women, and exhibited a high prevalence of vascular risk factors. The use of lipid-lowering therapy was highly prevalent, with most patients using the lipophilic statins simvastatin or atorvastatin. The mean body mass index, total cholesterol, and LDL cholesterol were significantly lower after one year of treatment with statins, however, no significant differences were observed in the mean values of triglycerides, VLDL cholesterol, or HDL cholesterol.

Table 2 shows the results of the genetic analyses. Although all variants were in Hardy–Weinberg equilibrium, all patients had the same rs3846662 (*HMGCR*) genotype (AA). Among them, 53.4% were *APOE*-ε4 carriers and 46.6% were *APOE*-ε4 non-carriers. All *CETP* haplotypes were represented in the sample, however, the low variability of genotypes of rs11669576 (*LDLR8*) led to the underrepresentation of some *LDLR* haplotypes.

Table 3 shows the lipid profile variations at one year according to the indiscriminate use of a statin and specific genetic variants. Overall, statin therapy led to lower total cholesterol and LDL-cholesterol levels after one year along with elevated HDL-cholesterol levels, an effect that did not survive correction for false discovery rates. No significant effects were observed for triglycerides. Carriers of the insertion allele of *ACE* had cumulatively lower total cholesterol and LDL-cholesterol levels regardless of statin therapy compared to non-carriers (**Figure 1**). No other genetic variants had any significant effects on lipid profile variations.

Table 4 shows the lipid profile variations at one year according to the use of simvastatin, atorvastatin or no statin and specific genetic variants. Overall, simvastatin therapy resulted in lower total cholesterol and LDL-cholesterol levels, and higher HDL-cholesterol levels after one year, while atorvastatin therapy resulted in lower total cholesterol, LDL-cholesterol and HDL-cholesterol levels after one year. Additionally, atorvastatin therapy resulted in lower triglyceride level variations than simvastatin therapy after one year.

Regarding genetic variants, the following findings remained significant after corrections for false discovery rates (**Figure 2**): *APOE*-ε4 carriers had a better response to atorvastatin, in terms of elevated HDL-cholesterol levels, than *APOE*-ε4 non-carriers. Carriers of the insertion allele of *ACE* had cumulatively lower

Table 1. Demographics and Clinical Features

Assessed Variables, *n* = 189		*n* (%)	Mean ± SD	P*
Sex	Women	124 (65.6%)	–	–
	Men	65 (34.4%)	–	–
Estimated age at dementia onset		–	73.24 ± 6.5 years-old	–
Baseline age		–	78.24 ± 5.9 years	–
Body mass index	Baseline values	–	25.61 ± 4.3 kg/m²	P < **0.0001**
	Final values	–	24.96 ± 4.6 kg/m²	
	Variation	–	−0.65 ± 2.2 kg/m²	–
Arterial hypertension		152 (80.4%)	–	–
Diabetes *mellitus*		52 (27.5%)	–	–
Dyslipidemia		141 (74.6%)	–	–
Lifetime alcohol use		45 (23.8%)	62.24 ± 72.6 L/year[a]	–
Current alcohol use		2 (1.1%)	102.50 ± 109.6 L/year[a]	–
Lifetime smoking		65 (34.4%)	133.48 ± 154.2 packs/year[b]	–
Current smoking		7 (3.7%)	101.43 ± 62 packs/year[b]	–
Statin therapy	Simvastatin	124 (65.6%)	18.79 ± 9.4 mg/day[c]	–
	Atorvastatin	14 (7.4%)	28.57 ± 22.8 mg/day[c]	–
	Rosuvastatin	2 (1.1%)	10 ± 0 mg/day[c]	–
No lipid-lowering therapy		49 (25.9%)	–	–
Total cholesterol	Baseline values	–	197.96 ± 46.7 mg/dL	P < **0.0001**
	Final values	–	181.25 ± 38.2 mg/dL	
	Variation	–	−16.71 ± 36.8 mg/dL	–
LDL-cholesterol	Baseline values	–	118.02 ± 40.4 mg/dL	P < **0.0001**
	Final values	–	101.59 ± 29.5 mg/dL	
	Variation	–	−16.43 ± 33.1 mg/dL	–
HDL-cholesterol	Baseline values	–	53.08 ± 14.6 mg/dL	P = 0.843
	Final values	–	53.22 ± 15.2 mg/dL	
	Variation	–	0.14 ± 9.7 mg/dL	–
VLDL-cholesterol	Baseline values	–	26.85 ± 12.1 mg/dL	P = 0.612
	Final values	–	26.44 ± 12.7 mg/dL	
	Variation	–	−0.42 ± 11.3 mg/dL	–
Triglycerides	Baseline values	–	134.26 ± 60.7 mg/dL	P = 0.606
	Final values	–	132.14 ± 63.3 mg/dL	
	Variation	–	−2.12 ± 56.3 mg/dL	–

SD = standard deviation. * Paired Student's ***t***-test. [a] Mean l/year only for those with a history of alcohol use. [b] Mean packs/year only for those with a history of smoking. [c] Mean mg/day only for those who used the drug.

total cholesterol and LDL-cholesterol levels when using simvastatin than when using atorvastatin. Conversely, they had cumulatively lower triglyceride levels when using atorvastatin than when using simvastatin, although carriers of the D/D genotype exhibited elevated triglyceride levels with atorvastatin. Carriers of the insertion allele of *ACE* had cumulatively lower triglycerides when using atorvastatin in comparison to those who used simvastatin, although carriers of the D/D genotype exhibited lower triglycerides only when using simvastatin. Carriers of the G allele of rs11669576 (*LDLR8*) had lower total cholesterol and LDL cholesterol levels with simvastatin, but lower total cholesterol and triglyceride levels with atorvastatin. Carriers of the GG genotype of rs5882 (*CETP*) had lower LDL-cholesterol levels with atorvastatin than carriers of the AA genotype using a statin or not. Carriers of the AA and GG genotypes of rs5882 (*CETP*) had lower triglyceride levels with atorvastatin than with simvastatin, although the effects were stronger for GG carriers. Carriers of the A allele of rs708272 (*CETP*) had higher increases in HDL cholesterol levels with atorvastatin, whereas carriers of the GG genotype had lower HDL cholesterol levels with atorvastatin.

Table 5 shows the lipid profile variations at one year according to the indiscriminate use of a statin or not and specific *CETP* haplotypes. Statin therapy resulted in lower total cholesterol and LDL-cholesterol levels after one year. Notably, carriers of *CETP* haplotypes with the A allele of rs5882 and the A allele of rs708272 benefitted the most from statins in terms of lower total cholesterol and elevated HDL-cholesterol levels.

Table 6 shows the lipid profile variations at one year according to the use of simvastatin, atorvastatin or no statin and specific *CETP* haplotypes. Simvastatin therapy resulted in lower total cholesterol

Table 2. Genotypes and Haplotypes

Genetic Variants, n = 189			***n* (%)**	**P***
APOE haplotypes	*APOE*-ε4 carriers, *n* = 101	ε4/ε4	21 (11.1%)	–
		ε4/ε3	72 (38.1%)	–
		ε4/ε2	8 (4.2%)	–
	APOE-ε4 non-carriers, *n* = 88	ε3/ε3	81 (42.9%)	–
		ε3/ε2	7 (3.7%)	–
		ε2/ε2	0 (0%)	–
ACE Alu repeat insertion/deletion genotypes		I/I	50 (26.5%)	P = 0.345
		I/D	88 (46.6%)	
		D/D	51 (26.9%)	
HMGCR (intron 13): rs3846662 genotypes		AA	189 (100%)	P = 1
		AG	0 (0%)	
		GG	0 (0%)	
LDLR single nucleotide polymorphisms	exon 8: rs11669576 genotypes	AA	1 (0.5%)	P = 0.850
		AG	28 (14.8%)	
		GG	160 (84.7%)	
	exon 10: rs5930 genotypes	AA	23 (12.2%)	P = 0.749
		AG	83 (43.9%)	
		GG	83 (43.9%)	
LDLR haplotypes	rs11669576 AA/rs5930 AA		0 (0%)	–
	rs11669576 AA/rs5930 AG		0 (0%)	–
	rs11669576 AA/rs5930 GG		1 (0.5%)	–
	rs11669576 AG/rs5930 AA		0 (0%)	–
	rs11669576 AG/rs5930 AG		10 (5.3%)	–
	rs11669576 AG/rs5930 GG		18 (9.5%)	–
	rs11669576 GG/rs5930 AA		23 (12.2%)	–
	rs11669576 GG/rs5930 AG		73 (38.6%)	–
	rs11669576 GG/rs5930 GG		64 (33.9%)	–
NR1H2 (intron 2): rs2695121 genotypes		CC	72 (38.1%)	P = 0.954
		CT	89 (47.1%)	
		TT	28 (14.8%)	
CETP single nucleotide polymorphisms	rs5882 genotypes	AA	71 (37.6%)	P = 0.407
		AG	94 (49.7%)	
		GG	24 (12.7%)	
	rs708272 genotypes	AA	20 (10.6%)	P = 0.051
		AG	101 (53.4%)	
		GG	68 (36%)	
CETP haplotypes	rs5882 AA/rs708272 AA		3 (1.6%)	–
	rs5882 AA/rs708272 AG		34 (18%)	–
	rs5882 AA/rs708272 GG		34 (18%)	–
	rs5882 AG/rs708272 AA		10 (5.3%)	–
	rs5882 AG/rs708272 AG		56 (29.6%)	–
	rs5882 AG/rs708272 GG		28 (14.8%)	–
	rs5882 GG/rs708272 AA		7 (3.7%)	–
	rs5882 GG/rs708272 AG		11 (5.8%)	–
	rs5882 GG/rs708272 GG		6 (3.2%)	–

APOE = apolipoprotein E gene; *ACE* = angiotensin-converting enzyme gene; *HMGCR* = 3-hydroxy-3-methylglutaryl-CoA reductase gene; *LDLR* = low-density lipoprotein cholesterol receptor gene; *NR1H2* = nuclear receptor 1 type H2 gene (liver X receptor β gene); *CETP* = cholesteryl ester transfer protein gene.
* Hardy-Weinberg equilibrium (chi-square test).

and LDL-cholesterol levels for most patients after one year, while atorvastatin therapy resulted in lower triglyceride, total cholesterol and LDL-cholesterol levels for most patients after one year. Carriers of *CETP* haplotypes with the A allele of rs5882 and the A allele of rs708272 benefitted mostly from atorvastatin, which lowered triglyceride levels. Conversely, the effects of atorvastatin lowering total cholesterol and LDL-cholesterol levels were more pronounced for

carriers of rs5882 GG/rs708272 GG. No significant effects were found for changes in HDL-cholesterol levels after one year.

Since no genotype variations were observed in rs3846662 (*HMGCR*), this gene could not be included in the pharmacogenetic analyses. In addition, considering the low variability of the genotypes of rs11669576 (*LDLR8*), which led to the underrepresentation of some *LDLR* haplotypes, only *CETP* haplotypes were considered in the analyses.

Likewise, the isolated results of rosuvastatin versus no statin or other statins (atorvastatin or simvastatin) were not calculated because only two patients used rosuvastatin.

DISCUSSION

Overall, this study showed that lipophilic statin therapy was effective in reducing total cholesterol and LDL-cholesterol levels after one year, which is consistent with previous literature,[24]

Table 3. Compared lipid profile variations at one year according to the use or not of a statin and specific genetic variants*

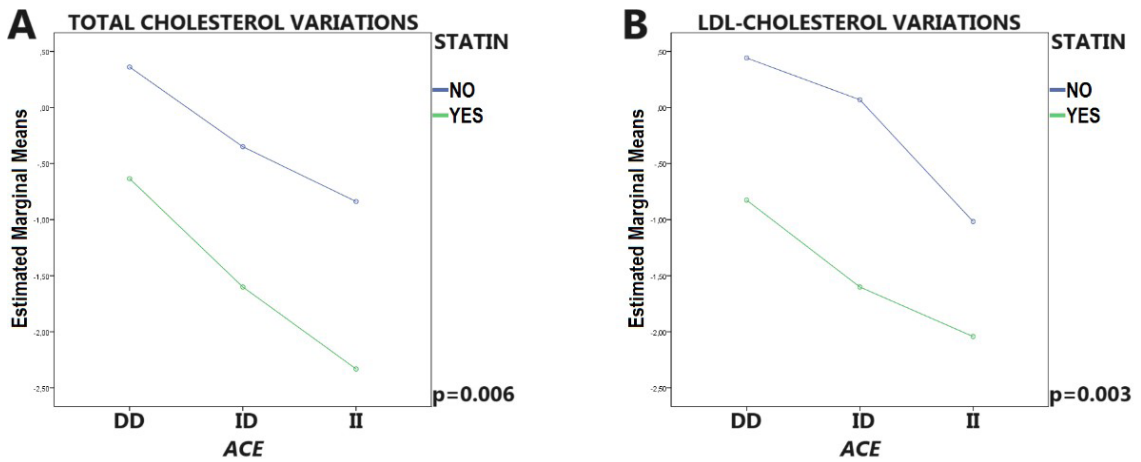
Genetic variants		Total cholesterol variations (mg/dl, mean ± SD)			LDL-cholesterol variations (mg/dl, mean ± SD)		
		Statin n = 140	no statin n = 49	Total n = 189	statin n = 140	no statin n = 49	Total n = 189
APOE haplotypes	*APOE*-ε4/ε4 n = 21	−40.47 ± 43.5[a1]	−4.4 ± 7.8[a1]	−31.89 ± 41	−36.36 ± 50.6[b1]	−4.96 ± 9.1[b1]	−28.89 ± 46.1
	APOE-ε4+/ε4-, n = 80	−19.68 ± 37.5[a1]	1.83 ± 28[a1]	−13.5 ± 36.2	−20.36 ± 30.2[b1]	2.83 ± 17[b1]	−13.69 ± 29
	APOE-ε4-/ε4-, n = 88	−20.58 ± 39.6[a1]	−1.38 ± 12.2[a1]	−16 ± 35.9	−20.32 ± 35.9[b1]	−1.99 ± 11.9[b1]	−15.94 ± 32.7
ACE Alu repeat insertion/deletion genotypes	I/I, n = 50	−31.2 ± 41.6[a2]	−5.4 ± 10.5[a2]	−23.46 ± 37.1[a2]	−26.9 ± 36[b2]	−5.31 ± 9.4[b2]	−20.42 ± 32[b2]
	I/D, n = 88	−21.58 ± 34.7[a2]	1.60 ± 26.8[a2]	−14.99 ± 34.2[a2]	−21.84 ± 32.9[b2]	1.68 ± 17.2[b2]	−15.16 ± 31.1[b2]
	D/D, n = 51	−16.59 ± 43.8[a2]	3.56 ± 13[a2]	−13.04 ± 40.8[a2]	−18.72 ± 40[b2]	4 ± 10.8[b2]	−14.71 ± 37.5[b2]
LDLR8: rs11669576 genotypes	AA, n = 1	0 ± 0[a3]	–	0 ± 0	0 ± 0[b3]	–	0 ± 0
	AG, n = 28	−29.83 ± 50.2[a3]	−2.6 ± 5.9[a3]	−20.11 ± 42.1	−31.27 ± 44.6[b3]	−3.88 ± 7.5[b3]	−21.49 ± 38.1
	GG, n = 160	−21.58 ± 37.9[a3]	0.44 ± 23.1[a3]	−16.22 ± 36.1	−20.99 ± 34.5[b3]	0.95 ± 15.6[b3]	−15.65 ± 32.3
LDLR10: rs5930 genotypes	AA, n = 23	−21.37 ± 32.4[c3]	−5 ± 16.7[c3]	−17.81 ± 30.2	−21.74 ± 29.3[d3]	−2 ± 13.4[d3]	−17.45 ± 27.7
	AG, n = 83	−23.45 ± 44.3[c3]	−3.21 ± 9.6[c3]	−18.82 ± 40	−25.08 ± 39.2[d3]	−1.09 ± 13[d3]	−19.59 ± 36.3
	GG, n = 83	−21.78 ± 36.2[c3]	3.08 ± 26.9[c3]	−14.29 ± 35.5	−19.09 ± 34[d3]	1.17 ± 15.9[d3]	−12.99 ± 31.1
NR1H2 (intron 2): rs2695121 genotypes	CC, n = 72	−29.19 ± 39.1[a4]	−4.50 ± 8.2[a4]	−25.08 ± 36.9	−26.19 ± 37.1[b4]	0.43 ± 11.5[b4]	−21.75 ± 35.6
	CT, n = 89	−18.37 ± 38.7[a4]	−2.5 ± 12.5[a4]	−13.73 ± 33.9	−19.96 ± 34.6[b4]	−2.63 ± 11.7[b4]	−14.89 ± 30.7
	TT, n = 28	−14.12 ± 42.1[a4]	10 ± 38.2[a4]	−4.64 ± 41.7	−16.18 ± 36.2[b4]	5.60 ± 21.2[b4]	−7.62 ± 32.6
CETP: rs5882 genotypes	AA, n = 71	−12.61 ± 38.7[a5]	1.24 ± 10.9[a5]	−9.29 ± 34.6	−15.22 ± 33.1[b5]	2.64 ± 8.6[b5]	−10.95 ± 30.1
	AG, n = 94	−27.75 ± 39.4[a5]	−0.46 ± 27.2[a5]	−20.2 ± 38.3	−25.36 ± 37.5[b5]	−0.83 ± 18[b5]	−18.58 ± 34.9
	GG, n = 24	−32.25 ± 37.6[a5]	−3 ± 7.3[a5]	−24.94 ± 35	−30.95 ± 35.7[b5]	−4.13 ± 8.4[b5]	−24.24 ± 33.2
CETP: rs708272 genotypes	AA, n = 20	−30.53 ± 36.6[c5]	−6 ± 10.4[c5]	−26.85 ± 34.9	−26.22 ± 33.8[d5]	−7 ± 12.1[d5]	−23.34 ± 32.1
	AG, n = 101	−22.46 ± 40.8[c5]	−3.06 ± 8.2[c5]	−15.93 ± 34.7	−23.84 ± 37.5[d5]	−1.51 ± 10.4[d5]	−16.32 ± 32.8
	GG, n = 68	−20.08 ± 39.1[c5]	9.42 ± 39.1[c5]	−14.88 ± 40.4	−18.94 ± 34.7[d5]	5.88 ± 22.1[d5]	−14.56 ± 34
Final variations at one year, n = 189		−22.49 ± 39.4[a]	−0.18 ± 20.8[a]	−16.71 ± 36.8	−22.17 ± 35.8[b]	−0.03 ± 14.4[b]	−16.43 ± 33.1

Continue...

Table 3. Continuation.

Genetic variants		HDL-cholesterol variations (mg/dl, mean ± SD)			Triglyceride variations (mg/dl, mean ± SD)		
		statin n = 140	no statin n = 49	Total n = 189	statin n = 140	no statin n = 49	Total n = 189
APOE haplotypes	*APOE*-ε4/ε4 n = 21	−3.59 ± 11.4	−1.6 ± 4.8	−3.11 ± 10.2	−1.9 ± 94.9	10.8 ± 21.5	1.12 ± 83
	APOE-ε4+/ε4-, n = 80	1.12 ± 10.2	−3.78 ± 9.3	−0.29 ± 10.2	−2.3 ± 58.5	14 ± 68.6	2.38 ± 61.6
	APOE-ε4-/ε4-, n = 88	1.54 ± 10	0.57 ± 4.2	1.31 ± 9	−9.22 ± 48	0.14 ± 8	-6.99 ± 42.2
ACE Alu repeat insertion/deletion genotypes	I/I, n = 50	0.63 ± 10.9	−0.8 ± 4.6	0.2 ± 9.4	−24.77 ± 67.1	3.67 ± 20.3	-16.24 ± 58.5
	I/D, n = 88	−0.55 ± 9.4	−2.88 ± 9.5	−1.21 ± 9.4	4.19 ± 49.6	13.72 ± 64.7	6.9 ± 54.1
	D/D, n = 51	2.91 ± 10.9	0.11 ± 2.3	2.41 ± 10.1	−4.21 ± 61.8	−2.11 ± 9.8	-3.84 ± 56.1
*LDLR*8: rs11669576 genotypes	AA, n = 1	0 ± 0	–	0 ± 0	0 ± 0	–	0 ± 0
	AG, n = 28	−1.5 ± 12.5	0.6 ± 1.7	−0.75 ± 10	14.39 ± 82.8	3.8 ± 12.6	10.61 ± 66.3
	GG, n = 160	1.13 ± 10	−2.28 ± 8.1	0.29 ± 9.7	−8.58 ± 54.5	8.74 ± 53.1	−4.36 ± 54.5
*LDLR*10: rs5930 genotypes	AA, n = 23	1.31 ± 8.9	−3.4 ± 5.6	0.29 ± 8.5	−4.3 ± 59.9	1.6 ± 6.7	−3.02 ± 52.8
	AG, n = 83	1.27 ± 11.2	−1.68 ± 10.6	0.59 ± 11.1	1.8 ± 61.3	−2.05 ± 9.5	0.92 ± 53.9
	GG, n = 83	0.08 ± 9.8	−1.36 ± 4	−0.35 ± 8.5	−14.09 ± 55.6	16.4 ± 65.6	−4.91 ± 60
NR1H2 (intron 2): rs2695121 genotypes	CC, n = 72	−1.26 ± 9.4[c4]	−6 ± 12.6[c4]	−2.05 ± 10.1	−8.84 ± 60.3	5.33 ± 19.3	−6.48 ± 55.7
	CT, n = 89	1.5 ± 10.7[c4]	−0.04 ± 4.2[c4]	1.05 ± 9.3	0.43 ± 55.9	0.85 ± 9.8	0.55 ± 47.2
	TT, n = 28	5.29 ± 10.7[c4]	−0.91 ± 2.8[c4]	2.86 ± 8.9	−16.24 ± 65.1	26.64 ± 98.6	0.61 ± 81.1
CETP: rs5882 genotypes	AA, n = 71	2.29 ± 10.9	−2.18 ± 6.6	1.23 ± 10.2	1.46 ± 59.8	4.06 ± 15.9	2.08 ± 52.6
	AG, n = 94	0.19 ± 9.6	−2.04 ± 8.5	−0.43 ± 9.3	−12.96 ± 53.9	11.96 ± 64.4	−6.07 ± 57.8
	GG, n = 24	−1.52 ± 11	1.17 ± 2	−0.85 ± 9.6	1.26 ± 72.6	−0.17 ± 3.5	0.9 ± 62.6
CETP: rs708272 genotypes	AA, n = 20	2 ± 10.5	0.67 ± 1.1	1.80 ± 9.7	−32.41 ± 64.7	1.67 ± 2.9	−27.3 ± 60.7
	AG, n = 101	0.64 ± 9.5	−1.44 ± 8.1	−0.06 ± 9.1	3.6 ± 55.9	−0.62 ± 10.4	2.18 ± 45.9
	GG, n = 68	0.58 ± 11.3	−3 ± 5.9	−0.05 ± 10.6	−8.39 ± 58.6	32.92 ± 93.1	−1.1 ± 67
Final variations at one year, n = 189		0.78 ± 10.3[c]	−1.69 ± 7.3[c]	0.14 ± 9.7	−5.57 ± 58.8	7.73 ± 47.6	−2.12 ± 56.3

* General linear model adjusted for sex, baseline age, and body mass index variations at one year. SD = standard deviation; *APOE* = apolipoprotein E gene: [a1] statin versus no statin, uncorrected P = 0.032; [b1] statin versus no statin, uncorrected P = 0.030; *ACE* = angiotensin-converting enzyme gene: [a2] genotype effect, statin versus no statin, significant P = 0.006 (corrected for false discovery rates); [b2] genotype effect, statin versus no statin, significant P = 0.003 (corrected for false discovery rates); *LDLR* = low-density lipoprotein cholesterol receptor gene: [a3] statin versus no statin, uncorrected P = 0.024; [b3] statin versus no statin, uncorrected P = 0.016; [c3] statin versus no statin, uncorrected P = 0.032; [d3] statin versus no statin, significant P = 0.011 (corrected for false discovery rates); *NR1H2* = nuclear receptor 1 type H2 gene (liver X receptor β gene): [a4] statin versus no statin, uncorrected P = 0.039; [b4] statin versus no statin, significant P = 0.006 (corrected for false discovery rates); [c4] statin versus no statin, uncorrected P = 0.036; *CETP* = cholesteryl ester transfer protein gene: [a5] statin versus no statin, uncorrected P = 0.014; [b5] statin versus no statin, significant P = 0.005 (corrected for false discovery rates); [c5] statin versus no statin, uncorrected P = 0.035; [d5] statin versus no statin, uncorrected P = 0.031; Final variations at one year: [a] statin versus no statin, significant P = 0.006 (corrected for false discovery rates); [b] statin versus no statin, significant P = 0.003 (corrected for false discovery rates); [c] statin versus no statin, uncorrected P = 0.036.



A = Carriers of the ACE insertion allele had cumulatively lower total cholesterol levels after one year, particularly when using a statin (corrected P = 0.006); B = Carriers of the ACE insertion allele had cumulatively lower LDL-cholesterol levels after one year, particularly when using a statin (corrected P = 0.003).

Figure 1. Graphical representations of significant pharmacogenetic findings concerning logarithmically transformed lipid profile variations at one year according to the use or not of a statin and specific genetic variants

because lipophilicity eases the distribution of statins into hepatocytes.[25] Particularly, simvastatin was more effective in elevating HDL-cholesterol levels, while atorvastatin was more effective in reducing triglyceride levels. Notably, the only genetic variant that resulted in significant lipid profile variation, regardless of statin therapy, was the insertion allele of *ACE*, which cumulatively lowered total cholesterol and LDL cholesterol. The effect of the insertion allele of *ACE* on lowering serum levels of the angiotensin-converting enzyme could explain its protective effect on lipid profiles, considering that angiotensin II upregulates the enzyme 3-hydroxy-3-methylglutaryl CoA reductase.[14]

Additionally, *APOE*-ε4 carriers showed a better response to atorvastatin in terms of elevated HDL-cholesterol levels compared to *APOE*-ε4 non-carriers. While Chinese *APOE*-ε2 carriers seem to have healthier lipid profiles even longitudinally,[26] a Brazilian study showed that *APOE*-ε4 alleles do not interfere with the pharmacological efficacy of simvastatin.[6] Furthermore, most studies show that *APOE*-ε4 carriers have higher total cholesterol and LDL-cholesterol, which are modifiable by diets.[5] In contrast, the evidence for HDL-cholesterol is inconsistent.[27] Rising HDL-cholesterol levels are also associated with functional harm for *APOE*-ε4 non-carriers with Alzheimer's disease, probably because of reduced lipid availability to protect neuronal membranes.[28] Moreover, the prospective side effects of psychotropic drugs are more intense according to *APOE*-ε4 carrier status.[29] Likewise, *APOE*-ε4 carrier status is a key element in the modulation of effects of cerebrovascular metabolism modulators.[30] Thus, stratification of patient samples according to *APOE*-ε4 carrier status may be the best alternative.[31]

APOE-ε4 carriers with Alzheimer's disease appear to have a distinct lipid profile that may lead to higher susceptibility to the disease,[27] with increased intestinal cholesterol absorption[32] and decreased plasma levels of the less lipidated apolipoprotein E4. In contrast, *APOE*-ε2 carriers have increased levels of the more lipidated apolipoprotein E2 which binds with lower affinity to the low-density lipoprotein receptor than the other isoforms.[13] In addition, patients with Alzheimer's disease have abnormal insulin metabolism associated with the apolipoprotein E,[33] corroborated by reports of reduced hippocampal insulin-degrading enzyme (an amyloid-β-degrading metalloprotease) in *APOE*-ε4 carriers.[34]

In this study, simvastatin use resulted in cumulatively lower total cholesterol and LDL-cholesterol levels in carriers of the insertion allele of *ACE*, as well as lower triglyceride levels in carriers of the D/D genotype. Conversely, atorvastatin lowered triglyceride levels, mostly in carriers of the insertion allele of *ACE*. A previous study showed that carriers of the deletion allele had a better response to fluvastatin in terms of reductions in triglycerides and LDL-cholesterol.[25] Although the insertion allele is associated with improved pharmacological protection against creatinine clearance variations,[8] which allele would confer protective effects against the lipid-lowering effects of statins remains controversial, considering that both alleles can behave in the same way under different conditions.

Simvastatin use resulted in lower total cholesterol and LDL-cholesterol levels whereas atorvastatin use resulted in lower total cholesterol and triglyceride levels in carriers of the G allele of rs11669576 (*LDLR8*). However, no significant results were found for rs5930 (*LDLR10*), even though the A allele has been described as

protective in terms of behavioral features[15] and creatinine clearance variations.[8] Moreover, presence of the G allele of rs11669576 or the G allele of rs5930 is associated with upregulation of an abnormal low-density lipoprotein receptor that inhibits the internalization of the apolipoprotein E (thus reducing its transfer to lipoproteins for clearance), resulting in increased synthesis of low-density lipoproteins and reduced storage of intracellular cholesterol while precluding recycling of the low-density lipoprotein receptor.[13] Furthermore, no significant differences were observed in rs2695121 (*NR1H2*). Although the T allele is associated with a higher behavioral burden[15] and lower blood pressure, conflicting results have been reported regarding its association with the metabolic syndrome.[8]

When using atorvastatin, carriers of the GG genotype of rs5882 (*CETP*) had lower LDL cholesterol and triglyceride levels,

Table 4. Compared lipid profile variations at one year according to the use of Simvastatin or Atorvastatin or no statin and specific genetic variants*

Genetic variants		Total cholesterol variations (mg/dl, mean ± SD)			LDL-cholesterol variations (mg/dl, mean ± SD)		
		Simvastatin, n = 124	Atorvastatin, n = 14	no statin, n = 49	Simvastatin, n = 124	Atorvastatin, n = 14	no statin, n = 49
APOE haplotypes	*APOE*-ε4/ε4, n = 21	−40.47 ± 43.5[a1]	–	−4.40 ± 7.8[a1]	−36.36 ± 50.6[b1]	–	−4.96 ± 9.1[b1]
	APOE-ε4+/ε4-, n = 78	−23.19 ± 37.3[a1]	0.5 ± 7.3	1.83 ± 28[a1]	−22.94 ± 30.8[b1]	−3.25 ± 4.1	2.83 ± 17[b1]
	APOE-ε4-/ε4-, n = 88	−17.72 ± 39.4[a1]	−36.9 ± 38.6	−1.38 ± 12.2[a1]	−19.46 ± 35.4[b1]	−25.2 ± 40.4	−1.99 ± 11.9[b1]
ACE Alu repeat insertion/deletion genotypes	I/I, n = 50	−32.06 ± 42.8[a2]	−22 ± 29.9	−5.4 ± 10.5[a2]	−28.99 ± 36.4[b2]	−4.67 ± 26.6	−5.31 ± 9.4[b2]
	I/D, n = 88	−20.5 ± 34.6[a2]	−29 ± 36.5	1.6 ± 26.8[a2]	−21.93 ± 32.7[b2]	−21.25 ± 36.8	1.68 ± 17.2[b2]
	D/D, n = 49	−18.57 ± 42.9[a2]	−23 ± 56.3	3.56 ± 13[a2]	−19.66 ± 40.5[b2]	−27 ± 46.8	4 ± 10.8[b2]
LDLR8: rs11669576 genotypes	AA, n = 1	0 ± 0	–	–	0 ± 0	–	–
	AG, n = 28	−26.65 ± 49.8[a3]	−84 ± 0[b3]	−2.6 ± 5.9[a3,b3]	−30.05 ± 45.7[c3]	−52 ± 0[d3]	−3.88 ± 7.5[c3,d3]
	GG, n = 158	−22.52 ± 37.9[a3]	−21.77 ± 34.2[b3]	0.44 ± 23.1[a3,b3]	−22.17 ± 34.5[c3]	−16.38 ± 35.3[d3]	0.95 ± 15.6[c3,d3]
LDLR10: rs5930 genotypes	AA, n = 23	−23.54 ± 33.9[f3]	−13.75 ± 29.4[g3]	−5 ± 16.7[f3,g3]	−26.52 ± 30.2[h3]	−5 ± 20.5	−2 ± 13.4[h3]
	AG, n = 82	−24.63 ± 43.9[f3]	−26.75 ± 40.9[g3]	−3.21 ± 9.6[f3,g3]	−26.29 ± 39.1[h3]	−19 ± 42.9	−1.09 ± 13.0[h3]
	GG, n = 82	−20.75 ± 35.9[f3]	−34.17 ± 42.3[g3]	3.08 ± 26.9[f3,g3]	−18.4 ± 33.7[h3]	−28.17 ± 40.1	1.17 ± 15.9[h3]
NR1H2 (intron 2): rs2695121 genotypes	CC, n = 72	−29.81 ± 38.9[a4]	−26.1 ± 41.7[b4]	−4.5 ± 8.2[a4,b4]	−27.68 ± 36.4[c4]	−18.7 ± 41.5	0.43 ± 11.5[c4]
	CT, n = 87	−19.83 ± 38.5[a4]	−22 ± 29.4[b4]	−2.5 ± 12.5[a4,b4]	−21.22 ± 35.4[c4]	−16.33 ± 15.3	−2.63 ± 11.7[c4]
	TT, n = 28	−12.5 ± 42.9[a4]	−40 ± 0.0[b4]	10 ± 38.2[a4,b4]	−15.38 ± 37.3[c4]	−29 ± 0	5.60 ± 21.2[c4]
CETP: rs5882 genotypes	AA, n = 70	−13.98 ± 39.0[a5]	−13.75 ± 17.9[b5]	1.24 ± 10.9[a5,b5]	−16.88 ± 33.8[c5]	−4.25 ± 19.7[d5]	2.64 ± 8.6[c5,d5]
	AG, n = 93	−28.2 ± 39.6[a5]	−27.87 ± 41.7[b5]	−0.46 ± 27.2[a5,b5]	−26.03 ± 37.3[c5]	−23.62 ± 42.6	−0.83 ± 18.0[c5]
	GG, n = 24	−30.72 ± 37.1[a5]	−44.5 ± 55.9[b5]	−3 ± 7.3[a5,b5]	−31.13 ± 37.1[c5]	−29.5 ± 31.8[d5]	−4.13 ± 8.4[c5,d5]
CETP: rs708272 genotypes	AA, n = 20	−31.69 ± 36.8[f5]	−26.75 ± 40.9[g5]	−6 ± 10.4[f5,g5]	−28.14 ± 32.7[h5]	−20 ± 41.8	−7 ± 12.1[h5]
	AG, n = 100	−23.64 ± 39.9[f5]	−24.4 ± 43.5[g5]	−3.06 ± 8.2[f5,g5]	−24.47 ± 37.4[h5]	−25.4 ± 41.7	−1.51 ± 10.4[h5]
	GG, n = 67	−19.74 ± 39.9[f5]	−27.6 ± 35.3[g5]	9.42 ± 39.1[f5,g5]	−20.05 ± 35.6[h5]	−11.6 ± 29.3	5.88 ± 22.1[h5]
Final variations at one year, n = 187		−22.91 ± 39.5[a]	−26.21 ± 36.8[b]	−0.18 ± 20.8[a,b]	−23.07 ± 36.0[c]	−18.93 ± 35.2[d]	−0.03 ± 14.4[c,d]

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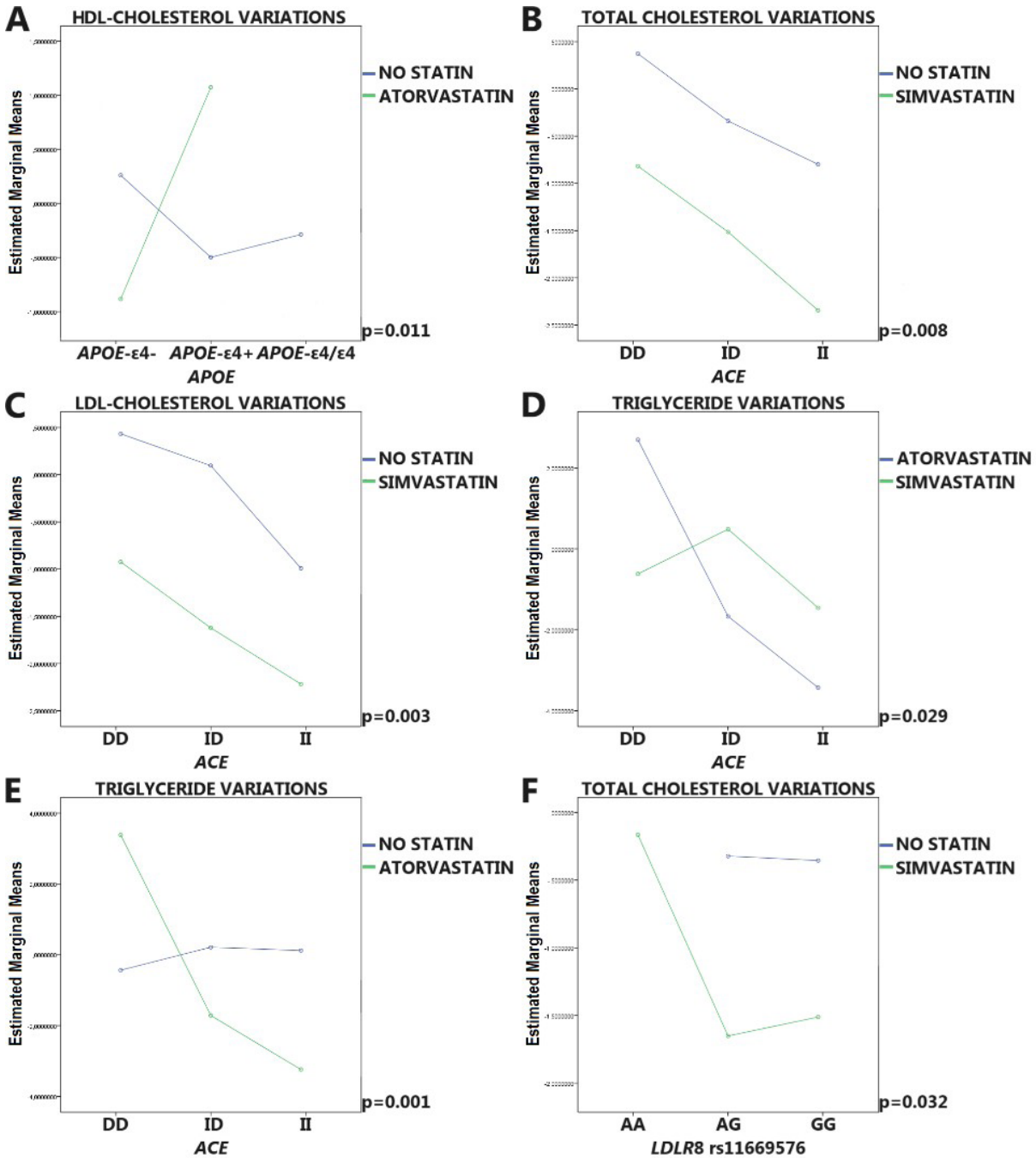
Table 4. Continuation.

Genetic variants		HDL-cholesterol variations (mg/dl, mean ± SD)			Triglyceride variations (mg/dl, mean ± SD)		
		Simvastatin, n = 124	Atorvastatin, n = 14	no statin, n = 49	Simvastatin, n = 124	Atorvastatin, n = 14	no statin, n = 49
APOE haplotypes	*APOE*-ε4/ε4, n = 21	−3.59 ± 11.4	–	−1.6 ± 4.8	−1.9 ± 94.9	–	10.8 ± 21.5
	APOE-ε4+/ε4-, n = 78	0.17 ± 9.2	4 ± 4.2[c1]	−3.78 ± 9.3[c1]	−2.18 ± 61.7	−2.5 ± 23.8	14 ± 68.6
	APOE-ε4-/ε4-, n = 88	2.54 ± 9.9	−4.2 ± 9.3[c1]	0.57 ± 4.2[c1]	−4.07 ± 44.3	−38.6 ± 59.9	0.14 ± 8
ACE Alu repeat insertion/deletion genotypes	I/I, n = 50	0.91 ± 11.4	−2.33 ± 1.2	−0.8 ± 4.6	−19.88 ± 66.3[c2]	−77 ± 63.4[c2,d2]	3.67 ± 20.3[d2]
	I/D, n = 88	−0.3 ± 9.3	−2.25 ± 10.5	−2.88 ± 9.5	8.88 ± 48.6[c2]	−28 ± 46.7[c2,d2]	13.72 ± 64.7[d2]
	D/D, n = 49	2.27 ± 9.6	−0.33 ± 11	0.11 ± 2.3	−6.11 ± 65.4[c2]	19.67 ± 13.6[c2,d2]	−2.11 ± 9.8[d2]
LDLR8: rs11669576 genotypes	AA, n = 1	0 ± 0	–	–	0 ± 0	–	–
	AG, n = 28	−0.53 ± 12.1	−18 ± 0	0.6 ± 1.7	19.35 ± 82.5	−70 ± 0[e3]	3.8 ± 12.6[e3]
	GG, n = 158	0.99 ± 9.7	−0.62 ± 7.9	−2.28 ± 8.1	−6.63 ± 54.9	−25.08 ± 54.6[e3]	8.74 ± 53.1[e3]
LDLR10: rs5930 genotypes	AA, n = 23	1.47 ± 10	0.75 ± 4.3	−3.4 ± 5.6	8.54 ± 52.1	−49.25 ± 71.7	1.6 ± 6.7
	AG, n = 82	0.81 ± 10.4	−1.5 ± 8.9	−1.68 ± 10.6	4.37 ± 61.2	−33.25 ± 67.3	−2.05 ± 9.5
	GG, n = 82	0.55 ± 9.6	−3.83 ± 11.7	−1.36 ± 4.0	−14.73 ± 58.4	−11 ± 31.7	16.40 ± 65.6
NR1H2 (intron 2): rs2695121 genotypes	CC, n = 72	−0.95 ± 9.4[d4]	−2.8 ± 9.7	−6 ± 12.6[d4]	−5.73 ± 60.5	−24.4 ± 59.9[f4]	5.33 ± 19.3[f4]
	CT, n = 87	0.79 ± 9.9[d4]	3.33 ± 5.7[e4]	−0.04 ± 4.2[d4,e4]	2.98 ± 56.7	−45.33 ± 45.9[f4]	0.85 ± 9.8[f4]
	TT, n = 28	6.12 ± 10.5[d4]	−8 ± 0[e4]	−0.91 ± 2.8[d4,e4]	−16.25 ± 67.3	−16 ± 0[f4]	26.64 ± 98.6[f4]
CETP: rs5882 genotypes	AA, n = 70	1.88 ± 9.9	−1.75 ± 5.4	−2.18 ± 6.6	5.06 ± 59.7	−39.75 ± 58.9[e5]	4.06 ± 15.9[e5]
	AG, n = 93	0.37 ± 9.8	−1.12 ± 9.1	−2.04 ± 8.5	−12.61 ± 54.3	−17.12 ± 58.5[e5]	11.96 ± 64.4[e5]
	GG, n = 24	−1.09 ± 10.6	−5 ± 18.4	1.17 ± 2.0	7.66 ± 74.4	−50 ± 28.3[e5]	−0.17 ± 3.5[e5]
CETP: rs708272 genotypes	AA, n = 20	2.85 ± 11.2	−0.75 ± 8.8[i5]	0.67 ± 1.1[i5]	−32.62 ± 67.4	−31.75 ± 64.5[j5]	1.67 ± 2.9[j5]
	AG, n = 100	−0.21 ± 8.5	3.4 ± 5.2[i5]	−1.44 ± 8.1[i5]	5.24 ± 57.1	−13.6 ± 42.3[j5]	−0.62 ± 10.4[j5]
	GG, n = 67	1.45 ± 11.3	−8 ± 9.5[i5]	−3 ± 5.9[i5]	−5.38 ± 58.8	−40.2 ± 59.2[j5]	32.92 ± 93.1[j5]
Final variations at one year, n = 187		0.78 ± 9.9[e]	−1.86 ± 8.9[f]	−1.69 ± 7.3[e,f]	−3.01 ± 59.5[g]	−28.29 ± 53.8[g,h]	7.73 ± 47.6[h]

*General linear model adjusted for sex, baseline age, and body mass index variations at one year. SD = standard deviation; *APOE* = apolipoprotein E gene: [a1]Simvastatin versus no statin, significant P = 0.004 (corrected for false discovery rates); [b1]Simvastatin versus no statin, significant P = 0.019 (corrected for false discovery rates); [c1]*APOE*-ε4+/ε4- versus *APOE*-ε4-/ε4-, Atorvastatin versus no statin, significant P = 0.011 (corrected for false discovery rates); *ACE* = angiotensin-converting enzyme gene: [a2] genotype effect, Simvastatin versus no statin, significant P = 0.008 (corrected for false discovery rates); [b2]genotype effect, Simvastatin versus no statin, significant P = 0.003 (corrected for false discovery rates); [c2]genotype effect, Simvastatin versus Atorvastatin, significant P = 0.029 (corrected for false discovery rates); [d2]genotype effect, Atorvastatin versus no statin, significant P = 0.001 (corrected for false discovery rates); *LDLR* = low-density lipoprotein cholesterol receptor gene: [a3]AG versus GG, Simvastatin versus no statin, significant P = 0.032 (corrected for false discovery rates); [b3]AG versus GG, Atorvastatin versus no statin, significant P = 0.011 (corrected for false discovery rates); [c3]AG versus GG, Simvastatin versus no statin, significant P = 0.016 (corrected for false discovery rates); [d3]AG versus GG, Atorvastatin versus no statin, uncorrected P = 0.036; [e3]AG versus GG, Atorvastatin versus no statin, significant P = 0.019 (corrected for false discovery rates); [f3]Simvastatin versus no statin, significant P = 0.024 (corrected for false discovery rates); [g3]Atorvastatin versus no statin, significant P = 0.027 (corrected for false discovery rates); [h3]Simvastatin versus no statin, significant P = 0.006 (corrected for false discovery rates); *NR1H2* = nuclear receptor 1 type H2 gene (liver X receptor β gene): [a4] genotype effect, Simvastatin versus no statin, uncorrected P = 0.033; [b4] Atorvastatin versus no statin, significant P = 0.019 (corrected for false discovery rates); [c4] Simvastatin versus no statin, significant P = 0.005 (corrected for false discovery rates); [d4]Simvastatin versus no statin, significant P = 0.026 (corrected for false discovery rates); [e4]CT versus TT, Atorvastatin versus no statin, uncorrected P = 0.040; [f4]Atorvastatin versus no statin, uncorrected P = 0.041; *CETP* = cholesteryl ester transfer protein gene: [a5]Simvastatin versus no statin, significant P = 0.015 (corrected for false discovery rates); [b5]Atorvastatin versus no statin, significant P = 0.013 (corrected for false discovery rates); [c5]Simvastatin versus no statin, significant P = 0.004 (corrected for false discovery rates); [d5]AA versus GG, Atorvastatin versus no statin, significant P = 0.032 (corrected for false discovery rates); [e5]genotype effect, Atorvastatin versus no statin, significant P = 0.014 (corrected for false discovery rates); [f5]Simvastatin versus no statin, uncorrected P = 0.034; [g5]Atorvastatin versus no statin, significant P = 0.022 (corrected for false discovery rates); [h5]Simvastatin versus no statin, significant P = 0.021 (corrected for false discovery rates); [i5]genotype effect, Atorvastatin versus no statin, significant P = 0.020 (corrected for false discovery rates); [j5]Atorvastatin versus no statin, uncorrected P = 0.035; Final variations at one year: [a]Simvastatin versus no statin, significant P = 0.008 (corrected for false discovery rates); [b]Atorvastatin versus no statin, significant P = 0.013 (corrected for false discovery rates); [c]Simvastatin versus no statin, significant P = 0.003 (corrected for false discovery rates); [d]Atorvastatin versus no statin, significant P = 0.032 (corrected for false discovery rates); [e]Simvastatin versus no statin, significant P = 0.026 (corrected for false discovery rates); [f]Atorvastatin versus no statin, significant P = 0.011 (corrected for false discovery rates); [g]Simvastatin versus Atorvastatin, significant P = 0.029 (corrected for false discovery rates); [h] Atorvastatin versus no statin, uncorrected P = 0.035.



A = APOE–ε4 carriers had higher HDL–cholesterol levels when using atorvastatin, while APOE–ε4 non–carriers had lower HDL–cholesterol levels (corrected P = 0.011); B = Carriers of the ACE insertion allele had cumulatively lower total cholesterol levels after one year, particularly when using simvastatin (corrected P = 0.008); C = Carriers of the ACE insertion allele had cumulatively lower LDL-cholesterol levels after one year, and particularly when using simvastatin (corrected P = 0.003); D = Carriers of the ACE insertion allele had cumulatively lower triglycerides when using atorvastatin compared to those using simvastatin, while carriers of the D/D genotype had lower triglycerides only when using simvastatin (corrected P = 0.029); E = Carriers of the ACE insertion allele had cumulatively lower triglycerides when using atorvastatin, whereas carriers of the D/D genotype had elevated triglyceride levels (corrected P = 0.001). F = Carriers of the G allele of rs11669576 (LDLR8) had lower total cholesterol levels after one year when using simvastatin (corrected P = 0.032).

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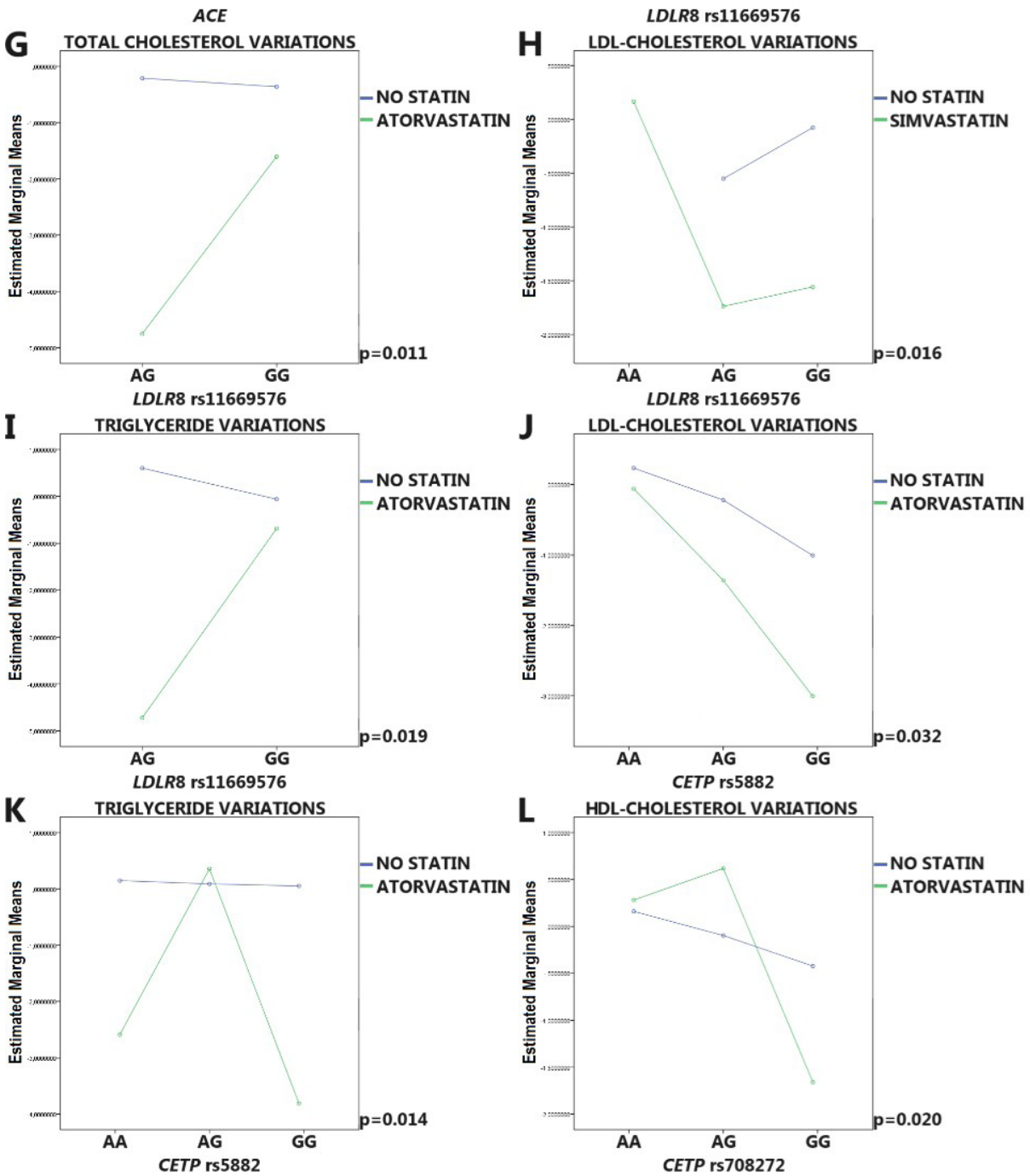
Figure 2. Graphical representations of significant pharmacogenetic findings concerning logarithmically transformed lipid profile variations at one year according to the use or not of simvastatin or atorvastatin and specific genetic variants



G = Carriers of the G allele of rs11669576 (LDLR8) had lower total cholesterol levels after one year with atorvastatin (corrected P = 0.011); H = Carriers of the G allele of rs11669576 (LDLR8) had lower LDL–cholesterol levels after one year with simvastatin (corrected P = 0.016); I = Carriers of the G allele of rs11669576 (LDLR8) had lower triglycerides after one year with atorvastatin (corrected P = 0.019); J = Carriers of the G allele of rs5882 (CETP) had cumulatively lower LDL–cholesterol levels after one year, while carriers of the GG genotype had lower LDL–cholesterol levels with atorvastatin compared to carriers of the AA genotype (corrected P = 0.032); K = Carriers of the AA and GG genotypes of rs5882 (CETP) had lower triglyceride levels after one year with atorvastatin, although effects were stronger for GG carriers (corrected P = 0.014); L = Carriers of the A allele of rs708272 (CETP) had higher HDL–cholesterol levels after one year with atorvastatin, while carriers of the GG genotype had lower HDL–cholesterol levels (corrected P = 0.020).

Figure 2. Continuation.

Table 5. Compared lipid profile variations at one year according to the use or not of a statin and specific *CETP* haplotypes*

***CETP* haplotypes**		**Total cholesterol variations (mg/dl, mean ± SD)**			**LDL-cholesterol variations (mg/dl, mean ± SD)**		
		statin *n* = 140	**no statin, *n* = 49**	**Total, *n* = 189**	**statin, *n* = 140**	**no statin, *n* = 49**	**Total, *n* = 189**
rs5882 AA/	Yes, *n* = 3	−58 ± 0	0 ± 0	−19.33 ± 33.5	−26 ± 0	0 ± 0	−8.67 ± 15
rs708272 AA	No, *n* = 186	−22.23 ± 39.5	−0.19 ± 21.2	−16.66 ± 36.9	−22.14 ± 35.9	−0.03 ± 14.7	−16.56 ± 33.3
rs5882 AA/	Yes, *n* = 34	−11.92 ± 36.1	−0.33 ± 6.6	−8.85 ± 31.3	−17.72 ± 30.9[a]	1.62 ± 4.5[a]	−12.6 ± 27.8
rs708272 AG	No, *n* = 155	−24.79 ± 39.9	−0.15 ± 22.9	−18.43 ± 37.8	−23.14 ± 36.9[a]	−0.41 ± 15.8[a]	−17.27 ± 34.2
rs5882 AA/	Yes, *n* = 34	−11.61 ± 41.3	4 ± 17.3	−8.85 ± 38.4	−12.61 ± 35.8[b]	5.03 ± 13.9[b]	−9.49 ± 33.6
rs708272 GG	No, *n* = 155	−25.21 ± 38.7	−0.77 ± 21.4	−18.43 ± 36.4	−24.56 ± 35.6[b]	−0.74 ± 14.4[b]	−17.95 ± 32.9
rs5882 AG/	Yes, *n* = 10	−36.8 ± 41[c]	–	−36.8 ± 41	−32.08 ± 41.8	–	−32.08 ± 41.8
rs708272 AA	No, *n* = 179	−21.39 ± 39.3[c]	−0.18 ± 20.8[c]	−15.58 ± 36.4	−21.41 ± 35.4[d]	−0.03 ± 14.4[d]	−15.56 ± 32.5
rs5882 AG/	Yes, *n* = 56	−25.49 ± 42.7[e]	−5.05 ± 9.5[e]	−18.19 ± 35.9	−22.59 ± 38.1[f]	−3.1 ± 13.2[f]	−15.63 ± 32.7
rs708272 AG	No, *n* = 133	−21.45 ± 38.4[e]	3.17 ± 25.5[e]	−16.08 ± 37.4	−22.02 ± 35.2[f]	2.08 ± 14.9[f]	−16.77 ± 33.4
rs5882 AG/	Yes, *n* = 28	−27.32 ± 33.9[g]	14.83 ± 54.7[g]	−18.29 ± 41.9	−26.84 ± 35.7[h]	6.73 ± 29.6[h]	−19.64 ± 36.7
rs708272 GG	No, *n* = 161	−21.59 ± 40.5[g]	−2.28 ± 10.1[g]	−16.43 ± 36	−21.30 ± 35.9[h]	−0.98 ± 11.1[h]	−15.87 ± 32.5
rs5882 GG/	Yes, *n* = 7	−15.5 ± 27.3	−18 ± 0	−15.86 ± 24.9	−16.5 ± 18.5	−21 ± 0	−17.14 ± 16.9
rs708272 AA	No, *n* = 182	−22.8 ± 39.9	0.19 ± 20.8	−16.74 ± 37.3	−22.42 ± 36.4	0.4 ± 14.2	−16.4 ± 33.6
rs5882 GG/	Yes, *n* = 11	−48.17 ± 39.5[i]	0 ± 0[i]	−26.27 ± 37.6	−56.83 ± 48.3[j]	−0.76 ± 1.7[j]	−31.35 ± 45
rs708272 AG	No, *n* = 178	−21.34 ± 39.2[i]	−0.2 ± 21.9[i]	−16.12 ± 36.8	−20.62 ± 34.6[j]	0.05 ± 15.2[j]	−15.51 ± 32.2
rs5882 GG/	Yes, *n* = 6	−33.09 ± 43.2	–	−33.09 ± 43.2	−19.51 ± 21.2	–	−19.51 ± 21.2
rs708272 GG	No, *n* = 183	−22.01 ± 39.4[k]	−0.18 ± 20.8[k]	−16.17 ± 36.6	−22.29 ± 36.4[l]	−0.03 ± 14.4[l]	−16.33 ± 33.5

Continue...

Table 5. Continuation.

***CETP* haplotypes**		**HDL-cholesterol variations (mg/dl, mean ± SD)**			**Triglyceride variations (mg/dl, mean ± SD)**		
		statin, *n* = 140	**no statin, *n* = 49**	**Total, *n* = 189**	**statin, *n* = 140**	**no statin, *n* = 49**	**Total, *n* = 189**
rs5882 AA/	Yes, *n* = 3	13 ± 0	0 ± 0	4.33 ± 7.5	−226 ± 0	0 ± 0	−75.33 ± 130.5
rs708272 AA	No, *n* = 186	0.69 ± 10.3	−1.77 ± 7.5	−0.07 ± 9.7	−3.98 ± 55.9	8.06 ± 48.6	−0.94 ± 54.3
rs5882 AA/	Yes, *n* = 34	2.68 ± 10.6	−2.22 ± 7.4	1.38 ± 9.9	15.40 ± 46.6	1.11 ± 7.8	11.62 ± 40.5
rs708272 AG	No, *n* = 155	0.37 ± 10.3	−1.58 ± 7.4	−0.13 ± 9.6	−10.13 ± 60.4	9.23 ± 52.6	−5.13 ± 58.9
rs5882 AA/	Yes, *n* = 34	1.57 ± 11.3	−2.83 ± 6.9	0.79 ± 10.7	−2.86 ± 54.4	9.83 ± 25.5	−0.62 ± 50.5
rs708272 GG	No, *n* = 155	0.58 ± 10.1	−1.53 ± 7.4	−0.01 ± 9.5	−6.25 ± 60.1	7.44 ± 50.2	−2.45 ± 57.7
rs5882 AG/	Yes, *n* = 10	−0.6 ± 10.5	–	−0.6 ± 10.5	−21.4 ± 51.4	–	−21.4 ± 51.4
rs708272 AA	No, *n* = 179	0.89 ± 10.3	−1.69 ± 7.3	0.18 ± 9.7	−4.35 ± 59.4	7.73 ± 47.7	−1.04 ± 56.5
rs5882 AG/	Yes, *n* = 56	−0.66 ± 9.1	−1.70 ± 9.3	−1.03 ± 9.1	−11.18 ± 50.1	−1.25 ± 12.6	−7.63 ± 40.9
rs708272 AG	No, *n* = 133	1.28 ± 10.7	−1.69 ± 5.7	0.63 ± 9.9	−3.63 ± 61.7	13.93 ± 60.7	0.2 ± 61.7
rs5882 AG/	Yes, *n* = 28	1.94 ± 10.3	−3.17 ± 5.4	0.85 ± 9.6	−12.03 ± 62.7	56 ± 130.9	2.55 ± 83.9
rs708272 GG	No, *n* = 161	0.57 ± 10.4	−1.49 ± 7.6	0.02 ± 9.7	−4.36 ± 58.3	1 ± 13.3	−2.93 ± 50.4
rs5882 GG/	Yes, *n* = 7	4.5 ± 10.5	2 ± 0	4.14 ± 9.7	−18.5 ± 25.9	5 ± 0	−15.14 ± 25.3
rs708272 AA	No, *n* = 182	0.62 ± 10.3	−1.77 ± 7.4	−0.01 ± 9.7	−4.99 ± 59.9	7.79 ± 48.2	−1.62 ± 57.2
rs5882 GG/	Yes, *n* = 11	0 ± 6.8	1 ± 2.2	0.45 ± 5.1	43.17 ± 96	−1.2 ± 2.7	23 ± 71.8
rs708272 AG	No, *n* = 178	0.82 ± 10.5	−2 ± 7.6	0.12 ± 9.9	−7.75 ± 56.2	8.75 ± 50.2	−3.67 ± 55.1
rs5882 GG/	Yes, *n* = 6	−9.06 ± 11.9[m]	–	−9.06 ± 11.9	−20.9 ± 69.8	–	−20.9 ± 69.8
rs708272 GG	No, *n* = 183	1.22 ± 10.1[m]	−1.69 ± 7.3[m]	0.44 ± 9.5	−4.88 ± 58.5	7.73 ± 47.7	−1.51 ± 55.9

* General linear model adjusted for sex, baseline age, and body mass index variations at one year. SD: standard deviation. ***CETP***: Cholesteryl ester transfer protein [a] statin versus no statin, P = 0.019 (corrected for false discovery rates). [b] statin versus no statin, uncorrected P = 0.047. [c] haplotype effect, statin versus no statin, significant P = 0.004 (corrected for false discovery rates). [d] statin versus no statin, P = 0.008 (corrected for false discovery rates). [e] statin versus no statin, P = 0.042 (corrected for false discovery rates). [f] statin versus no statin, significant P = 0.015 (corrected for false discovery rates). [g] statin versus no statin, P = 0.035 (corrected for false discovery rates). [h] statin versus no statin, P = 0.031 (corrected for false discovery rates). [i] statin versus no statin, P = 0.038 (corrected for false discovery rates). [j] statin versus no statin, significant P = 0.027 (corrected for false discovery rates). [k] statin versus no statin, P = 0.019 (corrected for false discovery rates). [l] statin vs. no statin, P = 0.008 (corrected for false discovery rates). [m] Haplotype effect, statin versus no statin, P = 0.046 (corrected for false discovery rates)

Table 6. Compared lipid profile variations at one year according to the use of Simvastatin or Atorvastatin or no statin and specific *CETP* haplotypes*

CETP haplotypes		Total cholesterol variations (mg/dl, mean ± SD)			LDL–cholesterol variations (mg/dl, mean ± SD)		
		Simvastatin, *n* = 124	Atorvastatin, *n* = 14	no statin, *n* = 49	Simvastatin, *n* = 124	Atorvastatin, *n* = 14	no statin, *n* = 49
rs5882 AA/ rs708272 AA	Yes, *n* = 3	−58 ± 0	–	0 ± 0	−26 ± 0	–	0 ± 0
	No, *n* = 184	−22.62 ± 39.5	–	−0.19 ± 21.2	−23.05 ± 36.2	–	−0.03 ± 14.7
rs5882 AA/ rs708272 AG	Yes, *n* = 34	−15.52 ± 34.2[a]	0 ± 0	−0.33 ± 6.6[a]	−20.31 ± 30.8[b]	2 ± 0	1.62 ± 4.5[b]
	No, *n* = 153	−24.59 ± 40.5[a]	−28.23 ± 37.5	−0.15 ± 22.9[a]	−23.70 ± 37.2[b]	−20.54 ± 36.1	−0.41 ± 15.8[b]
rs5882 AA/ rs708272 GG	Yes, *n* = 34	−10.80 ± 43.4	−18.33 ± 18.9[c]	4 ± 17.3[c]	−13.36 ± 37.3[d]	−6.33 ± 23.5	5.03 ± 13.9[d]
	No, *n* = 153	−25.97 ± 38	−28.36 ± 40.8[c]	−0.77 ± 21.4[c]	−25.52 ± 35.5[d]	−22.36 ± 37.9	−0.74 ± 14.4[d]
rs5882 AG/ rs708272 AA	Yes, *n* = 10	−38 ± 42.3[f]	−34 ± 46.9[g]	–	−35.40 ± 41.7[h]	−24.33 ± 50.1	–
	No, *n* = 177	−22 ± 39.3[f]	−24.09 ± 36[g]	−0.18 ± 20.8[f,g]	−22.33 ± 35.7[h]	−17.45 ± 33.2	−0.03 ± 14.4[h]
rs5882 AG/ rs708272 AG	Yes, *n* = 56	−24.87 ± 42.8[i]	−30.50 ± 47.7[j]	−5.05 ± 9.5[i,j]	−21.39 ± 37.8[k]	−32.25 ± 44.8	−3.10 ± 13.2[k]
	No, *n* = 131	−22.22 ± 38.4[i]	−24.50 ± 34.4[j]	3.17 ± 25.5[i,j]	−23.66 ± 35.6[k]	−13.60 ± 31.8	2.08 ± 14.9[k]
rs5882 AG/ rs708272 GG	Yes, *n* = 28	−30.11 ± 34.4[m]	1 ± 0	14.83 ± 54.7[m]	−30.17 ± 35.7[n]	13 ± 0	6.73 ± 29.6[n]
	No, *n* = 159	−21.52 ± 40.4[m]	−28.31 ± 37.4	−2.28 ± 10.1[m]	−21.71 ± 36.1[n]	−21.38 ± 35.4	−0.98 ± 11.1[n]
rs5882 GG/ rs708272 AA	Yes, *n* = 7	−17.60 ± 29.9	−5 ± 0	−18 ± 0	−18.40 ± 20	−7 ± 0	−21 ± 0
	No, *n* = 180	−23.13 ± 39.9	−27.85 ± 37.8	0.19 ± 20.8	−23.27 ± 36.6	−19.85 ± 36.5	0.40 ± 14.2
rs5882 GG/ rs708272 AG	Yes, *n* = 10	−48.17 ± 39.5[p]	–	0 ± 0[p]	−56.83 ± 48.3[q]	–	−0.76 ± 1.7[q]
	No, *n* = 177	−21.62 ± 39.2[p]	–	−0.20 ± 21.9[p]	−21.36 ± 34.7[q]	–	0.05 ± 15.2[q]
rs5882 GG/ rs708272 GG	Yes, *n* = 5	−22.92 ± 39.4[r]	−84 ± 0[s]	–	−13.01 ± 15.7[t]	−52 ± 0[u]	–
	No, *n* = 182	−22.91 ± 39.6[r]	−21.77 ± 34.2[s]	−0.18 ± 20.8[r,s]	−23.49 ± 36.6[t]	−16.38 ± 35.3[u]	−0.03 ± 14.4[t,u]

Continue...

Table 6. Continuation.

CETP haplotypes		HDL–cholesterol variations (mg/dl, mean ± SD)			Triglyceride variations (mg/dl, mean ± SD)		
		Simvastatin, *n* = 124	Atorvastatin, *n* = 14	no statin, *n* = 49	Simvastatin, *n* = 124	Atorvastatin, *n* = 14	no statin, *n* = 49
rs5882 AA/rs708272 AA	Yes, *n* = 3	13 ± 0	–	0 ± 0	−226 ± 0	–	0 ± 0
	No, *n* = 184	0.68 ± 9.9	–	−1.77 ± 7.5	−1.20 ± 56.2	–	8.06 ± 48.6
rs5882 AA/rs708272 AG	Yes, *n* = 34	1.26 ± 7.7	−1 ± 0	−2.22 ± 7.4	17.43 ± 48.1	−6 ± 0	1.11 ± 7.8
	No, *n* = 153	0.67 ± 10.4	−1.92 ± 9.3	−1.58 ± 7.4	−7.67 ± 61	−30 ± 55.6	9.23 ± 52.6
rs5882 AA/rs708272 GG	Yes, *n* = 34	2 ± 11.8	−2 ± 6.6	−2.83 ± 6.9	2.92 ± 51.3	−51 ± 66.8[e]	9.83 ± 25.5[e]
	No, *n* = 153	0.47 ± 9.5	−1.82 ± 9.7	−1.53 ± 7.4	−4.51 ± 61.5	−22.09 ± 51.8[e]	7.44 ± 50.2[e]
rs5882 AG/rs708272 AA	Yes, *n* = 10	0.71 ± 11.7	−3.67 ± 8.1	–	−16.71 ± 42.5	−32.33 ± 79	–
	No, *n* = 177	0.78 ± 9.9	−1.36 ± 9.4	−1.69 ± 7.3	−2.19 ± 60.4	−27.18 ± 50.1	7.73 ± 47.7
rs5882 AG/rs708272 AG	Yes, *n* = 56	−1.31 ± 9.3	4.50 ± 5.3	−1.70 ± 9.3	−10.64 ± 50.3	−15.50 ± 55.5[l]	−1.25 ± 12.6[l]
	No, *n* = 131	1.50 ± 10.1	−4.40 ± 8.9	−1.69 ± 5.7	−0.36 ± 62.4	−33.40 ± 55.3[l]	13.93 ± 60.7[l]
rs5882 AG/rs708272 GG	Yes, *n* = 28	2.94 ± 9.9	−16 ± 0	−3.17 ± 5.4	−14.34 ± 65.3	22 ± 0[o]	56 ± 130.9[o]
	No, *n* = 159	0.36 ± 9.9	−0.77 ± 8.2	−1.49 ± 7.6	−0.84 ± 58.4	−32.15 ± 54[o]	1 ± 13.3[o]
rs5882 GG/rs708272 AA	Yes, *n* = 7	3.80 ± 11.6	8 ± 0	2 ± 0	−16.20 ± 28.3	−30 ± 0	5 ± 0
	No, *n* = 180	0.65 ± 9.9	−2.62 ± 8.8	−1.77 ± 7.4	−2.46 ± 60.5	−28.15 ± 56	7.79 ± 48.2
rs5882 GG/rs708272 AG	Yes, *n* = 10	0 ± 6.8	–	1 ± 2.2	43.17 ± 96	–	−1.20 ± 2.7
	No, *n* = 177	0.82 ± 10.1	–	−2 ± 7.6	−5.36 ± 56.7	–	8.75 ± 50.2
rs5882 GG/rs708272 GG	Yes, *n* = 5	−7.27 ± 12.3	−18 ± 0	–	−11.08 ± 73.3	−70 ± 0[v]	–
	No, *n* = 182	1.12 ± 9.8	−0.62 ± 7.9	−1.69 ± 7.3	−2.67 ± 59.2	−25.08 ± 54.6[v]	7.73 ± 47.7[v]

* General linear model adjusted for sex, baseline age, and body mass index variations at one year. SD: standard deviation. CETP: Cholesteryl ester transfer protein a Simvastatin versus no statin, significant P = 0.048 (corrected for false discovery rates). b Simvastatin versus no statin, significant P = 0.007 (corrected for false discovery rates). c Atorvastatin versus no statin, significant, P = 0.025 (corrected for false discovery rates). d Simvastatin versus no statin, significant P = 0.045 (corrected for false discovery rates). e Atorvastatin versus no statin, significant, P = 0.032 (corrected for false discovery rates). f Simvastatin versus no statin, significant, P = 0.018 (corrected for false discovery rates). g atorvastatin vs. no statin; P = 0.011 (corrected for false discovery rates). h simvastatin vs. no statin, P = 0.004 (corrected for false discovery rates). i Simvastatin versus no statin, P = 0.036 (corrected for false discovery rates). j Atorvastatin versus no statin; uncorrected P = 0.049. k Simvastatin versus no statin, significant, P = 0.007 (corrected for false discovery rates). l Haplotype effect, atorvastatin versus no statin, significant P = 0.043 (corrected for false discovery rates). m simvastatin versus no statin; P = 0.027 (corrected for false discovery rates). n Simvastatin versus no statin, P = 0.011 (corrected for false discovery rates). o Haplotype effect, atorvastatin versus no statin, significant P = 0.041 (corrected for false discovery rates). p Simvastatin versus no statin, P = 0.029 (corrected for false discovery rates). q Simvastatin versus no statin, significant P = 0.018 (corrected for false discovery rates). r Simvastatin versus no statin, P = 0.023 (corrected for false discovery rates). s Haplotype effect, atorvastatin versus no statin, P = 0.011 (corrected for false discovery rates). t Simvastatin versus no statin, P = 0.002 (corrected for false discovery rates). u Atorvastatin versus no statin, significant P = 0.039 (corrected for false discovery rates). v Atorvastatin versus no statin, significant P = 0.034 (corrected for false discovery rates).

whereas carriers of the A allele of rs708272 (*CETP*) had higher increases in HDL cholesterol levels. Conversely, carriers of the GG genotype of rs708272 (*CETP*) showed lower HDL-cholesterol levels when using atorvastatin. The G allele of rs5882 (*CETP*) is associated with lower serum cholesteryl ester transfer protein levels and greater white matter integrity in young adults, as well as preserved cognitive function in longevity,[15] while the A allele of rs708272 (*CETP*) is associated with lower serum cholesteryl ester transfer protein activity and lower coronary heart disease risk.[35] Although one meta-analysis showed no associations between rs708272 (*CETP*) and the lipid-lowering effects of statin therapy,[36] rs708272 (*CETP*) may explain up to 10% of the variation in HDL-cholesterol levels,[37] with rs5882 (*CETP*) playing a similar role.[38]

Carriers of *CETP* haplotypes that included the A allele of rs5882 and the A allele of rs708272 benefited from statins, which lowered total cholesterol and raised HDL-cholesterol. Specifically, they benefitted from lower triglyceride levels with atorvastatin. However, the most pronounced effects of atorvastatin on lowering total cholesterol and LDL cholesterol were observed in carriers of rs5882 GG/rs708272 GG. The G alleles of both single nucleotide polymorphisms are associated with fewer frontal behaviors,[15] suggesting that *CETP* haplotypes associated with neuroprotection may also confer lower total cholesterol levels with atorvastatin therapy.

Nonetheless, this study has some limitations. First, it is limited by its convenience sample with no measurements of dietary patterns that could result in different lipid profiles according to specific genetic variants.[39] Second, although our longitudinal reading could identify significant differences for several associations, despite the absence of variability in rs3846662 (*HMGCR*) genotypes, the modest sample size is an inherent problem in most single-center studies. Despite this, almost three-quarters of our patients had dyslipidemia, confirming the burden of this vascular risk factor on patients with Alzheimer's disease. Third, interpretation of the results is limited by the lack of randomization of the sample or stratification according to environmental factors, as well as the absence of measures of the proteins that are translated by the studied genes. However, whether statins would be more efficacious at the start of lipid-lowering therapy is unknown, since many patients were already under treatment when they were included in the study, which may have resulted in better lipid outcomes.[40] Finally, physical activity could also be a confounding factor for our results; however, most patients were sedentary, thus, the effects of exercise were probably minimal.

CONCLUSION

Our study showed that the genetic determinants of lipid profiles affected the individual variability in the response to statins in patients with Alzheimer's disease, confirming the high heritability of lipid profile variations. Our findings may provide useful information for risk prediction and interventions in lipid disorders. Future genome-wide studies with larger sample sizes should address the effects of a greater variety of statins on lipid profile variations in patients of diverse age ranges.

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A modified stapled hemorrhoidectomy technique to optimize mucosectomy specimen and improve outcomes

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ABSTRACT

BACKGROUND: Surgical treatment of hemorrhoidal disease has undergone numerous modifications in recent decades. Among the technical options, stapled hemorrhoidopexy is currently considered an optimal alternative because it provides a less painful recovery. However, many reports have associated this technique with higher recurrence rates than excisional techniques.

OBJECTIVES: This manuscript presents a technical modification that aims to provide more extensive mucosectomy with mechanical hemorrhoidopexy.

DESIGN AND SETTING: The present technical modification was developed and has been recently used in two hospitals in São Paulo (SP), Brazil.

METHODS: To achieve this, we placed a circumferential submucosal suture at the 3 o'clock position in the clockwise direction. When the left lateral position (9 o'clock) was reached, a loop of 2-0 non-absorbable suture thread was passed around the continuous suture and retracted to the left. Subsequently, the original suture progressed towards the point on the right lateral side, where it was started.

RESULTS: Specifically, the modification consists of establishing two traction points from the pursestring suture; thus, the rectal mucosa entering the stapler head will be more uniform, and the retrieved mucosal strip will present a greater height. These features may play a role in effectively reducing mucosal prolapse and alleviating the symptoms.

CONCLUSIONS: The proposed modification of the original operative technique is simple and aims to improve postoperative results by increasing the height of the mucosal specimen to be resected, thereby reducing long-term recurrence. In the future, this hypothesis will be tested in a randomized study comparing the mucosectomy height and postoperative outcomes of both technical options (classical and present).

INTRODUCTION

Hemorrhoidal disease (HD) affects a large portion of the population and may significantly impact quality of life. While many patients benefit from clinical guidance on managing constipation, improving hygiene, and modifying personal habits, a substantial group still requires procedures to control symptoms[1]

Hemorrhoid excision techniques remain the gold standard for HD management. Ongoing discussions about the optimal surgical approach have focused on early postoperative outcomes such as bleeding, wound healing, and pain, particularly in comparative studies of the Milligan-Morgan and Ferguson techniques.[2]

The introduction of non-excisional procedures such as Stapled Hemorrhoidectomy (SH) and Doppler-Guided Hemorrhoidal Dearterialization with mucopexy (DG-HAL) has offered patients with grade III and IV disease an alternative that often allows a more comfortable recovery. As a result, attention has shifted toward evaluating long-term outcomes, especially symptom persistence or recurrence.[3]

Stapled Hemorrhoidectomy (also known as stapled hemorrhoidopexy or mechanical anopexy), introduced by Longo in 1998,[4] uses a circular stapler to remove a circumferential strip of rectal mucosa. This procedure aims to reduce prolapse by repositioning the hemorrhoidal cushions to their original anatomical location, based on the hypothesis that internal rectal prolapse contributes to disease development.[5]

By correcting the prolapse, the stapler-induced mucosectomy may also reduce submucosal blood flow to the hemorrhoidal plexus while preserving the anoderm from surgical trauma. This technique seeks to relieve symptoms while maintaining normal anatomy and physiological function. Today, SH is considered a major and innovative advancement in HD treatment.[6]

OBJECTIVE

The present study aims to present this idea and discuss the supposed advantages of a technical modification to classical mucosectomy provided by mechanical staplers.

METHODS

Our routine preparation involved an in-hospital procedure performed under sedation and spinal anesthesia. Preoperative measures included a rectal washout and intravenous antibiotics administered one hour before surgery. With the patient in the lithotomy and Trendelenburg positions, internal anorectal prolapse was assessed through digital examination and by inserting and retrieving endoanal gauze, applying inward traction during the maneuver.

A Circular Anal Dilator (CAD33, Ethicon Endo-Surgery, Raritan, New Jersey, United States) was inserted endoanally and secured to the perianal skin with stay sutures. A Purse-String Suture Anoscope was then introduced to facilitate the creation of a submucosal continuous purse-string suture using 2-0 Prolene.

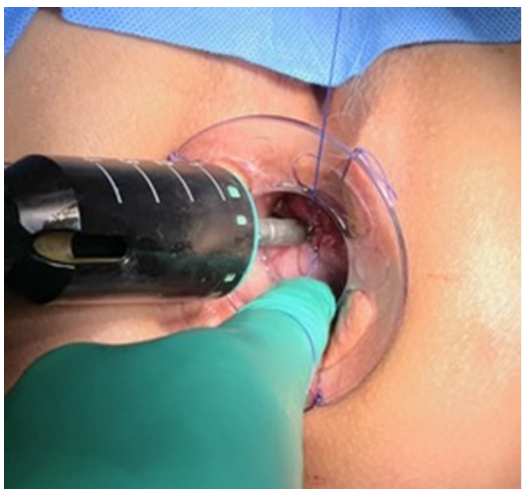
RESULTS

The proposed modification involves starting the circumferential submucosal suture at the 3 o'clock position and progressing in a clockwise direction (**Figure 1**). When the left lateral position (9 o'clock) was reached, a loop of 2-0 non-absorbable suture thread was passed around the Prolene suture and positioned under traction to the left side (**Figure 2**). Subsequently, the original Prolene suture continued toward the initial point on the right lateral side where it had started.

At this point, two traction points were identified in opposite positions: the Prolene suture on the right (3 o'clock) and the secondary thread on the left (9 o'clock). A 33 mm Circular Stapler (HCS33; Ethicon Endo-Surgery, Raritan, New Jersey, United States) was then opened, and its head was introduced beyond the purse-string suture, which was tied to allow mucosal approximation around the anvil axis (**Figure 3**). The surgeon pulled both threads through the lateral holes of the stapler, guiding the rectal mucosa enclosed by the purse-string suture to fit inside the stapler head (**Figures 4A** and **4B**). After confirming the position of the posterior vaginal wall, the stapler was closed and fired. It was then opened and removed from the rectum, allowing inspection of the staple line and the mucosectomy specimen within the stapler head (**Figure 5**).

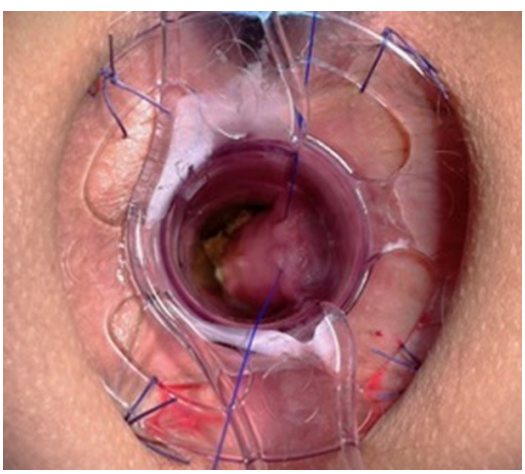
DISCUSSION

A stapled hemorrhoidectomy was performed to minimize postoperative pain. Over the years, this procedure has been increasingly adopted, despite concerns about disease recurrence. As understanding of patient needs has evolved, the technique has been combined with excisional methods to improve outcomes while still reducing pain and discomfort.[7]



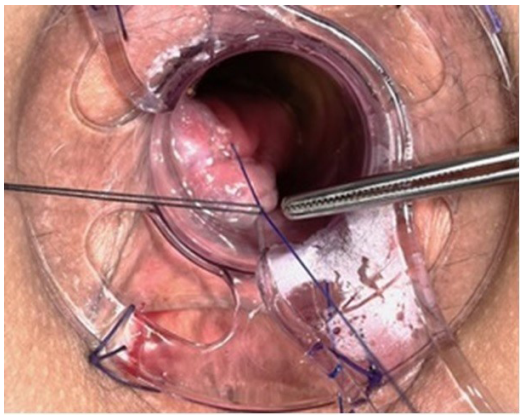
The circumferential submucosal suture was started at the 3 o'clock position and progressed in a clockwise direction, 2.5 cm above the dentate line.

Figure 1. Start of the circumferential suture.



Once the left lateral position (9 o'clock) was reached, a loop of 2-0 non-absorbable suture thread was passed around the Prolene suture and positioned under traction to the left side.

Figure 2. Technical modification.



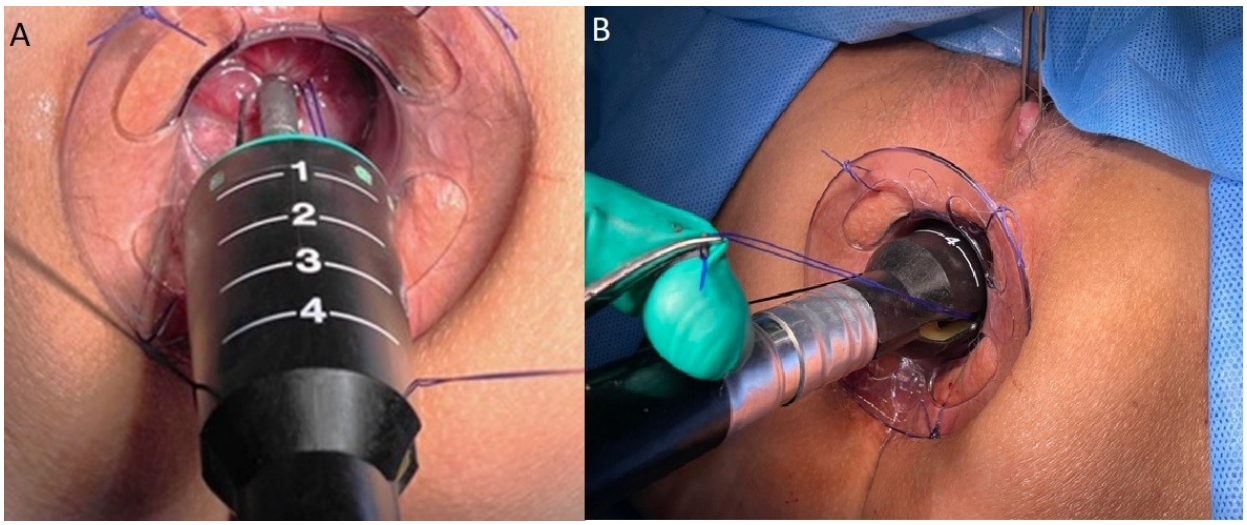
The 33 mm Circular Stapler (HCS33; Ethicon Endo-Surgery) was opened, and its head was inserted beyond the purse-string suture, which was tied to allow mucosal approximation around the anvil axis.

Figure 3. Insertion of the circular stapler.

Ongoing debate about long-term results underscores the importance of informing patients about the technical aspects and key considerations involved in this surgical choice. Patients must be fully informed about the benefits, risks, and expected outcomes.

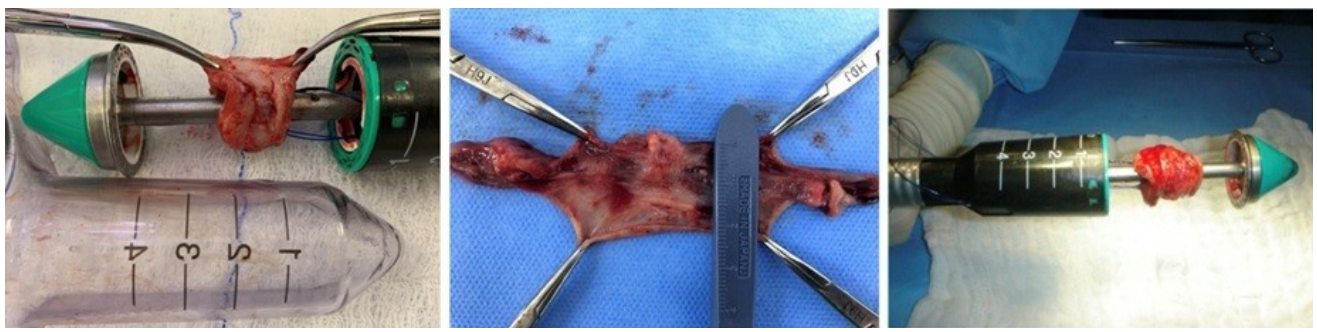
Mechanical hemorrhoidectomy is intended to perform a circumferential mucosectomy to restore local anatomy and correct prolapse that occurs during defecation. Careful attention to technical detail is essential for minimizing complications and achieving optimal outcomes. In this context, colorectal surgeons have recognized that the purse-string suture should ideally be placed 2.5-3.5 cm above the dentate line.[8]

Historically, when Procedure for Prolapse and Hemorrhoids (PPH) was introduced, the initial approach involved placing the suture higher in the rectum. However, this failed to adequately address the external hemorrhoidal components. On the other hand, placing the suture too low risks incorporating the dentate



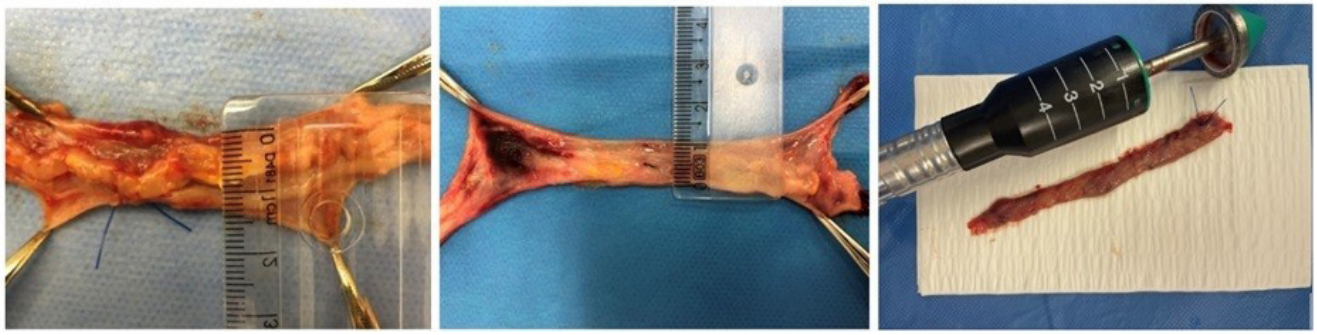
The surgeon pulled both threads through the lateral holes of the stapler and tied them together (Figure 4A). Outside traction forces the rectal mucosa to be tied using a pursestring suture to accommodate the inside of the stapler head (Figure 4B).

Figure 4. Traction of both threads.



After verifying the posterior vaginal wall, the stapler was closed and fired. It was then opened and removed from the rectum, allowing the surgeon to check the staple line and the mucosectomy specimen inside the stapler head.

Figure 5. Opening of the stapler to retrieve the mucosectomy specimen.



A more robust surgical specimen (approximately 2 cm) can be obtained using the proposed technical modification, whereas the original technique may have a height of 1 cm.

Figure 6. A better specimen is observed.

line into the mucosectomy, which can result in postoperative pain and dysfunction. It is now understood that the purse-string suture must not be placed too high (to ensure effective prolapse correction), too deep (to avoid including muscle fibers), or too low (to prevent stapling near the sensitive dentate line).[7]

The technique described here represents a modification designed to optimize the mucosectomy achieved with the stapler. In the classical approach, both threads are pulled from the 12 o'clock position, meaning traction is applied from a single (anterior) point. This can result in uneven mucosal entry into the stapler head. By contrast, applying suture traction from two opposite points is more likely to produce a regular, symmetric mucosal strip and increase the amount of tissue drawn into the stapler.

This modified method often results in a more substantial surgical specimen, as indicated by its gross appearance and height. A high-quality specimen may measure around 2 cm in height, compared to the roughly 1 cm height typical of specimens from the classical technique. **Figure 6** illustrates specimens obtained using both methods.

It is reasonable to conclude that the height and uniformity of the mucosectomy may influence surgical success. These factors likely affect the ability to correct prolapse effectively. We believe that the described maneuver helps produce a better mucosectomy specimen, as can be verified by examining the final outcome during surgery. In turn, this "optimized" mucosectomy may contribute to lower recurrence rates and greater patient satisfaction. A comparative trial is planned to explore this further by measuring specimen height and assessing the consistency of results between the two techniques.

CONCLUSIONS

The proposed modification of the original operative technique is simple and aims to improve postoperative results by increasing the height of the mucosal specimen to be resected, thereby reducing long-term recurrence. In the future, this hypothesis will be tested in a randomized study comparing the mucosectomy height and postoperative outcomes of both technical options (classical and present).

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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 postgraduate 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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