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Stroke prevention within primary care: management of atrial fibrillation using oral anticoagulation

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Chronic atrial fibrillation is a common heart arrhythmia closely associated with aging.[1] In Brazil, in the city of São Paulo, a door-to-door survey conducted in 2006 in the catchment area of a primary care unit showed that the prevalence of atrial fibrillation was 2.7% among people aged 65 years and older.[2,3] Another study in the state of Minas Gerais with data from primary care units showed similar prevalence,[4] and these surveys revealed that the frequency of atrial fibrillation increased exponentially according to age strata, such that, for example, for people aged 80 to 89, the prevalence ranged from 7.5% to 9.8%.[2-4] Despite this epidemiological profile, atrial fibrillation is still not included as a public health issue, due to the paucity of epidemiological studies addressing elderly individuals' health issues.[1]

On the other hand, accurate clinical knowledge of atrial fibrillation (formerly known as auricular fibrillation) has been available since the 1930s. In the seminal textbook *Heart Disease*, by Paul Dudley-White (1886-1973), published in 1937, the author stated "that the establishment of auricular fibrillation is often obscure in its origin. In most cases, there is a significant degree or type of heart disease or an important toxic agent or disease of some other nature, but sometimes there is no such cause and these individuals seem to be perfectly healthy without heart disease". Dudley-White, who is regarded as the father of American cardiology, explained that palpitation was "the characteristic symptom of atrial fibrillation", and he stated that "the observer, whether nurse or doctor, must be taught in such cases to record not only the radial pulse rate but, what is much more important, the apex heart rate also". Interestingly, Dudley-White recognized that "electrocardiogram is of the greatest assistance in confirming the diagnosis of auricular fibrillation, although this confirmation is often unnecessary".[5]

Over the next five decades, atrial fibrillation was only correlated with aging and was treated through relieving the symptoms. However, in the 1990s, the first results from the Framingham Heart Study threw new light on this condition.[6] Firstly, the scientists analyzing this cohort were able to identify from among the risk factors for atrial fibrillation that age was the most significant risk factor for atrial fibrillation, in comparisons with body mass index, intake of alcoholic beverages, smoking, diabetes and hypertension.[7] In the most recent analysis on the cohort (1998-2007), in comparison with individuals aged 50 to 59 years, the chance of having atrial fibrillation was five times greater for participants aged 60 to 69, seven times greater for those aged 70 to 79 and nine times greater for those aged 80 to 89 years.[6] Secondly, the outcomes of atrial fibrillation were very severe because this increased the incidence of stroke, heart failure and dementia. Cerebrovascular events consequent to atrial fibrillation are more often fatal or disabling, and present greater risk of dementia than do stroke events with etiologies that do not include atrial fibrillation.[8,9]

Randomized controlled trials comparing warfarin to placebo or control that were conducted two decades ago clearly showed that warfarin reduced ischemic stroke by 64% and all-cause deaths by one quarter.[10] Inevitably, use of warfarin and other vitamin K antagonists (VKA) became the standard of care for stroke prevention. However, use of warfarin is ambivalent, in that it requires precise anticoagulation monitoring of INR (international normalized ratio) prothrombin time, to keep this within a relatively narrow therapeutic range and avoid the risk of severe bleeding.[11] Hence, the emphasis in prevention of stroke due to atrial fibrillation is on identifying high-risk atrial fibrillation patients for whom the benefit of anticoagulation surpasses the risk of life-threatening bleeding

events. More recently, the introduction of non-VKA oral anticoagulants (NOACs), including direct thrombin inhibitors and factor Xa inhibitors, without the need to control for prothrombin time should have led to changes to the perspectives for the approaches towards atrial fibrillation and stroke.[11] However, most public healthcare systems cannot afford to supply NOACs for long-term use.

Consequently, in the Brazilian National Health System, there is a need to introduce a pragmatic program to deal with atrial fibrillation in association with anticoagulation that does not necessarily involve screening individually for atrial fibrillation but enables greater depth of evaluation on people who seek care complaining of palpitations. To reach this objective, one of the tasks should be to teach healthcare providers to examine the radial and apex heart rate in people who have been identified as presenting high risk of stroke, such as in the way proposed by Paul Dudley-White.[5] Moreover, all programs relating to hypertension and diabetes control need to identify people with atrial fibrillation by means of pulse palpation accompanied by a 12-lead resting electrocardiogram.[11]

To achieve this, all healthcare providers within the primary care setting will need to introduce the following mnemonic procedures:

1. Estimation of stroke risk among people with atrial fibrillation, using tools such as CHA2DS2-VASc, i.e. "Congestive heart failure, Hypertension, Age ≥ 75, Age between 65 and 74, Diabetes mellitus, prior Stroke, Transient Ischemic Attack [TIA] or Thromboembolism, Vascular disease, Sex female".[13]
2. Estimation of bleeding risk by applying HAS-BLED scores, i.e. "Hypertension, Abnormal renal or liver function, Stroke, Bleeding, Labile INR, Elderly (age > 65 years) and Drugs (alcohol)".[13]
3. Prediction of poor control through warfarin therapy by applying the SAMe-TT2R2 tool, i.e. "Sex (female), Age (<60 years), Me (medical history), Treatment (interacting drugs, e.g. amiodarone for rhythm control), Tobacco use (within two years), Race (non-European ancestry)".[14]

Several algorithms that enable safe adoption of anticoagulation within primary care are available. However, the approach towards people with atrial fibrillation implies reduction of the symptoms through prescribing beta-blockers and decreasing the burden of other risk factors like hypertension and dyslipidemia.[11]

A more detailed nationwide registry of atrial fibrillation is underway at 80 sites in Brazil, with follow-up on 5,000 patients with this arrhythmia. This will help to clarify some aspects of its prevention at primary care level.[15]

However, the challenge pointed out previously in this Journal is to put cardiovascular prevention into primary care.[16] This is an action that will effectively reduce the social gap regarding mortality,[17] morbidity[18] and disability[19] due to stroke, which is still a neglected disease in Brazil.[20]

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Validity and reproducibility of retinal arteriole and venule diameter measurements: ELSA-Brasil study. A cross-sectional study

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KEY WORDS:
Microvessels.
Retinal artery.
Retinal vein.
Reproducibility of results.

ABSTRACT

BACKGROUND: Investigation of alterations to retinal microvasculature may contribute towards understanding the role of such changes in the pathophysiology of several chronic non-communicable diseases. The objective here was to evaluate the validity and reproducibility of retinal arteriole and venule diameter measurements made by Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) graders.

DESIGN AND SETTING: Cross-sectional study at six teaching and research institutions.

METHODS: To evaluate validity, each of 25 retinal images from the University of Wisconsin (gold standard) was measured by five ELSA-Brasil graders. To evaluate reproducibility, 105 images across the spectrum of vessel diameters were selected from 12,257 retinal images that had been obtained between 2010 and 2012, and each image was reexamined by the same grader and by an independent grader. All measurements were made using the Interactive Vessel Analysis (IVAN) software. Bland-Altman plots, paired t tests and intraclass correlation coefficients (ICCs) were analyzed.

RESULTS: Mean differences between ELSA-Brasil and gold-standard readings were 0.16 µm (95% CI -0.17-0.50; P = 0.31) for central retinal artery equivalent (CRAE), -0.21 µm (95% CI -0.56-0.14; P = 0.22) for central retinal vein equivalent (CRVE) and 0.0005 (95% CI -0.008-0.009; P = 0.55) for arteriole/venule ratio (AVR). Intragrader ICCs were 0.77 (95% CI 0.67-0.86) for CRAE, 0.90 (95% CI 0.780.96) for CRVE and 0.70 (0.55-0.83) for AVR. Intergrader ICCs were 0.75 (95% CI 0.64-0.85) for CRAE, 0.90 (95% CI 0.79-0.96) for CRVE and 0.68 (95% CI 0.55-0.82) for AVR.

CONCLUSIONS: Retinal microvascular diameter measurements are valid and present moderate to high intra and intergrader reproducibility in ELSA-Brasil.

INTRODUCTION

Metabolic and vascular changes are inherent to the pathophysiology of diabetes and cardiovascular diseases, and their complications may cause damage to the microvasculature. This is reflected in changes to the diameters of retinal microvessels.[1-10] In this regard, the microvasculature of the retina is considered to be a non-invasive window to the microvascular system, which thus allows inferences to be made in relation to its involvement in the etiology of chronic diseases.[11] Furthermore, changes to retinal vessel caliber have been correlated with incident hypertension, diabetes and cerebral vascular disease.[12-14]

To be useful, microvascular measurements of the retina need to be accurate and precise. The approaches towards making such measurements on the retina are still not well described in the literature, most probably because some studies have not defined well which tools (software and mathematical calculations) are used to measure these data. In this regard, the aim of the present study was to evaluate the validity and reproducibility of retinal microvascular measurements that were made within the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil).

METHODS

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a multicenter study on 15,105 volunteer staff members (aged 35-74 years) at public institutions of higher education in six Brazilian state capital cities who were enrolled between 2008 and 2012. The overall objective of ELSA-Brasil was to investigate the epidemiological, clinical and molecular aspects of non-communicable chronic diseases, especially cardiovascular diseases and diabetes. ELSA Brasil was approved by the Ethics Committees of Hospital de Clínicas de Porto Alegre (under the registration number 06-194), Hospital Universitário da Universidade de São Paulo (669/06), Fundação Oswaldo Cruz (343/06), Universidade Federal de Minas Gerais (186/06), Universidade Federal da Bahia (027-06) and Universidade Federal do Espírito Santo (041/06), and all participants consented to their participation in the research.[15,16]

Sociodemographic and health data from the ELSA participants were collected through interviews and examinations, as previously described in greater detail.[17] Retinal images were obtained using a Canon CR-1 digital non-mydriatic retinal camera, coupled to a Canon EOS 40D digital camera (10 MP), and the images were shot at a 45º angle. Photographs of the macula and optic disc were acquired from both eyes of each participant. The retinal images were obtained in accordance with pre-established protocols,[18] without compression or zooming of images, so as not to lose quality.[1,19,20] The images were then transferred in Digital Imaging and Communications in Medicine (DICOM) format to the study's central server, from which they were retrieved by the reading center. A small fraction of the images were stored in .jpeg format, without compression, for transfer.

The supervisory ophthalmologist of the ELSA-Brasil retinal reading center was trained and certified by professionals in the Ocular Epidemiology Research Group of the Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison (UW), to make microvascular retinal measurements using the Interactive Vessel Analysis (IVAN) software.[20] He then trained and supervised the graders who performed the analyses in ELSA-Brasil, who were healthcare professionals without prior knowledge of ocular fundus images. The five readers who performed the greatest numbers of readings (28% of the total) were chosen to participate in the evaluations of validity and reproducibility.

For the validity evaluation, 25 retinal images received from the Ocular Epidemiology Research Group (UW) that had previously been read were considered to be the gold standard for ELSA-Brasil readings. Five evaluators from the ELSA-Brasil study read each of the 25 images received, totaling 125 readings.

For the reproducibility evaluation, images from ELSA-Brasil participants that had previously been read by these five graders were selected for new readings. In order to select images representing the range of values obtained, the images were classified into quintiles of their values, separately for central retinal artery equivalent (CRAE), central retinal vein equivalent (CRVE) and arterio-venous ratio (AVR, i.e. CRAE/CRVE). Seven images were selected from each quintile of each measurement for the study, thus totaling 105 images, of which 21 had been originally measured by each of the five graders.

Since a high correlation of retinal vessel caliber between the right and left eyes (0.78 to 0.99 intra and intergrader) had previously been shown,[21] optical disc images of a single eye were used. The right eye was given preference for this. In this selection, images that did not open in the software, or which did not meet the minimum criteria for grading (low light, poor visual quality or less than four venules and/or arterioles present) were excluded.

As mentioned earlier, to assess reproducibility, each of the five graders re-read each of their 21 selected images, thus totaling 105 repeat readings. To evaluate intergrader reproducibility, each image was re-read by another grader, again totaling 105 repeated readings. Thus, considering both the original readings and the intra and intergrader repeated readings, the reproducibility evaluation was based on 315 readings (105 original readings + 105 readings repeated by the same reader + 105 readings repeated by another reader). The third readings were performed on average two years after the first readings (on the same images). The readers on this occasion were blinded with regard to the identity of the previous reader and to the values previously obtained.

Statistical analysis

Shapiro-Wilk and Kolmogorov-Smirnov tests were used to evaluate the normality of the data. Differences between the readings were evaluated through Bland-Altman plots and were tested using the t test for paired samples.

The intraclass correlation coefficient (ICC) was used to evaluate reproducibility. Since each image was read twice by one grader and once by another grader, we used a two-factor random effects model (not nested), with estimation of variance components by means of the method of moments,[22] using an in-house SAS 9.4 routine (SAS Institute, Inc., Cary, North Carolina, USA) that had been developed for this purpose (supplementary material). The ICCs were calculated from the variance components in the usual manner: overall grading ICC (for repeated readings with different graders) = (1 - [(between-grader variance + within-grader variance)/(total variance)]); and within-grader ICC = (1 – [(within-grader variance)/(total variance)]). Ninety-five percent confidence intervals were calculated by means of bootstrapping, with 2000 bootstrap samples of the images.[23] Reproducibility was classified as proposed by Hinkle and Wiersma, as moderate

(ICC 0.50 to 0.70), high (ICC 0.70 to 0.90) or very high (ICC 0.90 to 1.00).[24]

Statistical analyses were performed using the SAS statistical software, version 9.4 (for ICC calculations) and the Statistical Package for the Social Sciences (SPSS), version 18 (for creation of Bland-Altman plots).

RESULTS

Validity

The retinal microvascular measurements of the sample of 25 images provided by the University of Wisconsin Ocular Epidemiology Research Group were normally distributed, as presented in **Table 1**. The mean CRAEs measured by the ELSA-Brasil and the UW graders were 138.2 ± 11.6 µm and 138.0 ± 11.5 µm, respectively, with a mean difference of 0.16 µm (95% CI -0.17 to 0.50; P = 0.31). The mean CRVEs were 198.3 ± 21.7 µm and 198.5 ± 21.7 µm, respectively, resulting in a mean difference of -0.21 µm (95% CI -0.56 to 0.14; P = 0.22). The mean AVRs were both 0.70 ± 0.07, with a mean difference of 0.0005 µm (95% CI -0.008 to 0.009; P = 0.55).

The Bland-Altman graph (**Figure 1**) showed that the differences obtained between the means of the microvascular readings performed by the five ELSA-Brasil graders, in comparison with the Wisconsin readers, were distributed in a similar way over the spectrum of values of these measurements. The maximum value for the difference was 1.78 µm for the arteriole diameter (CRAE) and, in 84% of the images, the differences did not reach 1.0 µm. Venule measurements (CRVE) showed a maximum difference of 1.87 µm and 80% of the images had differences smaller than 1.0 µm. For AVRs, the maximum difference was 0.009 and, for 76% of the images, the differences were smaller than 0.005.

Table 1. Comparison of microvascular measurements of the retina in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) and at the University of Wisconsin Department of Ophthalmology (gold standard), n = 25

	ELSA-Brasil	Gold standard	Difference	P
	Mean (SE)	Mean (SE)	Mean (95% CI)	
Central retinal artery equivalent (µm)	138.2 (2.33)	138.0 (2.29)	0.16 (-0.17-0.50)	0.31
Central retinal vein equivalent (µm)	198.3 (4.34)	198.5 (4.33)	-0.21 (-0.56-0.14)	0.22
Arteriole/venule ratio	0.70 (0.013)	0.70 (0.013)	0.0005 (-0.008-0.009)	0.55

P-value of the t test for paired data. SE = standard error; CI = confidence interval.

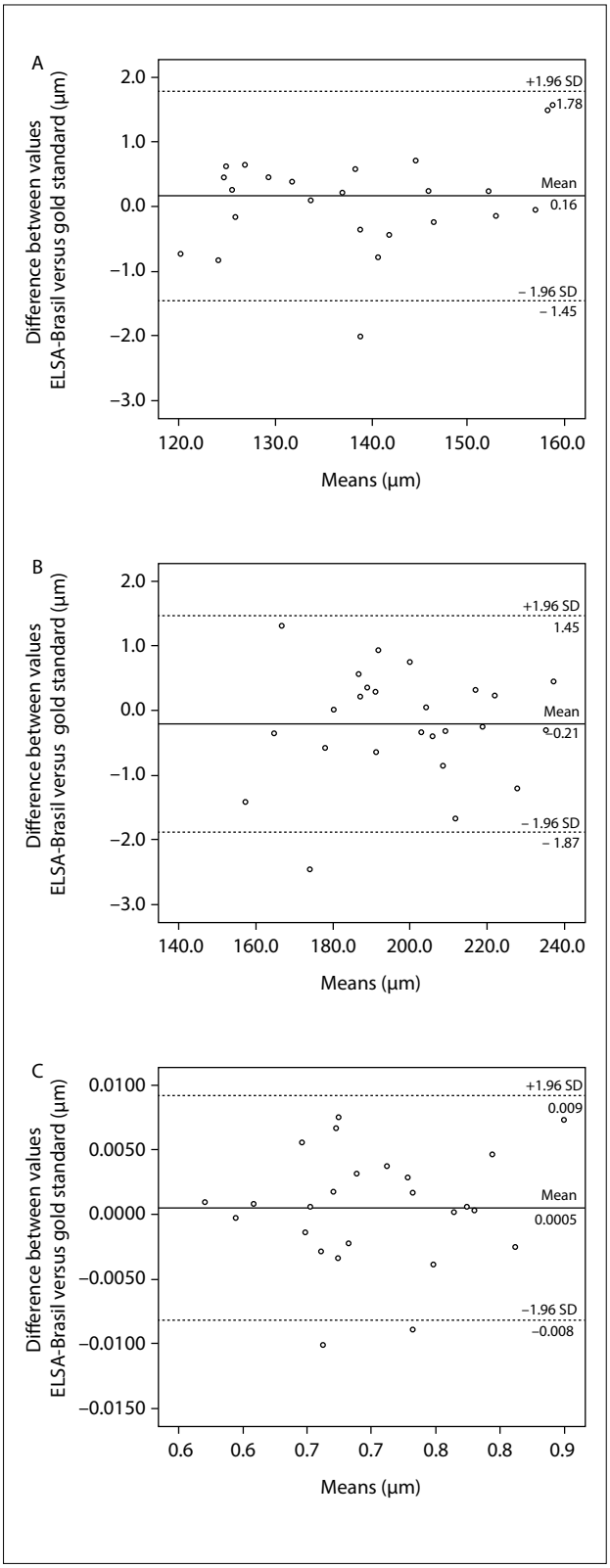

Figure 1. Validity evaluation on Bland-Altman plots of retinal microvascular measurements, comparing Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) graders with those of the University of Wisconsin. Dashed lines indicate the 95% confidence interval (CI) of the mean difference, which is indicated by the black line.

Reproducibility

Among the 105 images selected, 63.8% were from women, 44.8% from whites, 48.6% from participants aged 45-54 years and 61.0% from those who had completed a university degree; 25.7% were from individuals with hypertension and 20.0% from individuals with diabetes (**Table 2**).

The distribution of retinal microvascular values in the reproducibility sample (n = 105) approximated the values found overall in ELSA-Brasil (n = 12,257), as shown in **Table 3**. Additionally, although the average differences between the original and repeat readings were at times statistically significant, they were always less than 3%, as follows: -2.55 μm (P = 0.02) for CRAE; 0.65 μm (P = 0.54) for CRVE; and -0.0015 μm (P = 0.02) for AVR.

In the reproducibility evaluation, as seen in **Table 4**, the intragrader ICCs for CRAE, CRVE and AVR were 0.77 (95% CI 0.67 to 0.86), 0.90 (95% CI 0.78 to 0.96) and 0.70 (95% CI 0.55 to 0.83), respectively, and the intergrader ICCs were 0.75 (95% CI 0.64 to 0.85), 0.90 (95% CI 0.79 to 0.96) and 0.68 (95% CI 0.55-0.82), respectively.

Bland-Altman plots (**Figure 2**) showed that the differences obtained between the repeated readings in ELSA-Brasil were distributed in a similar way over the spectrum of values. In the intragrader measurements, the differences were < 10 μm in 75.2% of the arteriole measurements (CRAE) and in 86.7% of the venous measurements (CRVE). For the variable arteriole/venule ratio (AVR), 90.5% of the sample showed a difference < 0.1. In the intergrader measurements, the differences did not reach 10 μm in 71.4% of the arteriole measurements (CRAE) and 74.3% of the venule measurements (CRVE). For the variable arteriole/venule ratio (AVR), 89.5% of the sample showed a difference < 0.1.

Table 2. Sociodemographic, anthropometric and disease characteristics of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) sample that was used to evaluate microvascular measurement reproducibility, n = 105

Characteristic	N (%) or mean (standard deviation)	
Sex		
Female	67	(63.8)
Skin color/race		
White	47	(44.8)
Brown	33	(31.4)
Black	20	(19.1)
Others	5	(4.8)
Age (years)	51.4	(7.9)
Age strata		
35-44	22	(21.0)
45-54	51	(48.6)
55-64	24	(22.9)
65-74	8	(7.6)
Educational level		
Elementary school incomplete	2	(1.9)
Completed primary education	4	(3.8)
Completed high school	35	(33.3)
Completed college/university	64	(61.0)
Hypertension		
Yes	27	(25.7)
Diabetes mellitus		
Yes	21	(20.0)
Weight (kg)	72.5	(13.6)
Height (cm)	165.6	(9.7)
Body mass index (kg/m^2)		
Men	26.3	(4.1)
Women	26.3	(4.3)
Systolic blood pressure (mmHg)	117.9	(16.5)
Diastolic blood pressure (mmHg)	74.9	(10.7)

Table 3. Distribution of retinal microvascular measurements in the sample that was used to calculate reproducibility (n = 105) and in the whole cohort of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) (n = 12,257)

Variables	Sample mean (standard deviation)	Overall mean (standard deviation)
Central retinal artery equivalent (μm)	144.6 (15.5)	146.9 (15.0)
Central retinal vein equivalent (μm)	219.4 (24.7)	218.3 (20.6)
Arteriole/venule ratio	0.65 (0.08)	0.68 (0.07)

Table 4. Intragrader and intergrader reproducibility of microvascular measurements of the retina in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), n = 105

Measurement	Intragrader	Intergrader
	ICC (95% CI)	ICC (95% CI)
CRAE	0.77 (0.67-0.86)	0.75 (0.64-0.85)
CRVE	0.90 (0.78-0.96)	0.90 (0.79-0.96)
AVR	0.70 (0.55-0.83)	0.68 (0.55-0.82)

ICC = intraclass correlation coefficients; CI = confidence interval; CRAE = central retinal artery equivalent, CRVE = central retinal vein equivalent, AVR = arteriole/venule ratio.

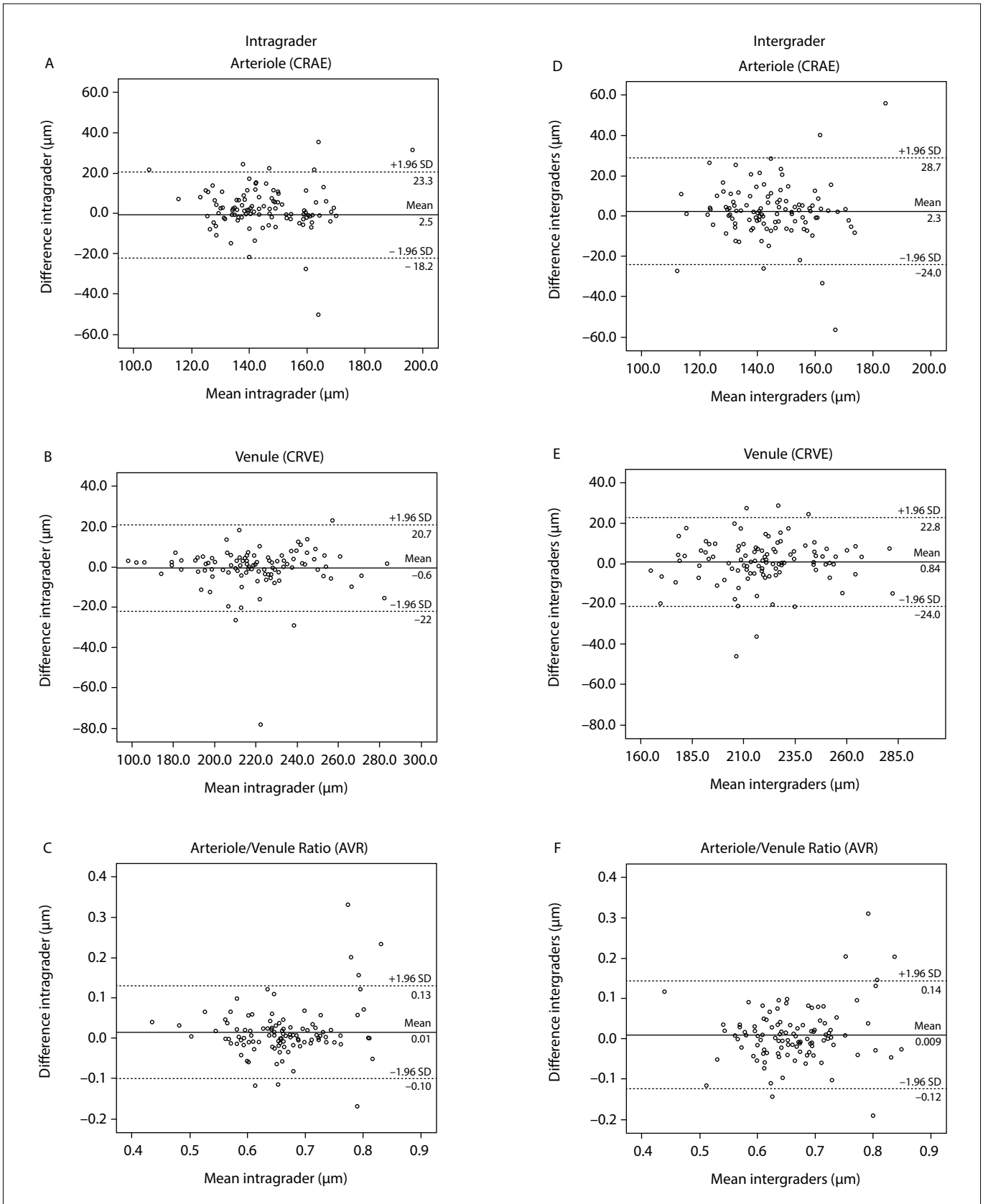

Figure 2. Reproducibility evaluation on Bland-Altman plots of the intra and intergrader differences in retinal microvascular measurements. Dashed lines indicate the 95% confidence interval (CI) of the mean difference, which is indicated by the black line.

DISCUSSION

The validity of the retinal vessel diameter measurements in ELSA-Brasil, based on repeated measurements on a sample of 25 images received from the Department of Ophthalmology and Visual Sciences of the University of Wisconsin, was excellent, such that only trivial differences were found. The reproducibility of determinations of these diameters, characterized through repeated grading of 105 images from the ELSA-Brasil study that were chosen to present values across the spectrum of those obtained in the study, was high for the basic measurements and moderate for their ratio.

Regarding validity, the differences in comparison with the gold standard were on average less than 1% of the absolute values of the diameters, as can be seen by comparing mean differences (Y axis) with mean values (X axis) in the Bland-Altman graphs. These differences, besides being epidemiologically irrelevant, were not statistically significant.

In general, the reproducibility of measurements in epidemiological studies involves both biological variability and the variability of the entire data collection method. This can include the biological variability of the retina between repeated measurements, variability in capturing the images through photography and variability in the process of grading those images. Thus, a hypothetical overall ICC would represent the correlation between repeated retinal evaluations on an individual involving photography at different times, use of different cameras, assessment of the various images by different graders and repeated gradings by each grader. In this study, we only considered the reproducibility of grading. Regarding this reproducibility, the agreement between measurements performed repeatedly by the same evaluator was high for both arteriolar and venular diameter, with ICC > 0.77 and ICC > 0.90 respectively, and the concordance between observers was high, with ICC > 0.70 for both measurements. The intra and intergrader agreements for the AVR (ICCs of 0.70 and 0.68, respectively) were at the top of the "moderate" range of agreement.

These results are compatible with those of other studies described in the literature. In the Atherosclerosis Risk Communities Study (ARIC), the reproducibility of the readings was estimated as 140 retinal images for intragrader agreement and 151 images for intergrader agreement. The concordance was high for the venous diameter, with ICCs of 0.89 and 0.77 for intra and intergrader agreement, respectively.[1] In the Beaver Dam Eye Study (BDES), reproducibility was evaluated in relation to 40 retinal images, with ICCs between 0.78 and 0.99 for inter and intragrader agreement,[25] presumably considering all three microvascular measurements. The Cardiovascular Health Study (CHS) described intra and intergrader reproducibility in relation to 71 and 69 subjects, respectively, with ICCs of 0.67 and 0.91, but also without indicating the microvascular measurement of the retina to which these related.[26] In the Singapore Cohort Study of Risk Factors for Myopia (SCORM), in relation to 50 images, the researchers evaluated intragrader reproducibility with only one evaluator. The ICC obtained for CRAE was 0.85 and for CRVE was 0.97, thus indicating high reproducibility for this grader.[27] In the Singapore Malay Eye Study (SIMES), 44 images were evaluated and intragrader ICCs of 0.88 for CRAE and 0.92 for CRVE were estimated, and intergrader ICCs of 0.88 for CRAE and 0.87 for CRVE.[20,28] Thus, we believe that our reproducibility results are consistent with those found in other studies, especially considering the relatively large time interval between measurements (about two years) and the larger number of readers who formed part of the team in ELSA-Brasil than were present in most of these other studies.

Retinal microvascular measurements are important for better comprehension of most chronic diseases. A growing number of published reports containing retinography data have expanded the range of metabolic and vascular diseases (cardiovascular diseases, diabetes mellitus, hypertension, stroke, obesity and dyslipidemia)[9,21,25,26] that seem to involve microvascular alterations in association with their development and/or progression. Aside from these better studied associations, retinal microvascular measurements have more recently also been shown to be associated with cognitive dysfunction,[31-32] the prevalence of complications in type 1 diabetes,[33] the internal carotid artery pulsation index[34] and changes to adiponectin levels.[35]

CONCLUSION

In summary, our study suggests that the retinal microvascular measurements in the ELSA-Brasil have strong validity and moderate to high reproducibility within and across graders. The capacity for generalization of these findings to other studies is restricted to the use of the same techniques, software and training procedures. Future studies analyzing associations of these microvascular retinal measurements with clinical and subclinical measurements of disease in ELSA-Brasil may contribute towards better understanding of changes within the disease-health process and towards prediction of the onset of these diseases.

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SUPPLEMENTARY MATERIAL

The statistical basis for measurement of the intraclass correlation coefficient when different numbers of measurements are read by each grader, but there is a constant number of readings per person, as developed by Dr. Lloyd E. Chambless, was used in this study. We applied the method of moments, as follows.

Y is the variable whose repeatability we study.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \in_{ijk}$$

$$\alpha \sim N(0, \sigma^2_{\alpha}), \qquad \beta \sim N(0, \sigma^2_{\beta}), \qquad \in \sim N(0, \sigma^2_{e})$$

People (α , ID)	Reader (β, R)	Repeats (∈ , e)
i = 1, …,n	j = 1, …p	k = 1, …, n_{ij}

For any ID =i, $m_i = \sum_{j=1}^{p} n_{ij}$ for all *i*, so m_i = obs per *i*.

Assume m_i=*m* for all ID. (Exclude observations for which this is false or keep only m if there are more).

We will work with the mean sums of squares:

$$MSS_{\alpha} = \frac{1}{n-1}\left[\left(\sum_{i=1}^{n}\frac{Y^2_{i..}}{m}\right) - \frac{Y^2_{...}}{nm}\right]$$

$$MSS_{\beta} = \frac{1}{p-1}\left[\left(\sum_{j=1}^{p}\frac{Y^2_{.j.}}{n_j}\right) - \frac{Y^2_{...}}{nm}\right]$$

$$MSS_{e} = \frac{1}{g}\left[\left(\sum_{i=1}^{n}\sum_{j=1}^{p} I(n_{ij} \geq 2)\left(\sum_{k=1}^{n_{ij}} Y^2_{ijk} - \frac{Y^2_{ij.}}{n_{ij}}\right)\right)\right]$$

where

$n_j = \sum_{i=1}^{n} n_{ij}$, $I(n_{ij} \geq 2)$ is 1 when $n_{ij} \geq 2$ and 0 otherwise, and

$$g = \sum_{i=1}^{n}\sum_{j=1}^{p} I(n_{ij} \geq 2)(n_{ij} - 1).$$

$$Y_{i..} = \sum_{j=1}^{p}\sum_{k=1}^{n_{ij}} Y_{ijk} = m(\mu + \alpha_i) + \sum_{j=1}^{p}\sum_{k=1}^{n_{ij}} \beta_j + \sum_{j=1}^{p}\sum_{k=1}^{n_{ij}} \in_{ijk}$$

$$= m(\mu + \alpha_i) + \sum_{j=1}^{p} n_{ij}\beta_j + \sum_{j=1}^{p}\sum_{k=1}^{n_{ij}} \in_{ijk}$$

$$Y_{...} = \sum_{i=1}^{n} Y_{i..} = m\,n\,\mu + m\,\alpha. + \sum_{i=1}^{n}\sum_{j=1}^{p} n_{ij}\beta_j + \sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{n_{ij}} \in_{ijk}$$

$$= m\,n\,\mu + m\,\alpha. + \sum_{j=1}^{p}\left(\sum_{i=1}^{n} \text{nij}\right)\beta_j + \Sigma_i\,\Sigma_j\,\Sigma_k \in_{ijk}$$

$$\text{for } \alpha. = \sum_{i=1}^{n} \alpha_i.$$

Note that $\left(\sum_{k=1}^{n_{ij}} \text{Y2ijk} - \frac{Y^2_{ij.}}{n_{ij}}\right) = 0$ when $n_{ij} = 1$.

$$E\,(MSS_\alpha) = \frac{1}{n-1}\left[\left(\sum_{i=1}^{n} \frac{E(Y^2_{i..})}{m}\right) - \frac{E(Y^2_{...})}{nm}\right]$$

$$Y^2_{i..} = (m\mu)^2 + \sum_{j=1}^{p} \text{n}_{\text{ij}}^{\ 2}\,\beta^2_{\text{j}} + m^2\alpha_i^{\,2} + \sum_{j=1}^{p}\sum_{k=1}^{n_{ij}} \in^2_{ijk}$$

+ cross-product terms with zero expectation.

$$E\,(Y^2_{i..}) = (m\mu)^2 + \left(\sum_{j=1}^{p} \text{n}^2_{\text{ij}}\,\sigma^2_{\beta}\right) + m^2\sigma^2_{\alpha} + m\sigma^2_{e}$$

$$Y^2\,... = m^2n^2\mu^2 + m^2\alpha^2. + \sum_{j=1}^{p}\left(\sum_{i=1}^{n} n_{ij}\right)^2 \beta^2_{\text{j}} + \sum_{i=1}^{n}\sum_{j=1}^{p}\sum_{k=1}^{n_{ij}} \in^2_{ijk}$$

+ terms with zero expectation.

$$E\,(Y^2\,...) = m^2n^2\mu^2 + m^2n\sigma^2_{\alpha} + \sum_{j=1}^{p}\left(\sum_{i=1}^{n} n_{ij}\right)^2 \sigma^2_{\beta} + n\,m\,\sigma^2_{\text{e}}$$

$$E\,(MSS_\alpha) = \frac{1}{m\,(n-1)}\left[nm^2\mu^2 + \left(\sum_{i=1}^{n}\sum_{j=1}^{p} n_{\text{ij}}^{\ 2}\right)\sigma^2_{\beta} + nm^2\sigma^2_{\alpha} + nm\sigma^2_{e} - \left(m^2n\mu^2 + \text{m}^2\sigma^2_{\alpha} + \frac{1}{n}\sum_{j=1}^{p}\left(\sum_{i=1}^{n} n_{\text{ij}}\right)^2 \sigma^2_{\beta} + m\sigma^2_{e}\right)\right]$$

$$= \frac{1}{m\,(n-1)}\left[(n-1)m^2\sigma^2_{\alpha} + \left(\sum_{i=1}^{n}\sum_{j=1}^{p} n^2_{\text{ij}} - \frac{1}{\text{n}}\sum_{j=1}^{p}\left(\sum_{i=1}^{n} n_{\text{ij}}\right)^2\right)\sigma^2_{\beta} + (n-1)m\sigma^2_{\text{e}}\right]$$

$$= m\,\sigma^2_{\alpha} + \frac{1}{m\,(n-1)}\left(\sum_{i=1}^{n}\sum_{j=1}^{p} n^2_{\text{ij}} - \frac{1}{\text{n}}\sum_{j=1}^{p}\left(\sum_{i=1}^{n} n_{\text{ij}}\right)^2\right)\sigma^2_{\beta} + \sigma^2_{\text{e}}$$

$$E\,(MSS_\alpha) = m\sigma^2_{\alpha} + \frac{1}{m\,(n-1)}\left(\sum_{i=1}^{n}\sum_{j=1}^{p} n^2_{\text{ij}} - \frac{1}{\text{n}}\sum_{j=1}^{p} n^2_{\text{j}}\right)\sigma^2_{\beta} + \sigma^2_{\text{e}} \quad \text{(A)}$$

Where $n_j = \sum_{i=1}^{n} n_{ij}$

$$Y_{.j.} = \sum_{i=1}^{n}\sum_{k=1}^{n_{ij}} Y_{ijk} = \sum_{i=1}^{n}\sum_{k=1}^{n_{ij}} (\mu + \alpha\text{i} + \beta_j + \in_{ijk})$$

$$= n_j\mu + \sum_{i=1}^{n} n_{ij}\alpha_i + n_j\beta_j + \sum_{i=1}^{n}\sum_{k=1}^{n_{ij}} \in_{ijk}$$

$$(Y_{.j.})^2 = n^2_j\mu^2 + \sum_{i=1}^{n} n^2_{ij}\alpha^2_{\ i} + n^2_j\beta^2_{\ j} + \sum_{i=1}^{n}\sum_{k=1}^{n_{ij}} \in^2_{ijk}$$

+ cross-product terms with zero expected value.

$$E\,((Y_{.j.})^2) = n^2_j\mu^2 + \left(\sum_{i=1}^{n} n^2_{ij}\right)\sigma^2_{\alpha} + n2_j\sigma^2_{\beta} + \text{n}_j\sigma^2_{e}$$

$$E\,(MSS_\beta) = \frac{1}{p-1}\left[\left(\sum_{j=1}^{p} \frac{E(Y.j^2.)}{n_j}\right) - \frac{E(Y^2...)}{nm}\right]$$

$$= \frac{1}{p-1}\left[\sum_{j=1}^{p}\left(n_j\mu^2 + \frac{\left(\sum_{i=1}^{n} n^2_{ij}\right)}{n_j}\,\sigma^2_{\alpha} + n_j\sigma^2_{\beta} + \sigma^2_{\text{e}}\right) - \left(m\,n\,\mu^2 + \text{m}\,\sigma^2_{\alpha} + \sum_{j=1}^{p} n^2_{\text{j}}\,\frac{\sigma^2_{\beta}}{mn} + \sigma^2_{\text{e}}\right)\right]$$

$$= \frac{1}{p-1}\left[\left(\sum_{j=1}^{p} n_{ij}\right)\mu^2 + \sum_{j=1}^{p}\frac{\left(\sum_{i=1}^{n} n^2_{ij}\right)}{n_j}\,\sigma^2_{\alpha} + \frac{\left(\sum_{j=1}^{p} n_j\right)}{nm}\,\sigma^2_{\beta} + \text{p}\,\delta^2_{e} - \left(m\,n\,\mu^2 + \text{m}\,\sigma^2_{\alpha} + \frac{\sum_{j=1}^{p} n^2_{\,j}}{nm}\,\sigma^2_{\beta} + \sigma^2_{\text{e}}\right)\right]$$

$$E\,(MSS_\beta) = \frac{1}{p-1}\left[\left(\sum_{j=1}^{p}\frac{\left(\sum_{i=1}^{n} n^2_{ij}\right)}{n_j} - m\right)\sigma^2_{\alpha} + \frac{1}{p-1}\left(n\,m - \frac{\sum_{j=1}^{p} n^2_{\,j}}{m\,n}\right)\sigma^2_{\beta} + (p-1)\,\sigma^2_{\text{e}}\right]$$

$$E\,(MSS_\beta) = \frac{1}{(p-1)}\left(\sum_{j=1}^{p}\frac{\left(\sum_{i=1}^{n} n^2_{ij}\right)}{n_j} - m\right)\sigma^2_\alpha + \frac{1}{(p-1)}\left(mn - \frac{\sum_{j=1}^{p} n^2_j}{mn}\right)\sigma^2_\beta + \sigma^2_e \quad \text{(B)}$$

Suppose n_{ij} = w for all i, j. Then $m = \sum_{j=1}^{p} n_{ij} = pw$ = *OBS per person*,

u = nj = $\sum_{i=1}^{n}$ nij = nw = OBS per reader, and mn = pwn = pu.

The coefficient of σ^2_β in the $E\,(MSS_\beta)$ formula simplifies as follows:

$$\frac{1}{(p-1)}\left(mn\,\frac{\left(\sum_{j=1}^{p} n^2_j\right)}{mn}\right) = \frac{1}{(p-1)}\left(pu - \frac{pu^2}{pu}\right)$$

$$= \frac{1}{(p-1)}\,(pu-u) = \frac{1}{(p-1)}\,(p-1)\,u = u$$

What is the coefficient of σ^2_β in the $E\,(MSS_\alpha)$ formula if $n_{ij} = w$ for all *i, j*?

u = *OBS per reader* = *nw*, w = *OBS per reader x ID*, m = *OBS per person* = *pw*

$$\frac{1}{m\,(n-1)}\left(\sum_{i=1}^{n}\sum_{j=1}^{p} w^2 - \frac{1}{\mathrm{n}}\sum_{j=1}^{p} u^2\right) = \frac{1}{m\,(n-1)}\left(pnw^2 - \frac{1}{n}\,pu^2\right)$$

$$= \frac{1}{pw\,(n-1)}\left(puw - \frac{1}{n}\,pu^2\right)$$

$$= \frac{1}{pw\,(n-1)}\,pu\left(w - \frac{1}{n}\right) = \left(w - \frac{nw}{w}\right) = 0$$

What is the coefficient of σ^2_α in the $E\,(MSS_\beta)$ formula $n_{ij} = w$ for all *i, j*?

$$\frac{1}{p-1}\left(\sum_{j=1}^{p}\frac{\left(\sum_{i=1}^{n} n^2_{ij}\right)}{n_j} - m\right) = \frac{1}{(p-1)}\left(\sum_{j=1}^{p}\frac{\left(\sum_{i=1}^{n} w^2\right)}{nw} - pw\right)$$

$$= \frac{1}{p-1}\left(\sum_{j=1}^{p}\frac{nw^2}{nw} - pw\right) = \frac{1}{p-1}\left(\sum_{j=1}^{p} w - pw\right)$$

$$= \frac{1}{p-1}\,(pw - pw) = 0\,!$$

Thus, if $n_{ij} = w$ for all *i, j* then the formula for $E\,(MSS_\beta)$ and $E\,(MSS_\alpha)$ simplifies to what SAS procglm would get.

We return to the other expected squares.

$Y^2_{ijk} = \mu^2 + \alpha^2_i + \beta^2_j + \in^2_{ijk}$ + cross-product terms with zero expectation.

$$E\,(Y^2_{ijk}) = \mu^2 + \sigma^2_\alpha + \sigma^2_\beta + \sigma^2_e$$

$$Y_{ij} = \sum_{k=1}^{n_{ij}} Y_{ijk} = \sum_{k=1}^{n_{ij}} (\mu + \alpha_i + \beta_j + \in_{ijk})$$

$$= n_{ij}\mu + n_{ij}\alpha_i + n_{ij}\beta_j + \sum_{k=1}^{n_{ij}} \in_{ijk}$$

$$Y^2_{ij.} = n^2_{ij}\mu^2 + n^2_{ij}\alpha^2_i + n^2_{ij}\beta^2_j + \sum_{k=1}^{n_{ij}} \in^2_{ijk}$$

+ cross-product terms with zero expectation.

$$E(Y^2_{ij.}) = (n^2_{ij}\mu^2 + n^2_{ij}\sigma^2_\alpha + n^2_{ij}\sigma^2_\beta + n_{ij}\sigma^2_e$$

With $I(n_{ij} \geq 2) = 1$ when $n_{ij} \geq 2$ and 0 otherwise,

$$E(MSS_e) = \frac{1}{g}\sum_{i=1}^{n}\sum_{j=1}^{p} I(n_{ij} \geq 2)\left(\sum_{k=1}^{n_{ij}} (E(Y^2_{ijk}) - \frac{E\,(Y2_{ij.})}{n_{ij}}\right)$$

$$= \frac{1}{g}\sum_{i=1}^{n}\sum_{j=1}^{p} I(n_{ij} \geq 2)\left(\sum_{k=1}^{n_{ij}} (\mu^2 + \sigma^2_\alpha + \sigma^2_\beta + \sigma^2_e) - (n_{ij}\mu^2 + n_{ij}\sigma^2_\alpha + n_{ij}\sigma^2_\beta + n_{ij}\sigma^2_e)\right)$$

$$\frac{1}{g}\sum_{i=1}^{n}\sum_{j=1}^{p} I\,I(n_{ij} \geq 2)$$

$$((n_{ij}\mu^2 + n_{ij}\sigma^2_\alpha + n_{ij}\sigma^2_\beta + n_{ij}\sigma^2_e) - (n_{ij}\mu^2 + n_{ij}\sigma^2_\alpha + n_{ij}\sigma^2_\beta + \sigma^2_e))$$

$$= \frac{1}{g}\sum_{i=1}^{n}\sum_{j=1}^{p} I(n_{ij} \geq 2)\,(n_{ij}-1)\,\sigma^2_e = \sigma^2_e, \text{ since}$$

$$g = \sum_{i=1}^{n}\sum_{j=1}^{p} I(n_{ij} \geq 2)\,(n_{ij}-1).$$

Thus, $E(MSS_e) = \sigma^2_e$. (C)

We use equations (A), (B), and (C) above to derive estimates for σ^2_α, σ^2_β, and σ^2_e, replacing the expected mean sums of squares by the observed values, and solving the equations.

From (C),

$$\hat{\sigma}^2_e = \left(\frac{1}{g}\right)\sum_{i=1}^{n}\sum_{j=1}^{p} I(n_{ij}-2)\left(\left(\sum_{k=1}^{n_{ij}} Y^2_{ijk}\right) - \frac{Y^2_{ij.}}{n_{ij}}\right)$$

From (B),

$$\frac{1}{(p-1)}\left(\sum_{j=1}^{p}\frac{\left(\sum_{i=1}^{n} n^2_{ij}\right)}{n_j} - m\right)\hat{\sigma}^2_{\alpha} + \frac{1}{(p-1)}\left(mn - \frac{\sum_{j=1}^{p} n^2_j}{mn}\right)\hat{\sigma}^2_{\beta} = MSS_{\beta} - \hat{\sigma}^2_{e} \qquad \text{(D)}$$

From (A),

$$m\hat{\sigma}^2_{\alpha} + \frac{1}{m(n-1)}\left(\sum_{i=1}^{n}\sum_{j=1}^{p} n^2_{ij} - \frac{1}{n}\sum_{j=1}^{p} n^2_j\right)\hat{\sigma}^2_{\beta} = MSS_{\alpha} - \hat{\sigma}^2_{e} \qquad \text{(E)}$$

Thus, with (D) and (E) we have two equations in two unknowns $\hat{\sigma}^2_{\alpha}$ and $\hat{\sigma}^2_{\beta}$. We have to solve this two equations for the unknown variances.

Then, $\hat{\sigma}^2_{total} = \hat{\sigma}^2_{\alpha} + \hat{\sigma}^2_{\beta} + \hat{\sigma}^2_{e}$, for $\hat{\sigma}^2_{\alpha}$ the between-person variance, $\hat{\sigma}^2_{\beta}$ the between-reader variance, and $\hat{\sigma}^2_{e}$ the error variance, which combines within-reader and within-person variability.

The ICC: $\text{Corr}(Y_{ijk}, Y_{ij'k'}) = \hat{\sigma}^2_{\alpha} / \hat{\sigma}^2_{total}$ for $j \neq j'$ (different readers)

$\text{Corr}(Y_{ijk}, Y_{ijk'}) = (\hat{\sigma}^2_{\alpha} + \hat{\sigma}^2_{\beta}) / \hat{\sigma}^2_{total}$ for $k \neq k'$ (same reader).

Experience of treatment of prosthetic valve endocarditis: a retrospective single-center cross-sectional study

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ABSTRACT

BACKGROUND: The aim of this study was to describe the experience of treatment of early prosthetic valve endocarditis at a heart center.

DESIGN AND SETTING: Retrospective single-center study on data collected from electronic medical records covering the period from January 2009 to December 2015.

METHODS: Over the study period, 1,557 consecutive valve operations were performed on adult patients. The study population comprised 32 patients (2%) who were diagnosed with prosthetic valve endocarditis within 12 months after the index surgery. Medical records were retrieved from electronic hospital records, retrospectively. Descriptive clinical, echocardiographic, microbiological and treatment-type data were used. Risk factors for early mortality were studied through univariate and multivariate analyses.

RESULTS: The main clinical manifestation of infective endocarditis was fever, and this was present in all patients. Most of the prostheses were affected in the aortic position (40.6% of cases). The most commonly cultured microorganisms were *Staphylococcus epidermidis* and *Staphylococcus aureus*. Twenty-six patients (81.3%) underwent surgical treatment and six (18.7%) underwent exclusive clinical treatment. The prevalence of postoperative complications was 31.3% and hospital mortality occurred in seven cases (21.9%). The mortality rate was 50% among the patients who underwent medical treatment and 15.4% among those who underwent surgery. There were no independent risk factors for mortality.

CONCLUSION: Prosthetic valve endocarditis is an infrequent complication of valve replacement. Surgical treatment has mortality rates compatible with the severity of patients' conditions. Surgical indication should not be delayed when clinical treatment has been ineffective.

INTRODUCTION

Infective endocarditis is one of the most serious diseases that can affect heart valves. Despite technological developments that have been incorporated into new diagnostic methods and surgical techniques, the morbidity and mortality rates relating to infective endocarditis are still significant.[1,2]

Regarding native valve endocarditis, studies have shown that early surgical treatment is associated with lower complication rates and greater possibility of valve preservation, with obvious long-term benefits.[3,4] However, early surgical treatment requires early diagnosis, which is not always possible. Presence of large valve destruction, ring invasion, abscesses, fistulas, distal embolization and multiorgan involvement may be frequent in cases of late diagnosis. The variability of the initial presentation to the surgeon gives rise to difficulties in patient management, and certainly impacts surgical results.[5,6]

Therefore, although the surgical indication for infective endocarditis has classic criteria that have been described in specific guidelines,[1] there is controversy regarding the best moment to make the indication, when applicable. The patient's clinical situation, the technical aspects of the operation and presence of extracardiac complications influence the therapeutic decision. Advanced age, important comorbidities and multiorgan dysfunction may be contraindications for surgical treatment.[5]

It is important to consider whether the infectious process reaches native valves or prostheses, since previously reported results have suggested that the evolution is worse in prosthetic valve endocarditis.[7,8] Infective endocarditis of prosthetic valves presents a more complicated diagnosis, with less sensitivity to the Duke criteria.[9] In addition, prosthetic valve infective endocarditis may be accompanied by perivalvular or myocardial abscess and valve dysfunction, thus presenting higher mortality than native valve infective endocarditis.[10,11] However, there is a shortage of studies assessing operative outcomes from prosthetic valve endocarditis,[12,13] and controversy

remains regarding whether operative or conservative treatment should be indicated.

The objective of this study was to describe the experience of a cardiological center in managing early prosthetic valve endocarditis, regardless of the treatment instituted.

METHODS

A retrospective single-center study was conducted through data-gathering from electronic medical records covering the period from January 2009 to December 2015. During this period, 1,557 consecutive valve operations were performed on adult patients, among whom 32 (2%) evolved with prosthetic valve endocarditis within 12 months after the index surgery. These patients constituted the population of our study. Patients who underwent surgery for correction or palliation of congenital heart diseases and native valve endocarditis were excluded.

The diagnosis of infective endocarditis was made in accordance with the modified Duke criteria.[14] The classification of early prosthetic valve endocarditis was supported by the definition of the American Heart Association, which considers infectious involvement of the heart valve prosthesis within 12 months after the index surgery.[1] In the cases evaluated here, once diagnostic suspicion had been established, cultures were collected and broad-spectrum empirical antimicrobial treatment was started while awaiting the culture results. In cases with a positive culture, antimicrobial treatment was guided by an antibiogram. The decision regarding surgical treatment and the best time to implement it was made following discussion within a team composed of a clinical cardiologist, a cardiovascular surgeon and an infectiologist. These decisions were made following wide-ranging discussion of individual cases.

Statistical analysis was performed using the JMP software (SAS version 9). Categorical variables were expressed as frequencies and percentages, and continuous variables as means and standard deviation. Multivariate analysis was performed by means of logistic regression to evaluate risk factors. The statistical significance level was taken to be 5%.

This study was approved by our institution's research ethics committee (ICDF Ethics Committee; approved on August 29, 2013, under number 376261), in accordance with the Helsinki standards.

RESULTS

Clinical and echocardiographic characteristics

Over the study period, 1,557 consecutive valve operations were performed and 32 patients were diagnosed as having prosthetic valve endocarditis. Their mean age was 42.9 years and 16 of them (50%) were male. The clinical and echocardiographic characteristics of the patients are described in **Table 1**. The main cause associated with initial valve dysfunction was rheumatic heart disease, which was present in 28.1% of the cases.

Endocarditis occurred within 30 days after the initial surgical procedure in 18 patients (56.3%), while 14 patients (43.7%) presented onset of infective endocarditis between one month and one year after the operation. Fever was the main clinical manifestation, and this was present in all patients.

The largest proportion of the prostheses were affected in the aortic position: 40.6%, comprising 11 cases in the aortic position alone and two cases in valved tubes. This was followed by cases affected in the mitral position (11; 34.4%) and in the mitroaortic position (6; 18.7%). The types of prostheses used in index surgery were biological in 18 cases (56.2%), followed by mechanical prostheses in 13 cases (40.6%) and a cryopreserved homograft in one patient. The prevalences of periprosthetic leakage, valve ring abscess and fistula were 34.4%, 9.4% and 3.1%, respectively.

Microbiology

Blood culture positivity was low (6 patients; 18.8%). The percentage of patients undergoing antibiotic therapy was unknown. The following agents were identified: *Streptococcus sanguinis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus capitis* and *Clostridium* sp. Among the patients who underwent surgical treatment, six patients whose blood culture was negative had a positive valve prosthesis culture. The microorganisms of these cultures were: *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Candida albicans* (**Table 2**).

Treatment

Out of the 32 patients diagnosed with prosthetic valve endocarditis, 26 (81.3%) underwent surgical treatment and 6 (18.7%) underwent exclusively clinical treatment. The decision on the type of therapy was based on the patients' clinical conditions, the echocardiographic characteristics of the heart valves, possible complications and presence of important comorbidities.

Among the patients who were treated clinically, three individuals had clinical comorbidities that made the operation unfeasible

Table 1. Patients' clinical characteristics

Mean age (years)	42.9 ± 15.9
Age > 60 years	4 (12.5%)
Males	16 (50%)
Weight (kg)	63.9 ± 11.9
Height (cm)	165 ± 11.3
Body mass index (kg/m^2)	23.6 ± 3.8
Systemic arterial hypertension	10 (31.3%)
Chronic kidney disease	6 (18.8%)
Previous stroke	3 (9.4%)
Rheumatic fever	9 (28.1%)
NYHA Functional Classification IV	4 (12.5%)
Diagnosis of infective endocarditis made within 30 days	18 (56.3%)

(such as severe sepsis and cardiogenic shock) and evolved to death; two patients were receiving antimicrobial treatment for another condition (sepsis and wound infection) and improved; and one patient presented an initial operative indication but evolved with improvement after around one week of antibiotic therapy.

In the surgically treated group, the median length of time between diagnosis and surgery was 13.5 days. Regarding the initial valve operation (pre-endocarditis), the mean time taken for the cardiopulmonary bypass (cardiopulmonary bypass) procedure was 141.1 ± 54.1 minutes (median of 130.5 minutes). The time taken to perform the cardiopulmonary bypass was greater than 120 minutes in 19 patients (59.4%). The duration of intubation was over 48 hours in four patients (12.5%). Blood products were transfused in 21 patients (65.6%), with poly-transfusion in 11 cases. Postoperative hemodialysis was required in six patients (18.8%) (**Table 3**).

The prevalence of postoperative complications was 31.3%. Complete atrioventricular block occurred in four patients (12.5%), stroke in three (9.4%), sepsis in two (6.3%), limb amputation in two (6.3%) and acute myocardial infarction in one (3.1%).

Mortality risk factors

Hospital mortality occurred in the cases of seven individuals (21.9%), thus representing 50% of the patients undergoing exclusively (**Table 3**) clinical treatment and 15.4% of the patients undergoing surgical treatment. Considering the echocardiographic and microbiological findings, along with the clinical factors associated with the initial operation, we did not identify any statistically significant risk factors for mortality through multivariate logistic regression analysis. This was probably due to the limited number of patients.

Table 2. Blood and prosthetic valve cultures

Microorganism	Frequency
Staphylococcus epidermidis	4 (12.5%)
Staphylococcus aureus	3 (9.4%)
Staphylococcus capitis	1 (3.1%)
Staphylococcus saprophyticus	1 (3.1%)
Streptococcus sanguinis	1 (3.1%)
Clostridium sp.	1 (3.1%)
Candida albicans	1 (3.1%)
Negative cultures	20 (62.5%)

Table 3. Echocardiographic data before operation for infective endocarditis

Mean ejection fraction (EF) (%)	59.6 ± 14.3
Left ventricle dysfunction (EF < 50%)	6 (18.8%)
Right ventricle dysfunction	10 (31.3%)
Pulmonary hypertension (**PASP > 40 mmHg**)	12 (37.5%)
Presence of vegetative growth	30 (93.8%)
Presence of periprosthetic leakage	11 (34.4%)
Presence of fistula	1 (3.1%)
Presence of valve ring abscess	3 (9.4%)
Site of involvement	
Aortic	13 (40.6%)
Mitral	11 (34.4%)
Mitroaortic	6 (18,7%)
Other	2 (6.3%)

PASP = pulmonary artery systolic pressure.

DISCUSSION

The frequency of prosthetic valve endocarditis, considering total valve operations, was approximately 2%. This proportion is close to what was reported in the study by Pomerantzeff et al.,[13] in which 28 patients presented prosthetic valve infective endocarditis out of a total of 1,512 valve operations (1.58%).

The diagnosis of infective endocarditis was established in accordance with the modified Duke criteria.[14] Fever was the main sign that guided the suspicion of infective endocarditis in the postoperative period, and this was present in 100% of the cases. Other studies that assessed clinical manifestations of postoperative infective endocarditis have also highlighted fever as the most prevalent sign, present in 87% to 96% of the cases.[13,15]

Echocardiography plays an important role in making the diagnosis of infective endocarditis. Its findings may include perivalvular vegetations and abscesses, which may become more complicated through pseudoaneurysms and fistulization, or dehiscence of the prosthetic valve, which may result in perivalvular leakage.[16] In our study, echocardiographic evaluation was fundamental for the diagnostic confirmation of infective endocarditis, and vegetative growths were detected in 93.8% of the cases. This frequency was higher than the 60% reported in another study.[13]

The aortic valve was the one most affected (40.6%), followed by the mitral valve (34.4%). This was concordant with previous studies that have mentioned that these valves are the ones predominantly affected by the infectious process.[13,17] Mechanical and biological valves were similarly involved, which is also consistent with previous findings.[18]

Our study found that the positivity rate among the blood cultures was low (18.8%). Even considering prosthetic valve cultures, 56.3% of the patients did not present positive microbiological findings. Internationally, the estimated frequency of negative cultures in confirmed cases of infective endocarditis, considering both native valves and prostheses, is approximately 20%.[19] Taking into account studies conducted in Brazil, the frequency of negative blood cultures ranges from 40% to 58%.[13,15,17,20] Among these studies, only Pomerantzeff et al.[13] specifically evaluated prosthesis endocarditis, and they reported that 50% of the blood cultures were negative (with the limitation of not describing prosthetic valve cultures).

The possible causes of high rates of negative blood cultures are inadequate culture techniques, uncultivated infectious agents and administration of antibiotics prior to sample collection for culture.[21] Improvement of collection and culture techniques could

increase the microbiological positivity indexes and optimize the clinical treatment targeted. In this context, the polymerase chain reaction (PCR) on valve tissue has also been highlighted as an effective method for microbiological confirmation, especially in cases of negative blood culture.[22]

In positive cultures, the most commonly isolated microorganisms were *Staphylococcus epidermidis* and *S. aureus*. In the literature, it has also been reported that early-stage prosthetic valve infective endocarditis (within one year after the index operation) is predominantly caused by coagulase-negative staphylococci or by *S. aureus*.[18]

In our series, the majority of the patients (about 80%) underwent operative treatment for prosthesis infective endocarditis. In an international multicenter cohort, the proportion of patients who underwent operations during the active phase of prosthetic valve infective endocarditis was approximately 50%.[12]

Despite the important role of surgery in the therapeutic management of infective endocarditis, only one randomized study of relatively small proportions has evaluated the role of valve operation in the treatment of endocarditis. That study demonstrated that the incidence of thromboembolic events was lower in the group that underwent surgery, but it was limited to only assessing patients with native valve infective endocarditis.[23] There are no randomized studies of larger proportions that can provide unequivocal assurance regarding the benefit of early operation in treating infective endocarditis. Indications for early surgery are still based fundamentally on observational studies.

Surgical indication needs to be individualized based on a number of factors, such as age, clinical comorbidities, infectious agent, response to antimicrobial treatment, extent of vegetative growth, presence of perivalvular infection, presence of embolism or heart failure, and the surgeon's experience.[1,6] In the present study, six cases were managed exclusively with clinical treatment because of clinical comorbidities that made the operation unfeasible (such as severe sepsis and cardiogenic shock) or a good response after the initial antibiotic therapy.

Assessment of mortality through comparing surgical and clinical treatment is complicated by the fact that patients in a better clinical condition are more susceptible to undergoing surgery than those with early mortality (survival bias).[1] The mortality rates of the exclusively clinical treatment and surgical treatment groups reported in this study (50% versus 15%) cannot be properly compared because of survival bias. A prospective multinational observational cohort showed that there was no difference in mortality between a group that underwent exclusively clinical treatment and a group that underwent early operation, after adjusting for survival bias.[12]

The prevalence of complications from the infective endocarditis operation was close to 30%, which was lower than the rate of 64% described in another study.[15] We reported occurrences of complete atrioventricular block, thromboembolic events and sepsis, thus reaffirming the findings of previous studies.[13] The operative mortality in our series was 15.4%, i.e. lower than that previously reported (27%),[12] but this comparison is limited by the absence of a correction for the surgical mortality risk scores of the patients involved in the different studies.

Overall mortality (22%) was lower than the 30% mortality rate due to prosthetic valve endocarditis that has been reported in the literature from other countries.[5] Brazilian studies have reported overall mortality rates ranging from 14% to 40%.[13,15,17] The present study sought to establish echocardiographic findings and factors associated with the initial operation (**Tables 1** and **2**) that could characterize risk factors for mortality, but no statistically significant independent risk factors were identified. This was probably due to the small number of events.

Our study presented limitations regarding the retrospective collection, its single-center nature and its small sample. The relevance of the findings described here comes from the absence of randomized studies evaluating outcomes from operative treatment of prosthesis infective endocarditis, which means that management of patients affected by this condition is still based on observational studies.

CONCLUSION

Prosthetic valve infective endocarditis is an infrequent complication of valve replacement operations, but it is associated with significant morbidity and mortality. Surgical treatment gives rise to mortality rates that are compatible with the severity of the patients' conditions. The findings of our study reaffirm that surgical indications should not be delayed when clinical treatment is seen to be ineffective.

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Sensitivity and specificity of ultrasonography in diagnosing supraspinatus lesions: a prospective accuracy diagnostic study

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ABSTRACT

BACKGROUND: This study was designed to define the accuracy of shoulder ultrasonography for diagnosing supraspinatus tendon tears. This examination is routinely used by orthopedists and may do away with the need for other examinations for diagnosing these tendon injuries. The aim of this study was to evaluate the sensitivity and specificity of shoulder ultrasonography for diagnosing supraspinatus tendon injuries, using magnetic resonance imaging as the reference.

DESIGN AND SETTING: Prospective accuracy study at a single center: the Shoulder and Elbow Surgery Clinic of the Department of Orthopedics and Traumatology.

METHODS: Shoulder ultrasonography was performed on 80 patients of both genders, over 18 years of age, with complaints of shoulder pain and clinically suspected supraspinatus tendon lesions. Jobe's test and a full can test were performed. In addition, they underwent magnetic resonance imaging in a 3.0-tesla machine, as the reference standard. The examinations were performed and interpreted by radiologists.

RESULTS: Ultrasonography showed sensitivity of 36.3% and specificity of 91.7% for supraspinatus tears overall: sensitivity of 25.8% and specificity of 91.8% for partial tears and sensitivity of 46.2% and specificity of 100% for full-thickness tears. Ultrasonography showed high accuracy for diagnosing full-thickness tears: 91.3%. The p-values were 0.003 for tears overall, 0.031 for partial tears and < 0.001 for full-thickness tears.

CONCLUSIONS: Ultrasonography showed low sensitivity for detecting supraspinatus tears, but high specificity for both partial and full-thickness tears.

INTRODUCTION

Rotator cuff tears (RCTs) are the main cause of shoulder pain in adults and the supraspinatus tendon is the element most affected.[1,2] Studies have shown that the prevalence of RCTs ranges from 5% to 40% and that it is directly related to increasing age.[3]

Clinical tests and imaging examinations are routinely performed to diagnose lesions of this tendon.[3] In a study conducted to elucidate the prevalence of rotator cuff tears in the general population, using ultrasonography as the reference standard, Yamamoto et al. found that the prevalence of rotator cuff tears was 20.7%, through examining 683 patients (total of 1,366 shoulders). They showed that the frequency of rotator cuff tears increased with age and that these lesions were most common in elderly male patients.[4]

Ultrasonography of the shoulder is a diagnostic method used in clinical practice by orthopedists. It is a non-invasive method that is accessible for most patients (both in primary and in tertiary-level healthcare services). It has low cost and high acceptability and allows viewing of rotator cuff tendons.[5] However, it is a diagnostic method with potential risks of pitfalls, depending on the examiner's technique and experience.[6]

Several studies assessing the accuracy of ultrasonography have been published. However, the literature still presents inconsistencies and variability regarding the sensitivity and specificity of this test in making the diagnosis of rotator cuff lesions.[7] It has been shown that the sensitivity and specificity of ultrasonography are very similar to those of magnetic resonance imaging in diagnosing supraspinatus lesions, but orthopedists have reported some discrepancies in clinical practice.[8]

In our orthopedic practice, it is common to find disagreements between magnetic resonance and ultrasonography in making the diagnosis of supraspinatus tendon tears. Thus, the objective of this study was to assess the accuracy of shoulder ultrasonography for diagnosing tears of the supraspinatus tendon, taking magnetic resonance imaging of the shoulder as a reference standard.

METHODS

Study design, setting and ethics

We conducted a prospective accuracy study that included adult patients followed up at a single center, the Shoulder and Elbow Surgery Clinic of the Department of Orthopedics and Traumatology, Federal University of São Paulo (Universidade Federal de São Paulo, UNIFESP), between November 2016 and May 2017. This project was approved by our institution's Research Ethics Committee under the number 0557/2017. All patients included had previously agreed to participate and had signed a written consent form, after being informed about the prognosis for and potential complications of their condition and the objectives of the study. We used the STARD checklist to guide the information in this article.

Participants

The inclusion criteria were that the patients could be of either gender, needed to be over 18 years of age and had presented complaints of shoulder pain for at least one month. The exclusion criteria were occurrences of loss of passive movement of the shoulder (severe osteoarthrosis or adhesive capsulitis); sensory and motor deficits of the affected limb; fractures, dislocations, neoplastic lesions or previous surgery on the affected limb; absence of the results from the imaging tests (ultrasonography and magnetic resonance imaging); and time between ultrasonography and magnetic resonance imaging of over three months.

We recruited patients based on their presentation of symptoms of shoulder pain. These patients came for medical appointments at the shoulder and elbow clinic between November 2016 and May 2017. At our institution, there is a state-level referral center for shoulder diseases, for patients with referrals from this hospital and from other regions of the city and state. The patients included in this study were evaluated clinically using special tests for supraspinatus lesions (Jobe's test and the full can test), and after the ultrasound examination (index test) had been performed by a radiologist belonging to the hospital team, magnetic resonance imaging (reference standard) was requested.

Ultrasound and magnetic resonance evaluation methods

A total of five radiologists participated in the ultrasound and magnetic resonance imaging (MRI) evaluations, as specified below.

Ultrasound examination (index test): The ultrasonographic evaluation on each patient was performed by one of two radiologists who were not musculoskeletal specialists. A single ultrasound examination was performed per patient. Imaging was produced in the coronal, axial and sagittal planes, using a 10-MHz linear transducer.

Magnetic resonance imaging (reference standard): another three radiologists reported on the MRI examinations, i.e. not the same ones who performed the ultrasound examinations. Each patient was positioned in horizontal dorsal decubitus, with slight elevation of the unaffected shoulder. The arm of the affected side was kept alongside the body in slight external rotation. The affected shoulder was positioned as close as possible to the center of the magnet. Three acquisition planes were imaged, using T2 weighting with saturation of the fat signal:

- Axial plane from the apex of the acromioclavicular joint to the lower recess of the glenohumeral joint;
- Coronal oblique plane parallel to the supraspinatus and covering the scapulohumeral joint;
- Oblique sagittal plane perpendicular to the supraspinatus, from the distal end of the tendon to the middle of the rotator cuff muscle belly.

Using T2 weighting, 16 to 20 slices were obtained from each acquisition plane, in which the thickness was less than or equal to 4 mm, with a gap of the order of 10%. Two T1-weighted planes were imaged without saturation of the fat signal, with thicknesses of 4 mm to 5 mm and centered on the rotator cuff muscles:

- Oblique coronal plane: 12 to 16 slices, parallel to the supraspinatus and covering the scapulohumeral joint;
- Sagittal plane: 12 to 16 slices, covering from the tuberosity to the medial third of the scapula.

Examination review and variables

The lesions were characterized in terms of the presence of tendinopathy and partial or full-thickness tears of the supraspinatus tendon. The radiologists took the following to be indicative of tears: absence of tendon visualization on ultrasonography; and hypersignal on all T2-weighted slices of the MRI of the shoulder.

The ultrasonography and magnetic resonance imaging of the shoulders that were performed by the radiologist team (formed by five radiologists: two who performed ultrasonography and three who performed MRI) were assessed. These radiologists were experienced medical staff members who were accustomed to performing these examinations but were not specialists in musculoskeletal disorders. There was no communication between the two radiologists who performed US and the other three who performed MRI. The diagnoses were made at the radiologists' discretion and no previous training was provided. Thus, they detected lesions in accordance with their own learning and experience. The time that elapsed between ultrasonography and magnetic resonance imaging was a maximum of three months, and the MRI was always performed on another day and in another place, after the ultrasonography.

The lesions of the supraspinatus tendon were classified as:

1. Tendinopathy;
2. Presence of tears:
   2A. partial tear;
   2B. full-thickness tear.

Statistical analysis

After collecting the data, we drew up double-entry tables of the ultrasound results as a function of the magnetic resonance results and then we calculated the sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios and accuracy. The significance was assessed at the 5% level and the chi-square test was used to find the significance of the study parameters on a categorical scale between two or more groups.

The sample size was calculated as follows:

It was firstly estimated that the target population would be 96 patients over the study period, based on previous numbers of monthly visits to our institution's outpatient clinic, which formed the source for our subjects. We assumed a sampling error of approximately 5% and took a 95% confidence interval. We then used the formula contained in the following website to calculate the sample size: https://pt.surveymonkey.com/mp/sample-size-calculator. In this manner, we found that the total sample size would need to be 77 patients. Based on the central limit theorem and the laws of large numbers, this sample size was sufficient to ensure that the statistical analyses would be reliable.

RESULTS

Eighty-five patients were attended at the Shoulder and Elbow Surgery Sector between November 2016 and May 2017. Five were excluded because their examination results were not available (ultrasonography or magnetic resonance imaging). There were no adverse events from performing the index tests or the reference standard. The ultrasonography and magnetic resonance imaging were performed with a maximum of three months between them. The patients who were excluded were referred to the shoulder and elbow clinic for clinical reassessment. Out of the 80 patients assessed, 51 (63.75%) were male and 29 (36.25%) female. The male-to-female ratio was 1.75:1. The ages of the participants ranged from 19 to 72 and the mean age was 48.9 ± 2.5 years. The mean length of time with shoulder pain symptoms was 33.9 ± 11.4 months. There were six patients (7.5%) with a history of smoking. Nine (11.25%) of the patients had suffered trauma. The right side was affected in 47 of the participants (58.75%) and the left side in 33 (41.25%); the dominant side was affected in 53 (66.3%) of them (**Table 1**).

In our study, six patients (7.5%) did not have any injury to the supraspinatus tendon, 30 (37.5%) presented tendinopathy of the supraspinatus, 31 (38.75%) had partial tears and 13 (16.2%) had full-thickness tears (**Table 1**). We evaluated the prevalence of supraspinatus tears according to the patients' age group, and we observed that only 21.7% of the patients aged ≤ 40 years had these lesions, while 78.3% of the patients in this age group did not present tears of this tendon; among the patients over 40 years of age, 68.4% had supraspinatus tears. We also found that the female participants presented higher prevalence (65.5%) than the males (49%); and that the left side (57.6%) and the non-dominant side (63.0%) were mostly affected (**Table 2**).

Table 3 shows the calculations of sensitivity, specificity, positive predictive value, negative predictive value, accuracy, positive likelihood ratio and negative likelihood ratio.

Ultrasonography showed sensitivity of 36.3% and specificity of 91.7% for supraspinatus tears overall: sensitivity of 25.8% and specificity of 91.8% for partial tears and sensitivity of 46.2% and specificity of 100% for full-thickness tears. The sensitivity and specificity for tendinopathy were 53.3% and 58.0%, respectively; and for all lesions (tears and tendinopathy) of the supraspinatus, the sensitivity was 36.4% and the specificity was 91.7%. The highest accuracy and specificity were seen for full-thickness tears (91.3% and 100%, respectively).

The positive likelihood ratios (PLRs) for tears, partial tears, tendinopathy and all lesions (tears and tendinopathy) were respectively 4.36, 3.16, 1.26 and 4.36. We also calculated the negative likelihood ratios (NLRs) for all tears, partial tears, full-thickness tears, tendinopathy and all lesions: 0.69, 0.80, 0.53, 0.80 and 0.69 (**Table 3**).

Table 1. Study patients' characteristics

Gender	Male	n = 51 (63.75%)
	Female	n = 29 (36.25%)
Age	Average age	48.9 ± 2.5 years
Duration of shoulder pain	Average	33.9 ± 11.4 months
Side	Right	n = 47 (58.75%)
	Left	n = 33 (41.25%)
Dominance	Dominant side	n = 53 (66.3%)
	Non-dominant side	n = 27 (33.8%)
Supraspinatus lesions as detected using magnetic resonance imaging	No injuries	n = 6 (7.5%)
	Tendinopathy	n = 30 (37.5%)
	Partial tears	n = 31 (38.75%)
	Full-thickness tears	n = 13 (16.2%)

Table 2. Prevalence of supraspinatus tears as detected using magnetic resonance imaging (MRI)

Supraspinatus tears	Yes		No	
	n	%	n	%
≤ 40 years	5	21.7	18	78.3
> 40 years	39	68.4	18	31.6
Male	25	49.0	26	51.0
Female	19	65.5	10	34.5
Right side	25	53.2	22	46.8
Left side	19	57.6	14	42.4
Dominant side	27	50.9	26	49.1
Non-dominant side	17	63.0	10	37.0

DISCUSSION

The etiology of rotator cuff tears is multifactorial, and age-related tendon degeneration is the most important risk factor associated with these lesions.[3,7] In our study, we observed that the frequency of supraspinatus tears became higher with increasing age among the patients: 48.7% of the patients over 40 years of age presented lesions while only 6.2% of the patients aged 40 years or younger had these lesions.

Male patients presented higher prevalence of lesions (31.2%), and the right and dominant sides were more affected (31.2% and 33.7%, respectively). These results are concordant with those from previous accuracy studies and epidemiological studies and thus demonstrate that the sample was representative.[3,4]

Evaluation of the accuracy of shoulder ultrasonography for diagnosing these lesions allowed us to compare our results with those found in the worldwide literature. The present study assessed the sensitivity and specificity of ultrasonography for diagnosing supraspinatus tendon injuries, using magnetic resonance imaging as the reference method. We conducted a prospective accuracy study in which 80 patients with shoulder pain were evaluated and, of these, 38.75% presented partial tears and 16.2% presented full-thickness tears. Partial tears were more common in our study, with prevalence almost three times greater than that of full-thickness tears.

We found that ultrasonography had low sensitivity for diagnosing supraspinatus lesions. However, it had high specificity for tears overall (both partial tears and full-thickness tears), such that the ultrasound results were negative in more than 90% of cases of patients without supraspinatus tears. Among patients with tears, we found a high positive likelihood ratio, thus showing that it was four times more likely to find a tear through ultrasonography in patients who really had such lesions than in those who really did not have them.

Regarding full-thickness lesions, we found high accuracy (91.3%), with specificity and positive predictive value of 100%. This shows that ultrasonography is an excellent examination for diagnosing complete rupture of the supraspinatus tendon. Regarding tendinopathy, ultrasound was not a good examination for diagnosing it, with low sensitivity, specificity and positive and negative likelihood ratios.

A systematic review published by Dinnes et al. showed that despite the heterogeneous results in the 38 studies evaluated (total of 2435 patients), ultrasonography showed high sensitivity (S = 0.87; 95% confidence interval, CI: 0.84-0.89) and specificity (SP = 0.96; 95% CI: 0.94-0.97) in making the diagnosis of full-thickness tears of the supraspinatus tendon. For partial tears, ultrasonography showed lower sensitivity (S = 0.67; 95% CI: 0.61-0.73) but higher specificity (SP = 0.94; 95% CI: 0.92-0.96), compared with magnetic resonance imaging. Those authors concluded that ultrasonography and magnetic resonance imaging were equivalent in making the diagnosis of complete rupture of the rotator cuff, and that a negative result from ultrasonography was more likely to rule out a rotator cuff injury than was magnetic resonance imaging.[9] In our study, we found that ultrasonography had high specificity for both partial and complete supraspinatus tears, but that it had low

Table 3. Accuracy of ultrasonographic examination

			Magnetic resonance imaging			
			Yes	**No**	**Total**	**P- value**
Ultrasonography	All tears	Yes	16	3	19	0.003
		No	28	33	61	
		Total	44	36	80	
		Sensitivity = 36.3%; specificity = 91.7%; PPV = 54.1%; NPV = 84.2%; accuracy = 61.3%; PLR = 4.36; NLR = 0.69				
	Partial tears	Yes	8	4	12	0.031
		No	23	45	68	
		Total	31	49	80	
		Sensitivity = 25,8%; specificity = 91.8%; PPV = 66.7%; NPV = 66.2%; accuracy = 66.3%; PLR = 3.16; NLR = 0.8				
	Full-thickness tears	Yes	6	0	6	< 0.001
		No	7	67	74	
		Total	13	67	80	
		Sensitivity = 46.2%; specificity = 100%; PPV = 100%; NPV = 90.5%; accuracy = 91.3%; PLR = n.c.; NLR = 0.53				
	Tendinopathy	Yes	16	21	37	0.325
		No	14	29	43	
		Total	30	50	80	
		Sensitivity = 53.3%; specificity = 58%; PPV = 43.2%; NPV = 67.4%; accuracy = 56.2%; PLR = 1.26; NLR = 0.8				
	All lesions (tears and tendinopathy)	Yes	16	3	19	0.002
		No	28	33	61	
		Total	44	36	80	
		Sensitivity = 36.4%; specificity = 91.7%; PPV = 84.2%; NPV = 54.1%; accuracy = 61.3%; PLR = 4.36; NLR = 0.69				

PPV = positive predictive value; NPV = negative predictive value; PLR = positive likelihood ratio; n.c. = not calculated; NLR = negative likelihood ratio.

sensitivity for diagnosing tendon lesions. In the systematic review by Dinnes et al., retrospective studies were also included, and lower sensitivity scores were found for the prospective studies included in the review than for the retrospective studies. Retrospective studies present implicit forms of bias, such as selection and information biases, which relate to the way in which the information was collected. This feature of retrospective studies may lead to results that differ from those of prospective studies. In addition, in the systematic review by Dinnes et al., some studies were carried out in specific radiology centers, using data gathered by radiologists who were specialists in musculoskeletal disorders. This may be one of the reasons for the difference in sensitivity found between our study and the systematic review by Dinnes et al.[9]

Magnetic resonance imaging has sensitivity and specificity that range from 84% to 96%. It is the reference test used in clinical practice for diagnosing ruptures of the rotator cuff. In two systematic reviews on the accuracy of ultrasonography for diagnosing rotator cuff lesions, published by Lenza et al. and Roy et al., it was concluded that ultrasonography and magnetic resonance imaging presented similar accuracy for diagnosing full-thickness tears and low sensitivity for diagnosing partial ruptures of the rotator cuff.[8,10]

Some studies published on this subject evaluated the accuracy of ultrasonography only among patients with a diagnosis of supraspinatus tears.[7,11] In our study, we also evaluated patients with no lesions and with tendinopathy of this tendon and we calculated the accuracy of ultrasonography for making the diagnosis of supraspinatus tendinopathy (accuracy = 56.2%), i.e. not only for partial and total tears.

In our study, the analysis was performed based on a sample of patients who had shoulder pain. In addition, the reports on the examinations were made by several evaluators (five radiologists) who were not specialists in musculoskeletal disorders, thereby increasing the external validity of our study. The limitations of our study were that the data were collected in a single center and the number of patients was small (N = 80), among whom only 13 presented complete rupture of the supraspinatus. We did not use arthroscopy as a reference test (magnetic resonance imaging was used); and the level of interobserver agreement between the evaluators was not calculated. However, despite these limitations, we found results regarding the accuracy and specificity of complete supraspinatus rupture that were similar to what has been reported in the published literature.

It is common in our practice to find situations in which, for example, an ultrasound examination may show tendinopathy while magnetic resonance imaging on the same patient may show a rotator cuff tear. Such situations can be explained by the low sensitivity found in our study. They may occur because it is infrequent in our setting for radiology centers to have radiologists who are also specialists in musculoskeletal disorders, for performing ultrasound examinations. Such specialists would have greater training and would be accustomed to performing ultrasound examinations of the shoulder. Our situation thus differed from the situation of the studies included in the systematic review conducted by Dinnes et al.[9]

Accuracy studies provide the best evidence for making a diagnostic evaluation on a test or examination. However, the lack of standardization found in older studies is one of the reasons for the variability of the published results. We conducted a standardized prospective accuracy study on ultrasound examinations, and we found high accuracy in making the diagnosis of full-thickness tears of the supraspinatus tendon. However, ultrasonography presented low sensitivity for detecting lesions of this tendon. We therefore recommend that further studies including larger numbers of patients should be conducted to evaluate the sensitivity and specificity of ultrasonography for diagnosing injuries of the supraspinatus tendon and other tendons.[12]

CONCLUSION

Ultrasonography showed low sensitivity for detecting supraspinatus tears, but high specificity for both partial and full-thickness tears.

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Incorrect use of inhalation devices among patients with bronchial asthma. A hospital-based cross-sectional study in Rio de Janeiro, Brazil

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ABSTRACT

CONTEXT AND OBJECTIVE: Treatment of asthma implies inhalation of specific drugs to reach high concentrations in the respiratory tree and ensure low drug bioavailability and few adverse effects. This study aimed to evaluate the effectiveness of the inhalation technique among outpatients with asthma.

DESIGN AND SETTING: Tertiary-care hospital-based cross-sectional study in Rio de Janeiro.

METHODS: We evaluated inhalation practices in a convenience sample. A questionnaire was used to investigate sociodemographic data and assess disease control level, duration of use of the inhalation device, length of treatment and previous instructions provided by the prescribing physician. Patients demonstrated their inhalation technique using empty devices, and their technique was considered correct when all steps were appropriately performed or when errors did not interfere with the treatment outcome.

RESULTS: Among the 71 participants, 53 (74.7%) had been using the same inhaler device for at least two years and 41 (57.8%) had been under treatment for two years or more. Twelve (17.1%) said that they had been taught once and 57 (81.4%) at least twice, while one (1.4%) reported not having received any guidance regarding use of inhaler devices. Eighteen patients (25.3%) presented controlled asthma and 28 (39.5%) performed the inhalation technique correctly. Incorrect technique was associated with fewer evaluations of the inhalation technique (P =0.04) and uncontrolled asthma (P = 0.01).

CONCLUSIONS: Less than half of the sample performed the inhalation technique correctly. Incorrect inhalation technique was related to lower number of evaluations of the use of the inhalation device and uncontrolled asthma.

INTRODUCTION

Asthma affects up to 300 million people worldwide[1] and, in 2015, caused one to two deaths per day in Brazil.[2]

Use of inhaler therapy allows drugs to rapidly reach high concentrations in the airways, with therapeutic effects even with low drug bioavailability and few adverse effects. Inhaler devices are therefore the main route of administration for treatments for asthma.[3,4] The goals of asthma treatment are symptom control and future risk reduction.[5,6] However, improper use of an inhaler device can substantially reduce treatment effectiveness, thus resulting in uncontrolled disease, side effects, higher costs,[7,8] greater need for rescue medications, more emergency visits and hospitalizations, and low adherence to treatment.[9,10] Previous studies have shown an association between disease control and proper use of inhaler devices.[11,12]

The guidelines recommend regular monitoring of the inhalation technique.[6,13] Despite this, sometimes only 25% of patients receive verbal and visual guidance regarding the inhalation technique, and when guidance occurs, it is often of poor quality.[14,15] Lack of time, use of poorly comprehensible language and neglect of reassessment interfere with the inhalation technique instructions given by healthcare professionals, and these factors increase the risk of misunderstandings.[13]

This study aimed to evaluate the effectiveness of the inhalation technique among outpatients with asthma.

METHODS

Study design, setting and ethics

This was a cross-sectional observational study on a convenience sample of patients with bronchial asthma who were being treated at one of the outpatient clinics of the Antônio Pedro University Hospital (Hospital Universitário Antônio Pedro, HUAP) in Niterói, state of Rio de Janeiro, Brazil. This study was approved by the local ethics committee of our institution (Universidade Federal Fluminense, UFF), under the number CAAE 56248816.1.0000.5243. Patients signed informed consent forms for their participation in this study.

Participants

Patients were eligible if they were 18 years of age or older, were not at their first medical appointment and were using the following inhaler devices containing any corticoids, long-acting bronchodilators (or combinations of them) or even a short-acting bronchodilator: Aerolizer, Aerocaps, Diskus and metered-dose inhaler (MDI), without the aid of a spacer. Patients in a phase of exacerbation of symptoms were excluded and referred to the emergency room for immediate treatment. Patients whose diagnoses of asthma were not confirmed during the treatment and those who regularly received any help from others for using the inhaler device were also excluded.

Recruitment was performed sequentially, according to the scheduling dates of patients' medical appointments. After signing an informed consent form, patients gave responses to a self-administered questionnaire that sought sociodemographic data. They had to demonstrate to researchers how they used the device.

Patients' degree of disease control was evaluated in accordance with the Global Initiative for Asthma (GINA)[6] document, and their length of time of use of the inhaler device, their treatment duration, the existence of instructions regarding use of the device issued by the prescribing physician and the existence of regular supervision of the inhalation technique at subsequent visits were assessed. Spirometry was performed and assessed in accordance with the Brazilian Guidelines for Pulmonary Function Tests.[16]

In our outpatient clinic, patients are given guidance for the inhalation technique, reinforced at all visits. The physician always demonstrates the technique using an empty device equivalent to the one used by the patient. It is always requested that patients should bring their empty devices for training, and when they do, they can practice the inhalation technique in front of the physician, and if there are any errors, these are always corrected.

The inhalation technique was assessed according to the following check-list: for Diskus users, the user was required to open the inhaler, push the lever back completely, exhale to residual volume, inhale deeply, hold breath for 10 seconds, exhale away from mouthpiece and close the inhaler. For MDI users, it was necessary to remove the mouthpiece cover, hold the inhaler upright, exhale to residual volume, press the canister and inhale deeply, hold breath for 10 seconds, exhale away from the mouthpiece and close the inhaler. For Aerolizer or Aerocaps users, it was necessary to remove the inhaler cover, open the capsule compartment, place the capsule in the appropriate chamber, close the capsule compartment, press the button(s) of the inhaler, exhale to residual volume, inhale deeply, hold breath for 10 seconds, exhale away from the mouthpiece and close the inhaler.

Evaluations

Only the inhalation technique that was performed using the device that patients considered to be their main device was evaluated in this study. The patients demonstrated their technique using empty devices, to at least two evaluators (one pulmonologist and one of a group of six medical students who had been trained by the pulmonologist). The inhalation technique demonstrations were filmed for reevaluation, for the eventuality of any disagreement among the evaluators. In such cases, evaluation by a third evaluator was mandatory.

The evaluation of the inhalation technique was based on the leaflet provided by the producer of each device. The technique was considered correct when all steps were performed properly or when the misconceptions probably did not interfere with the outcome of the treatment (e.g. not closing the device at the end of the demonstration). The technique was considered incorrect when one or more errors were observed in any of the following steps: preparation of the inhalation device for use, expiration, aspiration or apnea, regardless of the severity of the error. Whenever there were misconceptions relating to the inhalation technique, the patient received instructions on the correct use of the inhalation device.

Statistical analysis

The data obtained were digitized in a Microsoft Excel 2010 worksheet and were exported, for statistical analysis, into the Epi Info 7.2 data analysis software. Central trend measurements (average and median) and dispersion measurements were used for descriptive analysis on the numerical data. Simple and relative frequency calculations were used for questions with categorical variables. The chi-square test or Fisher's exact test was used to verify associations between the quality of the inhalation technique and patient characteristics, asthma control, severity of obstruction, number of evaluations of inhalation technique that had been made and duration of treatment. P-values (descriptive levels) lower than 0.05 were taken to indicate significant associations. A binary logistic regression model was used to identify characteristics that were predictive of incorrect technique. Variables with a significance level of less than 0.1 in univariate analysis, after adjustment for gender and age, were included in a logistic regression model.

RESULTS

Between August 2, 2016, and March 10, 2017, 75 patients were enrolled. However, four were excluded: one had an immobilized arm, which prevented unaided demonstration of the inhalation technique; one was subsequently confirmed to have a diagnosis of chronic obstructive pulmonary disease (instead of asthma); and two used the Aerocaps device when they should have been using Aerolizer to administer their medications (due to modification of the medications during the study, but using the "old" inhaler, inappropriately).

The final sample was thus composed of 71 participants between 19 and 81 years old, with a mean age of 57.7 ± 13.9 years, of whom 61 (85.9%) were female. Fourteen (19.7%) were single, 31 (43.7%) were married and 26 (36.6%) were separated, divorced or widowed. Thirty-seven (52.1%) had at most completed elementary education (9 years of schooling), 29 (40.8%) had at most obtained a high school diploma and five (7.1%) had obtained a university degree at bachelor or higher level. Regarding personal income, 49 (70%) received a maximum of one Brazilian minimum salary per month (equivalent to approximately US$ 247,46), whereas 20 (28.6%) received between one and three minimum salaries and one (1.4%) earned between three and ten minimum salaries. Regarding family income, 28 (40%) received up to one minimum salary, 38 (54.3%) one to three minimum salaries and four (5.7%) between three and ten minimum salaries.

According to the GINA document, two participants (2.8%) were in stage 1 of the treatment of bronchial asthma (as needed short-acting beta2-agonist), 14 (19.7%) in stage 2 (low dose of inhaled corticosteroids), 11 (15.5%) in stage 3 (low dose of inhaled corticosteroids and long-acting bronchodilator), 43 (60.6%) in stage 4 (moderate or high dose of inhaled corticosteroids and long-acting bronchodilator) and one (1.4%) in stage 5 (oral corticosteroids and/or anti IgE associated to Inhaled medications used in Stage 4). With regard to the asthma control level, only 18 patients (25.35%) presented controlled asthma, while 20 (28.15%) exhibited partial control over the disease and 33 (46.5%) had uncontrolled asthma. Concerning the severity of the obstructive disorder, 50 (70.42%) had spirometry findings that were considered normal or presented mild obstruction, 13 (18.31%) presented moderate disorder and six (8.45%) had severe obstruction. Two patients (2.82%) were not able to perform the test reliably. Fifty-six patients (78.9%) reported using the prescribed medication in accordance with the physician's recommendation and nine (12.7%) only rarely failed to use the drugs.

Regarding the inhaler devices, 10 patients (14.1%) used Aerolizer, 36 (50.7%) Aerocaps, 6 (8.5%) Diskus and 19 (26.7%) MDI. Fifty-three patients (74.7%) had been using the same device for at least two years and forty-one (57.8%) had been undergoing treatment at the outpatient clinic for two years or more, always coordinated by the same pulmonology specialist.

Only one participant (1.4%) reported not receiving any guidance at any time regarding use of the inhalation device, while 13 (18.3%) were never reassessed after their first instruction, 25 (80.3%) were reassessed once at an appointment subsequent to their first instruction and 32 (45.1%) were reassessed at least twice at medical appointments subsequent to their first instruction. This last group was therefore instructed at least three times.

Concerning the quality of the inhalation technique, 28 (39.5%) performed it correctly. In the few cases in which there was initial

Table 1. Factors relating to the quality of the inhalation technique

Variable	Inhalation technique		P- value
	Incorrect (n/%)	Correct (n/%)	
Asthma control level			
Controlled/partially controlled	18 (25.3)	20 (28.2)	0.01*
Uncontrolled	25 (35.2)	8 (11.3)	
Gender			
Male	6 (8.5)	4 (5.6)	0.61
Female	37 (52.1)	24 (33.8)	
Age (years)			
< 50	14 (19.7)	5 (7.0)	0.17
≥ 50	29 (40.9)	23 (32.4)	
Marital Status			
Married	17 (24.0)	14 (19.7)	0.38
Single/divorced/separated/ widowed	26 (36.6)	14 (19.7)	
Educational level			
Elementary education at most	26 (36.6)	11 (15.5)	0.08
Incomplete high school at least	17 (23.95)	17 (23.95)	
Personal income			
One minimum salary/month at most	32 (45.7)	17 (24.3)	0.16
More than one minimum salary/month	10 (14.3)	11 (15.7)	
Family income			
One minimum salary/month at most	19 (27.15)	9 (12.85)	0.27
More than one minimum salary/month	23(32.9)	19 (27.1)	
Severity of obstructive disorder			
Mild	28 (40.6)	22 (31.9)	0.34
Moderate or severe	13 (18.8)	6 (8.7)	
Years of use of inhalation device			
≥ 2	29 (40.9)	24 (33.8)	0.07
< 2	14 (19.7)	4 (5.6)	
Number of orientations			
≥ 2	31 (44.3)	26 (37.1)	0.04*
≤ 1	11 (15.7)	2 (2.9)	

*Significance level P < 0.05.

disagreement among the evaluators, the video review always unified opinions about the assessment of the inhalation technique. Incorrect inhalation technique showed associations with the asthma control level (P = 0.01) and the number of instructions (P = 0.04). However, there were no associations between incorrect inhalation technique and gender, age, marital status, personal and family income, level of education, duration of treatment orseverity of obstructive disturbance (**Table 1**). After controlling for all other factors, as described in the methods section, only the risk factors of uncontrolled asthma and fewer instructions remained associated with inadequate use of the device.

DISCUSSION

Lack of information is the main cause of improper use of inhalers,[17] and a simple statement given by a patient that he performs the inhalation technique properly, without any demonstration, is not necessarily a guarantee of good performance.[14] Reports of absence of instruction and reassessment of the inhalation technique are not uncommon. In at least two studies,[14,18] only approximately 66% of the patients were initially taught; in another study,[19] 90% were instructed at the first visit, but only 14% were reassessed on other occasions. In our study, almost all patients reported having been given guidance at least once and approximately 80% had been trained at least twice, but only 40% of the patients performed the inhalation technique properly. This result is similar to what has already been published from some studies,[17,20,21] but the percentage reported here is greater than what has been reported in several other studies, in which only 6% to 31% of the participants performed it correctly.[13,15,22,23,24]

Unlike several previous studies,[11,18,25,26] no relationship was found in our study between old age and incorrect inhalation technique. This was also reported in one other study.[22] The continued practice that is provided at our outpatient center probably helps to explain this outcome, because training among elderly patients also results in significant improvement in the inhalation technique.[8] Previously, a relationship between inadequate inhalation technique and widowhood[22] was also demonstrated, but this was not confirmed in our current study. There is evidence for associations between incorrect technique and low educational and income levels.[9,18,22] However, we did not observe these associations. We could not confirm the relationship between obstructive disorders of greater severity and incorrect inhalation technique that has previously been noted.[25] Patients with severe and moderate obstructions made errors more frequently than did those with mild obstruction or normal results in the spirometry test, but this difference was not statistically significant. Errors in inhalation technique were frequent, regardless of the socioeconomic, educational or obstruction level, which emphasizes the need to supervise the quality of the technique for any patient profile.

The length of time for which the treatment had been used did not interfere with the quality of the inhalation technique, as already reported.[27] However, there was an effect from the number of evaluations performed. This observation was supported by previous studies,[17,26] which showed that patients who had never been given any guidance frequently made errors in the inhalation technique. Furthermore, a single explanation of the technique may be insufficient. In such cases, at most 48.4% performed the technique correctly, but only if they had received extensive orientation at the first visit.[28] However, they reached their maximum ability if three instruction sessions were provided, such that the error rate might be less than 10%.[20] In the present study population, only 45.6% of the patients who were given guidance twice or more showed correct technique. Takaku et al.[20] worked with patients who received a prescription of an inhaled drug for the first time, for whom the technique was taught and supervised successively for between two and five times at intervals of two weeks to one month. This was a shorter period with more intensive training than in our study. Nevertheless, both studies emphasize the need for instructions given on multiple occasions, in order to achieve the proper inhalation technique. It is possible that instruction repetition is the main key to achieving asthma control.

Regarding the limitations of our study, besides its cross-sectional design and the sample size, the presence of comorbidities was not considered in this investigation. The evaluations were not performed under blinded conditions, and the investigators always analyzed the quality of the inhalation technique together. Adherence to treatment was investigated only through basic questions, and non-adherence might be a confounding factor that, in addition to inadequate technique, may have contributed to the causes of uncontrolled asthma. However, if it is assumed that the patients answered honestly regarding their adherence to treatment, more than 90% were performing the treatment properly. This is an excellent result and therefore would not be a reason for asthma control not to be obtained. The logistic regression model used did not change the results previously obtained, perhaps because of the sample size. Finally, our study was based on a convenience sample in a hospital that is equipped to deal with cases of high complexity, i.e. cases that are more difficult to control are commonly treated there. Thus, the results from this study may not represent the situations for other hospitals, and the ability to generalize from these results is limited.

This study did not show any association between the inhalation technique and any of the socioeconomic characteristics, the severity of obstruction, or even the duration of the treatment. One of the only relationships observed was between inadequate technique and uncontrolled asthma. Patients who received a greater number of orientations were also observed to be better at the inhalation technique. There seems to be a single

simple conclusion: if patients adhere to the treatment using the correct inhalation technique, their asthma will be controlled. For this to occur, caregivers must insist on teaching the technique regardless of the patient's profile. This seems to be the only way to achieve the main goal of treating asthma, namely, achievement of control.

CONCLUSIONS

The inhalation technique was correctly performed by 39.5% of the participants. Uncontrolled asthma was significantly associated with incorrect inhalation technique. The incorrect inhalation technique was observed frequently and had no relationship with age, gender, marital status, educational level, personal and family income, duration of use of the inhalation device or the severity of obstruction. This study emphasizes the need for quality supervision of the inhalation technique and suggests that the larger the number of reevaluations is, the closer it will be possible to come to proper execution of the technique and asthma control. Inhalation technique should be supervised for all patients, especially those who received fewer instructions and those with uncontrolled asthma.

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Pediatric Wilson's disease: findings in different presentations. A cross-sectional study

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ABSTRACT

BACKGROUND: Wilson's disease (WD) may present with different manifestations: from an asymptomatic state to liver cirrhosis. Here, we aimed to evaluate clinical presentations and laboratory findings and prognoses among WD cases.

DESIGN AND SETTING: Cross-sectional study based on patients' records from the university hospital, İnönü University, Malatya, Turkey.

METHODS: The medical records of 64 children with WD were evaluated focusing on the clinical, laboratory and liver biopsy findings in different clinical presentations.

RESULTS: The mean age at diagnosis was 8.6 ± 3.26 years (range 3.5-17) and mean length of follow-up was 2.49 years (range 0-9). There were 18 cases (28.1%), 12 (18.8%), 9 (14.1%) and 6 (9.4%) of chronic liver disease, fulminant liver failure, neurological WD and acute hepatitis, respectively. Nineteen (29.7%) were asymptomatic. The most common sign and laboratory finding were jaundice (45.3%) and hypertransaminasemia (85.9%), respectively. The lowest serum zinc level was found in the fulminant liver failure group (P = 0.035). Hepatosteatosis was detected in 35% of the 20 patients who underwent liver biopsy. Among those with hepatosteatosis, 57.1% were asymptomatic. While 35% had copper staining, 25% presented iron accumulation in liver biopsies. Nine cases underwent liver transplantation and seven of these presented fulminant liver failure (77.8%).

CONCLUSION: The presentation, symptoms and signs of our cases were similar to those in previously reported series, except for the high proportion of fulminant WD cases. Further studies are needed to clarify the relationship between zinc levels and development of a fulminant course and between iron status and WD.

INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism that occurs due to mutations in the copper-transporting ATP7B gene. Impaired excretion of copper through bile and decreased incorporation into ceruloplasmin cause excessive copper accumulation in different organs, including the liver, brain and cornea.[1-5] WD typically begins with a presymptomatic period, during which copper accumulation in the liver causes subclinical hepatitis, and progresses to liver cirrhosis and development of neuropsychiatric symptoms. There is no single diagnostic test that can rule out or confirm WD with certainty. Because of the lack of such a test, the diagnosis needs to be based on a combination of clinical features and laboratory, neuroradiological and pathological findings and also on the results from genetic analysis.[5-10]

Pharmacological treatment of WD has the aim of preventing further accumulation of copper, through either reducing its absorption or promoting its excretion in urine or bile, or both.[5,6] The indications for liver transplantation to cure WD include progressive liver failure, progressive neurological symptoms, complications from portal hypertension (despite medical and dietary therapy) and acute liver failure.[5,6]

There are not many detailed studies investigating pediatric WD series in the literature.[5-10] In the present study, we aimed to evaluate clinical presentations and laboratory findings among 64 children who were diagnosed with WD in our clinic over a nine-year period, and to determine their response to treatment and their prognosis from the follow-up.

METHOD

Study design, setting and ethical issues

This was a cross-sectional study based on retrospective evaluation of data from medical records, undertaken in the Pediatric Gastroenterology and Nutrition Department, İnönü Üniversitesi Tıp Fakültesi, Malatya, Turkey. Prior to the start of the work, local institutional ethics committee approval was obtained, as follows: ethics committee date: 2017; and ethics committee no: 22-2.

Participants

The medical records of 64 consecutive cases of pediatric WD that were diagnosed in the Pediatric Gastroenterology and Nutrition Department of our institution between January 2006 and December 2015 were evaluated.

The patients included were those who presented with elevated transaminases, acute or chronic liver failure or neuropsychiatric symptoms, or those who were asymptomatic siblings of index cases. The diagnosis of WD was based on the scoring system developed at the Eighth International Meeting on Wilson's disease, in Leipzig, 2001; a score > 4 was considered reasonable for making a diagnosis of WD.[6,11] No data on genetic mutational analysis could be provided. Other causes of liver disease such as autoimmune liver disease, chronic viral hepatitis, α1-anti-trypsin deficiency or other metabolic conditions were ruled out in all cases. Detailed follow-up data were collected in relation to each patient.

The clinical presentations were defined as follows:[5,6,12,13]

Asymptomatic WD: Symptom-free patients with or without hypertransaminasemia and/or hepatomegaly who had been diagnosed during family screening or routine check-up.

Acute WD: Acute-onset clinically and biochemically diagnosed liver disease in patients without any previously recognized liver disease.

Fulminant WD: Acute liver failure was defined in accordance with the criteria of the Pediatric Acute Liver Failure Study Group:

1. children with no evidence of chronic liver disease;
2. biochemical evidence of acute liver injury; or
3. hepatic-based coagulopathy.

This last criterion was defined as prothrombin time (PT) ≥ 15 s or international normalized ratio (INR) ≥ 1.5 that was not corrected by means of vitamin K in the presence of hepatic encephalopathy; or as PT ≥ 20 s or INR ≥ 2.0, regardless of the presence or absence of clinical encephalopathy.

Chronic liver disease: Patients with previously known/unknown WD who presented with clinical and laboratory findings of chronic liver failure.

Neurological WD: Patients presenting with neurological symptoms.

Histopathological examinations were performed on explant liver tissues from patients who underwent liver transplantation, and on percutaneous samples that had been provided from some patients.

Statistical analyses

We did not analyze the study power because all the patients in our group were evaluated retrospectively.

The data were analyzed statistically using the Statistical Package for the Social Sciences for Windows, version 16.0 (SPSS Inc, Chicago, USA). The continuous variables were reported as the mean ± standard deviation, whereas the categorical variables were defined as percentages. The data were tested for normal distribution using the Kolmogorov-Smirnov test. To compare the continuous variables, Student's t test, the one-way analysis of variance test or the Kruskal-Wallis test was used, as appropriate. When significant differences were observed between the groups based on the post-hoc analyses, either the Tukey or the Scheffe test was used to determine the differences between the groups. The chi-square test was used to compare the categorical variables. Statistical significance was defined as $P < 0.05$.

RESULTS

Out of the 64 patients evaluated, 39 (60.9%) were boys and 25 (39.1%) were girls. Their mean age at the time of the diagnosis was 8.6 ± 3.26 years (range 3.5-17 years) and the mean length of follow-up was 2.49 years (range 0-9 years). While the most common manifestation of the disease was jaundice (45.3%), the most common clinical findings were jaundice and splenomegaly (45.3%) followed by hepatomegaly (43.8%) and Kayser-Fleischer ring (KF) (40.6%) **(Table 1)**. Consanguinity and a familial history of WD were detected in 46 patients (71.9%) and 32 (50%), respectively. Among all the patients, 16 received the diagnosis through family screening (25%). Some of the clinical and laboratory findings are shown in **Table 1**.

Evaluation on the patients' clinical presentations showed that 19 (29.7%) were asymptomatic; and that 18 (28.1%), 12 (18.8%), 9 (14.1%) and 6 (9.4%) had chronic liver disease, fulminant liver failure, neurological WD and acute hepatitis, respectively. Among all the children, 19 (26%) presented both positive brain magnetic resonance imaging (MRI) findings and chronic liver disease findings. MRI examination revealed brain involvement in 26 out of 41 patients (63.4%); nine of them (34.6%) had symptomatic neurological WD and, among the remaining 17 patients, 11 (64.7%) had chronic liver disease, five (29.4%) had fulminant liver failure and one (5.8%) was asymptomatic at the time of the diagnosis. An association between brain MRI findings (intensity increase in basal ganglia) and chronic liver disease presentation was significant ($P < 0.0001$), while between asymptomatic WD and fulminant WD it was not.

Twenty-six patients (40.6%) presented KF rings. This was most common in cases of neurological WD (100%) and least common

in cases with fulminant liver failure (16.6%) **(Table 2).** KF was detected in 18 of the 21 patients (85.7%) who had MRI-detected brain involvement **(Table 1)**.

In 16 out of the 33 cases (48.5%) that underwent upper gastrointestinal endoscopy, esophageal varices were found.

The most common laboratory finding consisted of elevated transaminase levels (85.9%). Coombs-negative hemolytic anemia was detected in nine cases (14.8%): seven (77.8%) presented with fulminant liver failure whereas two (22.2%) had findings relating to chronic liver disease. There was a significant association between

Table 1. Some special clinical and laboratory findings regarding the presentation of Wilson's disease

Features	Asymptomatic 19	Acute 6	Chronic 18	Fulminant 12	Neurological 9	Total	P
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	
Kayser-Fleischer ring	4 (21)	2 (33.3)	9 (50)	2 (16.6)	9 (100)	26 (40.6)	0.00
Hemolytic anemia	0 (0)	0 (0)	2 (11.1)	7 (58.4)	0 (0)	9 (14)	0.00
Hypouricemia	5 (26.3)	1 (16.6)	14 (77.8)	12 (100)	5 (55.6)	37 (57.8)	0.00
Low albumin	3 (15.8)	2 (33.3)	11 (61.1)	11 (91.7)	1 (11.1)	28 (43.7)	0.00
High INR	3 (15.8)	0 (0)	14 (77.8)	12 (100)	3 (33.3)	32 (50)	0.00
High ammonium	0 (0)	0 (0)	5 (27.8)	12 (100)	0 (0)	17 (26.5)	0.00
Low HDL	5 (26.3)	1 (16.6)	12 (66.7)	10 (83.4)	4 (44.5)	32 (50)	0.03
Normal ceruloplasmin	2 (10.5)	1 (16.6)	3 (16.6)	0 (0)	1 (11.1)	7 (11)	0.41
Hepatosteatosis (n = 57) (USG)	2/15 (13.3)	4/6 (66.6)	0/17 (0)	1/11 (9)	0/8 (0)	7/57 (12.3)	0.00
Brain involvement (n = 41) (MRI)	1/6 (16.6)	0 (0)	11/14 (79)	5/8 (62.5)	9/9 (100)	26/41 (63.4)	0.00
Steatosis in biopsy (n = 20)	4/5 (80)	2/3 (66.6)	0/4 (0)	1/7 (14.3)	0/1 (0)	7/20 (35)	0.02
Iron accumulation in biopsy (n = 20)	0/5 (0)	0/3 (0)	2/3 (66.6)	3/7 (43)	0/1 (0)	5/20 (25)	0.11
Family history	14 (87.5)	3 (50)	6 (33.3)	4 (33.3)	5 (55.5)	32 (50)	0.103
Detected through screening	13 (68.4)	0	1 (5.5)	0	2 (16.7)	16 (25)	0.000
Hepatomegaly	3 (15.7)	4 (66.7)	17 (94.5)	10 (83.4)	2 (22.3)	36 (56.2)	0.000
Splenomegaly	1 (5.2)	1 (16.7)	15 (83.4)	9 (75)	3 (33.3)	29 (45.3)	0.000
Ascites	0	1 (16.7)	9 (50)	7 (58.3)	2 (22.3)	19 (29.6)	0.002
Jaundice	0	3 (50)	14 (77.8)	12 (100)	0	29 (45.3)	0.000
Encephalopathy	0	0	0	12 (100)	0	12 (18.7)	0.000

INR = international normalized ratio; HDL = high-density lipoprotein; USG = ultrasonography; MRI = magnetic resonance imaging.

Table 2. Laboratory findings at the time of diagnosis according to the type of presentation

Laboratory findings	Asymptomatic	Acute	Chronic	Fulminant	Neurological	Total	P
	Mean (Range)	Mean (range)	Mean (range)	Mean (range)	Mean (range)	Mean (range)	
WBC (10^3/ml)	7.4 (5-11.5)	10.2 (7.5-13.7)	6.5 (2.3-14.8) β	12 (4.4-34) α	5.3 (2.9-8.1) β	8 (2.3-34)	0.007
INR	1 (0.9-1.7) β	1 (1-1.3) β	2.2 (1-4.5) β	4.5 (2.5-8) α	1.48 (1-2.9) β	2.1 (0.9-8)	0.000
AST (U/l)	132.4 (20-302)	164.6 (82-400)	175.2 (23-609)	362.4 (66-1307) α	50.3 (29-95) β	182.7 (20-1307)	0.014
ALT (U/l)	187.2 (27-450)	184.5 (103-272)	120.2 (16-384)	259.2 (23-1345)	33.5 (13-79)	161.2 (13-1345)	0.095
AST/ALT	0.79 (0.5-1.5)	0.98 (0.34-2.03)	1.4 (0.85-2.45)	2.73 (1.09-9.1)	1.75 (1.16-2.61)	1.49 (0.34-9.1)	0.212
ALP (U/l)	559.5 (84-4042)	330.3 (202-514)	290.7 (57-537)	216 (19-511)	281 (129-613)	353.5 (19-4042)	0.394
T. bil (mg/dl)	0.5 (0.2-1) β	2 (0.2-5) β	6.8 (0.2-26.9) β	22.3 (1.9-43.8) α	0.7 (0.5-1) β	6.8 (0.2-43.5)	0.000
D. bil (mg/dl)	0.2 (0.1-0.4) β	0.66 (0.1-2) β	4.7 (0.1-23.6) β	14.8 (1-29) α	0.3 (0.1-0.7) β	4.4 (0.01-29)	0.000
Albumin(mg/dl)	3.9 (2.8-4.9) β	3.5 (2.4-4)	3 (1.7-5.8)	2.5 (1.8-3.7) α	3.8 (2.4-4.5) β	3.3 (1.7-5.8)	0.000
Uric acid (mg/dl)	2.5 (1-4) β	2.6 (1-3.6) β	1.5 (0.3-4)	0.9 (0.2-1.8) α	1.4 (0.5-2.5)	1.7 (0.2-4)	0.000
Zinc (mg/dl)	79.9 (46-128) β	80 (80) β	56.6 (28-70)	52 (46-56) α	60.2 (36-75)	66.5 (28-128)	0.035
Ferritin (ng/ml)	56.7 (10.2-125) β	39.5 (36-43)	163.2 (15.7-604)	600.4 (94-1500) α	53.6 (18.4-81) β	193.1 (10.2-1500)	0.038
Ceruloplasmin (g/l)	0.1 (0-0.3)	0.12 (0.2-2)	0.12 (0.1-0.4)	0.11 (0.01-0.19)	0.15 (0.05-0.5)	0.11 (0.00-0.5)	0.814
24-hour urinary Cu (µg/24 h)	329.6 (8-1287) β	368.3 (52-1319)	879.8 (25-4480)	1546.7(18-5280) α	521.7 (265-1066)	758.1 (8-5280)	0.047
24-hour urinary Cu after d-penicillamine (µg/24 h)	1153 (161-1984) β	1322 (170-4000)	833.5 (308-1120)	1495 (367-4096) α	1060 (1060)	1191 (161-4096)	0.93
Liver Cu level (µg/g)	855.6 (250-1244)	423 (275-510)	676.8 (277-1310)	-	292(292)	672 (250-1310)	0.31
Mortality score	2.76 (0-5) β	6.1 (3-14) β	9.3 (1-18) β	14.25 (11-17) α	2.5 (1-7) β	7.2 (0-18)	0.000

$P < 0.05$ values signify comparison of α to β in one-way analysis of variance. WBC = white blood cell; INR = international normalized ratio; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; T. bil = total bilirubin; D. bil = direct bilirubin; Cu = copper.

occurrences of fulminant liver failure and Coombs-negative hemolytic anemia (P < 0.0001).

Assessment of the association between laboratory findings and the clinical presentation showed that total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP) and ceruloplasmin levels were not significantly different according to presentation. However, the levels of albumin, aspartate aminotransferase (AST), total bilirubin, direct bilirubin, uric acid, white blood cells (WBCs), INR, 24-hour urinary copper and zinc differed significantly among the groups **(Table 2)**.

One year after the treatment, the AST, ALP, zinc and 24-hour urinary copper levels were not significantly different between the groups, but the albumin, ALT, total bilirubin, direct bilirubin, uric acid and INR levels were still different.

All of the fulminant cases had low levels of ceruloplasmin, whereas seven cases (11.3%) with other presentations had normal levels. Out of all the cases, four (6.4%) had normal levels of urinary copper whereas all of the neurological WD cases had high urinary copper levels.

Blood ferritin levels were significantly higher in fulminant WD cases than in asymptomatic and neurological WD cases (P = 0.038).

Liver samples from 20 patients were stained to ascertain iron levels; only five of these patients (25%) had iron deposits. Two of these patients (40%) presented with chronic liver disease and three (60%) with fulminant WD **(Table 1)**. Hepatosteatosis was found in seven cases (35%): four of these cases (57.1%) were asymptomatic, two (28.5%) had acute hepatitis and one (14.2%) had fulminant liver failure at the time of the diagnosis **(Table 1)**.

Nine cases underwent liver transplantation. Among these, seven had fulminant liver failure (77.8%), one had neurological WD (11.1%) and one had chronic hepatitis (11.1%) at the time of the diagnosis.

D-penicillamine and trientine were used for 23 and 14 patients, respectively, during the first year of treatment. For 18 children, their use of d-penicillamine was switched to trientine because of side effects or problems in obtaining the agent. Comparison of alterations to laboratory parameters between the d-penicillamine and trientine groups showed that the increase in serum albumin levels was significantly greater with trientine treatment (P = 0.013).

According to the mortality score of Dhawan et al.,[12] 21 patients had a mortality score of > 11. Twelve of those patients had fulminant hepatitis (57.2%), eight had chronic hepatitis (38%) and one had acute hepatitis (4.8%) at presentation. Only seven of those patients (30%) underwent liver transplantation. Two patients with a score of < 11 underwent transplantation: one of them had neurological WD and the other had chronic hepatitis at presentation. The sensitivity, specificity, positive and negative predictive values and accuracy of this index were detected as 77.78%, 74.5%, 33.34%, 95.3% and 75%, respectively.

DISCUSSION

Studies on childhood WD present limitations because the number of patients available to make up a relevant study group is frequently low, which precludes large cohort studies or randomized controlled trials. Machado et al.[14] evaluated 119 Brazilian patients, mostly adults, with a mean age of 19.3 at the time of diagnosis and reported that the major initial clinical manifestation was neurological. Our study is the most detailed single-centered study investigating the clinical and laboratory findings at admission and over the follow-up, among quite a large number of children with WD. The mean age (8.6 ± 3.26 years) and male dominance (60.9%) among our subjects were compatible with what has been described in the literature.[9] It has been reported that 10-33% of the cases detected through family screening were asymptomatic;[7,9,10] half of our cases had a family history and one-quarter of the cases were diagnosed through family screening.

There is no single diagnostic test that can rule out or confirm WD with certainty. A diagnosis of WD is based on a combination of clinical features, laboratory findings and genetic testing, if available.[9,15,16] In our study, the diagnosis of WD was based on the scoring system developed at the Eighth International Meeting on Wilson's disease, in Leipzig, 2001.[6,11] Genetic mutational analysis data could not be provided.

We found that jaundice was the most common symptom on physical examination (45.3%), along with organomegaly **(Table 1)**, which was consistent with reports in the literature.[9] Rukunuzzaman et al.[7] reported that 69% of 100 WD cases presented as hepatitis, 14% had both hepatic and neurological symptoms, 1% had psychiatric symptoms and 10% were asymptomatic. Similarly, most of our patients presented with hepatic symptoms (56.2%), but nearly one third of our patients were asymptomatic (29.7%) and none presented with psychiatric symptoms. In the abovementioned study,[7] among the hepatic presentations, 76% consisted of chronic hepatitis and 4% consisted of fulminant liver failure at presentation.[7] In our series, fulminant presentations were detected in 18.8% of all the cases: this was quite a high number and it might be attributable to the fact that our hospital is the largest liver transplantation center in the country.

We observed KF rings in 40.6% of all patients. Their presence has previously been reported in 38%-77%, most commonly in those with neurological WD (85-90%), followed by those with chronic hepatitis (52%-84%).[5,7,9,10] It was interesting to find a higher percentage of presence of KF rings in asymptomatic children than in the fulminant liver failure group (21% versus 16.7%) in our study. KF rings were present in 100% of the children with neurological WD, and this was consistent with the classical knowledge.

Normal ceruloplasmin levels, which were observed in 10.9% of our cases, have previously been reported in 10%-36% of the cases.[5,7,9,10,17] While the ratio was similar among different

presentations, it was remarkable that none of the fulminant liver failure cases had normal ceruloplasmin levels.

Magalhães et al.[18] reported that three of their 11 patients with WD (27%) had MRI findings without any clinical sign. We found that this ratio was 42.1% (8/19 patients) in our study. It is still not clear when the brain involvement becomes symptomatic and there is no consensus regarding use of MRI screening for neurological involvement in asymptomatic cases.

Twenty-four-hour urinary copper levels were reported to be higher than 200 μg in 99% of the patients after d-penicillamine challenge in a previous study.[7] El-Karaksy et al.[19] found that 53% of the cases had urinary copper > 100 μg and that after d-penicillamine challenge, 15% of them had urinary copper < 1000 μg/day. In our study, 6.4% of the cases had levels lower than 40 μg/day. Among the patients who underwent d-penicillamine challenge, 9% had urinary copper < 200 μg/day despite high liver copper content. Twenty-four-hour urinary copper levels were significantly higher in patients presenting with fulminant liver failure than in asymptomatic patients (P = 0.036). These findings might suggest that urinary copper levels are related to clinical presentation and that the d-penicillamine challenge test might not be 100% reliable for making diagnoses.

Rukunuzzaman et al.[7] reported that the ALT levels were elevated in 92% of their patients and that the mean AST levels were higher than the ALT levels in their study.[7] It was also shown that the AST/ALT ratio was > 2.2 in the patients who presented with fulminant hepatitis.[5,7] Our data supported the findings of the abovementioned studies, with higher AST than ALT and an AST/ALT ratio of 2.73 in the patients with fulminant hepatitis **(Table 2)**. Iorio et al.[8] reported that the ALT level might be normal in 9.5%; it was normal in 14% in our series.

A wide range of serum albumin and bilirubin levels has been reported in the literature.[7,19] In our fulminant cases, the mean albumin and cholesterol levels were lower, as expected, because of the impaired capacity of the liver for synthesis. Interestingly, the mean serum zinc level and 24-hour urinary copper level were significantly higher than in other presentations, which suggested that high levels of urinary copper and, especially, low levels of zinc might affect progression to fulminant liver failure. In a recent case report, a 2.5-year-old child with clinical signs of both Zn deficiency and WD was presented with the emphasis of the close relationship between Cu and Zn.[20] In a cohort of 18 children with WD, significant decrease in serum plasma Zn has been previously described.[20] Unfortunately, in that paper, it was not clear whether any of the patients had fulminant presentation or not.[21] It was also reported that in a subgroup of WD population with a mild liver disease, Zn serum level was normal or high at diagnosis.[22] These observations suggest that Zn serum levels could be related with clinical phenotype of patients with WD and its severity.[22]

The early histological findings in WD cases comprise micro or macrovesicular steatosis, glycogen accumulation in hepatocyte nuclei and focal hepatocellular necrosis.[5] Monolaki et al.[9] reported the presence of hepatosteatosis in 50% of their symptomatic and 87% of their asymptomatic patients. Its presence was reported as 54% and 24% in other two studies.[8,10] Hepatosteatosis was present in one-third of the 20 patients from whom hepatic specimens were available. 57% of these patients were asymptomatic. This finding emphasizes the importance of considering WD as a diagnosis in situations of incidentally found hepatosteatosis.

Another interesting finding was the iron accumulation in one-quarter of the patients from whom a liver biopsy had been obtained. There are only a few studies regarding iron accumulation in WD cases.[23,24] Shiono et al.[23] showed that histopathological iron accumulation occurred in two out of four cases before treatment and in all of them after treatment. They observed hypertransaminasemia and concurrent elevation of ferritin levels, which started to decrease after phlebotomy.[23] Another study revealed histological iron accumulation in seven out of ten patients, all of whom had hyperferritinemia. The authors of that study emphasized the possibility that cases might worsen after treatment.[24] In our study, three fulminant and two chronic hepatitis cases had histological iron accumulation. Three of them underwent liver transplantation and one of them had hyperferritinemia.

Dhawan et al.[17] prospectively applied a new Wilson mortality score, which showed that patients scoring ≥ 11 needed liver transplantations. Accordingly, 21 of our cases scored ≥ 11. However, only seven of these patients needed transplantation and there were two patients who underwent transplantation despite a score < 11: one with neurological WD and the other with chronic hepatitis. Although this scoring system was developed using data on children with WD who either survived or died, we used it for those who survived, those who died or those who underwent liver transplantation, and then grouped the latter two categories together. This was because the indication for liver transplantation in our series was presentation of decompensation findings that had been unresponsive to treatment. The lower sensitivity and specificity that we detected might have been due to the effectiveness of plasmapheresis in some cases with fulminant presentation in our series.

CONCLUSIONS

The presentation, symptoms and signs of our cases were similar to those in previously reported series, except for the high proportion of fulminant WD cases, which probably occurred because our institution is a liver transplantation center. Although the data were insufficient to conclude that decreased zinc levels have a role in the development of a fulminant course, we emphasize the need for further studies on this topic. We also emphasize the importance of incidentally detected hepatosteatosis in making an early diagnosis of WD and the probable relationship between iron status and WD.

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Relationship between socioeconomic and nutritional status in the Serbian adult population: a cross-sectional study

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ABSTRACT

BACKGROUND: Socioeconomic status is a well-known risk factor for obesity. The aim of this study was to assess the relationship between socioeconomic and nutritional status in the Serbian adult population.

DESIGN AND SETTING: Cross-sectional study on data from the 2013 National Health Survey performed in Serbia.

METHODS: The study population consisted of adults aged ≥ 20 years. Face-to-face interviews and anthropometric measurements were conducted by trained staff. Associations between body mass index and sociodemographic variables were analyzed using multivariable logistic regression analyses.

RESULTS: Out of 12,461 subjects of both sexes, 36.4% were overweight and 22.4% were obese. The prevalences of overweight and obesity differed significantly between the sexes, regarding all sociodemographic characteristics. Among women, educational attainment was associated with lower risk of being overweight (odds ratio, OR = 0.82; 95% confidence interval, CI: 0.69-0.98 for medium-level and OR = 0.77; CI: 0.62-0.97 for higher education) or obese (OR = 0.68; CI: 0.57-0.82 for medium-level and OR = 0.41; CI: 0.31-0.54 for higher education). In contrast, medium-level (OR = 1.28; CI: 1.08-1.52) and highly educated men (OR = 1.39; CI: 1.11-1.74) were more frequently overweight than were those with low education. Among men, grade I obesity was positively related to the richest wealth index group (OR = 1.27), while the opposite was true for grade II obesity among women (OR = 0.61).

CONCLUSION: This study showed significant socioeconomic inequalities in nutritional status between men and women. Continuous monitoring of socioeconomic patterns relating to weight is important, especially with further exploration of the link between education and obesity.

INTRODUCTION

Overweight and obesity are a growing public health problem worldwide. The prevalence is increasing rapidly in countries of all income levels (high, medium and low). Since 1975, obesity levels have nearly tripled worldwide. In 2016, 39% of adults aged 18 years and over were overweight and 13% were obese.[1] In Serbia, as globally, the increasing prevalence of overweight and obesity is an important public health challenge. In 2013, among adults aged 20 years and over, 56.3% were overweight and 21.2% were obese,[2] whereas in 2006 these figures were 54.5% and 18.3%, respectively.[3] The rise in obesity in Serbia, as in other middle-income countries, is likely due to a variety of factors, including increased food energy supply, economic transition, globalization of the world food market, and social and cultural changes.[4] However, there is currently a lack of detailed analysis of obesity in Serbia.

Socioeconomic status has been found to be an important factor associated with obesity. In most studies, occupation, education and income are used as socioeconomic status indicators and the results regarding their associations with obesity vary depending on the type of study, population selected, gender, age and indicators used.[5,6] In high-income countries, an inverse relationship between socioeconomic status and obesity has been reported,[7,8] while in middle and low-income countries, studies have shown a strong direct relationship between socioeconomic status and overweight/obesity both among men and among women.[9-12]

The objective of the present study was to assess the relationship between socioeconomic and nutritional status in the Serbian adult population.

METHODS

Setting, study design and ethical concerns

We used cross-sectional data on the Serbian adult population from the 2013 National Health Survey, which has been described in detail elsewhere.[13] The Ethics Review Board of the Institute of Public Health of Serbia (Decision number 5996/1, of October 1, 2013) and the Ministry of Health of the Republic of Serbia issued the necessary approval for undertaking this study.

Study population

The sample was chosen by using a stratified, two-stage nationally representative random sampling approach, using population data from census records that were generated in the year 2011. Four geographical regions of Serbia, including urban and rural areas (Vojvodina, Belgrade, Šumadija and Western Serbia, and Eastern and Southern Serbia) were identified in the sample. The units of the first stage of sampling were census enumeration areas, while the units of the second stage of sampling were randomly selected households.

A total of 670 census enumeration areas within each region with probability proportional to size were selected during the first stage. Households were selected using computer-generated simple random sampling without replacement. The number of households selected in each selected enumeration area was 10, plus three backup households. Representatives from backup households were interviewed only if individuals in some of the first 10 households were not found. If the members of a household refused to be interviewed, no backup household was contacted.

In the end, out of a total of 10,089 households that were contacted, members of 6,500 households agreed to participate in the survey, thus constituting a response rate of 64.4%, and personal visits were made to each one of these households. Out of the 12,722 members of these households who were aged 20 years and over, 12,461 were interviewed, which yielded a response rate of 97.9%. Face-to-face interviews and anthropometric measurements were carried out at participants' homes by trained staff, consisting of two interviewers and a healthcare worker. All respondents were informed about the purpose of the study and gave their written consent to participate.

Study variables

The variables assessed included sociodemographic characteristics (age, sex, region, education, marital status, employment status, wealth index, as calculated below, and type of settlement) and objective findings (weight, height and waist circumference). These were used to analyze socioeconomic inequalities according to sex and nutritional status, among the subjects selected. Standard procedures for measurements of weight, height and waist circumference were used. Body mass index was categorized according to the World Health Organization criteria.[14]

The following variables were used to reflect socioeconomic position in this study: educational level, defined in three categories, i.e. low (≤ 8 years), medium (8-12 years) and high (≥ 12 years); employment status, categorized as employed, economically inactive or unemployed; and wealth index, which was used to measure household wealth and thus categorized the respondents into three socioeconomic groups, i.e. low, medium and high on the basis of their assets. Generally, all items that could give a picture of socioeconomic position were used as variables in the wealth index calculation. These items included the number of bedrooms per household member; main material used for the floor, roof and walls of the house; main source of drinking water; means of sanitation; energy source used for heating; and possession of color television, mobile phone, refrigerator, computer, washing machine, dishwasher, air conditioning, central heating, car and internet access. Factor analysis and principal component analysis (PCA) were used to assign weights or factor scores to each variable.[15] Details about how the wealth index was calculated and other variables were determined have been provided elsewhere.[13,15,16]

Statistical analysis

Continuous variables were described in terms of means and standard deviations, while categorical variables were expressed as frequencies and percentages. Prevalence rates with appropriate 95% confidence intervals (CI) were estimated for the six categories of body mass index (BMI), separately for males and females. All reported age-adjusted estimates were weighted using probability-sampling weights. The impact on the precision of stratification of the sampling weights from the variance estimates and confidence intervals reported was accounted for by using Taylor-series linearization techniques for complex samples. The chi-square test, Student's t test, Mann-Whitney U test, Kruskal-Wallis test and one-way analysis of variance with the post-hoc Bonferroni test were used whenever appropriate.

Associations between the categories of body mass index and sociodemographic variables were analyzed using multivariable logistic regression analysis, separately for males and females. The dependent variables formed six different models: each body mass index category vs. normal weight as the referent category; and obesity (including all subjects with body mass index ≥ 30 kg/m^2) versus normal weight as the referent category. The independent variables were: age, region, type of settlement, educational level, marital status, employment status and wealth index. These were reported with odds ratios and their 95% confidence intervals, along with the probability P. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 software (SPSS Inc.,

Chicago, IL, USA) and STATA version 11.1 (StataCorp LP, College Station, TX, USA) and the complex sampling design was taken into account. Statistical significance was set at two-sided $P < 0.05$.

RESULTS

The study included 12,461 participants of both sexes, with mean age 48.8 ± 17.0 years. Slightly more females than males participated in the study (51.8% versus 48.2%) and the predominant age group among the participants was 20-44 years (42.9%). The highest proportion of the participants was from the Šumadija and Western Serbia region and the majority of the participants lived in urban settlements (60.2%). Most of the participants were married (65.2%), had had secondary education (57.5 %), were employed (37.2%) and belonged to the low wealth index group (41.1%). Out of the whole study population, 36.4% were overweight (BMI 25-29.9 kg/m^2) and 22.4% were obese (BMI ≥ 30 kg/m^2). The mean BMI was 26.6 kg/m^2. Overweight was significantly more frequent among men than among women (**Table 1**).

Significant differences were noticed for all variables according to age and gender (with the exception of gender distribution in certain geographical regions). Therefore, further analyses were performed separately for men and women and the prevalences of the BMI categories were adjusted according to age. The prevalences of overweight/obesity according to sociodemographic variables, after adjustment for age, are shown in **Table 2** (for men) and **Table 3** (for women).

There were significant differences ($P < 0.001$) in the distribution of overweight and obesity among men across age groups. Most of the overweight men were in the 20-44 age group and most of the obese men belonged to the 45-64 age group. The prevalences of overweight and obesity differed significantly in relation to all sociodemographic characteristics with the exception of geographical region (**Table 2**). Most of the overweight men were living in urban settlements, had high educational level, were married, were employed and had a high wealth index. Men with BMI ≥ 30 kg/m^2 more frequently lived in rural settlements, had secondary education, were married, were employed and had a low wealth index (**Table 2**).

Table 1. Characteristics of survey participants according to sex and age groups and their differences. Republic of Serbia, 2013

Characteristics	All n = 12,461	Sex		P‡	Age groups			P§
		Male n = 6,008 (48.2%)	Female n = 6,453 (51.8%)		20-44 n = 5,340 (42.9%)	45-64 n = 4,708 (37.8%)	≥ 65 n = 2,413 (19.3%)	
Age, mean ± SD	48.8 ± 17.0	47.9 ± 16.7	49.6 ± 17.2		32.3 ± 7.1	54.9 ± 5.7	73.4 ± 6.1	
Region, n (%)								
Vojvodina	3,317 (26.6)	1,586 (26.4)	1,731 (26.8)		1,422 (26.6)	1,257 (26.7)	639 (26.5)	
Belgrade	2,904 (23.3)	1,363 (22.7)	1,541 (23.9)	0.288	1,322 (24.8)	1,043 (22.2)	539 (22.3)	< 0.001
Šumadija and Western Serbia	3,419 (27.4)	1,672 (27.8)	1,747 (27.1)		1,466 (27.5)	1,344 (28.5)	609 (25.2)	
Eastern and Southern Serbia	2,821 (22.6)	1,387(23.1)	1,434 (22.2)		1,130 (21.2)	1,065 (22.6)	626 (25.9)	
Settlement, n (%)								
Urban	7,498 (60.2)	3,528 (58.7)	3,970 (61.5)	< 0.001	3,352 (62.8)	2,816 (59.8)	1,329 (55.1)	< 0.001
Rural	4,963 (39.8)	2,480 (41.3)	2,483 (38.5)		1,987 (37.2)	1,892 (40.2)	1,084 (44.9)	
Education level, n (%)								
Low	3,070 (24.6)	1,110 (18.5)	1,960 (30.4)	< 0.001	635 (11.9)	1,169 (24.8)	1,265 (52.4)	< 0.001
Medium	7,161 (57.5)	3,815 (63.5)	3,346 (51.9)		3,628 (67.9)	2,741 (58.2)	794 (32.9)	
High	2,230 (17.9)	1,083 (18.0)	1,147 (17.8)		1,077 (20.2)	798 (17.0)	354 (14.7)	
Marital status, n (%)								
Married/living with partner	8,123 (65.1)	4,045 (67.3)	4,078 (63.2)	< 0.001	3,069 (57.5)	3,675 (78.1)	1,379 (57.1)	< 0.001
Living without partner*	4,338 (34.9)	1,963 (32.7)	2,375 (36.8)		2,270 (42.5)	1,033 (21.9)	1,035 (42.9)	
Employment status, n (%)								
Employed	4,640 (37.2)	2,720 (45.3)	1,920 (29.8)	< 0.001	2,780 (52.1)	1,847 (39.2)	14 (0.6)	< 0.001
Inactive†	4,728 (38.0)	1,782 (29.6)	2,946 (45.6)		1,815 (43.7)	1,216 (25.8)	62 (2.6)	
Unemployed	3,093 (24.8)	1,506 (25.1)	1,587 (24.6)		225 (4.2)	1,645 (34.9)	2,337 (96.9)	
Wealth index, n (%)								
Low	5,116 (41.1)	2,523 (42.0)	2,593 (40.2)	0.117	1,799 (33.7)	1,997 (42.4)	1,322 (54.8)	< 0.001
Medium	2,485 (19.9)	1,183 (19.7)	1,302 (20.2)		1,061 (19.9)	996 (21.2)	428 (17.7)	
High	4,860 (39.0)	2,302 (38.3)	2,558 (39.6)		2,480 (46.4)	17,15 (39.5)	664 (27.5)	
BMI, mean ± SD	26.6 ± 5.0	26.9 ± 4.3	26.3 ± 5.6		25.0 ± 4.7	27.8 ± 4.9	27.8 ± 4.9	
BMI 25-29.9 kg/m^2, n (%)	4,541 (36.4)	2,605 (43.3)	1,936 (31.1)	< 0.001	2,384 (32.5)	3,261 (44.5)	1,688 (23.0)	< 0.001
BMI ≥ 30 kg/m^2, n (%)	2,793 (22.4)	1,285 (21.6)	1,506 (24.6)		5,340 (42.9)	4,704 (37.8)	2,413 (19.4)	

SD = standard deviation; BMI = body mass index.

*Unmarried, divorced or widowed; †Economically inactive (students, disabled people, pensioners and housewives); ‡According to chi-square test, t test or Mann-Whitney test, as appropriate; §According to chi-square test, one-way analysis of variance (ANOVA) or Kruskal-Wallis test, as appropriate.

The prevalences of both overweight and obese women were highest in the age groups ≥ 65 years age group (38.2% and 34.4% respectively, P < 0.001), and overweight and obesity differed significantly according to all sociodemographic characteristics. Most of the overweight and obese women were from Eastern and Southern Serbia (32.7% and 26.6% respectively) and were married (33.5% and 27.0%, respectively). Unlike the men, higher numbers of overweight and obese women lived in rural settlements (31.8% and 27.1% respectively) and were inactive (pensioners or housewives) (31.5% and 28.4% respectively). The largest proportion of the overweight women had received secondary education (32.1%) and were in the medium wealth index group (32.9%). Most of the obese women were of low education level (31.2%), were inactive (28.4%) and had a low wealth index (27.7%) (**Table 3**).

The associations of BMI categories with sociodemographic variables according to multivariable logistic regression are shown in **Tables 4 and 5** for men and women respectively. Among the men, the multivariable logistic regressions showed that the participants who belonged to the 45-64 age group were more obese than those aged 65 years and over, as were also those who lived in rural settlements. The men who were living without a partner and were economically inactive were less frequently obese than were the men who were married and employed, respectively. Grade I obesity was significantly positively related to the richest group according to the wealth index (**Table 4**). Among the men, the risk of being overweight was higher for those with medium and high education levels, as well as for those with medium and high wealth indexes, while the men living without

Table 2. Prevalence of body mass index (BMI) categories among men, in percentages with 95% confidence intervals (95% CI), adjusted for age, Republic of Serbia, 2013

Characteristics	BMI categories						P‡
	Underweight (BMI < 18.50)	Normal weight (BMI = 18.50-24.99)	Overweight (BMI = 25.00-29.99)	Obesity grade I (BMI = 30.00-34.99)	Obesity grade II (BMI = 35.00-39.99)	Obesity grade III (BMI ≥ 40.00)	
BMI, mean ± SE	17.5 ± 0.2	22.7 ± 0.0	27.3 ± 0.0	31.9 ± 0.0	36.7 ± 0.1	42.6 ± 0.2	
Age, mean ± SD	41.3 ± 17.7	44.8 ± 18.3	48.6 ± 15.9	51.6 ± 14.7	52.5 ± 14.4	51.1 ± 12.5	
All	1.1 (0.8-1.3)	34.0 (32.8-35.2)	43.3 (42.1-44.6)	17.3 (16.3-18.2)	3.6 (3.1-4.0)	0.7 (0.5-0.9)	
Age groups							
20-44	0.3 (0.0-1.1)	28.3 (24.6-32.0)	48.3 (44.4-52.1)	17.9 (14.9-20.8)	4.3 (2.9-5.8)	0.9 (0.3-1.6)	< 0.001
45-64	1.1 (0.6-1.6)	31.4 (29.1-33.6)	42.4 (40.0-44.8)	19.9 (18.1-21.7)	4.2 (3.3-5.1)	1.0 (0.6-1.4)	
≥ 65	2.6 (1.4-3.7)	49.9 (44.8-55.1)	35.0 (29.6-40.4)	11.6 (7.5-15.8)	1.0 (0.0-3.0)	0.1 (0.0-0.8)	
Region							
Vojvodina	0.8 (0.3-1.3)	34.4 (32.1-36.7)	44.3 (41.9-46.7)	15.8 (13.9-17.6)	3.9 (3.0-4.8)	0.8 (0.4-1.2)	
Belgrade	0.7 (0.1-1.3)	32.3 (29.7-34.9)	45.1 (42.3-47.8)	18.1 (16.0-17.6)	3.1 (2.1-4.2)	0.7 (0.2-1.1)	0.283
Šumadija and Western Serbia	1.1 (0.6-1.6)	34.1 (31.9-36.3)	42.6 (40.3-44.9)	17.7 (15.9-19.4)	3.7 (2.9-4.6)	0.9 (0.5-1.3)	
Eastern and Southern Serbia	1.6 (1.1-2.2)	34.9 (32.5-37.3)	41.7 (39.2-44.2)	17.8 (15.9-19.7)	3.4 (2.5-4.4)	0.5 (0.1-0.9)	
Settlement							
Urban	0.9 (0.5-1.2)	33.7 (32.1-35.2)	44.7 (43.1-46.4)	16.7 (15.4-17.9)	3.3 (2.6-3.9)	0.8 (0.5-1.1)	0.049
Rural	1.3 (0.9-1.7)	34.5 (32.7-36.3)	41.5 (39.6-43.4)	18.1 (16.7-19.5)	4.0 (3.3-4.7)	0.6 (0.3-1.0)	
Education level							
Low	1.8 (1.2-2.4)	40.4 (37.7-43.1)	37.2 (34.4-40.1)	16.0 (13.8-18.2)	3.9 (2.8-4.9)	0.7 (0.2-1.2)	< 0.001
Medium	1.0 (0.7-1.4)	32.5 (31.0-34.0)	44.2 (42.6-45.8)	17.6 (16.4-18.8)	3.8 (3.2-4.4)	0.8 (0.5-1.1)	
High	0.5 (0.0-1.1)	32.1 (29.3-34.9)	47.0 (44.1-50.0)	17.6 (15.3-19.8)	2.3 (1.2-3.4)	0.5 (0.0-1.0)	
Marital status							
Married/living with partner	0.6 (0.3-0.9)	29.7 (28.2-31.1)	45.2 (43.7-46.8)	19.5 (18.4-20.7)	4.1 (3.6-4.7)	0.8 (0.5-1.1)	< 0.001
Living without partner*	2.1 (1.6-2.6)	43.2 (41.1-45.3)	39.3 (37.0-41.6)	12.5 (10.8-14.2)	2.3 (1.5-3.2)	0.6 (0.2-1.0)	
Employment status							
Employed	0.5 (0.1-1.0)	27.2 (25.2-29.1)	48.0 (45.9-50.0)	19.7 (18.1-21.3)	3.8 (3.1-4.5)	0.8 (0.5-1.1)	< 0.001
Inactive†	1.4 (0.7-2.0)	41.4 (38.4-44.3)	39.3 (36.2-42.4)	14.4 (12.1-16.8)	3.9 (2.9-5.0)	0.8 (0.4-1.3)	
Unemployed	1.6 (1.1-2.2)	36.6 (34.2-39.1)	40.5 (37.9-43.1)	16.6 (14.7-18.6)	3.1 (2.4-3.9)	0.6 (0.2-0.9)	
Wealth index							
Low	1.7 (1.3-2.1)	37.7 (35.9-39.5)	39.3 (37.4-41.2)	16.7 (15.3-18.1)	7.9 (7.0-8.8)	2.6 (2.0-3.1)	< 0.001
Medium	0.7 (0.2-1.3)	31.6 (29.0-34.3)	46.1 (43.3-48.9)	16.7 (14.6-18.8)	5.7 (4.5-6.9)	2.1 (1.3-2.8)	
High	0.5 (0.0-0.9)	30.9 (28.9-32.8)	46.7 (44.6-48.8)	18.3 (16.7-19.9)	3.8 (2.8-4.7)	1.8 (1.2-2.4)	

SE = standard error; SD = standard deviation.

*Unmarried, divorced or widowed; †Economically inactive (students, disabled persons, pensioners and housewives); ‡According to chi-square test or analysis of variance (ANOVA), as appropriate.

a partner were less likely to be overweight. Underweight was significantly more frequent among the men who were living without a partner (**Table 4**).

Among the women, the risk of obesity increased with age, and it was significantly higher among those who were married, had low education level, were economically inactive and were unemployed (**Table 5**). High wealth index was associated with lower risk of grade II obesity among women (**Table 5**). Overweight was less frequent among women who were in the youngest age group, who had had secondary and higher education and were living without partner. Underweight was significantly more frequent among young women, women living without a partner, women who were economically inactive or unemployed and those with low wealth index (**Table 5**).

DISCUSSION

Our study showed that there were significant socioeconomic inequalities regarding nutritional status. These data, from a nationally representative sample of Serbian adults aged 20 years and over, suggest that low socioeconomic status, as measured according to education, employment and wealth index, was associated with obesity. Moreover, gender differences regarding the association between socioeconomic status and obesity were found.

The primary findings from many studies in middle and low-income countries have shown that there is a strong and direct relationship between socioeconomic status and overweight/obesity, both among men and among women.[9,10] This implies that the higher the socioeconomic status is, the more frequent obesity

Table 3. Prevalence of body mass index (BMI) categories among women, in percentages with 95% confidence intervals (95% CI), adjusted for age. Republic of Serbia, 2013

Characteristics	BMI categories						P‡
	Underweight (BMI < 18.50)	Normal weight (BMI = 18.50-24.99)	Overweight (BMI = 25.00-29.99)	Obesity grade I (BMI = 30.00-34.99)	Obesity grade II (BMI = 35.00-39.99)	Obesity grade III (BMI ≥ 40.00)	
BMI, mean ± SE	17.7 ± 0.1	22.2 ± 0.0	27.3 ± 0.0	32.0 ± 0.0	36.9 ± 0.1	43.0 ± 0.1	
Age, mean ± SD	37.7 ± 19.2	43.2 ± 16.7	54.2 ± 15.8	57.6 ± 14.0	57.0 ± 14.0	55.8 ± 13.6	
All	3.3 (2.9-3.8)	40.9 (39.8-42.1)	31.1 (30.0-32.2)	16.5 (15.6-17.4)	5.9 (5.4-6.5)	2.2 (1.8-2.5)	
Age groups							
20-44	6.6 (5.7-7.6)	61.8 (60.0-63.7)	20.7 (16.1-22.2)	7.1 (6.1-8.0)	2.6 (2.0-3.2)	1.0 (0.6-1.4)	< 0.001
45-64	1.0 (0.6-1.4)	32.4 (30.5-34.2)	35.7(33.8-37.6)	20.4 (18.8-22.0)	7.3 (6.3-8.4)	2.9 (2.3-3.6)	
≥ 65	2.1 (1.3-2.9)	25.1 (22.8-27.4)	38.2 (35.6-40.7)	23.6 (21.3-25.8)	8.4 (6.9-9.9)	2.4 (1.5-3.2)	
Region							
Vojvodina	3.3 (2.4-4.1)	39.9 (37.6-42.1)	31.5 (29.4-33.7)	16.6 (14.9-18.4)	6.3 (5.1-7.4)	2.4 (1.7-3.1)	
Belgrade	3.9 (3.0-4.8)	47.2 (44.9-49.6)	27.7 (25.4-30.1)	14.4 (12.5-16.2)	4.7 (3.4-5.9)	2.1 (1.4-2.9)	< 0.001
Šumadija and Western Serbia	3.2 (2.5-4.0)	39.9 (37.8-41.9)	31.9 (29.9-34.0)	17.4 (15.8-19.0)	5.6 (4.5-6.6)	2.0 (1.3-2.6)	
Eastern and Southern Serbia	3.1 (2.2-3.9)	37.7 (35.4-39.9)	32.7 (30.5-34.9)	17.2 (15.4-19.0)	7.2 (6.0-8.3)	2.2 (1.5-2.9)	
Settlement							
Urban	3.2 (2.7-3.8)	43.3 (41.9-44.8)	30.6 (29.2-32.1)	16.0 (14.8-17.1)	4.9 (4.2-5.6)	1.9 (1.5-2.4)	< 0.001
Rural	3.5 (2.9-4.2)	37.6 (35.9-39.3)	31.8 (30.1-33.4)	17.2 (15.9-18.6)	7.4 (6.5-8.2)	2.5 (2.0-3.1)	
Education level							
Low	4.2 (3.4-5.0)	34.7 (32.6-36.7)	29.9 (27.9-31.9)	19.3 (17.7-20.9)	8.2 (7.2-9.3)	3.7 (3.1-4.4)	< 0.001
Medium	2.9 (2.3-3.5)	41.5 (39.9-43.1)	32.1 (30.5-33.6)	16.4 (15.1-17.6)	5.5 (4.7-6.3)	1.7 (1.2-2.2)	
High	3.0 (1.9-4.0)	51.9 (49.2-54.7)	30.6 (27.9-33.3)	11.3 (9.2-13.5)	2.6 (1.2-3.9)	0.6 (0.0-1.5)	
Marital status							
Married/living with partner	1.9 (1.3-2.4)	37.5 (36.1-38.9)	33.5 (32.2-34.9)	18.1 (17.0-19.2)	6.7 (6.0-7.4)	2.2 (1.8-2.7)	< 0.001
Living without partner*	5.9 (5.2-6.6)	46.9 (45.0-48.7)	26.9 (25.1-28.7)	13.7 (12.3-15.1)	4.5 (3.6-5.5)	2.1 (1.5-2.7)	
Employment status							
Employed	1.3 (0.4-2.2)	49.5 (47.1-51.8)	31.1 (28.9-33.4)	13.3 (11.5-15.1)	3.7 (2.5-4.9)	1.1 (0.4-1.8)	< 0.001
Inactive†	3.9 (3.1-4.7)	36.2 (34.1-38.3)	31.5 (29.5-33.6)	19.0 (17.4-20.6)	6.7 (5.7-7.8)	2.7 (2.0-3.4)	
Unemployed	4.5 (0.5-5.4)	40.1 (34.1-38.3)	30.4 (28.1-32.8)	15.7 (13.8-17.5)	6.9 (5.7-8.1)	2.4 (1.7-3.2	
Wealth index							
Low	4.1 (3.5-4.8)	37.6 (35.8-39.3)	30.6 (28.9-32.3)	17.2 (15.9-18.6)	7.9 (7.0-8.8)	2.6 (2.0-3.1)	< 0.001
Medium	2.5 (1.6-3.5)	39.3 (36.8-41.7)	32.9 (30.4-35.3)	17.6 (15.6-19.5)	5.7 (4.5-6.9)	2.1 (1.3-2.8)	
High	2.9 (2.2-3.6)	45.8 (43.9-47.6)	30.7 (28.9-32.5)	15.1 (13.6-16.5)	3.8 (2.8-4.7)	1.8 (1.2-2.4)	

SE = standard error; SD = standard deviation.

*Unmarried, divorced or widowed; †Economically inactive (students, disabled persons, pensioners and housewives).; ‡According to chi-square test or analysis of variance (ANOVA), as appropriate.

Table 4. Associations of body mass index categories among men with demographic and socioeconomic variables, according to multivariable logistic regression. Republic of Serbia, 2013

Demographic and socioeconomic variables	Odds ratios (95% confidence intervals)*					
	Underweight versus normal	Overweight versus normal	Obesity I versus normal	Obesity II versus normal	Obesity III versus normal	Obesity (all) versus normal
Age groups						
20-44	0.48 (0.10-2.24)	0.82 (0.62-1.10)	0.86 (0.60-1.22)	1.21 (0.65-2.27)	1.19 (0.26-5.46)	0.89 (0.64-1.25)
45-64	0.50 (0.12-2.06)	1.14 (0.89-1.46)	1.55 (1.15-2.09)	2.07 (1.25-3.46)	2.64 (0.71-9.89)	1.63 (1.23-2.16)
> 65	1.00	1.00	1.00	1.00	1.00	1.00
Settlement						
Urban	1.00	1.00	1.00	1.00	1.00	1.00
Rural	0.83 (0.46-1.49)	1.09 (0.94-1.26)	1.24 (1.02-1.50)	1.25 (0.88-1.78)	0.71 (0.34-1.45)	1.20 (1.00-1.44)
Education level						
Low	1.00	1.00	1.00	1.00	1.00	1.00
Medium	0.84 (0.45-1.59)	1.28 (1.08-1.52)	1.16 (0.93-1.45)	1.18 (0.80-1.75)	1.23 (0.55-2.74)	1.16 (0.94-1.42)
High	0.53 (0.18-1.53)	1.39 (1.11-1.74)	1.21 (0.90-1.61)	0.84 (0.47-1.49)	0.77 (0.22-2.76)	1.12 (0.85-1.47)
Marital status						
Married/living with partner	1.00	1.00	1.00	1.00	1.00	1.00
Living without partner†	2.42 (1.36-4.33)	0.63 (0.55-0.72)	0.49 (0.41-0.60)	0.44 (0.30-0.64)	0.49 (0.22-1.09)	0.48 (0.41-0.57)
Employment status						
Employed	1.00	1.00	1.00	1.00	1.00	1.00
Inactive‡	1.53 (0.84-2.77)	0.74 (0.64-0.86)	0.71 (0.58-0.87)	0.69(0.48-1.03)	1.12 (0.55-2.28)	0.72 (0.60-0.87)
Unemployed	0.52 (0.12-2.17)	0.86 (0.68-1.09)	0.92 (0.69-1.23)	1.19 (0.73-1.93)	0.68 (0.23-2.01)	0.94 (0.72-1.24)
Wealth index						
Low	1.00	1.00	1.00	1.00	1.00	1.00
Medium	0.59 (0.27-1.28)	1.23 (1.04-1.47)	1.07 (0.85-1.35)	1.23 (0.82-1.85)	0.95 (0.42-2.17)	1.10 (0.89-1.36)
High	0.52 (0.24-1.14)	1.29 (1.09-1.53)	1.27 (1.00-1.61)	1.16 (0.74-1.80)	0.68 (0.27-1.68)	1.21 (0.98-1.51)

*Adjusted for region; †Unmarried, divorced or widowed; ‡Economically inactive (students, disabled persons, pensioners and housewives).
Normal (0) and other body mass index categories (1); 1.00: referent value; the dependent variables formed six different models: each body mass index category vs. normal weight as referent category, and obesity (including all subjects with body mass index ≥ 30 kg/m^2) versus normal weight as referent category.

Table 5. Associations of body mass index categories among women with demographic and socioeconomic variables, according to multivariable logistic regression. Republic of Serbia, 2013

Demographic and socioeconomic variables	Odds ratios (95% confidence intervals)*					
	Underweight versus normal	Overweight versus normal	Obesity I versus normal	Obesity II versus normal	Obesity III versus normal	Obesity (all) versus normal
Age groups						
20-44	2.65 (1.38-5.08)	0.38 (0.29-0.48)	0.32(0.24-0.44)	0.42 (0.26-0.65)	1.06(0.53-2.14)	0.37 (0.29-0.49)
45-64	0.75 (0.40-1.41)	0.91 (0.74-1.12)	0.99 (0.79-1.25)	1.17 (0.84-1.63)	2.38 (1.43-3.97)	1.10 (0.89-1.35)
> 65	1.00	1.00	1.00	1.00	1.00	1.00
Settlement						
Urban	1.00	1.00	1.00	1.00	1.00	1.00
Rural	1.01 (0.71-1.41)	1.01 (0.86-1.18)	0.96 (0.79-1.17)	0.96(0.73-1.28)	0.97 (0.62-1.52)	0.95 (0.80-1.13)
Education level						
Low	1.00	1.00	1.00	1.00	1.00	1.00
Medium	0.78 (0.51-1.18)	0.82 (0.69-0.98)	0.73 (0.59-0.90)	0.73 (0.55-0.98)	0.35 (0.22-0.55)	0.68 (0.57-0.82)
High	0.75 (0.44-1.27)	0.77 (0.62-0.97)	0.48 (0.35-0.65)	0.37 (0.22-0.62)	0.14 (0.05-0.36)	0.41 (0.31-0.54)
Marital status						
Married/living with partner	1.00	1.00	1.00	1.00	1.00	1.00
Living without partner†	1.95 (1.45-2.61)	0.62 (0.54-0.71)	0.62 (0.52-0.74)	0.56 (0.42-0.73)	0.85 (0.57-1.27)	0.60 (0.51-0.70)
Employment status						
Employed	1.00	1.00	1.00	1.00	1.00	1.00
Inactive‡	1.77 (1.02-3.07)	1.58 (1.31-1.92)	2.01 (0.57-2.56)	2.31 (1.57-3.42)	2.87 (1.52-5.41)	2.13 (1.71-2.65)
Unemployed	2.07 (1.46-2.92)	1.09 (0.92-1.29)	1.23 (0.97-1.55)	1.89 (1.30-2.76)	2.37 (1.28-4.42)	1.43 (1.16-1.76)
Wealth index						
Low	1.00	1.00	1.00	1.00	1.00	1.00
Medium	0.62 (0.40-0.95)	1.13 (0.94-1.36)	1.15 (0.92-1.45)	0.90 (0.65-1.26)	1.30 (0.77-2.19)	1.05 (0.86-1.29)
High	0.66 (0.44-0.98)	1.01 (0.84-1.22)	1.07 (0.85-1.35)	0.61 (0.42-0.87)	1.27 (0.74-2.18)	0.93 (0.75-1.14)

*Adjusted for region; †Unmarried, divorced or widowed; ‡Economically inactive (students, disabled persons, pensioners and housewives).
Normal (0) and other body mass index categories (1); 1.00: referent value; the dependent variables formed six different models: each body mass index category vs. normal weight as referent category, and obesity (including all subjects with body mass index ≥ 30 kg/m^2) versus normal weight as referent category.

also is. The results from high-income countries have implied an inverse relationship between socioeconomic status and obesity, both among men and among women.[7,8] Serbia belongs to the category of upper middle-income countries, with a poverty rate above 10%, and our results show that the prevalence of obesity was highest among those who belonged to the lowest socioeconomic class, among women but not among men.

This relationship between socioeconomic status and obesity, shown through our study, is similar to findings from several other studies in middle-income countries such as Thailand, the Philippines and China.[17-19] These studies have suggested that a transition is taking place regarding the relationship between socioeconomic status and obesity, from the pattern of middle-income countries to the pattern of high-income countries, and that this pattern was comparatively faster among women than among men. In Brazil, Monteiro et al. also suggested that obesity was increasing faster among low socioeconomic status groups.[20] Gender differences regarding the association between socioeconomic status and obesity are complex and may be explained by other factors such as health-related behavior and sociocultural norms.[21,22] Social pressure to be slim is stronger among women, particularly in higher socioeconomic status groups.[23] Among men, overweight and obesity occur more frequently among those who belong to middle and high social classes, according to the wealth index, and among those with middle and higher education levels. The absence of any association between low socioeconomic status and overweight/obesity among men can possibly be explained through the fact that men with low socioeconomic status are more likely to have physically demanding professions.

In our study, a low level of education was associated with higher risk of being overweight and obese, among women, while this was not the case among men. Many studies have found an inverse relationship between educational attainment and obesity, although direct, null and U-shaped associations have also been observed.[24] The EPIC Panacea study observed that in all the countries involved, body mass index was significantly lower in all higher education categories, compared with the lowest education level. The same study showed that among women, but not among men, the difference between highest and lowest education status was still statistically significant among non-obese subjects.[10] Sabanayagam et al.[25] showed in relation to an adult Chinese population that there was an inverse relationship between the level of education and the prevalence of overweigh/obesity among women, such that the highest prevalence of obesity was among the women whose education level was primary school or lower. In the same study, the prevalence of overweight/obesity was lowest among men with post-secondary education.[25]

In our study, both overweight and obese women who were economically inactive and obese women who were unemployed showed positive associations with body mass index. In contrast, there was a negative association with body mass index among men who were economically inactive.

Ball et al. demonstrated associations between employment status and body mass index among women, after controlling for age.[26] Women who were employed full-time had lower body mass indexes and presented lower risk of overweight than did women who scored lower regarding the employment factor. The relationships observed among these factors were less consistent for men. The relationship between employment and the risk of overweight was the reverse of that among women: men who scored lower in the employment domain were at lower risk of being overweight than were those with higher scores.

There are very few studies in the literature describing the relationship between socioeconomic status and being underweight.[27,28] In our study, underweight was more frequent among adults of both sexes who lived in rural settlements, had low education level, were living without a partner, were unemployed and had a low wealth index. Men older than 65 years were more frequently underweight, while among women, the frequency of underweight was highest among those aged 20-44 years.

The main strength of our study is that it used a large representative sample. However, there were several limitations. First, the cross-sectional study design does not allow the causality of the relationship between socioeconomic status and BMI to be considered. Second, the self-reporting of socioeconomic data may be biased and may lead to exposure misclassification. This may attenuate or overestimate relationships between socioeconomic status and obesity. Third, we did not explore mediating factors, such as dietary intake and sedentary behavior.

CONCLUSION

Our study showed gender-specific associations of socioeconomic status with body mass index among Serbian adults. The results from this study can be generalized for the entire adult population in Serbia, since a nationally representative sample was used. Continuous monitoring of socioeconomic patterns in relation to weight is important, especially for further exploration of the link between education and obesity, since this may lead to development of appropriate education-based policies to counteract recent trends regarding obesity. Further studies are needed to clarify the underlying mechanisms in the relationship between socioeconomic status and obesity.

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Prevalence of *Entamoeba histolytica* and other enteral parasitic diseases in the metropolitan region of Belo Horizonte, Brazil. A cross-sectional study

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ABSTRACT

BACKGROUND: Enteral parasitic diseases are a public health problem in nations with low economic development and in settings with poor sanitation. Amebiasis is the second most frequent form of parasitosis, with a high burden of disease. Knowledge of the prevalence of enteroparasitoses in a given region is useful for planning clinical decision-making. Thus, the aim of this study was to estimate the prevalence of enteral parasitic diseases, especially amebiasis, through analysis on stool samples from public and private laboratories in a metropolitan area in southeastern Brazil.

DESIGN AND SETTING: Cross-sectional study conducted in the metropolitan region of Belo Horizonte, Brazil.

METHODS: We evaluated 6,289 fecal samples from one private and one public laboratory. The samples were concentrated by means of spontaneous sedimentation, and those that were positive for *Entamoeba histolytica* or *Entamoeba dispar* in optical microscopy analyses were processed to obtain deoxyribonucleic acid, with subsequent identification through the polymerase chain reaction.

RESULTS: Among the stool samples, 942 (15.0%) had parasitic infections; 73 (1.2%) of these were helminthic infections and 847 (13.5%) were protozoan infections, caused mainly by *Escherichia coli* (6.0%), *Endolimax nana* (5.2%) and *Giardia lamblia* (1.2%). Infections due to *Entamoeba histolytica* or *Entamoeba dispar* occurred in 36 samples (0.6%) and the polymerase chain reaction revealed five (13.9%) as *Entamoeba histolytica*.

CONCLUSION: The prevalence of enteral parasitic diseases is high in the metropolitan region of Belo Horizonte, although amebiasis may not be a problem.

INTRODUCTION

Intestinal parasites affect approximately 3.5 billion people worldwide and are a public health problem, especially in developing countries, where almost one-third of the population live in conditions favorable to their dissemination.[1,2] Worldwide, amebiasis is the second most frequent parasitic disease, causing around 100,000 deaths each year and contributing towards the high global burden of diarrhea, notably in regions with low economic development and settings with poor sanitation.[2,4] Intestinal parasites are responsible for high levels of morbidity. The impact of intestinal nematode infections has been estimated to exceed five million disability-adjusted life years (DALYs), while amebiasis alone accounts for more than two million DALYs.[5]

The prevalence of enteroparasitosis in Brazil can reach over 60%[6] and varies across regions according to educational, economic and social levels, the population agglomeration index, sanitary conditions, soil contamination levels, contamination of water and food supplies and the virulence capacity of parasites.[7,8] These variations often make comparisons inadequate.

The prevalence of amebiasis ranges from 3.4% to 40.0%,[4,9] but these estimates may be imprecise because of the existence of *Entamoeba dispar*, which is morphologically indistinguishable from *Entamoeba histolytica*.[10] Differentiation between these species is very important for epidemiological and clinical purposes, such as selection of adequate therapy and investigation of other causes for gastrointestinal symptoms.[10] Brazilian studies aiming to characterize these two species in the general population are scarce,[4,11-13] and other studies have been conducted in specific populations such as schoolchildren[14] or regions without access to water,[15] or have compared the diagnostic methods available.[16]

The objective of the present study was to estimate the prevalence of enteral parasitic diseases in the general population using data from both public and private laboratories in the city of Belo Horizonte, Brazil, using molecular methods to distinguish between *Entamoeba histolytica* and *Entamoeba dispar* infections.

METHODS

Study design, setting, sampling and ethics evaluation

This was a cross-sectional study conducted in the metropolitan region of Belo Horizonte, Brazil. This study was approved by the ethics committee of the municipality of Belo Horizonte under the protocol number 021/2007.

Belo Horizonte, the capital of the state of Minas Gerais, is located in the southeastern region of Brazil. In the year 2008, when this study was conducted, Belo Horizonte had 2,399,920 inhabitants and had a human development index of 0.810, which is classified as high.[17]

Considering the population size of Belo Horizonte, an anticipated prevalence of *Entamoeba histolytica* or *Entamoeba dispar* of 20%, at a significance level of 5%, with 20% data loss, a sample of 296 patients would be required to estimate the prevalence of amebiasis in the city. However, due to the ease of access to the results from examinations, we included a total of 6,289 samples.

Stool sample origin

We included samples from the largest private laboratory in Belo Horizonte and from the city's municipal laboratory, from May to June 2008. The private laboratory processes approximately 800 fecal samples/day, from a wide variety of regions of Belo Horizonte and from adjacent regions. Because of the large number of stool examinations performed daily, samples from one week were included in the study.

The laboratory accredited to the network of clinical analysis laboratories of the municipality of Belo Horizonte attends communities on the outskirts of the city. We selected four low-income communities distributed in the northern, eastern, northeastern and central-southern regions. We chose one public and one private laboratory to evaluate possible differences in the prevalence of enteroparasitosis regarding socioeconomic conditions of their users. The network of public laboratories processes approximately 60 fecal samples/day and, therefore, samples covering one month were included in the study.

All fecal samples were conditioned at 4 °C and concentrated by means of spontaneous sedimentation not more than 24 hours after their receipt for analysis. All positive samples for *Entamoeba histolytica* and *Entamoeba dispar* were stored at 4 °C until they were sent to the amebiasis laboratory of the Federal University of Minas Gerais (Universidade Federal de Minas Gerais, UFMG) for identification by means of the polymerase chain reaction.

Method for deoxyribonucleic acid extraction

Deoxyribonucleic acid (DNA) was isolated by means of alkaline lysis in accordance with methodology that had previously been described,[18] with some modifications to minimize inhibition by fecal constituents. Polyvinylpolypyrrolidone (200 µl) was added to 200 µl of fecal sediment, and this mixture was incubated at 100 °C for 10 minutes. Lysis buffer (50 mM glucose, 25 mM Tris-HCl and 10 mM EDTA) was added to this material, with five minutes of incubation at room temperature. A solution of 0.2 mM NaOH was then added to the mixture together with 1% sodium dodecyl sulfate, and the mixture was then frozen in liquid N_2 and thawed, three times. Then 300 µl of 7.5 mM ammonium acetate was added, and this mixture was incubated in ice for 20 minutes, followed by centrifugation at 12,500 g for 5 minutes. The supernatant was then precipitated with 0.6 volumes of isopropanol. The purified deoxyribonucleic acid was resuspended in 20 µl of TE buffer (10 mM Tris-HCl and 1 mM EDTA).

Polymerase chain reaction

The polymerase chain reaction (PCR) target was a 300 bp ribosomal deoxyribonucleic acid segment that was amplified in *Entamoeba histolytica* and *Entamoeba dispar* in distinct reactions using different direct primers (Eh GTACAAAATGGCCAATTCATTC and Ed GTACAAAGTGGCCAATTTATG) and the same reverse primer (Eh/Ed GAATTGATTTTACTCAACTCTAG). The target of this polymerase chain reaction was a gene fragment of a small ribosomal subunit of ribonucleic acid. This target was chosen because it was present at a rate of around 200 copies per trophozoite,[19] thus providing greater sensitivity for making the diagnosis. The differences in the sequence of this gene between *Entamoeba histolytica* and *Entamoeba dispar* have made it possible to design specific primers for these two species.[20] The reaction took place over 50 cycles, with an annealing temperature of 57 °C and individual cycles of 30 seconds each.

Variables and statistical analysis

The numbers of positive results were described in terms of frequencies and proportions according to the type of parasite and were stratified according to the origin of the fecal sample, i.e. private or public laboratory. The chi-square test or Fisher's exact test was used, as appropriate, to compare proportions of infections between the laboratories. We considered differences with a P-value < 0.05 to be statistically significant. We used the OpenEpi v.3.01 software for the statistical analysis.

RESULTS

In the private laboratory, 5,695 stool examinations were evaluated during the data collection. The participants' mean age was 42.0 ± 34.0 years. In the public laboratory, 594 stool examinations were evaluated, and the patients' mean age was 29.6 ± 20.1 years.

The private laboratory registered 772 positive tests for enteral parasitic diseases (13.5%), while the public laboratory identified 170 positive samples (28.6%), and this difference in the proportions of positive samples was statistically significant (P < 0.001). The proportion of infections according to the type of parasite was also statistically lower in the private laboratory, such that helminthic infections accounted for 0.8% of the parasites, compared with 4.9% of the samples processed in the

public laboratory (P < 0.001). Protozoal infections occurred in 12.8% of the samples at the private laboratory and in 20.0% of those at the public laboratory, and this difference was statistically significant (P < 0.001).

The distribution of intestinal parasites according to the etiological agent and the laboratory is described in **Table 1**. In general, the same helminths presented greatest prevalence in both places, but with differences in magnitude. In the private laboratory, larvae of *Strongyloides stercoralis* and eggs of *Schistosoma mansoni* were the most prevalent forms (0.2%), followed by eggs of *Enterobius vermicularis, Ascaris lumbricoides* and *Taenia* (0.1%). In the public laboratory, eggs of *Ascaris lumbricoides* were the most prevalent (2.0%), followed by larvae of *Strongyloides stercoralis* (1.0%), eggs of *Trichuris trichiura* (0.5%), *Schistosoma mansoni* (0.5%), *Enterobius vermicularis* (0.3%), Ancylostomatidae and *Taenia* (0.2%). The prevalences of eggs of *Ascaris lumbricoides, Trichuris trichiura* and *Strongyloides stercoralis* larvae were statistically higher in the public laboratory (P < 0.05).

The most prevalent protozoon found in both laboratories was *Entamoeba coli*, which accounted for 5.6% and 9.4% of the cases in the private and public laboratories, respectively (P < 0.01). *Endolimax nana* ranked second in the private laboratory and third in the public laboratory, without a statistically significant difference between the groups (5.4% versus 3.5%; P = 0.07). In contrast, the prevalence of *Giardia lamblia* was higher in the public laboratory (0.9 versus 4.4%; P < 0.001), as also was the prevalence of *Entamoeba histolytica* or *Entamoeba dispar* (0.4 versus 2.2%; P < 0.001).

Polymerase chain reaction analysis on the samples that had been found to be positive through optical microscopy identified five cases of *Entamoeba histolytica* in the population studied, which was a prevalence of 0.08%. The proportion of cases due to *Entamoeba histolytica* in the *Entamoeba histolytica/Entamoeba dispar* complex was 15.0% (3/23) in the private laboratory and 15.4% (2/13) in the public laboratory, thus totaling 13.9% of the cases.

DISCUSSION

In this study, the prevalence of intestinal parasites was 15.0%, through optical microscopy. A similar rate was reported in a previous study in Belo Horizonte, and that study also highlighted high prevalence among members of the families of infected individuals.[21] Considering that a safe water supply was available in the region studied, other socioeconomic, behavioral and lifestyle characteristics may have played an important role in the transmission of intestinal parasites, such as consumption of uncooked food, low levels of personal hygiene and lack of usage of footwear.[6,21]

The proportion of the samples that was identified as infected at the public laboratory was almost twice what was identified at the private laboratory. Intestinal parasites are more common among low-income individuals with lower schooling levels.[22] This finding may therefore have been related to possible differences in the clientele between the two sites regarding socioeconomic conditions and inequalities of access to healthcare services.

In our study, most of the infections were produced by protozoa (13.5%). These results can be explained by the fact that the great majority of the constituents of the sampled group were adults, with an average age between 29 and 42 years. Helminth infections are more frequent in childhood[23] and may give rise to resistance to reinfection in adults. Another hypothesis explaining why most of the infection were protozoal is that massive screening for helminths and treatment with anthelmintic drugs have been implemented, as part of healthcare programs.[24] However, indiscriminate use of anthelmintic drugs is a matter of concern. This practice is rooted in popular culture in Brazil, regardless of the social or intellectual level of the population, and it has been reported in other studies.[12,25]

Among the 13.5% of the infections caused by protozoa, *Escherichia coli*, *Endolimax nana* and *Giardia lamblia* were the most common species, in accordance with previous studies.[21,25]

Table 1. Prevalence of intestinal parasites in Belo Horizonte, according to the type of laboratory (n = 6,289)

Parasite	Private laboratory (n = 5,695) n (%)	Public laboratory (n = 594) n (%)	Total n (%)	P-value
Blastocystis hominis	6 (0.1)	0 (0.0)	6 (0.1)	> 0.999*
Endolimax nana	305 (5.4)	21 (3.5)	326 (5.2)	0.071
Escherichia coli	321 (5.6)	56 (9.4)	377 (6.0)	0.000
Entamoeba histolytica/ Entamoeba dispar	23 (0.4)	13 (2.2)	36 (0.6)	0.000*
Giardia lamblia	52 (0.9)	26 (4.4)	78 (1.2)	< 0.000
Iodamoeba bütschlii	13 (0.2)	3 (0.5)	16 (0.3)	0.374*
E. hartmanni	-	0 (0.0)	0 (0.0)	-
Trichuris trichiura	1 (0.0)	3 (0.5)	4 (0.1)	0.006*
Ascaris lumbricoides	4 (0.1)	12 (2.0)	16 (0.3)	< 0.000*
Strongyloides stercoralis	13 (0.2)	6 (1.0)	19 (0.3)	0.012*
Hookworm	2 (0.0)	1 (0.2)	3 (0.0)	0.514
Cryptosporidium	-	0 (0.0)	0 (0.0)	-
Taenia	3 (0.1)	1 (0.2)	4 (0.1)	0.655*
Enterobius vermicularis	6 (0.1)	2 (0.3)	8 (0.1)	0.341*
Hymenolepis	1 (0.0)	1 (0.2)	2 (0.0)	0.360*
Schistosoma mansoni	10 (0.2)	3 (0.5)	13 (0.2)	0.235*

*Fisher's exact test; - = data not available.

However, the rates of infection in our study were smaller, probably due to differences in the population included. Only 4.2% of the protozoal infections were morphologically identified as *Entamoeba histolytica* or *Entamoeba dispar*. For the same route of infection, these results suggest that the dissemination efficiency of the other protozoa was better. The higher prevalence of the other protozoa may be related to the greater quantities and viability of their cysts, compared with those of *Entamoeba histolytica* or *Entamoeba dispar.*

The prevalence of the *Entamoeba histolytica/Entamoeba dispar* complex was 0.6% in the metropolitan region of Belo Horizonte. Studies conducted in Brazil have reported a range of prevalences of *Entamoeba histolytica* and *Entamoeba dispar* prevalence from 3.8%[4] to 46.3%,[14] depending on the study population, socioeconomic level and living conditions. In Minas Gerais, a prevalence of 4.4% was reported in urban slums,[21] and 14.3% among children and adolescents.[25] In an urban region in southeastern Brazil, the prevalence was 12.1%.[12] On the other hand, in regions with poor sanitation, parasites are endemic, and the prevalence of the infection is higher. In a recent study in northeastern Brazil, conducted in an urban slum, the prevalence of *Entamoeba histolytica* and *Entamoeba dispar* among children was 46.3%.[14]

Epidemiological studies have shown that cases of invasive amebiasis are caused by *Entamoeba histolytica* and that *Entamoeba dispar* is not found in intestinal or extraintestinal lesions. However, many people infected with *Entamoeba histolytica* are asymptomatic,[3,26] and host factors such as gene expression have a role in invasive amebiasis.[27] In an urban population of children under five, this type of infection was common and carried the risk of developing into severe cases of invasive amebiasis.[28] Simultaneous infections by both types of amoebae are often found in endemic areas, and people infected with *Entamoeba dispar* alone may manifest intestinal symptoms and high levels of anti-ameba antibodies, such that unnecessary treatment may occur.[29,30]

The polymerase chain reaction revealed that the prevalence of *Entamoeba histolytica* is very low, compared with that of *Entamoeba dispar*. Studies from different regions of the world have demonstrated that the frequency of *Entamoeba dispar* infection is higher, except for a few countries around the Pacific rim, where the frequency of infection by *Entamoeba histolytica* was higher.[11,28,31,32] Our data are in accordance with the findings from other studies conducted in Brazil, which also confirmed that the prevalence of *Entamoeba dispar* was higher.[4,11,14,15,30]

CONCLUSION

The prevalence of cases of parasitic infections in Belo Horizonte was 15.0%, and most of them were due to protozoa (13.5%). These were mainly *Escherichia coli* (6.0%), *Endolimax nana* (5.2%) and *Giardia lamblia* (1.2%). Only 0.6% of the samples were infected by *Entamoeba histolytica* or *Entamoeba dispar*, of which *Entamoeba histolytica* accounted for 13.9% of the cases. These results suggest that amebiasis may not be a problem in Belo Horizonte, and that clinicians should consider other causes of gastrointestinal disorders.

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Evidence for varicose vein treatment: an overview of systematic reviews

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Review [publication type].

ABSTRACT

BACKGROUND: Varicose veins affect nearly 30% of the world's population. This condition is a social problem and needs interventions to improve quality of life and reduce risks. Recently, new and less invasive methods for varicose vein treatment have emerged. There is a need to define the best treatment options and to reduce the risks and costs. Since there are cosmetic implications, treatments for which effectiveness remains unproven present risks to consumers and higher costs for stakeholders. These risks and costs justify conducting an overview of systematic reviews to summarize the evidence.

DESIGN AND SETTING: Overview of systematic reviews within the Discipline of Evidence-Based Health, at Universidade Federal de São Paulo (UNIFESP).

METHODS: Systematic reviews on clinical or surgical treatments for varicose veins were included, with no restrictions on language or publication date.

RESULTS: 51 reviews fulfilled the inclusion criteria. Outcomes and comparators were described, and a narrative review was conducted. Overall, there was no evidence that compression stockings should be recommended for patients as the initial treatment or after surgical interventions. There was low to moderate evidence that minimally invasive therapies (endovenous laser therapy, radiofrequency ablation or foam sclerotherapy) are as safe and effective as conventional surgery (ligation and stripping). Among these systematic reviews, only 18 were judged to present high quality.

CONCLUSIONS: There was evidence of low to moderate quality that minimally invasive treatments, including foam sclerotherapy, laser and radiofrequency therapy are comparable to conventional surgery, regarding effectiveness and safety for treatment of varicose veins.

INTRODUCTION

Varicose veins are enlarged and tortuous veins.[1] They are part of the chronic venous insufficiency syndrome[2] and are associated with complications such as edema, skin pigmentation, lower-limb ulcers, thrombophlebitis and bleeding.[3] This clinical variability has led to use of a classification system for chronic venous disorders (CEAP), as follows: C0 (no varicose veins); C1 (telangiectasias and reticular varicose veins up to 4 mm in diameter); C2 (trunk varicose veins); C3 (edema relating to varicose veins); C4 (skin pigmentation); C5 (healed venous ulcer); and C6 (active venous ulcer).[2] Eklöf revised the CEAP classification, including modification of the threshold for reticular varicose veins from 4 mm in diameter to a maximum of 3 mm.[4] However, there is no absolute consensus regarding the classification of varicose veins, which imposes limitations on comparisons of results between different studies.[5]

The prevalence of varicose veins reaches up to one-third of the Western population.[3] Prevalence rates vary due to different definitions in epidemiological studies, ranging from less than 1% to 73% among women, and from 2% to 56% among men.[6] In Brazil, the prevalence rate reaches around 50%, after excluding CEAP C1.[7,8] Lower-limb ulcers affect 1-2% of the world's population, and this has clinical and economic impacts.[8,9]

Treatment of varicose veins can be justified by its positive impact on quality of life.[3] The financial burden due to venous ulcers in the United States has been estimated to be 14.9 billion American dollars a year.[10] Moreover, because esthetic concerns impose a need for treatment, such concerns may lead to institution of ineffective and potentially harmful treatments. In Brazil, the cost of treatment increased four-hundredfold between 1995 and 2001.[8]

The high prevalence of this disease, the costs, the potential for complications attributed to its treatment and the need to disseminate science among stakeholders justify conducting a high-quality synthesis of systematic reviews on this topic, with the aim of mapping out the

current knowledge and identifying gaps in the literature to guide future sound research.

The primary objective of this study was to summarize evidence derived from systematic reviews focusing on interventions to treat varicose veins. In addition, the following secondary objectives were defined:

1. To describe comparisons applied in studies;
2. To verify outcomes chosen to evaluate treatment;
3. To assess the methodological quality of systematic reviews on the topic;
4. To describe the strength of evidence according to different outcomes.

METHODS

This study was an overview of systematic reviews, conducted within the Discipline of Evidence-Based Health, at the Federal University of São Paulo (Universidade Federal de São Paulo, UNIFESP).

The inclusion criterion for the systematic reviews was that they needed to focus on clinical or surgical interventions for lower-limb varicose veins, provided that the abstracts contained the terms systematic review and/or meta-analysis and that a full report was available. In cases of updates of the same review, only the most recent version was considered for inclusion. The following types of study were excluded: narrative reviews, conference proceedings, structured abstracts and systematic reviews focusing on the healing of lower limb ulcers without venous interventions.

A search strategy was run in the following databases: MEDLINE, EMBASE, LILACS and CENTRAL (last updated on September 3, 2017), applying the terms "varicose veins" or "varices" or "telangiectasias". Regarding the LILACS database, 286 references were retrieved using the term "varicose veins" and synonyms, thus dispensing with the need for filters. For all other databases, a filter that had been developed for retrieval of systematic reviews was used. There were no limitations regarding language or publication date. We conducted a hand search of references presented in the studies included in our review.

Two authors independently screened studies (RAO and ACPM), and any disagreements were resolved by a third author (RR), through use of Rayyan software.[11] Two independent authors conducted data extraction (RAO and ACPM), and disagreements were resolved by reaching a consensus.

The AMSTAR tool (assessment of multiple systematic reviews) was applied to assess the methodological quality of the systematic reviews included.[12] This tool encompasses 11 items for methodological evaluations, each scoring from 0 to 1. Studies with a total score of 0 to 4 were considered to present low methodological quality; 5 to 8, moderate quality; and 9 to 11, high quality.[13]

RESULTS

The search strategy yielded 1,245 studies. 107 studies were considered for inclusion after screening of titles and abstracts, with further retrieval of full texts. Among these, 51 reviews fulfilled the inclusion criteria (**Figure 1**).

The reviews included were combined into 13 distinct groups of interventions, which were described as follows:

1. **Clinical treatment of varicose veins:** Amsler and Blättler concluded that compression levels of 10 to 15 mmHg are effective in treating chronic venous insufficiency, despite the weakness of evidence due to heterogeneity across studies.[14] Two studies suggested that the effectiveness of compression stockings is overestimated, since adherence to treatment under real-world conditions is low, only reaching around 37% of the patients.[15,16] Thus, it was claimed that there was no high-quality evidence to support use of compression stockings as the initial type of treatment. Smyth et al. found that rutosides, reflexology and water immersion improved the symptoms in pregnant women with edema relating to varicose veins, although those findings were only based on a moderate level of evidence.[17] Boada and Nazco concluded that use of venotonics might alleviate the symptoms of fatigued legs. However, the quality of evidence was not assessed.[18]
2. **Techniques and complications relating to sclerotherapy:** Foam sclerotherapy is effective and safe, although the quality of studies has been considered to be low.[19] Cerebrovascular events associated with foam sclerotherapy are a rare but still a possible complication that has mostly been reported in the form of case reports.[20,21] These side effects seem to be mild, considering that it has been reported that the majority of patients were discharged from hospital without neurological sequelae. One study evaluated sclerosing agents to treat telangiectasias and concluded, based on very low-quality evidence, that one particular agent is not superior to another.[22]
3. **Liquid versus foam sclerotherapy:** Foam sclerotherapy increases the technical success rates (venous occlusion), in comparison with liquid sclerotherapy.[23] The quality of the evidence for this finding was not assessed in that report. Despite methodological limitations to evaluations on appropriate methods, dosages, formulations and compression levels, the current evidence supports the use of sclerotherapy in clinical practice.[24]
4. **Surgical techniques:** The CHIVA technique (ambulatory conservative hemodynamic correction of venous insufficiency) reduces disease recurrence in comparison with ligation and stripping and has been correlated with fewer adverse events.[25] These findings are based on a few studies with high risk of bias, because of the impossibility of blinding and the small number of incidents reported. Better esthetic results are achieved through use of transillumination, but with a higher number of hematomas and more intense pain in the postoperative period.[26] The quality of evidence for these findings was not assessed in that report. Studies with high risk of bias have suggested that use of tourniquets reduces bleeding.[27] Mumme et al. described

the valvuloplasty technique and concluded that it was suitable for preserving veins in specific patients who were at high risk of atherosclerotic disease. The quality of the evidence was not assessed.[28] Pearson et al. took the view that surgery should continue to be used to treat varicose veins in public healthcare systems, although without indicating the most cost-effective technique.[29] Due to the methodological limitations of the primary studies in that review, no meta-analysis was conducted. Rudström et al. assessed complications relating to the surgical approach and found that despite their infrequency, they were potentially harmful. The most common complication was bleeding after injury to the femoral vein or arterial lesions. The quality of the evidence was not appraised.[30]

5. **Surgery versus sclerotherapy:** There was no evidence that one treatment was superior to any other. However, it was suggested that sclerotherapy was associated with lower cost of treatment and better results after one year of follow-up.[31] Surgical outcomes are long-lasting, but it is unknown whether sclerotherapy outcomes also are. The overall quality of the studies included was considered low, mostly due to inadequate randomization. Complications relating to sclerotherapy were infrequent, but the data were deemed to be insufficient for conclusions to be drawn, and the methodological quality of the primary studies was considered low.[32]
6. **Surgery versus endolaser therapy (EVLT):** All studies concluded that EVLT was as safe as conventional surgery. Van den Bos et al.[33] and Darwood and Gough[34] found that rare but potentially harmful complications might be associated with EVLT treatment. The mild complications included ecchymosis, pain, superficial thrombophlebitis, nerve lesion, arteriovenous fistula and matting. The wavelengths applied in EVLT treatment ranged from 810 to 1320 nm, and these were associated with recanalization in 5% of the patients in the first year.[34] Liu et al.[35] and Pan et al.[36] concluded that the results from the two types of treatment were similar over a follow-up period of two years when fibers of 810 nm and 980 nm were used. The quality of the evidence was not appraised. Pan et al.[36] found that technical failure (saphenous reflux) was more frequent with EVLT, while Xiao et al.[37] concluded that there were no differences in the results from EVLT and conventional surgery. Risk of bias was assessed in this study, but not the quality of the evidence. Hoggan et al.[38] and Mundy et al.[39] came to contradictory conclusions, based on evidence that was of low quality because of ineffective randomization and losses during the follow-up.[38] Hoggan et al.[38] concluded that the rates of reflux resolution were comparable, and Mundy et al.[39] pointed out that EVLT was associated with higher rates of recanalization. Similarly, Lynch et al.[40] reported that there was a higher risk of

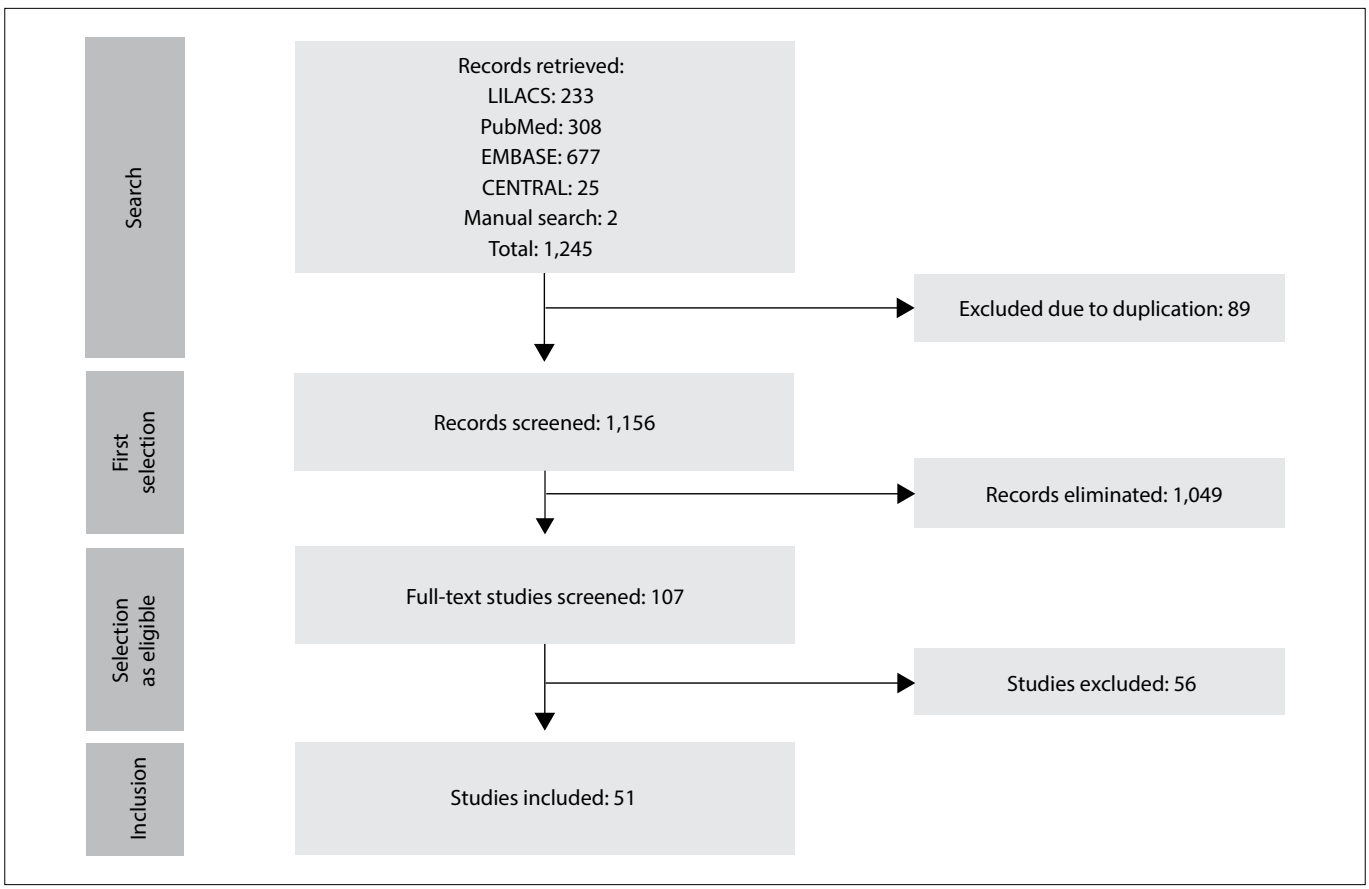

Figure 1. PRISMA flow chart for study selection process.

recanalization over a twelve-month period, although EVLT was less frequently associated with nerve lesions, infections and skin pigmentation. The findings of that study were based on low-quality evidence. Ruiz-Aragón et al.[41] also reported that there were fewer complications in the EVLT group, although it was assumed that a risk of bias existed due to exclusion of unpublished studies.

7. **Surgery versus radiofrequency ablation (RFA):** Radiofrequency ablation was found to be beneficial over the short term, due to lower risk of ecchymosis, hematoma and pain, a more positive impact on quality of life and faster return to work.[42] On the other hand, radiofrequency ablation increased the risk of recanalization after 12 months.[42] It was noteworthy that there was no reliable evidence supporting superiority of radiofrequency ablation over conventional surgery.[43] The rates of complications like deep venous thrombosis reached 1.8%, and recurrence remained to be clarified. Patient satisfaction and preference were found to favor surgery. In Canada, the costs of radiofrequency ablation were considered lower, based on evidence of low to moderate quality.[44]
8. **Surgery versus thermal ablation (EVLT or RFA):** Conventional surgery and thermal ablation were found to share comparable results over the long term,[45] with no difference in recurrent rates.[46] Compared with surgery, thermal ablation was considered safe and effective, with the advantage of being associated with faster recovery over the short and medium terms.[47] The quality of evidence was not appraised in any of these studies.
9. **EVLT versus RFA:** The outcomes were considered comparable over the short term[48] and over a longer term of five years.[49] He et al.[48] concluded that the quality of evidence to support these findings was low, while Balint et al.[49] did not appraise the quality of evidence.
10. **Surgery versus EVLT, RFA or foam sclerotherapy:** Van den Bos et al., Nesbitt et al., and Leopardi et al.[50-52] considered that minimally invasive techniques were as effective and safe as surgery. Thermal ablation was considered superior to surgery.[53] According to Murad et al.,[54] surgery and minimally invasive treatments were safe and effective, although minimally invasive procedures resulted in less disability and postoperative pain. Carrol et al.[55] concluded that alternative therapies were a possible substitute for surgery, and pointed out that foam sclerotherapy was probably more cost-effective. Paravastu et al.[56] found that the rate of recanalization of the small saphenous vein over the short term was higher in the conventional surgery group than in the EVLT group, and that the rate was uncertain for foam, compared with surgery. Overall, the quality of evidence either was considered low due to the small number of events and use of surrogate outcomes or was not appraised.
11. **Compression versus surgery for leg ulcers:** One author considered compression to be the first-line treatment for leg ulcers.[57]
12. **Surgery for leg ulcers:** Samuel et al.[58] did not identify any clinical trial. Mauck et al.[59] recommended surgery and considered that surgical treatment might improve healing. This finding was mostly based on observational studies. According to Howard et al.,[60] surgery was associated with rates of healing similar to those for compression alone, but presented lower levels of recurrence. The quality of evidence was not assessed.
13. **Any postoperative intervention:** Postoperative compression may reduce the extent of hematomas and incidence of thrombophlebitis in treatments for telangiectasias and reticular veins over a three-week period.[61] Conversely, Huang et al.[62] concluded that compressive therapy lasting for more than seven days was not associated with clinical benefits regarding pain, edema, complication rate and absenteeism. In two studies by El-Sheikha et al.,[63,64] no meta-analysis could be conducted because of substantial heterogeneity. Overall, the quality of evidence was either considered low or was not appraised.

The methodological quality of the systematic reviews described above was appraised through using the AMSTAR tool.[12] Out of these 51 reviews, 18 presented high methodological quality, 21 were of moderate quality and 12 were of low quality (**Annex 1**).[14-64]

Potential bias in conducting this overview

No study protocol was developed a priori for this analysis. However, we followed the goals and methods that were initially planned.

No additional search was conducted in the gray literature. However, we did conduct a hand search of references presented in the studies included in our review.

There may also be bias in relation to endolaser technology if studies using interventions at different stages of its development are compared.

DISCUSSION

This overview revealed heterogeneity in relation to many aspects of varicose disease, including terminology and classification. While some authors described varicose veins as enlarged veins of more than 3 mm in diameter,[4] others defined them as veins larger than 4 mm in diameter[2] or included telangiectasias and reticular veins within the definition.[5] There is still a need for standardization of terminology.[65]

Regarding prophylactic issues, Robertson et al.[66] did not find any good-quality studies that would enable assessment of whether lifestyle modifications might be useful as prophylaxis and for avoiding complications of varicose veins. Governments should prioritize topics like this when considering which studies to fund, since this issue may have practical implications at low cost, both for individuals and for healthcare systems.

Studies on surgical interventions frequently focus on ideal patients (with uncomplicated varicose veins of limited diameter,

Annex 1. Critical appraisal of studies included, through using the Assessment of Multiple Systematic Reviews (AMSTAR) tool.[12]

Review question	First author	AMSTAR											Total Score	Overall quality
		1	2	3	4	5	6	7	8	9	10	11		
Clinical treatment	Amsler[14]	0	U	0	U	1	1	0	1	0	0	1	**4**	L
	Palfreyman[15]	0	U	1	1	1	1	0	1	0	0	1	**6**	M
	Shingler[16]	1	1	1	1	1	1	1	1	1	NA	1	**10**	H
	Smyth[17]	1	1	1	1	1	1	1	1	1	NA	1	**10**	H
	Boada[18]	0	1	1	0	1	1	1	1	0	0	0	**6**	M
Techniques and complications relating to sclerotherapy	Rathbun[19]	0	1	1	0	1	0	0	0	0	0	1	**4**	L
	Sarvananthan[20]	0	1	1	1	1	1	1	1	1	NA	1	**9**	H
	Willenberg[21]	0	U	0	1	1	1	0	0	0	0	0	**3**	L
	Schwartz[22]	1	1	1	1	1	1	1	1	1	1	1	**11**	H
Foam versus liquid sclerotherapy	Hamel-Desnos[23]	0	U	1	1	1	1	0	0	0	0	0	**4**	L
	Tisi[24]	1	0	1	1	1	1	1	1	1	1	1	**10**	H
Surgical techniques	Bellmunt-Montoya[25]	1	1	1	1	1	1	1	1	1	NA	1	**10**	H
	Luebke[26]	0	0	1	U	1	0	1	0	1	0	0	**4**	L
	Rigby[27]	1	1	1	1	1	1	1	1	1	NA	1	**10**	H
	Mumme[28]	0	0	0	0	0	1	0	0	0	0	0	**1**	L
	Pearson[29]	0	U	1	U	0	1	1	1	1	0	0	**5**	M
	Rudström[30]	0	U	0	0	0	0	0	0	0	0	0	**0**	L
Surgery versus sclerotherapy	Rigby[31]	1	1	1	1	1	1	1	1	1	0	1	**10**	H
	Jia[32]	0	1	1	1	1	1	1	1	1	0	1	**9**	H
Surgery versus endolaser	Van Den Bos[33]	0	U	U	0	0	0	0	0	0	0	1	**1**	L
	Darwood[34]	0	U	0	0	1	1	0	0	0	0	1	**3**	L
	Liu[35]	0	1	1	1	1	1	1	1	1	0	0	**8**	M
	Pan[36]	0	U	1	1	1	1	1	0	1	0	0	**6**	M
	Xiao[37]	0	1	1	0	1	1	1	0	1	1	0	**7**	M
	Hoggan[38]	1	1	1	1	0	1	1	1	0	0	1	**8**	M
	Mundy[39]	0	1	1	1	1	0	1	1	0	0	0	**6**	M
	Lynch[40]	1	U	1	1	1	1	1	0	1	0	1	**8**	M
	Ruiz-Aragón[41]	0	1	1	1	1	1	1	1	0	1	1	**9**	L
Surgery versus radiofrequency	Luebke[42]	0	U	1	1	1	1	1	0	1	1	1	**8**	M
	Goodyear[43]	0	U	0	U	0	0	0	0	0	0	1	**1**	L
	Health Quality Ontario[44]	0	U	1	0	1	1	1	0	0	0	1	**5**	M
Surgery versus thermal ablation (laser and radiofrequency)	Xenos[45]	0	1	1	1	1	1	0	0	1	1	0	**7**	M
	O'Donnell[46]	0	1	1	1	1	1	0	0	0	0	1	**6**	M
	Brar[47]	0	0	1	0	1	1	1	0	1	0	0	**5**	M
Endolaser versus radiofrequency	He[48]	0	1	1	0	1	0	1	1	U	1	1	**7**	M
	Balint[49]	0	1	0	0	1	1	0	0	1	1	1	**6**	M
Surgery versus laser or radiofrequency or foam sclerotherapy	van den Bos[50]	0	1	1	0	1	0	1	0	0	0	1	**5**	M
	Nesbitt[51]	1	1	1	1	1	1	1	1	1	1	1	**11**	H
	Leopardi[52]	1	1	1	1	0	1	0	0	0	0	1	**6**	M
	Boersma[53]	0	1	1	0	1	1	1	0	1	0	1	**7**	M
	Murad[54]	1	1	1	1	1	1	1	1	1	0	1	**10**	H
	Carrol[55]	1	1	1	1	1	1	1	1	1	0	1	**10**	H
	Paravastu[56]	1	1	1	1	1	1	1	1	1	1	1	**11**	H
Compression versus surgery for lower-limb ulcers	de Carvalho[57]	0	U	1	0	1	1	0	0	0	0	1	**4**	L
Lower-limb ulcers	Samuel[58]	1	1	1	1	1	1	1	1	1	1	1	**11**	H
	Mauck[59]	0	1	1	1	1	1	1	1	1	1	1	**10**	H
	Howard[60]	0	U	1	1	1	1	0	0	0	0	1	**5**	M
Any postoperative intervention	Noppeney[61]	0	U	0	0	1	1	0	0	0	0	0	**1**	L
	Huang[62]	0	1	1	1	1	1	1	1	1	0	1	**9**	H
	El-Sheikha[63]	0	1	1	1	1	1	1	1	1	NA	1	**9**	H
	El-Sheikha[64]	0	1	1	1	1	1	0	0	0	0	1	**6**	M

H = high methodological quality; M = moderate methodological quality; L = low methodological quality; NA = not applicable; u = unclear.
Total score of 0 to 4 was considered to represent low methodological quality; 5 to 8, moderate quality; and 9 to 11, high quality.[12]

saphenous veins that are not very tortuous and absence of previous thrombophlebitis). In real life, patients present heterogeneous disease concomitantly in the same limb. Therefore, there is frequently a need to make use of a combination of techniques to achieve the best results,[31] based on the characteristics and clinical presentation of the varicose veins.[52] It is crucial to establish criteria for choosing the most suitable technique for different clinical scenarios.[45]

Sclerotherapy is currently considered to be the first-line treatment for telangiectasias. Other therapies have been proposed as alternatives, but evidence to justify their choice is sparse and indirect.[16,52,55] In fact, surrogate outcomes are frequently reported in trials. Thus, conclusions are based solely on technical parameters[38,67] for heterogeneous populations[68] with short follow-ups,[54] which serves to increase the uncertainties rather than to resolve them.

Ligation and stripping are frequently chosen as the comparator because of their safety, effectiveness, cost issues and time span, and these have been used as a gold standard.[55] The complications associated with surgery include nerve lesions, hematomas, postoperative pain and pigmentation. However, severe complications are rare.[30]

Minimally invasive treatments have been developed with the aim of reducing the risks and discomfort, as well as for reducing the time taken to return to work and optimizing cost-effectiveness. Their efficacy and effectiveness are comparable to those obtained through conventional surgery, regardless of the parameters chosen for this comparison. Minimally invasive therapies or surgery cannot always be applied to particular patients.[60]

However, foam sclerotherapy seems to be particularly useful in this context since it can be used alone or in combination with other interventions. For instance, it may improve the results after surgery, bearing in mind that no surgical technique is capable of eliminating all varicose veins. The limitations associated with foam sclerotherapy include higher risk of recanalization and pigmentation,[56] along with the need for multiple sessions in order to obtain satisfactory results. These restrictions are surpassed by the benefits regarding cost-effectiveness.[55] We therefore considered it odd that we did not find any studies focusing on foam sclerotherapy for leg ulcers. Since fibrotic tissue may prevent the possibility of stripping some varicose veins, which consequently could maintain the pathological condition and hence the ulcers, foam sclerotherapy might potentially be a better treatment for this population.

There is no evidence that compressive stockings might bring benefits for patients with primary varicose veins.[15,16] Questions arise regarding the technical attributes of stockings (the type of elastic material and level of compression), the anatomical characteristics of the lower limbs and patients' mobility while using these stockings.[69] Furthermore, there is low compliance due to discomfort, pruritus, skin irritation and edema.[70,71] Adherence to compressive treatment over a four-week period is as low as 40%,[70] thus compromising the accuracy of any estimates of treatment effect.[63] To date, the causal relationship between symptoms and varicose veins remains uncertain.[72] These factors may lead to many unnecessary treatments. On the positive side, stockings can be used to reduce the incidence of hematomas and thrombophlebitis[61] and leg ulcers,[57] thereby reducing the time taken for healing[73] and the recurrence rate. However, it is logical to claim that the best intervention should aim to treat the primary cause of leg ulcers. It has been found that surgery is just as effective in healing leg ulcers as are compression stockings, and it additionally reduces the recurrence rate.[60] This should always be considered in cases of leg ulcers that are associated with varicose disease.[74] Even though use of stockings in the postoperative period has been recommended by some authors,[63] the effectiveness of this intervention was not found to be superior over the short term (seven days) or medium term (three weeks).[62]

Regarding the implications for practice of our analysis, the important question to be formulated is how much longer should be waited before the paradigms for varicose vein treatment are changed.[75] This question remains to be answered, considering the current body of literature. According to Chalmers and Glasziou,[76] gaps in knowledge occur when study questions are not well formulated, studies are not well designed, studies are not published, or there is still a lack of data on a particular topic. Surgery seems to be the most frequent intervention for varicose vein disease in many countries, but new endovascular techniques may provide an alternative for reducing costs and risks. Nonetheless, the studies underpinning these observations have presented serious limitations that have had a negative impact on the strength of the derived evidence, due to the indirectness, low number of events and small sample sizes of these studies.

CONCLUSIONS

There is evidence of low to moderate quality to suggest that minimally invasive treatments, including foam sclerotherapy, laser and radiofrequency are comparable to conventional surgery, regarding their effectiveness and safety in treating lower-limb varicose veins.

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Self-reported assessment of female sexual function among Brazilian undergraduate healthcare students: a cross-sectional study (survey)

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ABSTRACT

BACKGROUND: The present study aimed to evaluate female sexual function among young undergraduate women.

DESIGN AND SETTING: Cross-sectional survey conducted among Brazilian undergraduate students.

METHODS: This study used online questionnaires to assess sociodemographic and health-related data and used the Brazilian version of the Female Sexual Function Index (FSFI) among female undergraduate students aged 18 to 25 years who were regularly enrolled in undergraduate healthcare courses. The FSFI is composed of 19 items that measure female sexual function over the last four weeks, in six domains: desire and subjective stimulation, sexual arousal, lubrication, orgasm, satisfaction and pain or discomfort.

RESULTS: Among the 149 female undergraduate students evaluated, 43 (28.8%) presented sexual dysfunction (score < 26.55). Health conditions were not associated with female sexual dysfunction. Among the women with sexual dysfunction, all domains of the sexual response cycle were affected ($P < 0.001$).

CONCLUSIONS: Sexual dysfunction was identified in at least a quarter of these young undergraduate women and it was not associated with gynecological problems, menstrual cycles, dysmenorrhea, contraceptive use or physical activity.

INTRODUCTION

Female sexuality was historically treated as taboo in some cultures and was deemed to be restricted to procreation and distant from pleasure. Today, women's sexuality is considered to be an integral part of their sexual rights and quality of life that is important not only for reproduction but also for longevity of their affective and pleasurable relationships, as well as being part of their health and wellbeing.[1]

Sexual function and dysfunction present multifactorial characteristics that lead to a range of psychological, interpersonal, sociocultural and neurobiological factors.[2] Female sexual dysfunction encompasses a wide variety of clinical conditions, including hypoactive sexual desire, sexual aversion disorder, sexual arousal disorder, orgasmic disorder and painful disorders such as dyspareunia and vaginismus.[3]

Although female sexual response has not been completely elucidated, it is known that female sexual function involves somatic, psychosocial and neurobiological factors.[4] Any disturbance or change in sexual function, such as pain and discomfort during sexual intercourse, can compromise women's wellbeing and quality of life.

The World Health Organization (WHO) recognizes female sexual dysfunction as a public health problem and recommends that it should be investigated in the event of important changes in quality of life.[5] Impaired female sexual function (problems with sexual desire, arousal, orgasm and sexual pain) causes high levels of personal or interpersonal distress.[6]

The majority of Brazilian studies on female sexual function have investigated this among women who had some disease or were in a specific reproductive period, such as pregnancy or the menopause.[7] Studies that evaluate female sexual function among Brazilian students are scarce, but it is known that the prevalence of sexual dysfunction increases with age and multiparity and after the menopause.[3]

There are differences in female sexual function relating to the demographic variables of different countries and between individuals, such that these affect individuals' behavior and the sexual practices that they adopt. Thus, the prevalence of sexual dysfunction varies. Therefore, evaluations on sexuality should not be generalized for the entire female population but should have a specific focus for each population studied.[5,8] It is important to know about the different female sexual responses within different populations, like young undergraduate women.

Hence, because of the scarcity of research addressing groups of young women in Brazil, the aim of the present study was to evaluate female sexual function among young undergraduate women.

METHODS

Study design, date, setting and ethical issues

This was a cross-sectional survey conducted on the Baixada Santista campus of the Federal University of São Paulo (Universidade Federal de São Paulo), in Santos, state of São Paulo, between August and December 2012. This study was approved by the Ethics Committee for Human Research (under number 32,649/2012) and all the participants provided written informed consent. The "STrengthening the Reporting of OBservational studies in Epidemiology" (STROBE) statement was used for reporting the study.

Participants

We included female undergraduate students aged 18 to 25 years who were regularly enrolled in some of the undergraduate healthcare courses (physiotherapy, occupational therapy, physical education, psychology, nutrition and social service focusing on healthcare interdisciplinarity) on the Baixada Santista campus, in Santos, were personally contacted and invited to participate in the study. Those who agreed to participate answered the questionnaires online.

We excluded women who had not had sexual intercourse within the previous four weeks, since this is a criterion for responses that are used to form the Female Sexual Function Index (FSFI). We also excluded women who had had children or who were pregnant because these conditions may interfere with sexual function. In addition, women who had never had sexual intercourse were excluded because experience of sexual intercourse is a condition for answering the FSFI questionnaire[5].

A recent study revealed that almost 40% of undergraduate students were at risk of female sexual dysfunction.[15] This frequency for the primary outcome was therefore taken as an assumption. The sample size for this study was calculated considering a 95% confidence level and a sample error of 10%. Thus, it was found that the sample size needed to be 93 women.

Data collection, variables and analysis

An online questionnaire was sent by e-mail to women with an interest in participating in the study. The information collected through this questionnaire comprised age, undergraduate course, age at menarche, gynecological problems, information on the menstrual cycle (regular or irregular), dysmenorrhea, contraceptives, age at first sexual intercourse and physical activity. The level of physical activity was classified as follows: sedentary (does not perform any physical activity for at least 10 continuous minutes during the week); irregularly active (performs physical activity, but insufficient to be classified as active, as this does not comply with the recommendations regarding frequency or duration); active (vigorous activity ≥ 3 days/week lasting ≥ 20 minutes/session, or moderate activity/walking ≥ 5 days/week lasting ≥ 30 minutes/session, or any added activity ≥ 5 days/week lasting ≥ 150 minutes/week); or very active (vigorous activity ≥ 5 days/week lasting ≥ 30 minutes/session, or vigorous activity ≥ 3 days/week lasting ≥ 20 minutes/session + moderate activity/walking ≥ 3 days/week lasting ≥ 30 minutes/session).[9]

Every participant also answered the online Brazilian version of the Female Sexual Function Index (FSFI) questionnaire, which had been adapted and validated for the purpose of assessing female sexual function.[5,10-12] This is a simple and objective questionnaire composed of 19 items that measure female sexual function over the last four weeks. The FSFI is the only index addressing affective, emotional and psychosocial issues and it includes the following six domains of female sexual response: desire and subjective stimulation, sexual arousal, lubrication, orgasm, satisfaction and pain or discomfort. In addition, this instrument has gone through a process of verification of its trustworthiness and reliability as an online version.[12]

The results from the questions that comprised each domain were multiplied by the factor for that domain. The scores from each domain were summed, resulting in the final FSFI score. The final score was obtained by summing the weighted scores of each domain. The final scores could range from 2 to 36, and higher scores represented better female sexual function. As previously established in the literature,[13] women with scores less than or equal to 26.55 were considered to have some sexual dysfunction.[5,8,10-12,14]

Statistical analysis

The participants' characteristics were analyzed using descriptive statistics. The chi-square test was used to verify whether there were any associations between presence of sexual dysfunction and the variables of interest. The Mann-Whitney test was used to verify whether there was any difference between women with sexual dysfunction and women without sexual dysfunction, in relation to the variables. The significance level used for every comparison was 0.05 ($P \leq 0.05$).

RESULTS

In total, 230 female undergraduate students were invited to participate, and 180 (78.2%) of these students answered the questionnaires online. Thirty-one were excluded because they had not had sexual intercourse within the last four weeks (**Figure 1**).[5] Thus, 149 undergraduate students (**Figure 1**) from the following healthcare courses were included: physiotherapy, occupational therapy, physical education, psychology, nutrition and social service focusing on healthcare interdisciplinarity. The participants' average age was 21 years (± 1.68) (**Table 1**) and, among them, 43 (28.8%) were categorized as having dysfunction because they presented a Female Sexual Function Index of less than 26.55.

The women with sexual dysfunction did not differ from the women without sexual dysfunction in relation to gynecological problems, menstrual cycles, contraceptives, dysmenorrhea or physical activity (**Table 2**). The age at the menarche was on average 12.13 years (± 1.14) for the women with sexual dysfunction and 12.17 years (± 1.55) for the women without sexual dysfunction (P = 0.922). In relation to the age at the first sexual intercourse, there was also no significant difference between the women with sexual dysfunction (17.02 ± 1.72 years) and those without sexual dysfunction (17.33 ± 2.06 years) (P = 0.485).

The total FSFI score (**Figure 2**) and the scores for each domain of the FSFI were compared among the women, both with and without sexual dysfunction. Among those with sexual dysfunction, it could be seen that all the FSFI domains were affected (**Table 3**).

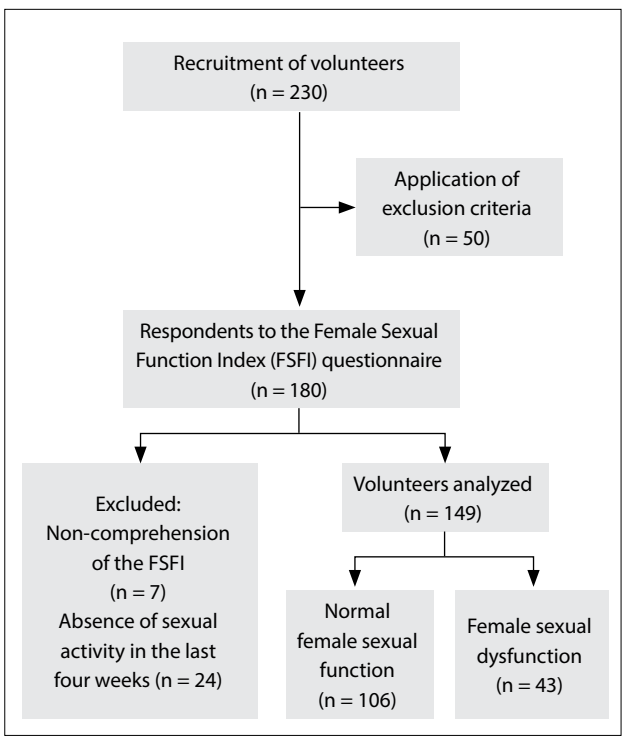

Figure 1. Recruitment and distribution of volunteers.

DISCUSSION

Sexual dysfunction was found in 28.8% of the undergraduate students who participated in this study, with a mean age of 20.9 years old. Moreover, every domain was affected among the women with sexual dysfunction, i.e. orgasm, desire, arousal, pain, lubrication and satisfaction.

Although there was an increase in interest in publishing data on female sexual function in Brazil between 2013 and 2015,[7] gaps in the literature still exist,[3] especially with regard to young women. A recent Brazilian systematic review[7] pointed out that few articles had good methodology and used validated questionnaires to assess

Table 1. Distribution of the number of volunteers according to course and undergraduate level

Characteristics	n	%
Undergraduate course		
Physiotherapy	25	16.78
Occupational therapy	25	16.78
Physical education	20	13.42
Social service focusing on healthcare interdisciplinarity	27	18.12
Psychology	26	17.45
Nutrition	26	17.45
Undergraduate level		
1st year	22	14.77
2nd year	31	20.81
3rd year	41	27.52
4th year	51	34.23
5th year	4	2.68

Table 2. Health conditions among women with and without sexual dysfunction

	With sexual dysfunction		Without sexual dysfunction		P*
	n	%	n	%	
Gynecological problems					
Present	5	11.63	15	14.15	0.68
Absent	38	88.37	91	85.85	
Menstrual cycle					
Regular	36	83.72	86	81.13	0.71
Irregular	7	16.28	20	18.87	
Dysmenorrhea					
Present	27	62.79	67	63.20	0.96
Absent	16	37.21	39	36.80	
Contraceptives					
Yes	33	76.74	73	68.87	0.34
No	10	23.26	33	31.13	
Physical activity					
Sedentary	5	11.63	20	18.87	0.26
Irregularly active	9	20.93	32	30.19	
Active	21	48.84	43	40.57	
Very active	8	18.60	11	10.38	

n = sample number; % = frequency (percentage); *chi-square test with significance level of P < 0.05.

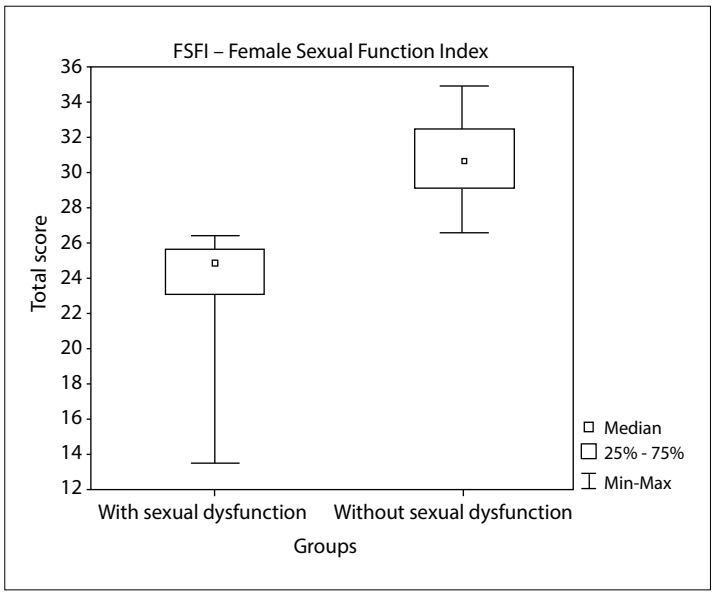

Figure 2. Comparison of the categories of with and without sexual dysfunction through the Brazilian version of the Female Sexual Function Index (FSFI) (P ≤ 0.001; Mann-Whitney test).

Table 3. Domains of Female Sexual Function Index among women with and without sexual dysfunction

Domains of Female Sexual Function Index	With sexual dysfunction		Without sexual dysfunction		P*
	Median	Interquartile range	Median	Interquartile range	
Desire	3.6	3-3.6	4.2	3.6-4.8	< 0.001
Arousal	4.2	3.6-4.5	5.4	5.1-5.7	< 0.001
Lubrication	4.8	3.9-5.1	5.4	5.1-6	< 0.001
Orgasm	3.6	2.8-4.4	5.2	4.4-5.6	< 0.001
Satisfaction	4.8	3.6-5.2	5.6	5.2-6	< 0.001
Pain	4.4	3.6-5.4	5.2	4.8-6	< 0.001
Total score	24.9	23.3-25.6	30.7	29.2-32.5	< 0.001

*Mann-Whitney test with significance level of P < 0.05.

sexual dysfunction among women. Among the articles included in that systematic review, the majority related to female sexual function among patients with some type of disease or investigated sexual function at specific reproductive periods, such as during pregnancy or at the menopause.

Young women, such as undergraduate students, are often considered healthy and do not show any impairment of their sexual health. Taking into account that the age at the first sexual intercourse in the present study was on average 17 years, the women included in this survey were at the beginning of their sexual life. Sexual dysfunction during this period affects the quality of life of young and healthy women.

In a recent study[15] involving female medical students in German-speaking countries with a mean age of 23.5 years, it was revealed that almost 40% were at risk of female sexual dysfunction. It was demonstrated that being in a steady relationship, having better physical fitness, being more active at work and having greater subjective positive self-acceptance were associated with higher total scores in the Female Sexual Function Index. Thus, individuals with these characteristics were at lower risk of presenting female sexual dysfunction.

Considering that the prevalence of sexual dysfunction increases with age and parity and after the menopause, investigation of the quality of sexual health among undergraduate students is necessary in order to support preventive action, health promotion and functional recuperation designed specifically for young Brazilian women.

The literature indicates that lubrication dysfunction is more often observed during the climacteric period, due to the various hormonal changes that occur in this period.[15] Another important point to be emphasized is that absence of sexual dysfunction does not necessarily imply sexual satisfaction.[2]

Regarding the health conditions investigated among the volunteers of the present study, it seems that there were no differences between the women with sexual dysfunction and the women without sexual dysfunction. However, further studies should be conducted to investigate the association of sexual dysfunction with health conditions throughout female sexual life. Recently, some studies have demonstrated the existence of associations between sexual dysfunction and other health conditions such as pubic pain,[17] type II diabetes[18] and conditions subsequent to conventional abdominal hysterectomy.[19]

Another point to be discussed is that the present study assessed self-reported sexual function among young women through the Female Sexual Function Index. This questionnaire is quick and easy to apply and has high reliability for diagnosing the presence or absence of sexual dysfunction subjectively, without presenting the causes of this disorder. Thus, it would be of interest to carry out a physical evaluation in order to complement the Female Sexual Function Index results and to identify possible physical causes of sexual dysfunction.

Unfortunately, there is a scarcity of studies evaluating the presence of sexual dysfunction in populations of young and healthy women in Brazil. Most studies in the literature relate to women in the climacteric or gestational period, or to women who presented some form of disorder or chronic disease.[7,16,20-23]

Higgins et al.[24] investigated the physiological and psychological satisfaction with sexual life among American undergraduate students. They found that several of the same individual, relationship and cultural-level factors correlated with sexual satisfaction among adults, regardless of gender. However, they highlighted some differences between the genders. For example, men were twice as

likely as women to report that they always or almost always experienced an orgasm during sexual intercourse.

Brazilian conservative society still sees female orgasm as unnecessary while male orgasm is synonymous with virility. In this regard, many women no longer resort to healthcare services in order to improve their sexual health because of the belief that lack of orgasmic experiences is inherent to the female organism.

The present study was not intended to differentiate women according to sexual orientation. Perhaps this is a limitation of the study, since the Brazilian version of the Female Sexual Function Index does not report the partner's sex as an inclusion criterion.[5,10-12] Therefore, new studies should be designed to provide validated instruments capable of assessing female sexual function regardless of sexual orientation.

Treatments for sexual dysfunction are essentially transdisciplinary and include not only collaboration among multidisciplinary professionals but also application of collaborative practice to ensure the highest level of provision of care.[2] For the physical aspects of sexual dysfunction, physiotherapy may be beneficial.

Among the possibilities for physiotherapeutic intervention, studies have shown the effectiveness of raising awareness of the pelvic floor muscles and strengthening them, which alters female sexual life positively. Furthermore, some studies have shown that awareness and proprioception of the musculature improve self-image, receptivity towards sexual activity and sexual performance.[25] Use of electrostimulation with biofeedback has also proved to be an effective technique for treating sexual dysfunctions, through increased learning and control of pelvic floor muscle contraction. This, together with regard for the contraction force provided by biofeedback, aids in improvement of performance and reduces pain.[26]

Thus, early diagnosing of female sexual dysfunction in young women, along with transdisciplinary intervention, may contribute towards improvement of sexual practice and quality of life. This approach may provide new possibilities for treatment for this younger population.

CONCLUSION

Sexual dysfunction was identified in at least a quarter of the young undergraduate women surveyed. It was not associated with gynecological problems, menstrual cycles, dysmenorrhea, contraceptive use or physical activity.

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Relationship between premenstrual syndrome and basic personality traits: a cross-sectional study

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Premenstrual syndrome.
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ABSTRACT

BACKGROUND: Although many studies have investigated premenstrual syndrome and related factors, there is still only a limited number of studies investigating the relationship between premenstrual syndrome and basic personality traits. This study was conducted to investigate the association between premenstrual syndrome and basic personality traits among university students.

DESIGN AND SETTING: Cross-sectional analytical study conducted in a city in western Turkey.

METHODS: The Premenstrual Syndrome Scale, the Basic Personality Traits Scale and a questionnaire on sociodemographic characteristics developed by the present researchers were applied to 490 female students at the College of Health Sciences of a state university.

RESULTS: Premenstrual syndrome was more common among students living in rural areas (65.1%), students with chronic diseases (74.1%), students who suffered from menstrual cramps (61.1%), students who used cigarettes (72.1%) and students with alcohol intake (65.5%). In the final model of the logistic regression analysis, presence of pain during the menstrual period increased the risk of presence of PMS by a factor of 1.554 (95% confidence interval, CI: 1.033-2.336; P = 0.034) and high scores on the total basic personality traits scale increased it by a factor of 1.016 (95% CI: 1.002-1.030; P = 0.029). The prevalence of premenstrual syndrome was found to be higher among students who were less extrovert (P = 0.007) and less conscientious (P = 0.001); and among students with higher neuroticism (P = 0.000) and negative valance (P = 0.000).

CONCLUSION: This study demonstrates that personality may be associated with premenstrual syndrome.

INTRODUCTION

The term premenstrual syndrome (PMS) is defined as a set of cognitive and emotional symptoms that start in the luteal phase of the menstrual cycle and swiftly disappear with the start of menstruation. Biological, psychological, physical and social factors have an impact on occurrences of PMS.[1-3] Premenstrual symptoms are considered to be affected by increased intensity of menstrual cramps, neurotic personality, increased body mass index, general self-perceived health and cultural differences.[4,5]

Personality traits play an important role in self-perceived health. Many health problems seen in adolescents have been found to be related to low self-esteem. Adolescents with low self-esteem were found to expect failure, be generally nervous, show less effort towards being successful, ignore important things in life and feel worthless and untalented when they failed.[6] Personality traits can affect the way in which people perceive daily events. It is known that some stimuli triggered by daily events also trigger PMS in women with sensitive personalities.[7,8]

However, the literature indicates that the findings from research on the relationship between premenstrual symptoms and some personality traits are complicated.[9] Gaion and Vieira[10] demonstrated that individuals with PMS displayed a strong need for performance but a low need for assistance, and also displayed introversion and low desire for change. On the other hand, individuals without PMS showed strong needs for denial and assistance, along with dominance and persistence. Sassoon et al.[11] found greater percentages of personality disorders (especially obsessive-compulsive personality disorder) among women with severe PMS than among women without PMS. The findings in the literature have indicated that neuroticism and agreeableness were positively related to PMS, while extroversion, conscientiousness, openness to experience and family communication were negatively related to PMS.[12,13] In an Iranian study, it was reported that one third of the women who had PMS presented borderline personality traits. Furthermore, the

most significant positive correlations were found between PMS and three personality types; neurotic, emotionally stable and introvert.[14]

In the literature, although many studies have investigated PMS frequency, symptoms and related factors,[1,2,4,14,15-21] there is still only a limited number of studies investigating the relationship between PMS and basic personality traits. Personality traits have an important role in coping with health problems. The directions (healthcare applications) needed will affect individuals positively in terms of quality of life. Moreover, no study in Turkey has yet investigated the relationship between PMS and personality traits. Increasing the level of awareness among young women studying nursing will be important, to enable them to provide relevant information to their peers with PMS complaints and provide support through positive coping methods.

The present study was conducted to investigate and define the factors affecting PMS and the relationship between PMS and basic personality traits, among students at the College of Health Sciences of a state university in Turkey.

METHODS

Study design, date, setting and ethical concerns

This cross-sectional study (survey) was conducted during the 2015-2016 spring term in the Nursing, Midwifery, Nutrition and Dietetics Departments of the College of Health Sciences, Aydin, Turkey. The study was approved by the Ethics Committee of Adnan Menderes University, Aydin (protocol no. 2015/98).

Participants

Out of the total of 716 female students in the college, 490 students participated: these were students who met the inclusion criteria and who were at school during the study period. No random sampling method was used. Students who did not have any diagnosed psychiatric disorder, declared that they had regular menstrual periods (every 22-35 days), did not have any diagnosed somatic diseases or gynecological or hormonal disorders and were not using any medication or contraceptive pills were included in the study. At the time of enrolling in health-related departments at universities in Turkey, students are required to present a health status document. Therefore, the students' on-file records were compared with their verbal declarations, and students who were eligible to participate in accordance with the inclusion criteria were checked especially regarding their health status.

Data collection

The data for this study were collected using a sociodemographic questionnaire and two scales: the Premenstrual Syndrome Scale and the Basic Personality Traits Scale.[22,23]

The questionnaire consisted of 31 questions that aimed to identify the students' sociodemographic characteristics and some attributes associated with the menstruation period (age at menarche in years, current menstrual status and menstrual cycle length in days), menstrual problems and management strategies for menstrual problems. The questions were developed based on the literature.[1,5,15,16]

The Premenstrual Syndrome Scale consists of 44 items and has nine sub-dimensions: depressive sensation, anxiety, fatigue, nervousness, depressive thoughts, pain, appetite changes, sleep pattern changes and bloating. The minimum score on the scale is 44 and the highest score is 220. High scores indicate that PMS symptoms are intense. In evaluating the findings from the scale, PMS is considered "present" if the total score exceeds 50% of the maximum score. The Cronbach's alpha value of the scale has been calculated as 0.75.[22]

The Basic Personality Traits Scale, which was developed by Gençöz and Öncül,[23] consists of 45 items that are each assessed using a five-point Likert scale. It evaluates six basic personality traits: extroversion, conscientiousness, agreeableness, neuroticism, openness to experience and negative valence. **Extroversion** is assessed using eight adjectives including "timid, withdrawn, shy, talkative, lethargic, enterprising, cold and passive"; **conscientiousness** is assessed using eight adjectives including "self-disciplined, tidy, hard-working, prudent, fussy, determined, irresponsible and lazy"; **agreeableness** is assessed using eight adjectives including "sincere, compassionate, genial, well intentioned, philanthropic, tolerant, sharer and sensitive"; **neuroticism** is assessed using nine adjectives including "nervous, aggressive, angry, temperamental, impatient, capricious, impetuous, touchy and worried"; **openness to experience** is assessed using six adjectives including "self-confident, self-assured, brave, creative, easy going and capable"; and **negative valence** is assessed using six adjectives including "ill-mannered, pretentious, rude, backstabbing, greedy and hidebound". Higher scores indicate that the person has that personality trait. The Cronbach's alpha coefficients of the sub-dimensions were as follows: 0.85, 0.74, 0.83, 0.81, 0.65 and 0.69.

Data analysis

The Statistical Package for the Social Sciences (SPSS) 17.0 for Windows was used for statistical analysis on the data. Descriptive statistics from the study were shown with percentage, mean and standard deviation values. Student's t test was used for intergroup comparisons of continuous variables and chi-square statistics were used to compare nonparametric categorical variables. Logistical regression analysis was used to determine the possible risk factors that could affect premenstrual syndrome. In this analysis, absence and presence of PMS were taken to be dependent variables, while residence, chronic disease, pain during menstrual period, alcohol consumption, smoking and basic personality traits score were taken to be independent variables. The

results from the regression analysis were showed as relative risk (odds ratio, OR) and 95% confidence interval (CI). The backward Wald method was used as the regression model.

RESULTS

The mean age of the participants was 20.20 ± 1.65 years; 96.6% of the students were single and 67.8% had an income equal to their expenditure. Out of the 490 students included in the study, 26.3% of them lived in rural areas; 48% of their mothers and 33.1% of their fathers were primary school graduates.

53.5% of the students lived in dormitories and 36.7% lived in shared apartments during their education. 11% had chronic diseases other than gynecological problems and 6.3% were continuously using medications. The age at the menarche was found to be 13.2 ± 1.22 years (minimum of 9 years and maximum of 17 years) and the average length of menstruation was 5.4 ± 1.32 days. 71.8% of the students reported that they had menstrual cramps. While 75.5% of the participants viewed menstruation as a natural process of the body, 16.7% of them considered it to be a way for the body to remove dirty blood. 12.7% of the students smoked and 10.8% of them used alcohol. They learned their first information about menstruation from their mothers. The foodstuff that was most consumed among the students before and during PMS was sweets/deserts (79.6%).

The most common symptom among the students that they experienced one week before menstruation was stress-uneasiness, with a rate of 80.6%. The other more common symptoms were breast fullness or swelling, headaches, bloating in the abdomen or "bloating" related to tightness in shoes, clothes or rings or feelings of weight intake, with a rate of 77.3%; and tiredness, lethargy and loss of energy, with a rate of 72.2% (**Table 1**).

Table 1. Symptoms experienced by students one week before menstruation

	n	%
Stress and uneasiness for an unknown reason	395	80.6
Breast fullness or swelling, headache, bloating in the abdomen or "bloating" or feelings of increased weight, experienced with tightness in shoes, clothes or rings	379	77.3
Tiredness, lethargy or loss of energy	354	72.2
Appetite changes manifested through overeating or craving for certain foods	329	67.1
Continuous uneasiness, feelings of anger and increased interpersonal conflicts	309	63.1
Feeling sad, hopeless or worthless	254	51.8
Feeling frustrated or lacking control	220	44.9
Hypersomnia or insomnia	213	43.5
Difficulty in focusing	200	40.8
Frequent desire to cry or bouts of crying	199	40.6
Avoidance of social relations and reduced interest in ordinary events	199	40.6

PMS was seen in 57.1% of the students in this study. No statistically significant relationship was found between occurrences of PMS and income level, parents' education level, continuous medication use, consumption of coffee, tea and cola drinks, use of oral contraceptives and pain experienced by their mothers or sisters during the premenstrual period. PMS was found to be more common among students who lived in rural areas (χ^2 = 4.546; P = 0.033); those who had chronic diseases other than gynecological diseases (χ^2 = 7.104; P = 0.008); those who had menstrual cramps (χ^2 = 7.909; P = 0.005); those who smoked (χ^2 = 9.520; P = 0.002); and those who consumed alcohol (χ^2 = 4.001; P = 0.045) (**Table 2**).

The prevalence of PMS was found to be higher among the students whose characteristics of extroversion and conscientiousness were of lesser degree (t = 2.701; P = 0.007; t = 3.208; P = 0.001 respectively), and whose neuroticism and negative valence were higher (t = -8.488; P = 0.000; t = -3.607; P = 0.000 respectively). Among the students' basic personality traits, no relationship was found between agreeableness and openness to experience in terms of the prevalence of PMS (**Table 3**).

In the final model of the logistic regression analysis, it was found that presence of pain during the menstrual period and the basic personality traits score both affected the presence of PMS. Pain during the menstrual period increased the risk of presence of PMS by a factor of 1.554 (95% CI: 1.033-2.336; P = 0.034) and high scores on the total basic personality traits scale increased the risk of presence of PMS by a factor of 1.016 (95% CI: 1.002-1.030; P = 0.029).

The most common methods that students used to cope with menstrual cramps included the following: analgesics (36.7%), keeping the abdomen warm (35.5%) and drinking herbal teas (27.3%). These were followed by taking a hot shower (19.6%), lack of continuity at school (16.3%), using intravenous analgesics (10.6%), exercise (9.2%) and going to the emergency department (6.9%).

DISCUSSION

The findings from this study showed that PMS was seen in 57.1% of the students and that PMS was more prevalent among students who were less extrovert and less conscientious and who had higher neuroticism and negative valance. In this study, the most common complaints that the students had during the premenstrual period were stress, uneasiness, "bloating" or feelings of gaining weight and fatigue or loss of energy. Like in our study, similar complaints such as bloating, fatigue, pain, etc.[1,24] have been reported in many other studies. Since these complaints affect students' social lives, family relations and success at school negatively, it is important to increase awareness of these issues among adolescents and inform them about methods for coping with these complaints.

Table 2. Factors affecting occurrences of premenstrual syndrome

Student characteristics	Premenstrual syndrome		No premenstrual syndrome		χ^2	P
	n	%	n	%		
Residence						
City	196	54.3	165	45.7	4.546	**0.033***
Rural area	84	65.1	45	34.9		
Family's income level						
Income is less than or equal to expenditure	252	58.3	180	41.7	2.112	0.146
Income is more than expenditure	28	48.3	30	51.7		
Mother's education level						
Elementary school or no education	153	54.8	126	45.2	1.405	0.236
Higher than elementary school	127	60.2	84	39.8		
Father's education level						
Elementary school or no education	99	55.3	80	44.7	0.388	0.533
Higher than elementary school	181	58.2	130	41.8		
Chronic disease						
No	240	55.0	196	45.0	7.104	**0.008****
Yes	40	74.1	14	25.9		
Continuous medication use						
No	257	56.0	201	44.0	5.212	0.074
Yes	23	74.2	8	25.8		
Pain during menstrual period						
No	65	47.1	73	52.9	7.909	**0.005****
Yes	215	61.1	137	38.9		
Frequency of drinking coffee						
1-2 cups a week	150	57.0	113	43.0	0.003	0.958
3 or more cups a day	130	57.3	97	42.7		
Frequency of drinking tea						
1-2 glasses a week	39	49.4	40	50.6	2.325	0.127
3 or more glasses a day	241	58.6	170	41.4		
Consumption frequency for cola drinks						
1-2 cans a week	157	56.7	120	43.3	0.056	0.813
3 or more cans a day	123	57.7	90	42.3		
Smoking						
Nonsmoker	218	54	186	46	9.520	**0.002****
Smoker	62	72.1	24	27.9		
Alcohol consumption						
No	208	54.7	172	45.3	4.001	**0.045***
Yes	72	65.5	38	34.5		
Oral contraceptive use						
No	12	41.4	198	43.0	.027	0.868
Yes	17	58.6	263	57.0		
Premenstrual pain experienced by mother or sister						
No	100	52.1	92	47.9	3.300	0.069
Yes	180	60.4	118	39.6		

*P < 0.05; **P < 0.01.

Table 3. Relationship between premenstrual syndrome and sub-dimensions of the Basic Personality Traits Scale

	Premenstrual syndrome		No premenstrual syndrome		t	P
	Mean	SD	Mean	SD		
Extroversion	27.35	6.41	28.86	5.78	2.701	**0.007***
Conscientiousness	28.67	5.54	30.25	5.20	3.208	**0.001***
Agreeableness	33.53	4.25	33.46	4.77	-0.199	0.843
Neuroticism	27.24	0.50	22.40	5.88	-8.488	**< 0.001****
Openness to experience	21.26	0.11	21.16	4.09	-0.260	0.795
Negative valence	10.13	3.49	9.04	0.10	-3.607	**< 0.001****

SD = Standard deviation. *P < 0.01; **P < 0.001.

PMS was more common among the students living in rural areas, students with chronic diseases, patients who suffered from menstrual cramps and students who used cigarettes and alcohol. Pınar and Öncel[25] and Kebabcılar et al.[26] reported that smoking was more prevalent among women with PMS in the 15-49 age groups. Other studies conducted in other countries have also reported that smoking and alcohol consumption increase PMS complaints.[27,28] Therefore, it is important for future healthcare providers to inform women about the relationship between PMS and smoking/alcohol consumption. Students can be informed about the importance of reducing/quitting smoking and alcohol consumption, increasing their quality of life and exercising to improve health. They can thus become informed about other healthy lifestyles for themselves and for those for whom they provide guidance.

This was the first study to use the Basic Personality Traits Scale in relation to PMS. The findings from studies that have evaluated premenstrual dysphoria in terms of personality traits have indicated the presence of neuroticism. In our study, in terms of personality, the prevalence of PMS was found to be higher among the students who were less extrovert and less conscientious; and among those with higher neuroticism and negative valence. Sassoon et al.[11] reported that the prevalence of personality disorders among women who had PMS was 27% and, additionally, they found that women with PMS were odd-eccentric, excitable-moody and had anxiety and fears. The most common personality disorder in the PMS group was reported to be obsessive-compulsive personality disorder (18%). Berlin et al.[29] found that women with PMS showed high levels of histrionic, obsessive-compulsive, self-defeating and dependent personality traits during the follicular and late luteal phases and had higher scores for schizoid, schizotypal, borderline, narcissistic and passive-aggressive personality disorder traits. Similarly, Firoozjaei et al.[14] reported that the level of borderline traits among women with PMS was 29.9%. They found a positive relationship between neurotic personality, neuroticism, emotional consistency and introversion. Additionally, they claimed that neuroticism accounted for 15% of the severity of PMS.

Some issues relating to women's health (like PMS) can be perceived differently in different cultures, and this may result in different approaches towards these issues. Especially in developing countries, PMS is not considered to be a health problem and, therefore, awareness about the issue may be insufficient. For this reason, it is important to conduct similar studies in diverse cultures. Moreover, personality is a concept that can be improved through factors such as better education and a better environment, etc. The following support measures can be suggested: participation in social groups for students who are introverts or have low levels of anger control and socialization; improvement of personality structure through using education; organizing of educational programs relating to coping with stress; and directing students to exercise programs and sportive activities.

Our study presents some limitations. It was conducted in only one university and only in its health science departments. This group of subjects can be considered to be a group with a high level of awareness of problem identification and coping methods (or how to seek coping methods). From this perspective, it is possible to suggest that the level of awareness of this problem among adolescents and within society in general may be lower. Therefore, PMS complaints in the general population may be ignored more often. In addition, being unable to reach absent students on the days on which the study was conducted may have had an impact on the study results.

Furthermore, PMS and personality were not evaluated through clinical examinations. Instead, they were evaluated using a scale. The fact that the inclusion criterion of having no psychiatric diagnoses was based on the participants' self-reports and on information in their medical records was another limitation. Because of stigma and other issues, many people might simply not report psychiatric diagnoses, especially if such conditions are under control and/or not severe; also, some psychiatric diagnoses like depression may be poorly noticed by the patients themselves and depression and psychiatric conditions may be underdiagnosed by physicians. Therefore, we cannot definitively rule out the possibility of psychiatric diagnoses among the participants in this study.

CONCLUSION

In this study, one out of every two students had PMS, and personality traits had an impact on occurrences of PMS. The basic approach towards health problems is based on selection of patient-specific treatments. Therefore, suitable treatment options based on basic personality traits need to be considered.

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Family functioning and quality of life among children with anxiety disorder and healthy controls. A cross-sectional study

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KEY WORDS:
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Child.
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Quality of life.

ABSTRACT

BACKGROUND: Studies have shown that children with anxiety disorders (ADs) present impaired family functioning and quality of life. We aimed to evaluate family functioning and quality of life among children with AD and healthy controls.

DESIGN AND SETTING: Cross-sectional study (survey) at two centers in Turkey.

METHODS: The study group comprised 42 children diagnosed with AD and 55 controls. The Screen for Child Anxiety-Related Emotional Disorders (SCARED) questionnaire was filled out by their parents to measure the severity of anxiety symptoms. Family functioning among the children was assessed using the Family Assessment Device (FAD) and Parental Attitude Research Instrument (PARI). The children's quality of life was assessed through the Pediatric Quality of Life Inventory (PedsQL).

RESULTS: The children's average age was 10.00 ± 0.21 years in the AD group and 9.98 ± 1.53 years among the controls. There were higher scores on all FAD subscales in the AD group (2.15 ± 0.52; 2.29 ± 0.44; 2.44 ± 0.55; 2.10 ± 0.61; 2.56 ± 0.40; 2.32 ± 0.33; and 2.29 ± 0.47). On PARI subscales, there were significant differences favoring the AD group ($p < 0.05$), except for democratic attitude. All PedsQL subscales differed significantly between the groups, favoring the AD group. A statistically significant relationship was found between all PedsQL subscales and SCARED scores in the AD group.

CONCLUSION: We found that both family functioning and quality of life among children with AD were negatively affected. However, further studies with larger sample sizes are required to reach stronger conclusions.

INTRODUCTION

Anxiety disorders (ADs) usually begin in childhood[1] and they cause serious impairment of academic performance, peer relationships and family functioning.[2] Early onset of AD tends especially to show a chronic course.[3] The presence of AD decreases with age and, by late adolescence or early adulthood, secondary psychopathological conditions such as depressive disorders or substance use disorders have frequently developed.[4]

In many studies focusing on adults, it has been shown that AD has a negative impact on quality of life.[5-7] Kang et al. evaluated the quality of life among individuals with panic disorder. Their findings suggested that evaluation of symptoms along with individual anxiety-related traits should be included in assessments of quality of life among panic patients.[8] Although many studies have evaluated quality of life among cases of psychiatric disorders during childhood,[9,10] only a few studies have evaluated quality of life among cases of childhood AD alone. In a review, it was shown that quality of life assessed through self-reported scales among cases of childhood mental and behavioral disorder is significantly reduced compared with that of healthy controls.[11] Martinsen et al. evaluated sad and anxious children in terms of quality of life. They found that internalization of symptoms such as depressive and anxious symptoms was associated with lower self-reported quality of life and self-esteem.[12]

In studies examining family functioning and parental attitudes in cases of AD, various features have been included. It has been shown that overprotective attitudes of families may be associated with AD in children.[13] A reciprocal relationship between parental overprotectiveness and anxiety among offspring has been seen in most models.[14] Towe Goodman et al. assessed the perceived family impact of preschool anxiety disorders. They found that preschool anxiety had an important, unique impact on family functioning, particularly parental adjustment, thus highlighting the family impairment linked to early anxiety.[15]

It is likely that AD is more common among children whose parents present anxiety.[16] Parents with anxiety may show more fear and anxiety reactions. This is a risk factor for development of AD in children.[17] In Turkey, anxiety levels have been investigated among children of both divorced and married parents. It was found that the anxiety scores among children with divorced parents were significantly higher than those among children living with both of their parents.[18]

We aimed to evaluate family functioning and quality of life among children with anxiety disorders (ADs) and among healthy controls.

METHODS

Study design, date, setting and ethical issues

This study was a cross-sectional analytical study (survey) with a healthy control group. It was conducted at two centers (Nevşehir State Hospital and Izmir Atatürk Training and Research Hospital), from August 2016 to December 2016. The research protocol was approved by the Research Ethics Committee of Izmir Katip Celebi University of Medical Sciences, on August 11, 2016, under number 223. All participants gave their informed consent to participate in the study. All of the study procedures were in accordance with the Declaration of Helsinki and with local laws and regulations.

Participants

The participants for the AD group comprised patients aged 8-12 years with anxiety symptoms who were consecutively admitted to these two centers between August 2016 and October 2016. The inclusion criteria for the AD group were that the subjects needed to: have a diagnosis of AD in accordance with the descriptions in the Diagnostic and Statistical Manual of Mental Disorders, version 5 (DSM-V); be treatment naïve; have learned to read and write in first grade of school and have clinically normal intelligence; be living with both of their parents; be free from chronic medical or neurological conditions requiring treatment (e.g. epilepsy or diabetes etc.); and provide informed consent for study participation. The exclusion criteria for the AD group were situations in which the children had received diagnoses of major depressive disorder, bipolar disorder, psychotic disorders, obsessive-compulsive disorder, post-traumatic stress disorder (PTSD) or mental retardation; the children were using psychotropic medication; the children had a divorce in the family or one/both parents had died; the mothers had undergone a parent training program; or the children had medical and neurological disorders. The children and their mothers were evaluated by the same child psychiatrist. The study flow chart for the AD group is shown in **Figure 1**.

The healthy control group was formed after the participants in the AD group had been chosen. It was composed of children aged between 8 and 12 years who were chosen from the pediatric clinics of the two centers between October 2016 and December 2016. Pediatricians identified children aged 8-12 years and asked the parents whether they would be interested in participating in the study. The inclusion criteria for participants in the healthy control group were that they needed to have come to either of the pediatric clinics with non-psychiatric symptoms between October 2016 and December 2016 and to be living with both of their parents. The exclusion criteria for the healthy control group were situations in which the subjects presented psychiatric disorders and were using psychotropic medication; the subjects had a divorce in the family or one/both parents had died; the subjects' mothers had undergone a parent training program; the subjects had chronic medical and neurological disorders; or the subjects had been admitted to a psychiatric clinic.

Data collection

Schedule for Affective Disorders and Schizophrenia for School Age Children Present and Life-time KIDDIE-SADS-PL

The comorbidities of the children in the AD group were examined by means of the Schedule for Affective Disorders and Schizophrenia for School-Age Children Present and Life-time (K-SADS-PL). The K-SADS-PL instrument is applied in the form of a semi-structured diagnostic interview that is designed to assess current and past episodes of psychopathological conditions in children and adolescents, in accordance with the DSM-III-R and DSM-IV criteria. Child and parent ratings are combined in a compound summary.[19]

The questionnaire consists of three sections: the questions in the first section seek information on sociodemographic characteristics, the second section asks about current and past episodes of

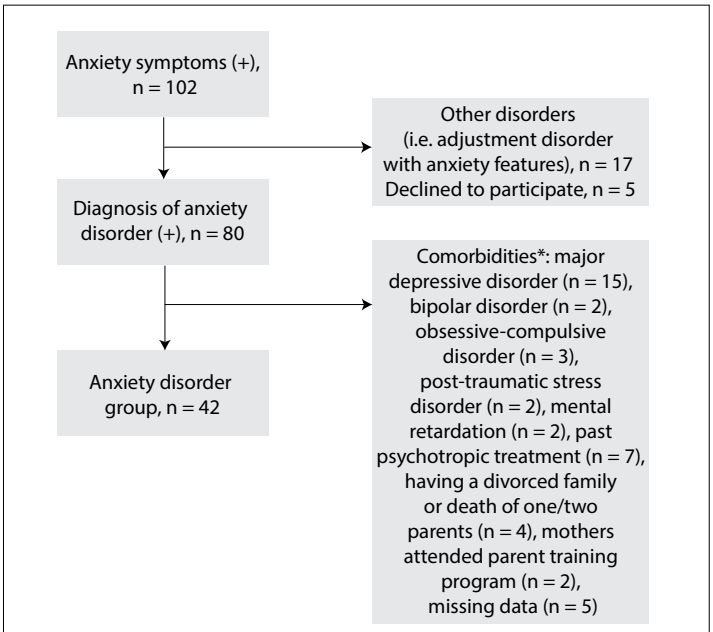

Figure 1. Study flowchart.

psychiatric symptoms and the third section evaluates the general functions of the children during the evaluation. Mood disorders, psychotic disorders, anxiety disorders, elimination disorders, disruptive behavior disorders, alcohol and drug use disorders, eating disorders and tic disorders are evaluated during the interview. It is administered by psychologists or child psychiatrists to both the children and their parents and produces a score that takes into account all the data collected from the various sources available (family, children, teachers, pediatricians, etc.).[19]

The Turkish translation of K-SADS-PL and a validity and reliability study on this translation were performed by Gökler et al.[20]

The Screen for Anxiety-Related Emotional Disorders (SCARED):

The SCARED scale was used to indicate the degree of veracity of descriptive phrases regarding how children may have felt over the course of the previous three months.

This scale is a 41-item standardized screen for anxiety that has been validated for use among children aged 8 to 16 years and also features a parent report form.[21] The child version, or self-report form, is answered by the child with regard to his/her own anxiety. The parent-reported SCARED form is completed by parents with regard to their child's anxiety.

Participants rate the items for each factor on a three-point scale (0 = not true or hardly ever true; 1 = sometimes true; or 2 = true or often true). The total score for the Screen for Child Anxiety-Related Emotional Disorders (SCARED) scale is obtained by summing the responses from the 41 items and can range from 0 to 82. The purpose of the instrument is to screen for signs of anxiety disorders in children. Higher scores indicate greater severity of anxiety. A composite score of 25 or higher suggests the presence of an anxiety disorder.[21]

The validity and reliability of the Turkish version of SCARED was ascertained by Cakmakci.[22]

The Pediatric Quality of life Inventory (PedsQL):

The PedsQL scale was used to assess problems within the multidimensional health-related quality of life in the last month.

This is a modular instrument that is designed to measure health-related quality of life (HRQOL) among children and adolescents aged 2 to 18 years.[23] It is answered by both children and their parents.

The 23-item PedsQL 4.0 Generic Core Scale encompasses the following: 1) physical functioning (8 items); 2) emotional functioning (5 items); 3) social functioning (5 items); and 4) school functioning (5 items). It was developed through focus groups and cognitive interviews. All its items have a five-point response scale (0 = never a problem; 1 = almost never a problem; 2 = sometimes a problem; 3 = often a problem; and 4 = almost always a problem), and they are reverse-scored and linearly transformed into a 0-100 scale, such that higher scores indicate better functioning. Each scale score is computed as the sum of the items divided by the number of items answered on the scale. If more than 50% of the items on a scale are missing, no score is computed.[23]

Cakın-Memik studied the reliability and validity of the Turkish version of PedsQL.[24]

Parental Attitude Research Instrument (PARI):

The PARI scale was used to evaluate the parental attitudes towards child rearing.

The questionnaire was developed by Schaefer and Bell in 1958[25] and consists of five sections. It is completed by parents and aims to rate parents' child-rearing attitudes. Furthermore, it rates five separate dimensions in the form of "overprotective mothership", "democratic treatment and granting of equality", "rejection of housewifery role", "incompatibility" and "rigid disciplining". It is a Likert-type scale and each item can be rated from 1 to 4 points. All items except for items 2, 29 and 44 are rated with a directly scored grade. The scores are summed separately for each factor dimension. Thus, five separate scores reflecting five distinct dimensions are calculated for each case instead of a single summed score. High scores for factors other than "democratic treatment and granting of equality" indicate negative parent attitudes. There is no total score but, rather, actor scores are taken into consideration.[25]

The validity and reliability of the scale were ascertained by Küçük.[26]

Family Assessment Device (FAD):

The FAD scale was used to determine problems relating to family functioning.

The instrument was developed by Epstein et al., in 1983, and its questions are answered by the parents.[27] It comprises seven sections, as follows. The first section addresses problem-solving skills; the second, intra-family communication; the third, roles in the family; the fourth, effective responsiveness against emotions such as sadness, anger, fear, joy, love and interest; the fifth, effective involvement of family members; the sixth, behavior control; and the seventh, general functions in the family. This instrument consists of 60 statements about a family, and subjects are required to rate the extent which the description of each statement is concordant with the situation in their own family. All items are rated on a four-point Likert scale on which the response choices range from 1 (strongly agree) to 4 (strongly disagree). Higher scores indicate worse levels of family functioning.

FAD has been widely used in both research and clinical practice. Its uses include: (1) screening to identify families experiencing problems; (2) screening to identify specific domains in which families are experiencing problems; and (3) assessment of changes following treatment.

Bulut et al. translated the Turkish version of this questionnaire and conducted a validity and reliability study on it.[28]

Children's success at school and peer relationships

The children's level of success at school was assessed based on their final grade point average (80-100 = good; 60-80 = medium; and 0-60 = poor).

Peer relationships were assessed through the children's declarations, by asking how well they got along with their friends. The children and their families were asked to give responses (yes/no) to the following statements regarding peer relationships: 1. Peers let this child play with them; 2. This child is chosen as a playmate by peers; 3. Peers approach this child; 4. This child is included in peers' activities; 5. This child is noticed by peers; and 6. This child is much liked by other children. If the response to more than half of the statements was yes, the peer relationships were deemed to be good. If the response to more than half of the statements was no, the peer relationships were deemed to be bad.

Statistical analysis

The data of this study were evaluated using the Statistical Package for the Social Sciences (SPSS), version 22.0. Continuous variables are presented by means of summary statistics. This (unless otherwise stated) consisted of the number of patients (n), mean and standard deviation (SD). Categorical data are presented using either absolute or relative frequencies. Demographic data were compared using the chi-square test. A continuity correction (Yates's correction) and Fisher's exact test were applied when required. The distribution of the data was evaluated using the Kolmogorov-Smirnov method. Since the data demonstrated normal distribution, two-up groups were evaluated by means of the parametric t test and triple groups were evaluated through the analysis of variance (ANOVA) test. Pearson correlation analysis was used to determine the relationships between continuous variables. All tests were two-tailed, and P-values < 0.05 were considered significant.

RESULTS

Within the study period (August 2016 to December 2016), 42 children with AD and 55 healthy control children were enrolled. During this period, 58 healthy children without psychiatric, chronic medical or neurological symptoms came to the two pediatric clinics. Three of them declined to participate in the study. Thus, the healthy control group comprised 55 children.

The average age of the children in the AD group was 10.00 ± 0.21 years, and the average age of the children in the healthy control group was 9.98 ± 1.53 years. No significant difference was found between the average ages of the groups (P = 0.114). Thirty children in the AD group and 44 children in the healthy control group were female. No statistically significant difference was found between the groups in terms of gender (P = 0.346). There were also no differences between the AD and healthy control groups in terms of the mother's age and educational level (P = 0.066 and P = 0.505, respectively). There were significant differences between the groups in terms of the level of success at school and peer relationships (P < 0.001 and P < 0.001, respectively). The sociodemographic data are presented in **Table 1**. The AD subtypes in the AD group are presented in **Table 2**.

The mean scores for the child form of SCARED were 38.57 ± 7.1 (range = 34) in the AD group and 6.18 ± 3.47 (range = 16) in the healthy control group. This difference between the two groups was significant (P < 0.001). The mean scores for the parent form of SCARED were 41.12 ± 6.67 (range = 33) in the AD group and 7.54 ± 3.40 (range = 14) in the healthy control group. This difference between the two groups was also significant (P < 0.001). The comparisons between the AD and healthy control groups using the PedsQL, FAD and PARI subscales are presented in **Table 3**.

In comparing the PedsQL scores with the child and parent SCARED scores, significant negative correlations were found between both the child and parent SCARED scores and all the

Table 1. Sociodemographic data of the AD and control groups

	AD group (n = 42)	Healthy control group (n = 55)	P
Age* (mean ± SD)	10.00 ± 0.21	9.98 ± 1.53	0.144
Gender n (%)**			
Male	12 (28.6)	11 (20.0)	0.346
Female	30 (71.4)	44 (80.0)	
Mother's mean age* (mean ± SD)	39.07 ± 3.99	36.98 ± 6.39	0.066
Maternal education n (%)**			
< 8 years	26 (61.9)	41 (74.5)	0.505
> 8 years	16 (38.1)	14 (25.5)	
School success n (%)**			
Good	29 (69.1)	47 (85.5)	
Medium	10 (23.8)	6 (10.9)	< 0.001
Poor	3 (7.1)	2 (3.6)	
Peer relationship n (%)**			
Good	30 (71.4)	50 (90.9)	< 0.001
Bad	12 (28.6)	5 (9.1)	

AD = anxiety disorder; SD = standard deviation. *evaluated using parametric t test, **evaluated using chi-square test.

Table 2. Anxiety disorder subtypes

	n	%
Generalized anxiety disorder	10	23.8
Separation anxiety disorder	9	21.4
Specific phobia	8	19.1
Social anxiety disorder	6	14.3
Panic disorder	3	7.1
Generalized anxiety disorder + specific phobia	2	4.8
Social anxiety disorder + separation anxiety disorder	3	7.1
Panic disorder + specific phobia	1	2.4

subscales of PedsQL (PedsQL school functioning subscale P = 0.028; and other subscales P = < 0.001) (**Table 4**). When the control group was compared in the same way, there was no significant correlation between the child SCARED scores and the PedsQL subscales (P > 0.05).

DISCUSSION

This study aimed to compare family functioning, parental attitudes and quality of life between AD and healthy control groups. We found that the children with a diagnosis of AD who had not yet been started on medication had more difficulty in family functioning and in relation to parental attitudes than did the children without any psychiatric diagnosis or chronic disease. Furthermore, we found that impairment of quality of life was more prominent among the children with a diagnosis of AD than among the controls. The findings from our study are similar to those of previous studies examining relationships between AD and family functioning and between AD and parental attitudes.[29-31]

Studies evaluating links between AD and family relationships have drawn attention both to familial risk factors for development of AD and to the attitudes of the family members of children with AD. In a meta-analysis within a study that evaluated parental factors associated with anxiety among young people, it was shown that the parental factors that gave rise to increased risk of anxiety included less warmth, more inter-parental conflict, over-involvement and aversiveness.[31] Furthermore, in the Duke Preschool Anxiety Study on preschoolers (ages 2-5 years), 917 parents were evaluated regarding the perceived impact of families on preschool children with AD. These parents were interviewed using the Preschool Age

Table 3. Comparison of the AD and control group in terms of quality of life, family functioning and parental attitude

Scale	AD group	Healthy control group	P	Cohen's d	Effect size
PedsQL - child form					
Physical functioning	67.09 ± 9.51	84.50 ± 9.13	**< 0.001**	-1.87	-0.62
Emotional functioning	52.62 ± 16.76	80.46 ± 7.42	**< 0.001**	-2.15	-0.73
Social functioning	69.05 ± 17.68	79.71 ± 8.40	**< 0.001**	-0.77	-0.36
School functioning	70.48 ± 18.90	80.09 ± 10.99	**0.018**	-0.62	-0.30
Total scale score	64.81 ± 14.24	81.51 ± 4.08	**< 0.001**	-1.59	-0.62
PedsQL - parent form					
Physical functioning	69.29 ± 20.12	84.27 ± 12.32	**< 0.001**	-0.90	-0.41
Emotional functioning	52.98 ± 18.15	80.37 ± 7.65	**< 0.001**	-1.97	-060
Social functioning	66.07 ± 17.62	75.91 ± 7.94	**0.001**	-0.72	-0.34
School functioning	69.76 ± 17.35	78.00 ± 11.81	**0.022**	-0.56	-0.27
Total scale score	64.52 ± 14.62	79.61 ± 5.15	**< 0.001**	-1.38	-0.57
PARI overprotective parenting attitude	41.70 ± 6.52	32.62 ± 5.05	**< 0.001**	1.56	0.61
PARI democratic attitude	23.14 ± 5.23	23.84 ± 2.89	0.858		
PARI rejection of homemaking attitude	33.71 ± 6.29	30.45 ± 5.05	**0.010**	0.57	0.27
PARI marital conflict	18.62 ± 4.15	15.11 ± 2.74	**< 0.001**	1.00	0.45
PARI strict discipline	32.21 ± 6.86	27.82 ± 4.79	**0.002**	0.74	0.35
FAD problem-solving	2.15 ± 0.52	2.07 ± 0.61	0.143		
FAD communication	2.29 ± 0.44	1.87 ± 0.53	**< 0.001**	0.86	0.40
FAD roles	2.44 ± 0.55	1.81 ± 0.64	**< 0.001**	1.07	0.47
FAD affective emotions	2.10 ± 0.61	1.67 ± 0.49	**< 0.001**	0.78	0.36
FAD affective attachment	2.56 ± 0.40	1.77 ± 0.51	**< 0.001**	1.72	0.65
FAD behavior control	2.32 ± 0.33	1.74 ± 0.64	**< 0.001**	1.14	0.49
FAD general functionality	2.29 ± 0.47	1.81 ± 0.45	**< 0.001**	1.04	0.46

AD = anxiety disorder; PedsQL = Pediatric Quality of Life Inventory; PARI = Parental Attitude Research Instrument; FAD = Family Assessment Device.

Table 4. Examination of the relationship of child and parent SCARED scores with children's quality of life scores in the AD group (Spearman correlation analysis)

		PedsQL PF	PedsQL EF	PedsQL SF	PedsQL ScF	PedsQL Total score
SCARED - child form	r	-0.541	-0.424	-0.447	-0.380	-0.561
	P	< 0.001	< 0.001	< 0.001	0.028	< 0.001
SCARED - parent form	r	-0.474	-0.528	-0.453	-0.276	-0.550
	P	< 0.001	< 0.001	< 0.001	0.076	< 0.001

SCARED = Screen for Anxiety-Related Emotional Disorders; AD = anxiety disorder; PedsQL = Pediatric Quality of Life Inventory; PF = Physical functioning; EF = Emotional functioning; SF = Social functioning; ScF = School functioning.

Psychiatric Assessment,[32] an interviewer-based diagnostic assessment for two to five-year-olds. It was found that preschool anxiety had an important, unprecedented effect on family functioning, particularly parental adjustment, thus highlighting the family impairment that was linked with early anxiety.[15] In the same study, it was found that generalized AD and separation AD were similar to the impaired family functioning in ADHD.[15]

Another finding in our study was that children with AD showed greater deterioration than healthy controls in all areas of the PedsQL, as assessed through self-reports both from the children and from their families. While there are many studies evaluating quality of life among adults with AD, the limitation of similar studies regarding childhood anxiety disorder is notable. In a study evaluating quality of life among individuals with panic disorder, which is a subtype of anxiety disorder, quality of life was assessed using the short form-36 scale,[33] which is similar to the PedsQL scale. Consequently, anxiety sensitivity and anxiety traits were found to be independent determinants of quality of life. Therefore, it was suggested that evaluation of quality of life in cases of panic disorder should include evaluation of the symptoms of the disease.[8] Weitkamp et al. evaluated 120 patients as part of an effectiveness trial for child and adolescent psychotherapy in Germany. They aimed to demonstrate a relationship between childhood mental disorders (45.1% with an anxiety disorder, 31.0% with an affective disorder, 25.7% with a PTSD, 15.9% with a disruptive disorder, and 33.6% with other disorders) and the quality of life, as assessed using the German Kidscreen,[34] which is similar to PedsQL. They reported that impairment of the quality of life was strongly associated with internalizing rather than externalizing pathological conditions, according to both self-reports and parental reports, and they also found a relationship between mental disorders and impairment of the quality of life.[35] In another study assessing 310 children (ages 6-18 years) at an outpatient child psychiatric clinic in Rotterdam, Netherlands, who had been referred because of psychiatric problems, the aim was to determine the relationship between the most prevalent child psychiatric diagnoses and quality of life measured through PedsQL. It was found that the overall quality of life of children diagnosed with psychiatric disorders, including AD, was more impaired than that of healthy controls.[10] The findings from our study and data in the literature suggest that the quality of life of children with AD is impaired significantly.

In our study, there was a significant negative correlation between anxiety symptom severity and all the quality of life sub-scores. This negative correlation showed that as AD symptom severity increased, quality of life became more impaired. In a study conducted by Ramsawh and Chavira in 2016, 73 children (aged 8-12 years) from district pediatric primary care practices were evaluated as part of a larger study focusing on mental health service utilization. This pediatric primary care sample was used to examine the relationships between child anxiety and quality of life measured using PedsQL. It was found that having more than one diagnosis of comorbid AD and having greater severity of anxiety symptoms were associated with reduced quality of life.[36] It has also been shown in studies that quality of life problems originating from AD can be improved through appropriate treatment. For example, Memik et al. (2014) studied the effects of sertraline on the quality of life of children and adolescents with AD and found that sertraline treatment improved the quality of life.[37]

There are some limitations to our study. First, the mothers' psychiatric status was not assessed. Second, our sample size may have limited the generalizability of our findings. Third, the children and their mothers were evaluated by the same child psychiatrist, which therefore gave rise to lack of blinding. Fourth, only using information from the mothers in our study may have affected the objectivity of our study. Information from the children's teachers might have provided greater objectivity for the results from the study. The children's mental capacity could have been assessed through objective tests. The data analyzed here were obtained before treatments were implemented for the children in this study; the changes achieved through the treatment could be examined in the future.

CONCLUSION

We found that both family functioning and the quality of life of children with AD were negatively affected and that, as the severity of anxiety symptoms increased, the quality of life of the children with AD diminished. Consequently, we consider that it is important to address family functioning and quality of life when planning AD treatments for children.

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What do Cochrane systematic reviews say about interventions for treating psoriasis?

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ABSTRACT

CONTEXT AND OBJECTIVE: Psoriasis is a common chronic inflammatory skin disease characterized by abnormal and increased growth of the cells that produce keratin and abnormal functioning of the immune system. We aimed to summarize the evidence available regarding interventions for patients with psoriasis.

DESIGN AND SETTING: Review of systematic reviews, developed in the Discipline of Evidence-Based Medicine, Escola Paulista de Medicina, Universidade Federal de São Paulo.

METHODS: A systematic search was conducted to identify Cochrane systematic reviews that fulfilled the eligibility criteria. Two authors screened titles and abstracts that had been retrieved through the search strategy. The results from all the Cochrane systematic reviews that were included were summarized and presented in a narrative synthesis.

RESULTS: We included six Cochrane systematic reviews assessing interventions for treating psoriasis. The findings from high-quality evidence were that (a) etanercept reduced the psoriasis severity index, compared with placebo and (b) steroids plus vitamin D, compared with vitamin D alone, improved the skin clearance rate, as assessed by investigators, but was associated with a higher proportion of participants who dropped out due to adverse events. For all other comparisons, the quality of the evidence ranged from moderate to very low.

CONCLUSION: This review included six Cochrane systematic reviews that provided evidence ranging in quality from unknown to high, regarding management of psoriasis. Further randomized controlled trials are imperative to reduce the uncertainties relating to several treatments that are already used in clinical practice.

INTRODUCTION

Psoriasis is a common chronic inflammatory skin condition affecting about 1% to 2% of the general population in the United States and United Kingdom.[1,2] The disease is characterized by abnormal and increased growth of the cells that produce keratin and abnormal functioning of the immune system, especially T lymphocytes.[3]

It may be triggered by several factors such as stress, alcohol consumption, drugs, smoking, sunlight, infections, local trauma, endocrine factors and genetic changes.[4] Psoriasis can be identified in different clinical presentations or subtypes, including nail psoriasis, palmoplantar psoriasis, psoriatic arthritis, plaque psoriasis, psoriatic erythroderma and generalized pustular psoriasis.[5] Around 20% of patients are diagnosed with severe types of the disease.[6]

There is no difference in prevalence between the sexes, although it is more common in adults than in children.[1] It may be presented in association with a variety of comorbidities such as arthritis, depression and cardiovascular diseases.[7] It can lead to fear of other reactions and social isolation, thus affecting the patients' quality of life.[8]

There continues to be no cure for psoriasis.[9] There are different options for treatments that can be used alone or in combination, such as systemic drugs, topical drugs and phototherapy.[10] In decision-making, physicians need to consider the disease severity, the patients' circumstances and the best evidence available.[11]

The aim of this review was to identify and summarize the evidence from Cochrane systematic reviews (SRs) relating to interventions for treating psoriasis.

OBJECTIVE

To synthesize the evidence from Cochrane SRs regarding interventions for treating psoriasis.

METHODS

Design and setting

This study was a review of Cochrane SRs and was conducted within the Discipline of Evidence-based Medicine of Escola Paulista de Medicina, Universidade Federal de São Paulo (EPM-UNIFESP). This article was written for the section Cochrane Highlights. This initiative is a formal collaboration between the São Paulo Medical Journal and Cochrane, and it is supported by Cochrane Brazil. The aim of this initiative is to disseminate the evidence from Cochrane SRs.

Inclusion criteria

Types of study

We only included SRs published in the Cochrane Database of Systematic Reviews. We excluded all protocols for SRs and withdrawn SRs. We also did not take previous versions of a single SR into consideration, or SRs that were being updated. We did not apply any date limit as an inclusion criterion.

Types of participants

The participants included were patients (regardless of age) who had been diagnosed with any form of psoriasis. SRs considering participants presenting psoriatic arthritis with no skin involvement were not included.

Types of intervention

We considered SRs assessing any intervention (whether pharmacological or not), which could either be single interventions or be in combination with other interventions, compared with any other intervention, an inactive comparator or no intervention (no treatment).

Types of outcomes

We considered any outcome (clinical or laboratory), as evaluated in the SRs that were included.

Search for reviews

We conducted a broad search in the Cochrane Database of Systematic Reviews (via Wiley) on June 17, 2018. The full search strategy is presented in **Table 1**.

Selection of reviews

Two authors (RLP and DVP) screened the titles and abstracts independently. Any disagreements were resolved by consulting a third author (ALCM). The SRs that met the inclusion criteria were selected and summarized by three authors (COCL, RR, RLP).

Presentation of results

We used a qualitative synthesis (narrative approach) to present the results from the search and from the SRs that were included.

RESULTS

Search results

Our search strategy retrieved 124 references. After screening the titles and abstracts, six systematic reviews were selected for full-text assessment.[12-17] During this selection phase, two SRs were excluded because they were very outdated versions of two reviews that are currently being updated.[18,19] Thus, we included six SRs for qualitative synthesis, given that they fulfilled our eligibility criteria.

Reviews included

We present a summary of each SR that was considered. Details regarding the characteristics of the interventions, comparisons, outcomes and the quality of evidence are presented in **Table 2**.

Anti-tumor necrosis factor agents for treating pediatric psoriasis

This review[12] had the aim of evaluating the use of anti-tumor necrosis factor (TNF) agents in pediatric patients with psoriasis. One RCT (211 participants) was included. This trial assessed etanercept (at dosages ranging from 0.8 to 50 mg per kilogram of body weight), compared with placebo. One of the main outcomes consisted of investigator-assessed improvement, measured as the proportion of participants achieving 75% reduction in the Psoriasis and Severity Index (PASI). After 12 weeks, the number of patients achieving this reduction was higher in the etanercept group (60/106 participants) than in the placebo group (12/105). This represented a relative risk [RR] of 4.95; 95% confidence interval [95% CI] 2.83 to 8.65; one RCT; 211 participants; high quality of evidence. There was also an improvement favoring the etanercept group regarding the quality-of-life assessment using the Children's Dermatology Life Quality Index (mean difference [MD] of 2.30; 95% CI 0.85 to 3.75; one RCT; 211 participants; moderate quality of evidence).

Regarding safety outcomes, three serious adverse events in the etanercept group were reported. The authors of the review concluded that use of etanercept seemed to be effective and safe for treating pediatric psoriasis. However, in relation to this conclusion, it needs to be borne in mind that the evidence came from a single industry-sponsored RCT. Further RCTs are imperative for

Table 1. Search strategy

#1 MeSH descriptor: [Psoriasis] explode all trees
#2 (Psoriasis) OR (Pustular Psoriasis of Palms and Soles) OR (Psoriases)
#3 #1 OR #2
#4 #3 Filter: in Cochrane Reviews

Table 2. Characteristics of interventions, comparisons, outcomes and quality of evidence

Intervention	Comparators	Population	Main findings	GRADE[20]
Anti-tumor necrosis factor (etanercept) (0.8 to 50 mg per kilogram of body weight)[12]	Placebo	Children with moderate to severe plaque psoriasis who did not respond to, had a contraindication against, or did not tolerate other systemic therapies or photo chemotherapy	*Favored etanercept:* • Reduction of 50%, 75%, or 90% in the PASI index • Health-related quality of life	High Moderate
Pharmacological and non-pharmacological interventions, including infliximab, golimumab and superficial radiotherapy[13]	Placebo and active interventions	Participants with nail psoriasis	• Infliximab and golimumab were superior to placebo for improvement in nail score over the short and medium terms • Superficial radiotherapy (SRT) was superior to placebo in short-term treatment	NA NA
Narrow-band ultraviolet B phototherapy (NB-UVB)[14]	Oral PUVA, bath PUVA, topical PUVA, selective broad band ultraviolet B	Patients with chronic plaque psoriasis, palmoplantar psoriasis or guttate psoriasis	NB-UVB versus oral PUVA: No difference in PASI 75 or in discontinuation due to side-effects NB-UVB versus bath PUVA: The clearance rate favored bath PUVA NB-UVB versus topical PUVA: No difference in the clearance rate NB-UVB versus selective broad-band UVB: No difference in the clearance rate or in discontinuation due to side effects.	Low Low Low Low Low
Oral fumaric acid esters[15]	Placebo or methotrexate	All subtypes of psoriasis	*Favored fumaric acid esters, compared with placebo:* • PASI 75 *No difference between fumaric acid esters and methotrexate:* • PASI 75 • Adverse events	Low Very low Very low
Topical interventions including steroids and vitamin D[16]	Other topical interventions	Chronic plaque psoriasis	Multiple comparisons and analyses. Heterogeneity between studies prevented pooled analysis	NA
Topical interventions, including steroids and vitamin D[17]	Other topical interventions	Scalp psoriasis	**Steroids versus vitamin D** *Favored steroids* • Proportion of patients achieving "clearance", according to investigators' global assessment *Favored vitamin D* • Proportion of patients discontinuing due to adverse events **Steroids plus vitamin D versus steroids alone** *Favored steroids plus vitamin D* • Proportion of patients achieving "clearance", according to investigators' global assessment *No difference between groups* • Proportion of patients discontinuing due to adverse events **Steroids plus vitamin D versus vitamin D** *Favored steroids plus vitamin D* • Proportion of patients achieving "clearance", according to investigators' global assessment • Proportion of participants discontinuing due to adverse events.	 Moderate Moderate Moderate Moderate High High

NA = not assessed; PASI = Psoriasis Area and Severity Index; PASI 75 = 75% reduction in the PASI index; NB-UVB = narrow-band ultraviolet B; PUVA = psoralen ultraviolet A photochemotherapy.

*GRADE (Grading of Recommendations Assessment, Development and Evaluation) aims to assess the quality of the evidence. Outcomes are classified as providing the following: (a) high quality of evidence (high confidence that the estimated effect is near the true effect); (b) moderate quality of evidence (it is very likely that the estimated effect is close to the real effect but there is a possibility that it is not); (c) low quality of evidence (limited confidence in the effect estimate); or (d) very low quality of evidence (the true effect is likely to be substantially different from the estimated effect).

reaching solid conclusions. For further details, refer to the original abstract, available from: http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD010017.pub2/full.

Interventions for treating nail psoriasis

This review[13] evaluated the efficacy and safety of the treatments for nail psoriasis and included 18 RCTs with 1,266 participants. It was not possible to pool the results because of the heterogeneity of many of the studies. The findings regarding the main outcome of improvement in nail score are presented below:

- Infliximab (5 mg/kg) was superior to placebo after medium-term treatment (57.2% improvement in nail score versus -4.1%; $P < 0.001$) and short-term treatment;
- Golimumab (50 mg and 100 mg) was superior to placebo after medium-term treatment (33% improvement in nail score versus 0% and 54% versus 0%, respectively; $P < 0.001$) and short-term treatment.
- Superficial radiotherapy (SRT) was superior to placebo after short-term treatment (20% improvement in nail score versus 0%; $P = 0.03$).
- Ciclosporin was similar to etretinate.
- Methotrexate was similar to ciclosporin.
- Ustekinumab was similar to placebo.
- 5-fluorouracil (1%) in Belanyx lotion as a vehicle was similar to Belanyx lotion alone.
- Tazarotene (0.1% cream) was similar to clobetasol propionate.
- Calcipotriol (50 μg/g) was similar to betamethasone dipropionate with salicylic acid.
- Calcipotriol (0.005%) was similar to betamethasone dipropionate.

Not all the studies included reported adverse events; those that did only reported mild adverse effects. Only one study reported the effect on quality of life, which limits the confidence in the results.

For further details, refer to the original abstract, available from http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD007633.pub2/full.

Narrow-band ultraviolet B phototherapy versus broad-band ultraviolet B or psoralen-ultraviolet A photochemotherapy for treating psoriasis

This SR[14] had the aim of assessing the effects of different ultraviolet therapy interventions in patients with psoriasis. Thirteen RCTs (662 participants) were included. The quality of the evidence was assessed regarding four comparisons of narrow-band ultraviolet B (NB-UVB): against oral psoralen ultraviolet A photochemotherapy (PUVA), bath PUVA, topical PUVA and selective broad-band ultraviolet B. There was also a combined-therapy comparison: NB-UVB plus retinoid compared with PUVA plus retinoid.

In the comparison between NB-UVB and oral PUVA for treating chronic plaque psoriasis, there was no statistical difference between the groups regarding the proportions of the participants reaching 75% reduction in PASI (RR 0.91; 95% CI 0.63 to 1.32; one RCT; 51 participants; low quality of evidence). There was also no difference regarding the outcome of dropping out due to side effects (RR 0.71; 95% CI 0.20 to 2.54; three RCTs; 247 participants; low quality of evidence).

In the comparison between NB-UVB and bath PUVA for patients with chronic plaque psoriasis, the only outcome reported was the clearance rate (clearance was defined as no lesions of psoriasis or minimal residual activity). The clearance rate in studies that made comparisons between patients was significantly better in the bath PUVA group (RR 0.18; 95% CI 0.05 to 0.71; one RCT; 36 participants; low quality of evidence).

In the comparison between NB-UVB and topical PUVA for treating palmoplantar psoriasis, there was no statistical difference in the clearance rate (RR 0.09; 95% 0.01 to 1.56; one RCT; 50 participants; low quality of evidence).

In the comparison between NB-UVB and selective broad-band ultraviolet B for treating chronic plaque psoriasis, there was also no difference in the clearance rate (RR 1.40; 95% CI 0.92 to 2.13; one RCT; 100 participants; low quality of evidence). Nor was there any difference regarding dropping out due to side effects (RR 3.0; 95% CI 0.32 to 27.87; one RCT; 100 participants; low quality of evidence).

In the comparison of combined therapy of NB-UVB plus retinoid versus PUVA plus retinoid for patients with chronic plaque guttate psoriasis, there was also no difference in the clearance rate (RR 0.93; 95% CI 0.79 to 1.10; two RCTs; 90 participants; low quality of evidence). Nor was there any difference regarding the proportion of patients reaching 75% reduction in PASI (RR 0.89; 95% 0.59 to 1.35; 60 participants; one RCT; low quality of evidence).

The authors of this review concluded that the current evidence was heterogenous and needed to be interpreted with caution. All the evidence presented was from head-to-head comparisons and no inactive comparator was considered. The overall quality of the evidence was low because of imprecision and risk of bias. Further studies are imperative in order to confirm the efficacy and safety of these interventions for psoriasis.

For further details, refer to the original abstract, available from http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD009481.pub2/full.

Oral fumaric acid esters for treating psoriasis

This review[15] assessed the use of oral fumaric acid esters (FAE) for patients with psoriasis. Six RCTs (544 participants) were included and use of FAE was compared with use of placebo or methotrexate. The studies included presented high clinical and methodological diversity, and this prevented pooled analysis.

The PASI scale was used by the investigators in each RCT to assess improvement in different ways, and the authors of the SR decided to report the results from each RCT narratively. Five RCTs reported data from use of FAE versus placebo. In three of them (418 patients), the PASI score measurements showed that there were benefits from using FAE. The proportion of the patients who achieved 75% reduction in PASI was reported by two RCTs in which use of FAE was also favored. A single RCT (175 participants), published as an abstract, assessed quality of life using the Skindex-29 scale, but the numerical data was poorly reported and no formal analysis comparing the two arms of the study could be performed. The quality of the evidence for all these outcomes was considered low, following GRADE assessment.

Regarding the comparison of FAE versus methotrexate, only one RCT (51 participants) was included. There was no statistical difference between the proportions of patients who achieved 75% reduction in PASI, in this comparison (5/26 versus 6/26; RR 0.80; 95% CI 0.26 to 2.29; one RCT; 51 participants; very low quality of evidence). There was also no statistical difference in total PASI scores between the groups (MD 3.80; 95% CI 0.66 to 6.92; one RCT; 51 participants; very low quality of evidence).

Adverse events were poorly reported in general. From the RCT comparing FAE with methotrexate, it was reported that one patient in the FAE group discontinued the treatment due to adverse events while five patients in the methotrexate group dropped out, but this difference did not reach statistical significance (RR 0.89; 95% CI 0.77 to 1.03; one RCT; 54 participants; very low quality of evidence).

This SR made it clear that further studies are imperative for decreasing the uncertainties regarding the efficacy and safety of FAE for management of psoriasis. Further RCTs should be better planned and fully reported, to avoid further waste of research resources. The numerical data from all the RCTs included were reported in the full version of the SR. For further details, refer to the original abstract, available from http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD010497.pub2/full.

Topical treatments for treating chronic plaque psoriasis

This SR[16] assessed any topical intervention for chronic plaque psoriasis and included 177 RCTs (34,808 participants). In this summary, we present only the comparisons that the authors of the SR considered most clinically relevant.

For topical treatment, vitamin D analogues were significantly better than placebo in an investigator's assessment of overall global improvement (IGA). Because many schemes for vitamin D administration were included, the authors of the SR presented results separated per scheme. They did not pool the results. For twice-daily use of becocalcidiol versus placebo, the standard mean difference [SMD] was -0.67 (95% CI -1.04 to -0.30; one RCT; 119 participants), which represents 0.8 points of improvement on an IGA scale from 0 to 6. For once-daily use of paricalcitol versus placebo, the SMD was -1.66 (95% CI -2.66 to -0.67; one RCT; 11 participants), which represents 1.9 points of improvement on an IGA scale from 0 to 6.

The authors of this SR also performed many analyses on corticosteroids alone or in combination with vitamin D compounds. In general, most corticosteroid interventions performed better then placebo, but this statement is not accurate for all corticosteroids. Each analysis needs to be considered when assessing the relevance of this intervention for treating chronic plaque psoriasis.

Many head-to-head comparisons were also included, and these data may be assessed in the full version of the SR. This SR did not include any evaluation of the quality of the evidence using the GRADE recommendations.

For further details and access to all the analyses, refer to the original abstract, available from http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD005028.pub3/full.

Topical treatments for treating scalp psoriasis

This SR[17] had the aim of evaluating all topical interventions for scalp psoriasis. Because of this wide eligibility criterion, 59 RCTs (11,561 participants) were included. In total, 15 comparisons were assessed. In this summary, we only report the following three comparisons, which the authors of this SR considered to be the "main comparisons": steroids versus vitamin D; steroids plus vitamin D versus steroids; and steroids plus vitamin D versus vitamin D.

1. Steroids versus vitamin D:
   - Proportion of patients achieving "clearance", according to the investigators' global assessment: higher in the steroid group (392/1,392 versus 129/813; RR 1.82; 95% CI 1.52 to 2.18; 4 RCTs; 2,180 participants; moderate quality evidence).
   - Proportion of patients discontinuing due to adverse events: higher in the steroid group (15/1422 versus 44/869; RR 0.22; 95% CI 0.11 to 0.42; 4 RCTs; 2,291 participants; moderate quality of evidence).
2. Steroids plus vitamin D versus steroids:
   - Proportion of patients achieving "clearance", according to the investigators' global assessment: higher for combined therapy group (429/1,231 versus 357/1,243 participants; RR 1.22; 95% CI 1.08 to 1.36; four RCTs; 2,474 participants; moderate quality of evidence).
   - Proportion of patients discontinuing due to adverse events: no difference between groups (13/1211 versus 15/1,222 participants), representing a RR of 0.88 (95% CI 0.42 to 1.88; three RCTs; 2,433 participants; moderate quality of evidence).
3. Steroids plus vitamin D versus vitamin D:
   - Proportion of patients achieving "clearance", according to the investigators' global assessment: higher for combined therapy (442/1330 versus 96/678 participants; RR 2.28; 95% CI 1.87 to 2.78; four RCTs; 2,008 participants; high quality of evidence).

- Proportion of participants discontinuing due to adverse events: higher for vitamin D alone (37/659 participants versus 14/1,311 participants; RR 0.19; 95% CI 0.11 to 0.36; three RCTs; 1,970 participants; high quality of evidence).

The authors of this SR concluded that use of steroids alone and combined with vitamin D presented more effective and safer results that did any other comparison assessed in the SR. These results should be considered in the context that all the results presented came from head-to-head comparisons and that no inactive treatment was considered in any of the three main comparisons. To check all other comparisons and analyses, refer to the full text. For further details, refer to the original abstract, available from http://cochranelibrary-wiley.com/doi/10.1002/14651858.CD009687.pub2/full.

DISCUSSION

This review included six Cochrane systematic reviews that evaluated interventions for patients with psoriasis. Three of them had broad criteria for interventions and considered any intervention for a specific condition (scalp,[17] chronic plaque[16] and nail psoriasis[13]). The other three assessed specific interventions (oral fumaric acid esters,[15] anti-TNF therapy[12] and narrow-band ultraviolet B phototherapy[14]). Only one SR limited the population criterion, through only including pediatric patients.[12]

The fact that some SRs used broad criteria for interventions resulted in inclusion of a high number of randomized clinical trials (RCTs). Therefore, there was high diversity of populations, interventions and outcomes, and multiple analysis had to be performed. This should be taken into consideration, in making recommendations for practice.

The number of systematic reviews published over the years has also been increasing. A rapid search of the literature, using the term "Psoriasis" [Mesh] and a filter for systematic reviews retrieved 5,310 abstracts from MEDLINE. The number of studies published per year since 1963 is presented in **Figure 1**.

This high number of published papers is a rough estimate. However, this volume of data does not necessarily mean that the quality of the data on interventions for treating psoriasis is good. The fact that Cochrane SRs follow rigid methodology and a special editorial process increases our confidence in the results included in this review.

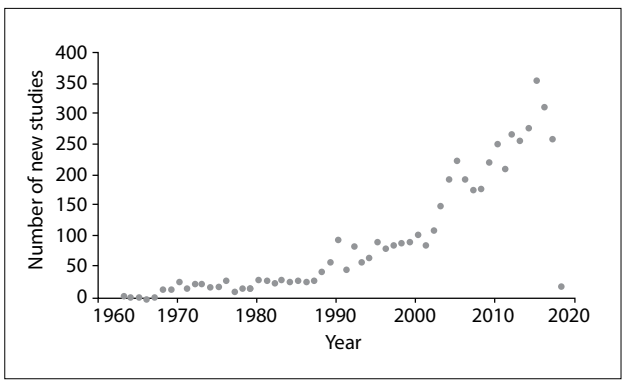

Figure 1. Number of studies indexed with "Psoriasis"[Mesh], published per year since 1963.

Our search in the Cochrane library retrieved six protocols that in the future may present results of relevance for the clinical question of this review. These protocols, which have been published in the Cochrane Library, will assess the following: interventions for guttate psoriasis;[18] methotrexate for psoriasis;[21] indoor salt water baths followed by artificial ultraviolet B light for chronic plaque psoriasis;[22] lifestyle changes for treating psoriasis;[23] antistreptococcal interventions for guttate and chronic plaque psoriasis[19]; and complementary therapies for chronic plaque psoriasis.[24]

Regarding clinical implications, only two Cochrane SRs provided high-quality evidence relating to use of anti-TNF agents for psoriasis in pediatric patients and use of combined therapy of steroids and vitamin D for scalp psoriasis. More specifically, there was high-quality evidence showing that use of etanercept was related to higher numbers of participants reaching PASI 75 (75% reduction in the PASI index) than was use of placebo. There was also high-quality evidence showing that combined therapy of steroids plus vitamin D was better for the clearance rate than was vitamin D alone, as determined through the investigators' global assessment, but that this combined therapy led to a higher proportion of participants dropping out due to adverse events. The evidence presented in **Table 2** may provide a guide for clinical practice, but all healthcare decision-makers and patients need to be aware that future studies could produce drastically changed results, in relation to the current studies.

Regarding research implications, further studies are needed in order to reduce the uncertainties surrounding the effects from several interventions for treating psoriasis. The reporting on all RCTs needs to follow the recommendations of the Consolidated Standards for Reporting Trials (CONSORT).[25] All studies need to be well designed and well conducted, in order to reduce imprecision and the risk of bias.

CONCLUSION

This review included six Cochrane systematic reviews that provided quality of evidence for management of psoriasis that ranged from unknown to high. High quality of evidence was found favoring use of anti-TNF (etanercept) treatment for pediatric psoriasis and use of combined therapy of steroids plus vitamin D rather than vitamin D alone. Further randomized controlled trials are imperative for reducing the uncertainties relating to several treatments.

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Complex karyotype including ring chromosome 11 in a patient with acute myeloid leukemia: case report

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KEY WORDS:
Leukemia, myeloid, acute.
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Obesity.
Chromosome aberrations.
Cytogenetic analysis.

ABSTRACT

CONTEXT: Complex karyotypes in acute myeloid leukemia (AML) are characterized by an overall low response rate with frequent relapses after clinical treatment.

CASE REPORT: Here, we describe the case of a 61-year-old obese female with clinically diagnosed AML who presented a complex karyotype involving an uncommon abnormality: ring chromosome 11. Immunophenotypic analysis confirmed the diagnosis. Classical and molecular cytogenetic analyses, using GTG banding and FISH (fluorescence in situ hybridization), revealed the presence of complex structural rearrangement involving r(11), add(12)(p13), der(5) and der(13).

CONCLUSION: Molecular cytogenetic analysis is suitable for better identification and characterization of chromosomal rearrangements in AML. Case reports like this, as well as population-based studies, are necessary for understanding the karyotypic changes that occur in humans.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous group of diseases. In some cases, patients have satisfactory survival, whereas in others, the course has a dismal prognosis. The risk factors include obesity and chromosomal aberrations. Recent studies have suggested that obesity is a risk factor associated with AML.[1-3]

Classically, karyotyping has formed a powerful independent prognostic indicator in this group of diseases.[4,5] It serves to identify biologically distinct subsets of disease and has been widely used to provide the framework for risk-adapted treatment approaches. Three subgroups can be distinguished:

1. AML with normal karyotype;
2. AML with primary balanced chromosomal aberrations; and
3. AML with unbalanced karyotype abnormalities characterized by gains and/or losses of usually larger regions of the genome and no known primary balanced abnormality.[4]

Complex karyotypes are defined as three or more independent chromosomal abnormalities in one genome. AML patients with such abnormalities are characterized by a low overall response rate, and often present relapses after clinical treatment.[5] Ring chromosomes are considered to be a rare finding in these diseases.[6]

Here, we describe a case with a complex karyotype involving uncommon chromosomal abnormalities in an obese patient. We also present a review of the literature focusing on studies in which ring chromosome 11 was found in AML cases.

CASE REPORT

A 61-year-old woman was registered at Pedro Ernesto University Hospital (HUPE) in December 2010, with a three-month history of dizziness, precordial pain and adynamia, a weight loss of 5.5 kg and fever. Her medical history was remarkable for obesity and hypertension. Physical examination showed pallor, rare petechiae and no palpable lymph nodes. Laboratory analysis revealed a

hemoglobin concentration of 5.5 g/dl, platelet count of 25 x 10^3/µl and leukocyte count of 19.58 x 10^3/µl. Serological tests were negative for anti-HIV1/2, anti-HTLV1/2, anti-HBc and anti-HBS. Bone marrow immunophenotypic analyses revealed 23% blasts; positivity for CD34, HLADR and CD33; and negativity for CD7, CD19, CD10, CD117, CD36 and CD15, characterizing acute myeloid leukemia (AML), FAB classification M4.

The patient was treated with one cycle of cytarabine and daunorubicin to induce remission and four consolidation cycles of cytarabine. Hematological remission was achieved after the first cycle of cytarabine/daunorubicin (one month afterwards).

Nevertheless, she relapsed in August 2011. At that time, a myelogram showed the presence of blasts (**Figure 1**) and an immunophenotypic analysis revealed that the blasts were 71.2% positive for HLADR, CD33, CD38, CD117, CD13, CD15, CD64, CD33, CD13 and CD14. The immunophenotypic profile of this case at relapse is shown in **Figure 2**. A molecular analysis was positive for AML-ETO/t(8;21) rearrangement and negative for PML-RARA/t(15;17) and CBFb-MyH11/inv(16)/t(16;16) rearrangements. A cytogenetic analysis using GTG banding revealed a complex karyotype of 46,XX, r(11), add(12)(p13), der(5?) and der(13?)[15] (**Figure 3**). For further clarification, fluorescence in situ hybridization (FISH) was performed, applying whole chromosome probes (wcp) for chromosomes 5, 11, 12 and 13 (**Figure 4**). The molecular cytogenetic results were as follows: 46,XX, del(5)(q), r(11), t(11?;12;13) and der(13)t(11?;12;13). A new conditioning regimen (FLAG) was started, but the patient died due to septic shock in September 2011, 293 days after diagnosis.

Ethics committee approval: CAAE #56621716.5.0000.5259.

Review of ring chromosome 11 and AML

The literature was reviewed through the MEDLINE (PubMed) and LILACS databases (see **Table 1** for details on strategy and results). For the LILACS database, the keywords in Portuguese were: "aberrações cromossômicas", "cariótipo complexo" and "leucemia mielóide aguda". In PubMed/MEDLINE we searched for "acute myeloid leukemia" and "chromosomic aberrations" and "ring chromosome" and "case reports".

Articles were selected if they met the following inclusion criteria:

1. Indexed articles published between January 1, 1975, and October 20, 2016;
2. Letters to the editor, case presentations, case series, original research reports and reviews;
3. Clinical research articles on adults;
4. Articles written in the following languages were included: English, French, Portuguese and Spanish;

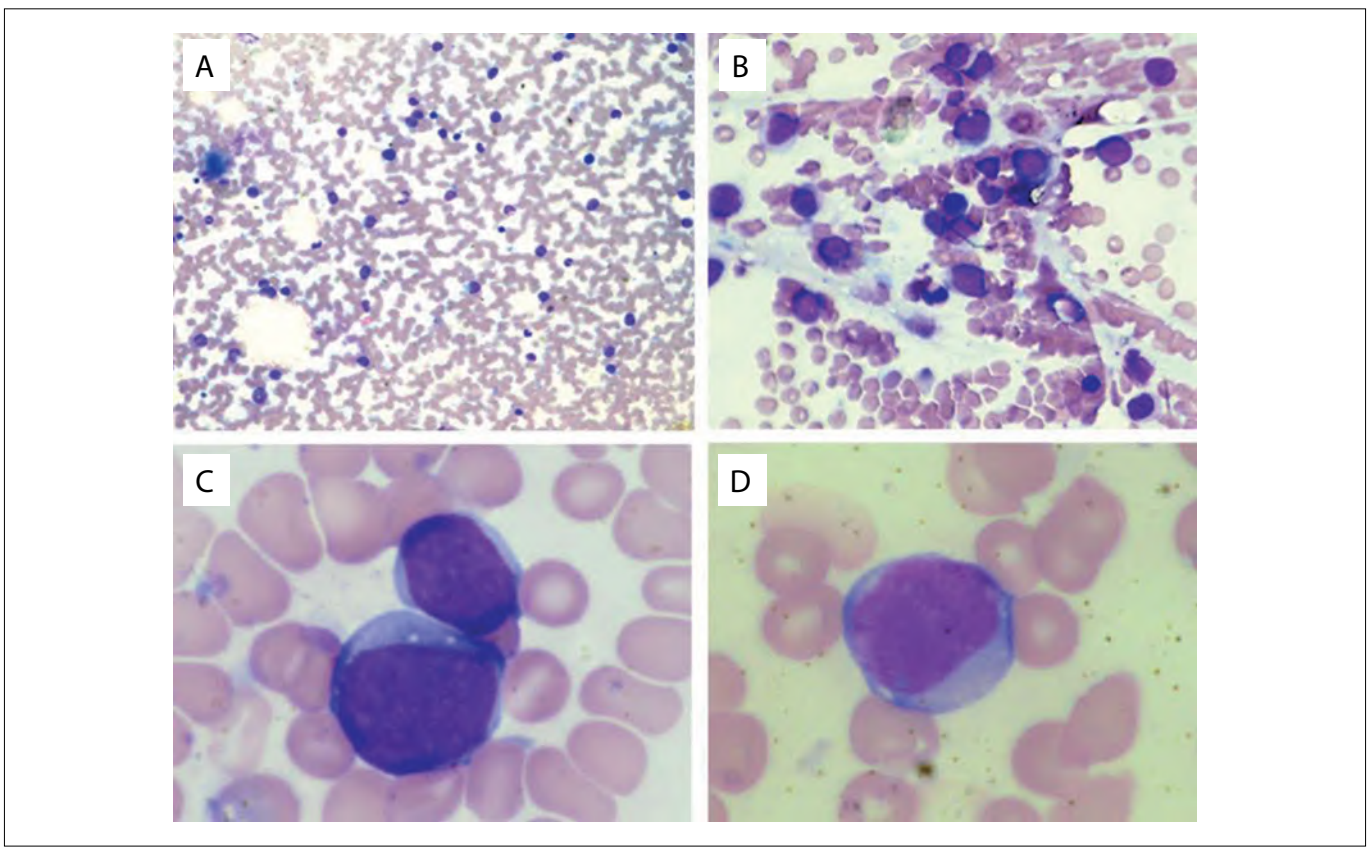

Figure 1. Bone marrow smears at the time of relapse of acute myeloid leukemia. A) Bone marrow presenting blastic cell infiltration, 20 X; B) Bone marrow presenting blastic cell infiltration, 40 X; C) Blast, 100 X; D) Blast, 100 X.

The exclusion criteria were:

1. Articles published outside of proposed period;
2. Other published articles not specified in the inclusion criteria;
3. Clinical research articles on children;
4. Experimental clinical research articles;
5. Articles written in languages not specified in the inclusion criteria.

Manuscripts that met the inclusion criteria were retained for full analysis. Any disagreements were resolved through further discussion involving an additional author.

The PubMed search identified 66,877 articles involving AML, of which 22 were eligible.[7-26] The search in LILACS identified 582 articles and none of them were eligible.

Among the 22 studies selected, 34 patients presenting ring chromosome 11 were described (**Table 2**). Gender and age were reported in 27/34 cases, with mean ages for women and men of 62 and 60 years and medians of 61 and 63 years, respectively. The French-American-British (FAB) classification was given for 20/34 patients, and the distribution was: M0 (1 male), M1 (2 females and 2 males), M2 (2 females and 3 males and 1 gender not informed), M4 (3 females and 4 males), M5a (1 female) and M6 (1 female). Along with ring chromosome 11, all the 34 patients presented a complex karyotype.[7-28]

DISCUSSION

Although our patient satisfactorily tolerated chemotherapy and achieved complete remission after one cycle, a relapse occurred eight months later. The observed resistance to chemotherapy might be a possible explanation for treatment failure, but the expression of multidrug-resistant genes was not tested.

According to our records, obesity was the only lifestyle-related risk factor in cancer presented by our patient. She was considered to have class II/III obesity (body mass index, BMI > 35). Obesity is a chronic inflammatory condition, characterized by increased production of pro-inflammatory cytokines and adipokines, presence of hyperinsulinemia and insulin resistance and elevated levels of insulin-like growth factors.[1-3,29] It has been suggested that obesity is an adverse prognostic marker in patients with cancer.[7,8,29,30] It is well known that overweight and obesity are associated with increased incidence and mortality due to cardiovascular disease, diabetes mellitus and certain types of cancer, including leukemia. Epidemiological, case control and meta-analysis studies have correlated obesity with poor prognosis for AML.[31-34] In addition, Finn et al. reported an association between obesity and cytogenetic categories.[35] Several studies have considered that obesity might confer poor prognosis in different ways.[3,32-39] For example, the mean elimination half-life of doxorubicin is longer in obese patients than

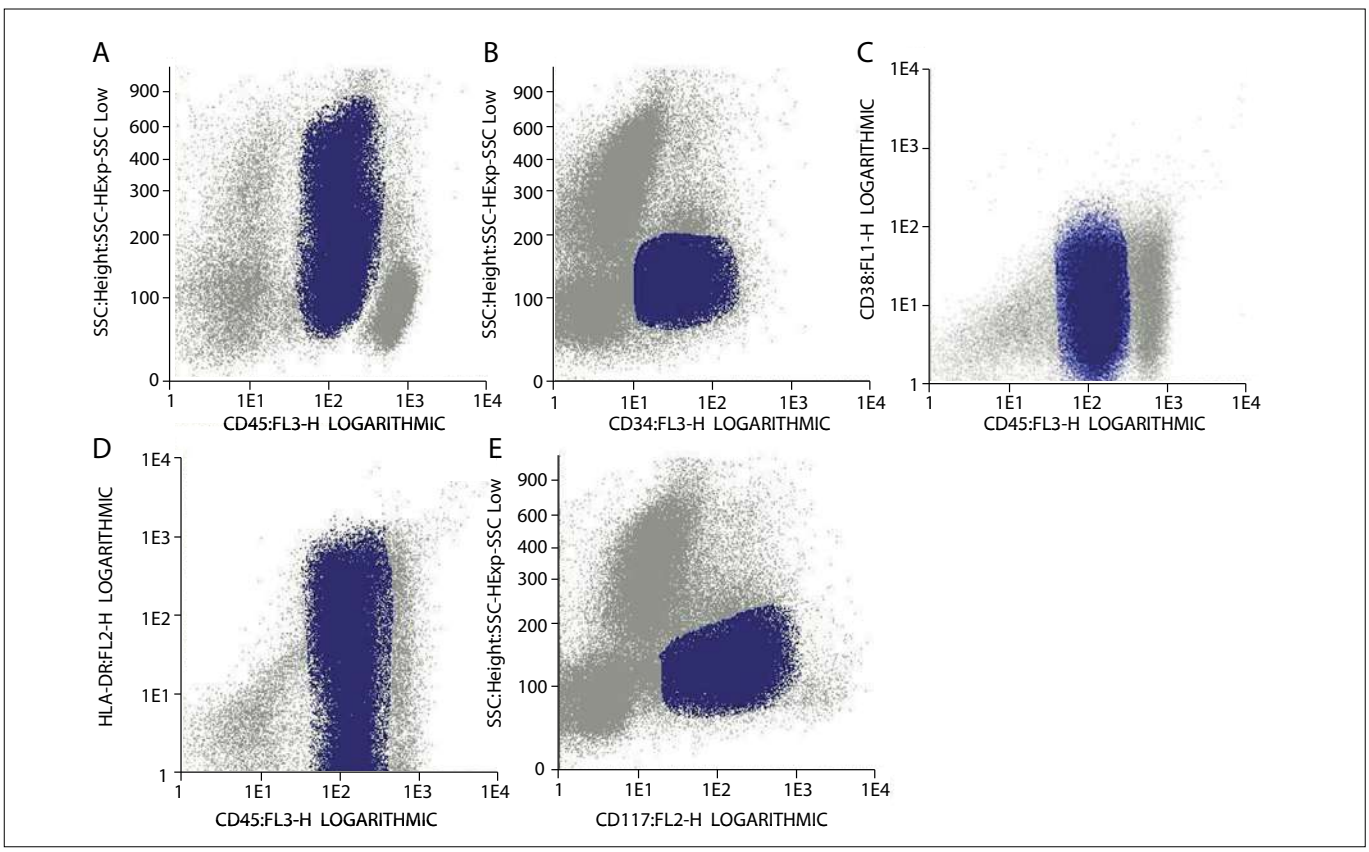

Figure 2. Immunophenotypic profile at the time of relapse. Dot plots revealing 71.2% of blasts positive for CD45 (A), CD34 (B), CD38 (C), HLADR (D) and (E) CD117.

in normal patients, thus increasing its toxicity to these patients. Adipocytes are also mesenchyme-derived cells and were previously considered to play only a passive "space filling" role in the bone marrow cavity. An inverse correlation between increasing numbers of adipocytes and active hematopoiesis in bone marrow is consistent with the recent identification of adipocytes as negative regulators of hematopoiesis.[29] Other proposed mechanisms for the negative association of obesity with AML include impaired immune function due to chronic elevation of tumor necrosis factor alpha (TNFα), decreased T lymphocyte production, increased leptin and increased insulin-like growth factor activity, which are involved in hematopoiesis and survival of myeloid cells.[33-35,37,38]

At the time of relapse, our patient also presented a complex chromosomal karyotype involving at least four chromosomes. She was positive at the molecular level for AML-ETO [t(8;21)] rearrangement, e.g. der(12), and the marker had the capacity to carry parts of chromosomes 8 or 21.[39-41] However, none of these points could be tested, because of the limitations of the material available. The AML1/ETO fusion protein is essential for development of t(8;21) AML and is well recognized for its dominant-negative effect on the coexisting wild-type protein AML1. It is associated with 12% of the cases of de novo AML and up to 40% of the cases of AML subtype M2 of the French-American-British classification. Furthermore, it has also been reported in a small portion of M0, M1 and M4 AML samples. Chromosome karyotyping and reverse transcription polymerase chain reaction (RT-PCR) results cannot be coincidental. The incidence of AML1/ETO is 5-10% higher when molecular biology approaches are used.[20,39-41]

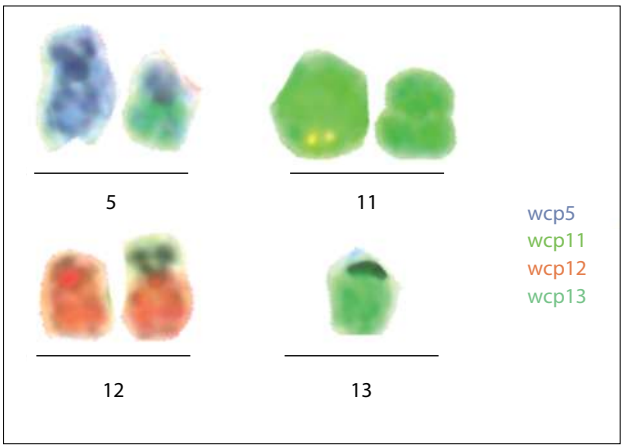

Figure 4. Fluorescence in situ hybridization (FISH) technique using whole chromosome painting (WCP). Chromosomes 5 (blue), 11 (green), 12 (red) and 13 (green), showing a complex translocation involving all chromosomes tested. Final karyotype: 46,XX, del(5)(q), r(11), t(11?;12;13) and der(13)t(11?;12;13).

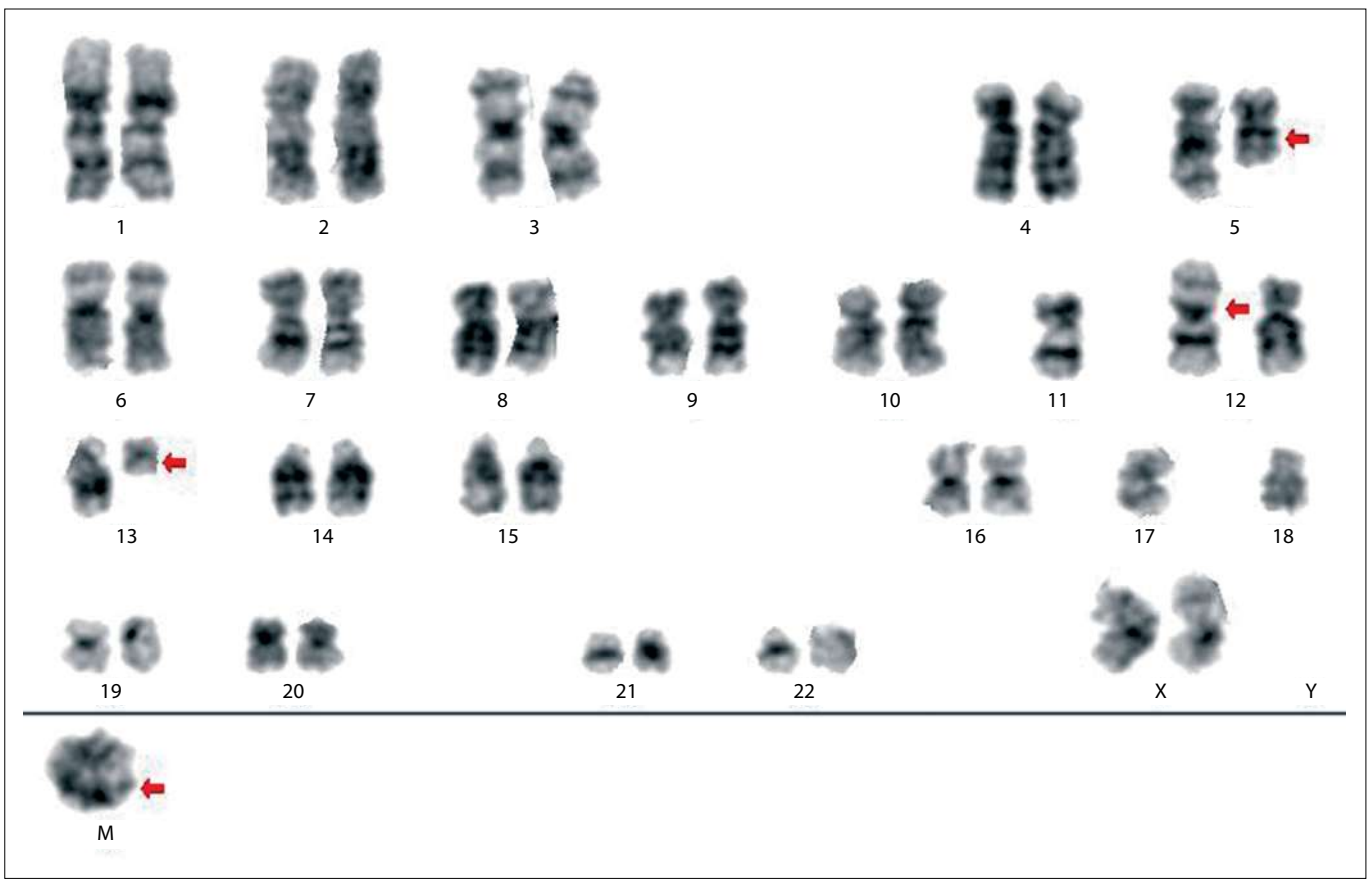

Figure 3. GTG banding. Complex karyotype determined through GTG banding: 46,XX, der(5), r(11), add(12)(p13) and der(13)[15].

Table 1. Search of the literature in medical databases for case reports on ring chromosome 11 in association with acute myeloid leukemia

Database	Search strategies	Papers found	Related papers
MEDLINE (via PubMed)	("ring chromosome", "chromosomic aberrations", "complex karyotype", "acute myeloid leukemia" AND "leukemia, myeloid, acute"[MeSH Terms]) OR ("leukemia"[All Fields] AND "myeloid"[All Fields] AND "acute"[All Fields]) OR ("acute myeloid leukemia"[All Fields]) OR ("acute"[All Fields] AND "myeloid"[All Fields] AND "leukemia"[All Fields] AND "ring"[All Fields] AND "11"[All Fields] AND ("chromosomes"[MeSH Terms] OR "chromosomes"[All Fields] OR "chromosome"[All Fields])	66,877	22
LILACS (via BVS)	"leucemia mieloide aguda" and "leucemia" and "mielóide", "aguda", "aberrações cromossômicas", "cariótipo complexo"	582	0

Table 2. Patients' characteristics and French-American-British (FAB) classification according the literature for acute myeloid leukemia involving ring chromosome 11

Reference	Gender/age	FAB	Outcome
Andreasson et al.[7]	F/62	Not informed	Not informed
Avet-Loiseau et al.[8]	F/53	M4	Died after diagnosis
Cigudosa et al.[9]	M/69	Not informed	Not informed
	F/59	Not informed	Not informed
Dastugue et al.[10]	M/57	Not informed	Died after 5.7 months
El-Rifai et al.[11]	F/52	M1	Still alive
Fischer et al.[12]	NI	Not informed	Not informed
Groupe Français de Cytogénétique Hématologique[13]	F/89	Not informed	Died after 1 month
Gisselsson et al.[14]	M/72	M4	Not informed
Johansson et al.[15]	M/72	M4	Not informed
Koka et al.[16]	F/61	Not informed	Died 7 days after treatment
Lindvall et al.[17]	F/74	M1	Not informed
	F/61	M4	Not informed
Liozon et al.[18]	M/65	M0	Died after 6 months
Mamuris et al.[19]	Not informed	M2	Not informed
Michaux et al.[20]	F/69	M6	Not informed
Mrózek et al.[21]	F/53	M4	Not informed
Poppe et al.[22]	M/63	M1	Not informed
	M/72	M1	Not informed
	F/46	M2	Not informed
Sárová et al.[23]	M/28	M2	Not informed
Schoch et al.[24]	NI	Not informed	Not informed
	NI	Not informed	Not informed
	NI	Not informed	Not informed
	NI	Not informed	Not informed
	NI	Not informed	Not informed
Streubel et al.[25]	M/54	M4	Not informed
Tanaka et al.[26]	M/72	M2	Not informed
Whang-Peng et al.[27]	M/45	M4	Died after 6 months
Zatkova et al.[28]	M/60	Not informed	Not informed
	M/50	Not informed	Not informed
	F/56	M5a	Not informed
	M/63	M2	Not informed
	F/76	M2	Not informed

Table showing 34 cases of acute myeloid leukemia involving ring chromosome 11.

The prognosis for AML/ETO-positive cases in the absence of t(8:21) has been reported to be poor, as was found in our case.[41]

For the chromosomal abnormalities found here, i.e. ring chromosome and translocations, it needs to be noted that ring chromosomes are considered to be rare in hematopoietic cancer (less than 10%).[6] With regard to ring chromosome 11, this abnormality has only been found in 34 AML patients (**Table 2**).[7-28] The outcomes of 26 of these 34 patients were not reported in the papers, but among the 8 with outcomes reported, 7 died, and only 1 was alive at the time of publication of the data. Our patient died nine months after admission. These data strongly suggest that presence of ring chromosome 11 is associated with poor prognosis in leukemia cases.

Ring chromosome 11 may carry the important leukemia-related gene MLL (mixed lineage leukemia)/KMT2A, which encodes a DNA-binding protein that methylates histone H3. MLL is a frequent target for recurrent translocations in acute leukemia cases, which can be classified as acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or mixed lineage (biphenotypic) leukemia (MLL). Interestingly, leukemia with translocations involving MLL shows poor prognosis. More than 50 different MLL fusion partners have been identified, and it has been observed that MLL fusion proteins lose H3K4 methyltransferase activity, thus generating transformation capacity.[42] Unfortunately, we were unable to study the breakpoint cluster region involved in this case, and future studies will be necessary to elucidate whether ring chromosome 11 in leukemia cases carries a rearranged MLL gene and what the mechanism underlying its gene expression are.

CONCLUSION

Molecular cytogenetic analysis is suitable for better identification and characterization of chromosomal rearrangements in acute leukemia. Single case reports, as well as population-based studies, are necessary for providing further insights into karyotypic changes that take place in human leukemogenesis.

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Report of a rare case of histiocytic necrotizing lymphadenitis with bilateral pleural effusion diagnosed via cervical lymph node biopsy

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KEY WORDS:
Histiocytic necrotizing lymphadenitis.
Pleural effusion.
Tuberculosis.
Lymph nodes
Biopsy.

PALAVRAS-CHAVE:
Linfadenite histiocítica necrosante.
Derrame pleural.
Tuberculose.
Linfonodos.
Biópsia.

ABSTRACT

CONTEXT: Histiocytic necrotizing lymphadenitis (HNL) is a rare disorder that is often benign and self-limiting. There have been reports of co-occurrence of HNL with other diseases, including systemic lupus erythematosus, hemophagocytic syndrome and antiphospholipid syndrome.

CASE REPORT: Here, we report a case in which a patient experienced unexplained fever, swelling of the cervical lymph node and bilateral pleural effusion and was ultimately diagnosed with HNL based on results from a lymph node biopsy. After treatment with glucocorticoid, the patient regained normal body temperature, the swelling of the lymph nodes disappeared and the pleural effusion was reabsorbed.

CONCLUSIONS: The pathogenesis of HNL remains unclear, and pleural effusion is rarely reported in HNL patients. We presented this case to improve diagnostic awareness of this condition among clinicians and help reduce the likelihood of misdiagnosis.

INTRODUCTION

Histiocytic necrotizing lymphadenitis (HNL) is a rare disorder, and its pathogenesis remains unclear. Some scholars believe that HNL is not an independent disease and that it may be a manifestation of an underlying autoimmune disorder; as such, the diagnosis and treatment for HNL should be targeted to the underlying disease instead. There have been reports of co-occurrence of HNL with other diseases, including systemic lupus erythematosus (SLE), hemophagocytic syndrome and antiphospholipid syndrome.[1,2]

However, pleural effusion has rarely been reported in HNL patients. Here, we report a case in which the patient experienced unexplained fever, swelling of the cervical lymph node and bilateral pleural effusion and was ultimately diagnosed with HNL based on the results from a lymph node biopsy.

CASE REPORT

A 26-year-old woman was hospitalized due to anorexia and fatigue that had lasted one month. Ten days before admission, the patient began to experience intermittent chills and a fever between 38.5 and 40 °C. Physical examination on admission revealed that both cervical lymph nodes were swollen and tender, more obviously so on the left side. Double lung auscultation detected lower breathing sounds.

Laboratory examinations revealed the following: white blood cell count, 2.45×10^9/l; neutrophils: 48.70%; lymphocytes: 42%; red blood cell count: 3.9×10^{12}/l; hemoglobin: 101 g/l; platelets: 141×10^9/l; erythrocyte sedimentation rate: 30 mm/h; procalcitonin: 0.095 ng/ml; and C-reactive protein: 19.11 mg/l. The results for tumor markers, autoimmune antibodies, anti-neutrophil cytoplasmic antibodies and blood cultures were normal. The T-SPOT and purified protein derivative tests for tuberculosis were negative, as were tests for pleural tuberculosis antibodies. Bone marrow cytological analysis revealed bone marrow hyperplasia.

Computed tomography (CT) of the chest revealed slight swelling of the mediastinal lymph nodes and a small amount of bilateral pleural effusion, which was slightly more severe on the

right side (**Figure 1**). Ultrasonography of the neck revealed multiple swollen bilateral supraclavicular lymph nodes, with some as large as 16 × 7 mm. Ultrasonography of the upper abdomen and uterus did not reveal any anomaly.

The pleural puncture technique was applied to drain excessive fluid, and Rivalta test results were positive. The pleural effusion pH was 7.35 (normal, 6.8-7.6), proteins were 32 g/l (normal, 0-30), and lactate dehydrogenase, adenosine deaminase and carcinoembryonic antigen levels were 2186 U/l (normal, 0-200), 30 U/l (normal, 0-40) and 1.64 ng/ml (normal, 0-6.5), respectively. The total number of nucleated cells in the pleural effusion was 3886×10^6/l (normal, 100-500 $\times 10^6$/l). Repeated pleural fluid cytological analysis revealed a large quantity of lymphocytes. Pleural fluid cultures were negative.

The patient underwent two weeks of anti-inflammatory treatment with 0.2% levofloxacin, 300 ml daily administered by means of intravenous drip, which was ineffective. She still experienced intermittent fever, and ultrasonography revealed continued pleural effusion.

A biopsy on the left cervical lymph node showed scattered fibrin deposition, a large amount of nuclear debris and large mononuclear cell aggregates in the necrotic area (**Figure 2**). The pathological diagnosis was histiocytic necrotizing lymphadenitis.

Antibiotics were discontinued after two weeks of treatment with intravenous infusion of levofloxacin, and 10 mg of oral prednisone was administered three times daily. Within three days, the patient's body temperature gradually returned to normal. After general improvement during a one-week observation period, she was discharged and was prescribed a reduced dose of oral prednisone. Three months later, a chest radiograph showed that the double lung texture disorder and bilateral pleural effusion had disappeared (**Figure 3**).

DISCUSSION

Histiocytic necrotizing lymphadenitis is a rare inflammatory disease of the lymph nodes. It is more common in young women and has a natural course of 1-4 months,[3] but relapse may occur. The cause of HNL remains unclear, but it is probably associated with infections and autoimmune disorders. A variety of viruses, such as adenovirus, Epstein-Barr virus and herpes virus, are reportedly associated with the disease. HNL onset is commonly associated with acute or subacute clinical manifestations, such as swollen lymph nodes, fever and neutropenia. The most commonly affected lymph nodes are cervical, followed by axillary. Intermittent fever, fatigue, joint pain, hepatosplenomegaly and skin rashes are also common symptoms. Rarely, HNL patients exhibit complex clinical manifestations involving multiple organ systems, similar to those for rheumatic diseases.

It has been reported in the literature that the clinical manifestations of HNL are similar to those of certain connective tissue diseases, such as adult-onset Still's disease and SLE. There are also reports of HNL co-occurring with these diseases, with the strongest link to SLE. Reports have suggested that HNL can occur before, after or concurrently with SLE pathogenesis.[4] In particular,

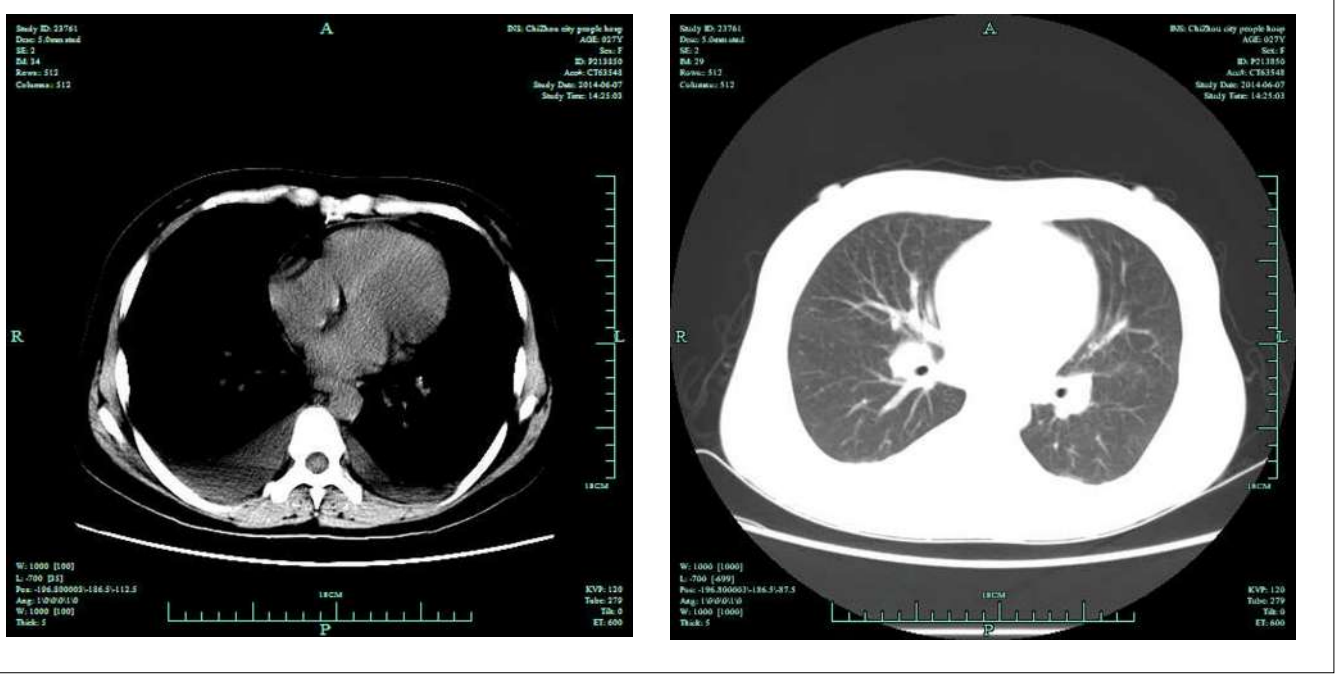

Figure 1. Computed tomography of the chest, revealing slight swelling of the mediastinal lymph nodes and a small amount of bilateral pleural effusion, with slightly more on the right side than on the left.

anti-nuclear antibody-positive HNL is more likely to develop into SLE, and certain scholars even hold that HNL is a unique manifestation of SLE.[5] Additionally, certain clinical manifestations of lymphoma are very similar to those of HNL; as such, HNL patients are often misdiagnosed as having malignant lymphoma.

Noninvasive diagnostic methods are limited in their effectiveness. At present, application of 18F-deoxyglucose (18F-FDG) positron emission tomography (PET)/CT is widely used as a tumor metabolism imaging technique for determining lymph node malignancy. However, studies using FDG imaging and PET/CT have suggested that in HNL patients, lymph node uptake values ranged from 2.05 to 13.94, with an average of 6.25 ± 3.32. Cervical lymph nodes often have a higher uptake value. Evidently, PET/CT scans alone are not sufficient to differentiate between HNL and lymphoma, but the biopsy site can be determined using the standard uptake value.[6]

Pathological and immunohistochemical examinations are important tools for differential diagnosis. In 1995, Kuo[7] further defined HNL into three subtypes: proliferative, necrotic and xanthoma-like, which may reflect the different stages of disease development, namely hyperplasia-necrosis-xanthoma or granulation-reabsorption-recovery. The pathological characteristics of HNL include a patchy lymph node disorder, commonly found in the juxtacortical area with visible fibrinoid necrosis and a large amount of nuclear debris. Necrotic areas are surrounded by proliferative cells, but granulocytes and plasma cells are rare or absent. Specialized or tufted CD68-positive and MPO-positive cells are important immunohistochemical features of HNL.[8] T-cells are the most commonly found lymphocytes, while B-cells and natural killer cells are rarer. Using an electron microscope, a large number of apoptotic bodies surrounded by mononuclear histiocytes and scattered T-cells undergoing apoptosis were found in the lymph nodes of HNL patients. These three observations are closely related, and they suggest that T-lymphocyte apoptosis may play an important role in the pathogenesis of HNL.[9]

The incidence of pleural effusion in HNL is low and rarely reported clinically, so we cannot reach any conclusion regarding the likelihood that HNL patients might have pleural effusion. In the present case, the patient had an exudate. The mechanism of its occurrence remains unclear, and it is possible that the disease itself may lead to pleural disruption, thus causing exudative pleurisy; however, there is a lack of histological evidence to support this concept. Pleural biopsies can easily miss the location of the lesion, since they can only collect a relatively small amount of the sample, and they may be prone to false negative results. On the other hand, thoracoscopy or thoracotomy examinations are highly invasive and are not very popular with most patients. Additionally, the possibility that HNL might be caused by an endogenous sex hormone dysfunction cannot be ruled out, since corticosteroid therapy has been observed to lead to complete reabsorption of pleural effusion. The mechanism of pleural effusion in HNL patients still need to be further investigated.

We searched for similar cases in different databases (PubMed, Embase and LILACS) using the following terms: "histiocytic necrotizing lymphadenitis" AND "pleural effusion" or "Kikuchi-Fujimoto disease" AND "pleural effusion" (**Table 1**). We found that only one case had been published.[10]

The treatments for HNL include non-steroidal anti-inflammatory drugs and corticosteroids; antibiotic treatment is often

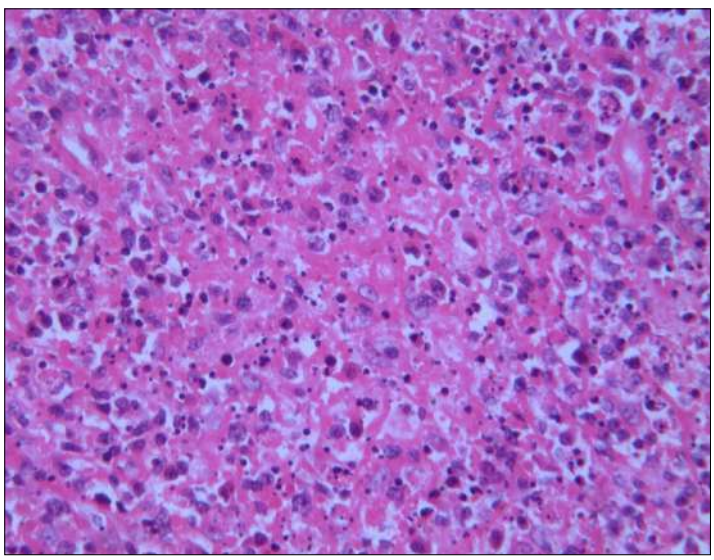

Figure 2. Biopsy specimen from the left cervical lymph node. Pathological manifestation showing fibrin deposition, a large amount of nuclear debris and large mononuclear cell aggregates within the necrotic regions (hematoxylin and eosin staining; magnification × 400).

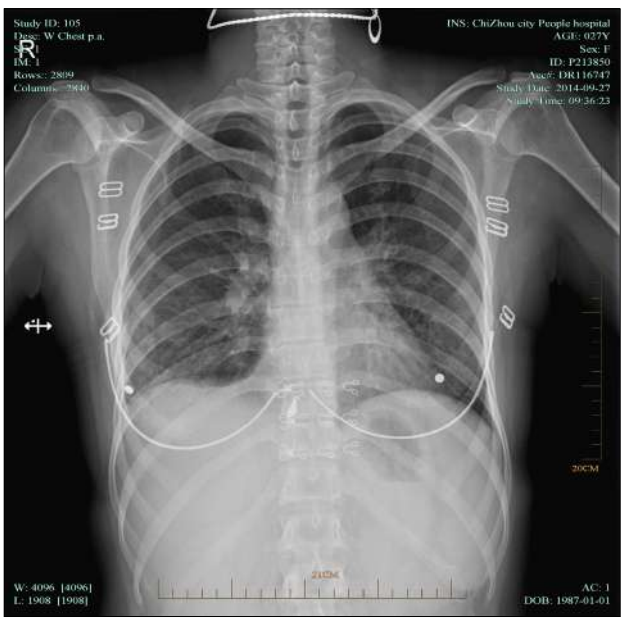

Figure 3. Chest radiograph, showing that the double lung texture disorder and bilateral pleural effusion had disappeared.

Table 1. Results from a search of the literature in medical databases, for case reports on "histiocytic necrotizing lymphadenitis with bilateral pleural effusion diagnosed via cervical lymph node biopsy". The search was conducted on December 22, 2016

Database	Search strategies	Papers found	Related papers
MEDLINE (via PubMed)	((histiocytic necrotizing lymphadenitis[Title]) AND pleural effusion[Title])	0	0
MEDLINE (via PubMed)	(((Kikuchi-Fujimoto disease[Title]) AND pleural effusion[Title])	1	1
Embase (via Elsevier)	((histiocytic necrotizing lymphadenitis[Title]) AND pleural effusion[Title])	0	0
LILACS (via BVS)	((histiocytic necrotizing lymphadenitis[Title]) AND pleural effusion[Title])	0	0

ineffective. Although non-steroidal anti-inflammatory drugs can elicit partial effects, there have been reports suggesting that this treatment may cause patients to be more prone to relapse.[11] It has been suggested that early glucocorticoid therapy should be used to treat HNL, and that the duration and intensity of the treatment should be based on the patient's response to hormone therapy and the results from follow-up examinations.[12]

CONCLUSIONS

Pleural effusion is a very uncommon manifestation of HNL that has rarely been reported in these patients. We presented a case of HNL with bilateral pleural effusion that was diagnosed using cervical lymph node biopsy and which was successfully treated with prednisone.

HNL patients are often admitted to a respiratory department, and it is easy to misdiagnose them as having tuberculous pleurisy or other diseases. We reported on this patient in order to improve clinicians' understanding of such diseases and help reduce the likelihood of misdiagnosis.

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Prolonged survival after surgical resection of cerebral metastasis from melanoma with multisystemic metastasis already present: a case report and literature review

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KEY WORDS:
Melanoma.
Neoplasm metastasis.
Glasgow coma scale.

ABSTRACT

CONTEXT: Malignant melanoma is the third most common cause of cerebral metastases after breast and lung cancer. Despite advances in therapeutic options, the prognosis for patients with cerebral metastases from melanoma remains poor, with a median survival time of six months after diagnosis.

CASE REPORT: A 65-year-old woman was diagnosed with a malignant melanoma on the third toe of her left foot. The tumorous spot was excised surgically. However, the melanoma reappeared after one year and skin biopsy confirmed recurrence of malignant melanoma. Investigations showed metastasis to the left pelvic region, left lobe of the liver and right lobe of the lung. The patient then received chemotherapy. Subsequently, the patient was brought to the emergency department with an altered level of consciousness (Glasgow coma scale: 9) and hemiplegia on the right side of her body. Computed tomography scans of the brain revealed hemorrhagic lesions in the parieto-occipital lobes of the brain. Urgent surgical evacuation was done to remove the lesion, following which the patient showed improvement in her score on the Glasgow coma scale and a concomitant decrease in weakness. She was discharged from hospital with full consciousness. The patient died of acute renal failure 14 months after the brain surgery and approximately 4 years after the initial presentation of the case.

CONCLUSION: This case outcome is rare and shows the effectiveness of surgery to treat cerebral metastasis from malignant melanoma in a situation with multisystem metastasis already present.

INTRODUCTION

Melanoma, the cancer of melanocytes, i.e. the pigment-forming cells of the body, is the least common but most lethal of all skin cancers.[1] The onset of the disease is marked by uncontrolled division of melanocytes between the epidermis and dermis, which is followed by radial invasive growth and vertical growth phases, respectively.[2] The main causative agent of melanoma is exposure to ultraviolet (UV) radiation, particularly UV-B.

Treatment of melanoma is palliative in nature. A clinical diagnosis is required, followed by wide-margin excision of the tumor. Excisional biopsies may remove the tumor, but further surgery is often necessary to reduce the risk of recurrence. Complete surgical excision with adequate surgical margins and assessment for the presence of detectable metastatic disease, along with short and long-term follow-up, is the standard treatment protocol.[2] Several treatment regimens including interferon therapy improve the prognosis but have severe side effects.

CASE REPORT

The patient was a 65-year-old woman who developed a dark skin lesion on the third toe of her left foot in February 2013. This skin was excised surgically in December 2013 but unfortunately was not sent for biopsy, and the lesion recurred at the same location in February 2014. A skin biopsy now confirmed the presence of a Clark's level II melanoma with surrounding hyperkeratosis and invasion of the epidermis and superficial dermis. Then, with further diagnostic workup, ultrasonography of the abdomen revealed normal liver, gall bladder, spleen, kidneys, urinary bladder, ovaries and pelvic lymph nodes. However, three lymph nodes in the left inguinal canal and a few in the right canal were enlarged, such that the largest one measured 35 × 29 × 17 mm. T1 and T2-weighted gadolinium-contrasted phase contrast magnetic resonance imaging (MRI) confirmed the results obtained from ultrasonography, thereby confirming the presence of metastasis to the inguinal lymph nodes.

A computed tomography (CT) scan of the chest on January 18, 2015, revealed left basal lung consolidation of heterogeneous density with spots of calcification, measuring 50 × 42 mm, which was suggestive of metastasis. Bilaterally enlarged axillary lymph nodes, measuring up to 13 × 6.5 mm, presence of fatty hilum and small hypodense lesions of 7 × 6 mm in the left lobe of the liver were also seen. MRI of the abdomen confirmed the presence of solitary, well-defined lesions in the left liver lobe with dimensions of 8 × 7 mm, which appeared hypo-intense in the T1-weighted and hyper-intense in the T2-weighted and STIR images, respectively.

The patient was administered chemotherapy comprising cisplatin, vinblastine and dacarbazine for three sessions starting at the end of January 2015, for three months, after which abdominal ultrasonography was performed to assess the status of the metastasis. This showed vascularized hypoechoic lesions in the left femoral region with a maximum size of 50 × 35 × 50 mm, thus indicating malignant lesions or lymphadenopathy. A pelvic MRI confirmed the presence of lymphadenopathy through detection of three enlarged lymph glands in the left femoral region.

This was followed by surgical removal of the diseased toe and resection of the inguinal lymph nodes in May 2015. Gross analysis on the amputated toe revealed ulceration up to the phalangeal bone, lymph node necrosis and hemorrhage. Histopathological analysis confirmed the presence of recurrent malignant melanoma of Clark's level V with malignant melanocytes present down to the subcutaneous layer, a Breslow thickness of > 4.0 mm with stage T4b ulceration, and total effacement of the inguinal lymph nodes with pigmented malignant melanocytes. The disease was in stage T4N1bM0 of the TNM classification of the American Joint Committee on Cancer (AJCC), and in pathological stage IIIC.

A CT scan of the chest was performed in June 2015, and this revealed two well-defined hypodense pulmonary lesions of 8 × 7 mm and 7 × 6 mm, respectively, which were indicative of secondary metastasis. However, there was a reduction in the size of the primary lung lesion that had been observed prior to chemotherapy. Nonetheless, the chemotherapeutic regimen was continued for another three months with a reduced dose.

On July 22, 2015, the patient suddenly complained of altered consciousness and hemiplegia on the right side of her body, with a score of 9 on the Glasgow coma scale (GCS). A CT scan revealed an intracerebral hemorrhagic mass, which was treated with urgent resection by means of parieto-occipital loop-assisted craniotomy on the same day (**Figure 1A-C**). Histopathological

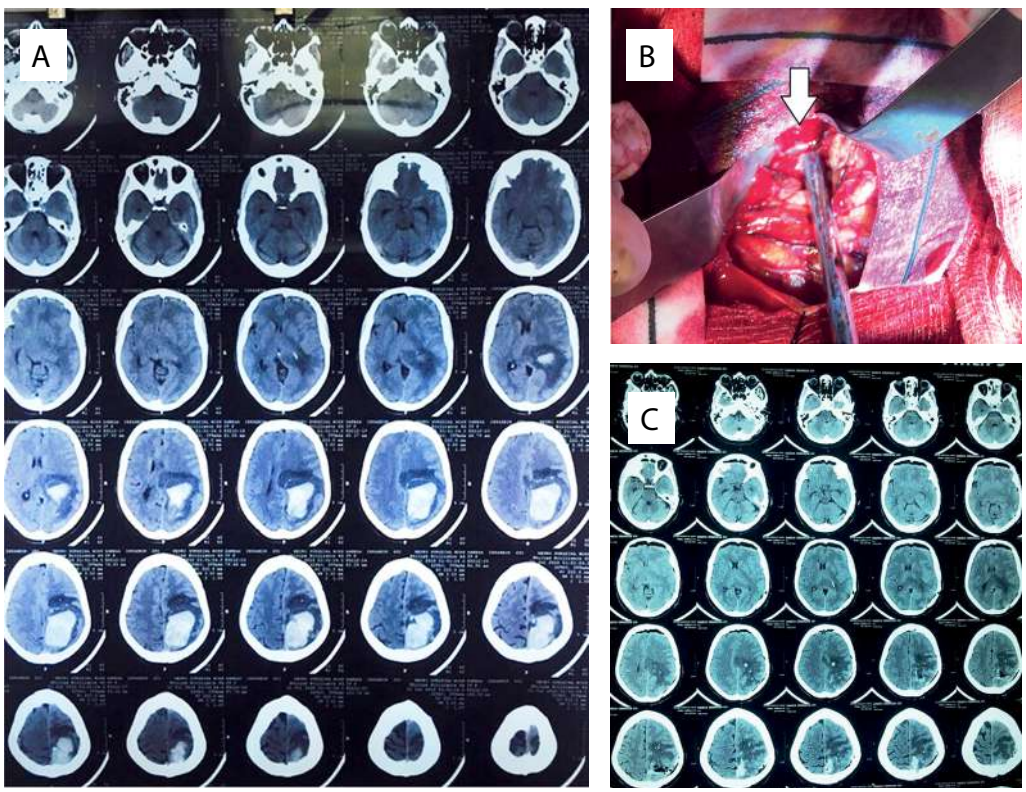

Figure 1. (A) Computed tomography (CT) scan images of the patient's brain, showing metastatic hemorrhagic mass lesion before the cranial surgery. (B) Intraoperative photo showing the hemorrhagic mass (white arrow). (C) Immediate postoperative brain CT scan, showing removal of the lesion with relative reversal of mass effect.

and cytological reports on the intracranial hematoma showed positivity for melan A and HMB-45. The patient was discharged after the surgery, on August 1, 2015, with improved consciousness (GCS 15).

Unfortunately, she died from renal failure on September 16, 2016, 14 months after the brain surgery and approximately 4 years after the first presentation of this case. This is a rare and unprecedented case among the among known occurrences of metastatic melanoma that were already multisystemic (**Table 1**).

DISCUSSION

Several imaging techniques such as X-ray, ultrasonography, magnetic resonance imaging (MRI), CT scan and positron emission tomography (PET) scan are regularly used to detect metastatic melanomas.[3,4] In the present case, combinations of all these methods were successfully used to evaluate the patient's condition.

Here, we found that the patient presented a partial response to chemotherapy consisting of three drugs with anti-mitogenic effects. Despite complete remission in the case of the hepatic lesion and partial regression of the primary pulmonary lesion, the melanoma rapidly metastasized to the brain, thus indicating a requirement for invasive interventional methods.

Radiation therapy is another palliative method of choice that has been shown to extend life span by 3-5 months. It is often combined with systemic treatments such as use of immunotherapeutic drugs, to improve the prognosis of cerebral metastasis from melanoma.[5,6] However, the precise location of the metastasis inside the brain (intra or extracranial) is a decisive factor for the extent to which the treatment can be used successfully with minimal side effects. Considering the drawbacks of the existing strategies, the search for better alternatives for treating brain metastasis from melanoma is still on (**Table 2**).

Here, we found that intracranial surgery significantly improved the clinical symptoms associated with brain metastasis from melanoma in our patient. The prognosis for brain metastasis from melanoma is poor, with a median survival span of 2-10 months.[3,4]

CONCLUSIONS

This is the first report highlighting success from intracranial surgery to alleviate the symptoms of a patient with brain metastasis from melanoma that had progressed with systemic dissemination for a considerable length of time. This case is unprecedented in the history of prognoses from multisystemic metastatic melanoma. The outcome from this case is rare and shows the effectiveness of surgery in improving the prognosis and treating brain melanoma that already has systemic metastasis.

Table 1. Diagnosis and treatment planning of the patient

2013 February	Black spot on left third toe.
2013 December	Excision of spot only without biopsy.
2014 February	Recurrence with biopsy done.
2015 January 18	Chest CT scan shows lung metastatic lesion.
2015 January	Start of chemotherapy: 3 sessions of 4 drugs, ending in March 2015.
2015 May	Left third toe excision + left inguinal lymph node resection.
2015 June	New chest CT scan shows a reduction in the size of the primary lung lesion that had been observed prior to chemotherapy.
2015 July 22	Sudden right-side weakness and disturbed consciousness, leading to urgent surgical evacuation by means of loop-assisted parieto-occipital craniotomy.
2015 August 1	Discharge with GCS 15, with improvement in weakness.
2016 September 16	Death due to renal failure.

CT = computed tomography; GCS = Glasgow coma scale.

Table 2. Search of the literature in medical databases for case reports on melanoma conducted on May 2, 2017

Database	Search strategies	Papers found	Related papers
MEDLINE (via PubMed)	Radiation AND therapy AND lymph AND node AND dissection	2,163	2
MEDLINE (via PubMed)	Metastatic melanoma: chemotherapy review	1,706	3
MEDLINE (via PubMed)	Temozolomide melanoma	480	4
MEDLINE (via PubMed)	Interleukin-2 therapy melanoma	2,172	5
MEDLINE (via PubMed)	Intracranial metastases of malignant melanoma	193	6

Total number of articles retrieved from PubMed, removing duplications, was 5.

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Pegylated interferon for treating severe recurrent respiratory papillomatosis in a child: case report

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KEY WORDS:
Papillomatosis.
Respiratory.
Peginterferon.
Child.

ABSTRACT

CONTEXT: Recurrent respiratory papillomatosis (RRP) is the most common laryngeal tumor. During childhood, it may present in extremely severe forms defined by the need for frequent surgical procedures to relieve respiratory distress and/or involvement of extralaryngeal sites such as lung involvement. Adjuvant therapies are indicated in these cases and interferon is one of the options. Pegylated interferon is more effective than conventional alpha interferon and, given its reported results in relation to treating hepatitis C over the past decade, we hypothesized that this might be more effective than conventional interferon also for treating respiratory papillomatosis. Use of a treatment strategy that eliminates the need for general anesthesia is particularly appealing, yet obtaining approval for use of medications that are not currently used for this purpose is challenging.

CASE REPORT: We report the case of a child with severe RRP that had been followed for the preceding six years, who was treated with pegylated interferon after failure of other adjuvant therapies. There was noticeable improvement in the frequency of surgical procedures, which was regarded very receptively, considering the child's history and previous response to other therapies.

CONCLUSION: Pegylated interferon may be a good option for diminishing the need for surgical intervention in severe cases of recurrent respiratory papillomatosis.

INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is the most common benign tumor of the larynx and occurs both in adults and in children. An estimated 4.5 cases per 100,000 occur in the United States.[1] Many medical and surgical treatments have been described but the cure remains unknown.

When the disease initially presents before the age of three years, the need for frequent surgical intervention is more common[1] and acute episodes of respiratory obstruction can be quite dramatic and even fatal. This gives rise to an emotional burden both for the child and for the family involved. Lesions that occur prematurely under the age of 12 months and infection with human papilloma virus type 11 are frequently associated with worse prognosis and greater severity of clinical presention.[2]

Surgical interventions are the only way to secure a patent airway and various instruments can be used depending on the surgeon's experience and the available technology. Cold instrumentation, carbon dioxide laser and microdebridement are the methods most frequently reported. Repeated surgical intervention facilitates disease dissemination to other sites of the airway and increases the risk of definitive scarring of the larynx, with significant sequelae for the voice. Adjuvant treatments may be needed in up to 20% of the cases.[1,2]

The recommended indications for adjuvant therapy are: the need for more than four surgical procedures per year; rapid growth of papillomata with airway compromise; or distal multisite spreading of disease.[1] Extralaryngeal involvement may occur in up to 30% of the cases,[3] with lesions occurring in the oral cavity, trachea, bronchi and esophagus. Malignant transformation, although rare, may occur in around 1.6-2% of cases.[3]

The adjuvant treatments for RRP include use of cidofovir, cis-retinoic acid, ribavirin, indole-3-carbinol, human papillomavirus (HPV) vaccine, bevacizumab, photodynamic therapy, celecoxib

and interferon.[4,5] The most commonly used adjuvant treatment is cidofovir,[4,5] and this involves intralesional application of medication that is performed under general anesthesia. Thus, despite improving the disease, it does not eliminate the need for hospitalization and use of anesthesia for microlaryngoscopy. Unfortunately, there is a general lack of evidence to support these therapies when not in the context of clinical research.

Alpha-interferon was the first adjuvant treatment used to treat respiratory papillomatosis, but the evidence has failed to support its use, based on limited case series reports and a few controlled studies that lacked sustainable results, despite the initial improvement observed when alpha-interferon therapy was combined with surgical removal of tumors.[6] Systemic side-effects and high costs are also a disadvantage of interferon.

Use of adjuvant treatments that avoid the need for surgical intervention and anesthesia is of particular interest, since repeated procedures and exposure to general anesthesia are a burden for these children and their families.

The advantage of pegylated interferon (PEG-IFN) over conventional interferon has been proven through its use for treating hepatitis C. This benefit is due mostly to its sustained levels in plasma, which translates into better treatment results. Since 2001,[7] significant improvement in treating hepatitis C through using PEG-IFN, in comparison with conventional alpha-interferon, has been continually reported. Use of PEG-IFN has revolutionized the treatment of hepatitis C worldwide.[7] There are no reports of use of pegylated interferon as an adjuvant treatment for recurrent respiratory papillomatosis in children.

The present study is a case report on a pediatric patient with recurrent respiratory papillomatosis who was treated with both surgical resection and adjuvant therapies, including use of PEG-IFN, with noticeable improvement in recurrence. Following the case report, we present a brief discussion and a systematic search of data in the PubMed, Cochrane, LILACS and Embase databases (**Table 1**). The articles included were either clinical trials on alpha-interferon or case reports on PEG-IFN. Duplicated studies and those that did not refer specifically to respiratory papillomatosis were excluded.

CASE REPORT

Institutional ethics committee approval was obtained (Ethics Committee of the School of Medical Sciences, State University of Campinas, UNICAMP) under protocol number 020/2016, subsequent to obtaining signed parental consent for submission of this communication.

A male child aged 14 months presenting recurrent episodes of suspected laryngitis and upper airway obstruction was diagnosed with laryngeal papillomatosis after nasofibroscopy revealed typical papillomatous lesions of the larynx (**Figure 1**).

The diagnosis was confirmed through a biopsy specimen and hybridization confirmed the presence of infection with HPV type 11. Initially, the treatment consisted of multiple airway procedures to remove obstructive lesions from the airway. The criterion for surgical removal of lesions was respiratory distress. After eight surgical procedures, at the age of 17 months the child underwent tracheostomy and adjuvant treatment with cidofovir was introduced, following parental informed consent. At this time, the lesions involved both of the vocal cords and also the laryngeal ventricles, false vocal cords, epiglottis and peristomal trachea (**Figure 2a** and **b**).

Intralesional applications of cidofovir were performed under general anesthesia and were scheduled every 2 to 4 weeks but no sustainable control of disease was achieved, with respiratory distress occurring repeatedly, three weeks after the application. Progression of papilloma lesions to the lower airway was also observed (**Figure 2c**) during the course of cidofovir treatment, which was halted after 12 applications.

A computed tomography (CT) scan was performed after use of cidofovir had been halted and after observation of papilloma lesions in the lower airway. It revealed multiple pulmonary nodules ranging from 3 mm to 14 mm in size; cavitary lesions with thick, irregular walls; multiple diffuse small centrilobular nodules; and bilateral hilar and right lower paratracheal and subcarinal lymphadenopathy (**Figure 3**). A biopsy on one of the pulmonary nodules confirmed the diagnosis of pulmonary papillomatosis.

Table 1. Description of yearly follow-up: number of procedures, instrumentation used, adjuvant treatments, presence of tracheostomy and interval between surgical procedures (mean in days)

	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Number of procedures	16	8	6	4	8	5
Surgical instrumentation	cold/laser	cold/mdb	mdb	mdb	mdb	mdb
Adjuvant treatment	cidofovir	bevacizumab	none	none	none	PEG-IFN
Tracheostomy	yes	yes	yes	yes	no	no
Interval between procedures (days)	22.3	45.5	61.3	83.2	43.8	77.2

mdb = microdebridement; PEG-IFN = pegylated interferon.

A trial using intralesional bevacizumab was attempted, but was also halted after three applications, since no noticeable improvement of recurrence rates was achieved and the patient presented moderate hair loss.

Up to the age of 5 years, the tracheostomy helped alleviate the patient's respiratory distress. The intervals between surgical procedures were as long as 83 days during the fourth year of follow-up (**Table 1**). Just before the patient reached six years of age, although the recurrence rates were still high, the significant growth of the airway allowed decannulation. After this, without the tracheostomy, deobstruction of the airway was required almost on a monthly basis (year 5 in **Table 1**) with profuse lesions involving the laryngeal inlet (**Figure 4a**), despite progressive improvement of the lesions in the lower airway (**Figure 4b**).

A six-month treatment with PEG-IFN α2 β IFN-α2b (1.0 μg/kg/wk) was proposed in an attempt to increase the intervals between surgical procedures. This was started after receiving informed parental consent. The child was six years old at the time. The detailed yearly follow-up of the child can be seen in **Figure 2**.

Subcutaneous injections of PEG-IFN (1 μg/kg) were given once a week. Fever, headaches and general flu-like symptoms were observed after the first applications during the initial 48 hours but were well managed with the usual antipyretic drugs. Complete blood count, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), albumin, international normalized ratio (INR), thyroxine (T4) and thyroid-stimulating hormone (TSH) were monitored monthly.

For more than three consecutive months, for the first time, surgical intervention was not needed. This interval between procedures had not been seen, even when the child still had a tracheostomy. After 102 days, surgery was indicated, for the first time, not due to respiratory distress or stridor but because of vocal demands and progressive moderate to severe dysphonia, which was a hazard at school.

Unfortunately, after a three-month treatment, a threefold elevation in liver transaminase levels was noted and treatment was halted. Despite withdrawing the treatment, the mean interval between procedures improved considerably over the months following PEG-IFN treatment (year 6), in comparison with the previous year (year 5).

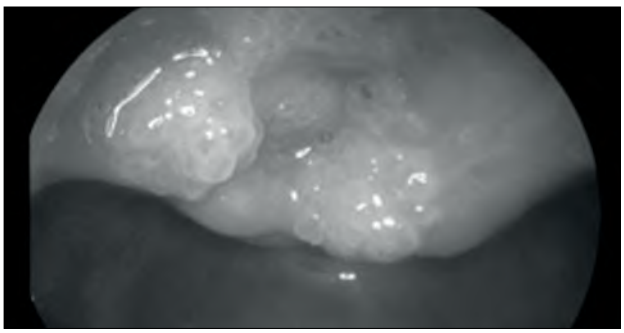

Figure 1. Laryngoscopic view of the laryngeal inlet obstructed by profuse papilloma lesions.

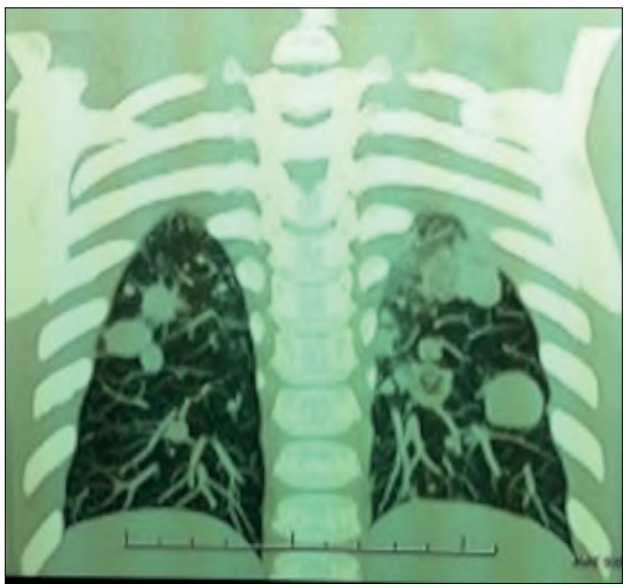

Figure 3. First pulmonary computed tomography scan showing numerous bilateral solid nodules, in the patient at the age of three years.

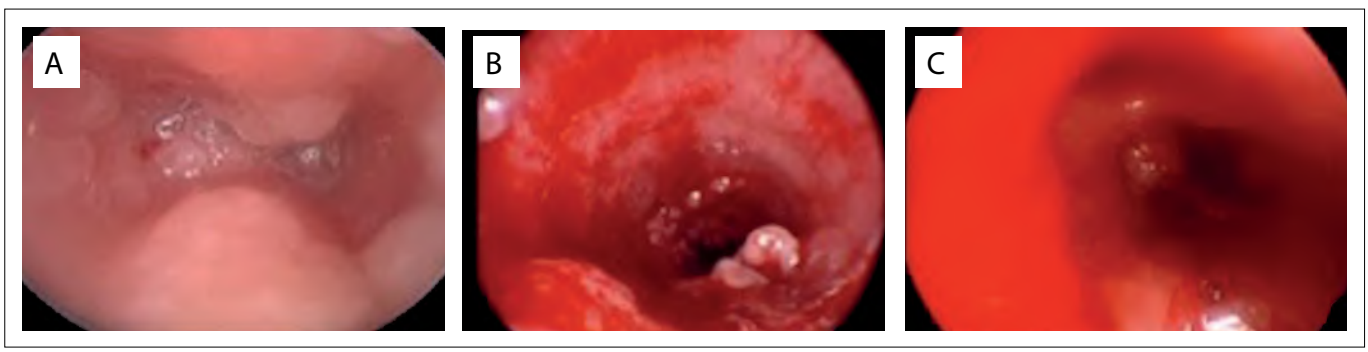

Figure 2. Laryngoscopic view of the upper trachea with papilloma lesions surrounding supra-stomal trachea (A), middle trachea (B) and bronchi (C).

CT scans performed at yearly intervals showed improvement in the number of solid nodules, particularly in years 5 and 6, in comparison with years 3 and 4 (**Figure 5**).

Throughout the years of follow-up, the child has maintained mild to moderate and sometimes severe dysphonia, depending on the progressive and intermittent infiltration of the vocal cords with papilloma lesions, but also secondary to the vocal cord scarring that has inevitably occurred. Pulmonary function has remained stable, despite persistent pulmonary lesions. Spirometry performed during follow-up in year 5 was normal.

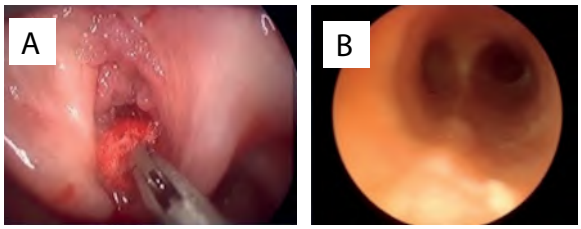

Figure 4. View of the larynx (A) and lower airway (B) during year 5.

DISCUSSION

Pegylated interferon has proven to be significantly more effective for treating hepatitis C than conventional alpha-interferon.[7,8] It is only natural to hypothesize that the pegylated form of interferon might be also more effective for treating respiratory papillomatosis than alpha-interferon.

Alpha-interferon is an immunomodulatory cytokine with antiviral and antiproliferative properties and was the first widely used adjuvant drug for treating RRP, with diverse results ranging from better prognosis to absence of response.[6,9] The evidence has failed to support its use, since the reports have mostly been based on case reports and clinical trials have shown varied results with limited follow-up.[6,9,10]

The association between interferon and polyethylene glycol, which is called pegylated interferon, increases the half-life of the drug, eliminates immunogenicity and improves the pharmacokinetics of the protein, such that it can be administered once a week. It potentially has greater antiviral activity than conventional alpha-interferon.[8] Since it was first introduced for treating hepatitis C, there has been a complete change of scenario for patients infected with the hepatitis C virus worldwide.

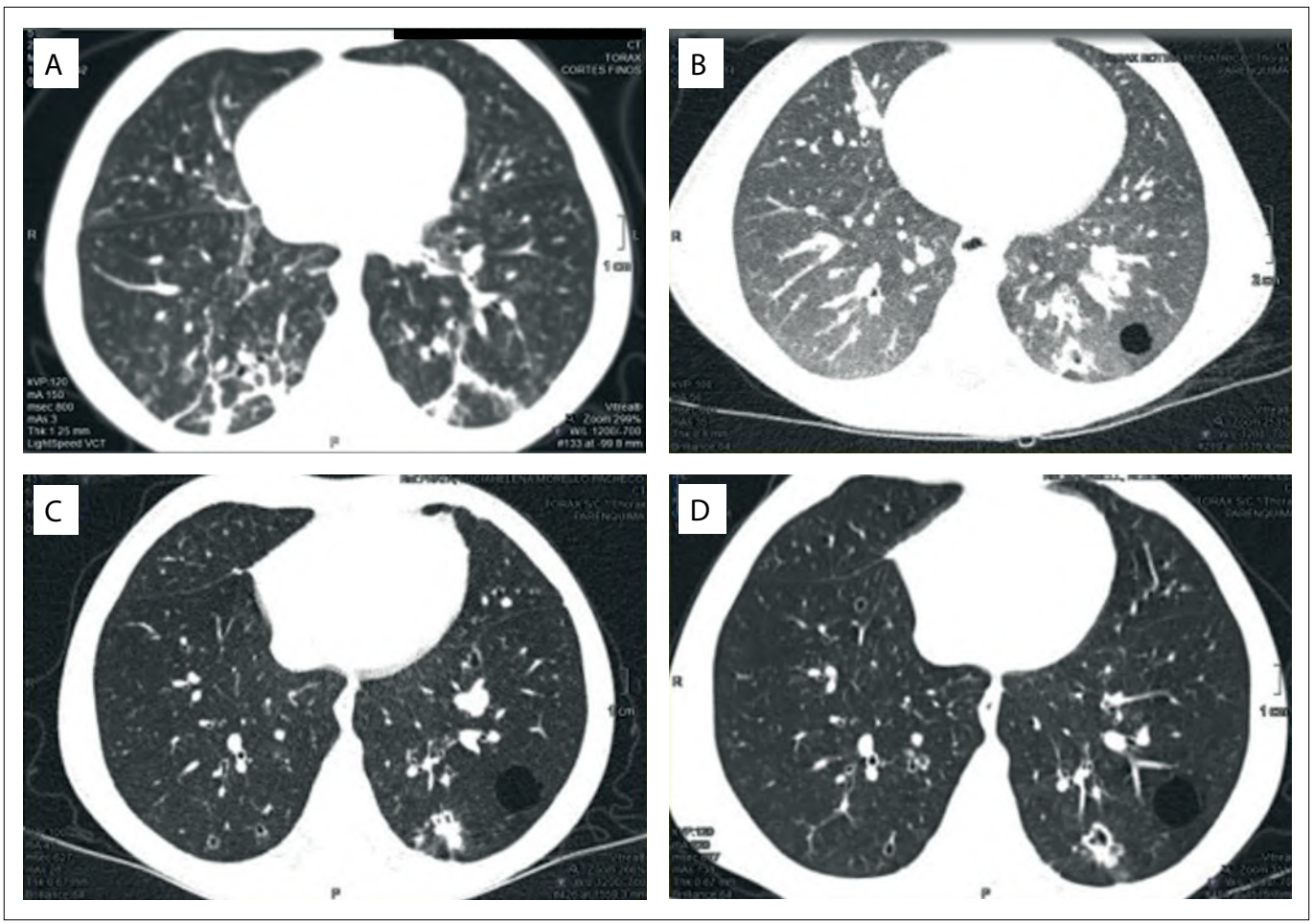

Figure 5. Computed tomography scans in year 3 (A), year 4 (B), year 5 (C) and year 6 (D).

Regarding use of alpha-interferon for treating respiratory papillomatosis, very few clinical trials could be found for this review (**Table 2**).

The most relevant clinical trial found in the Cochrane database is the one by Healy et al.[6] Their study consists of a randomized trial on 123 patients. Patients were assigned either to treatment with interferon and surgery or to surgery alone. They found that the papillomata growth rate was significantly reduced in the group receiving alpha-interferon over the first six months of the treatment. Nevertheless, in the second six months, there was no statistical difference among the groups. The authors concluded that interferon treatment was effective but could not be sustained. The second most relevant study consisted of a randomized crossover trial[9] describing 66 patients who were treated with interferon. Half of the patients were treated from the beginning of the study period and half waited six months to begin treatment lasting for the same length of time. Comparison of the groups during the first six months showed that there was better control over the disease in the group that underwent interferon therapy, with eight remissions occurring in this group, compared with none in the observation-alone group. Benjamin et al.[10] conducted a crossover trial on 10 patients and also reported that there was an apparent benefit from using interferon in association with surgical resection, in comparison with surgical resection alone. Complete or partial remission was seen in six of their 10 patients. Complete remission was sustained for two years. It is important to note that these clinical trials were all reported before pegylated interferon became available and therefore before any of the papers published by hepatologists or infectologists that demonstrated improvement in treatment of hepatitis C virus infection in comparison with conventional alpha-interferon.

The only published article describing a series of patients with respiratory papillomatosis who were treated with pegylated interferon reported on 11 adult patients who received PEG-IFN α2a.[11] They were treated for six months with weekly subcutaneous injections of 180 μg and in the third month were also given granulocyte-monocyte colony-stimulating factor. The authors reported that a significant improvement in voice quality and a reduction in the number of surgical interventions were achieved after a six-month treatment with a 12-month follow-up period.[11] Another single case report on a 73-year-old patient with longstanding juvenile respiratory papillomatosis described the use of PEG-IFN in an attempt at adjuvant treatment, among other treatments, in the patient's final years. The patient eventually succumbed to the disease following malignant transformation.[12]

The case that we described here was one with a dramatic burden on the family, since surgical debulking to restore airway patency was required every month, following decannulation. Early pulmonary involvement and progression was also a concern, and these were criteria for adjuvant treatment. In the years without tracheostomy (years 5 and 6), upper airway obstruction was inevitably potentially more symptomatic, with the possibility of a fatal outcome. However, there was a noticeable increase in the interval free from surgical interventions, from 43.8 days before treatment was needed to 77.2 days. This coincided with the pegylated interferon treatment.

Unfortunately, treatment had to be halted after three months, despite the initial plan for a six-month treatment period, due to significant elevation in transaminase levels. It is possible that a longer-lasting response would have been seen with six months of treatment. Nonetheless, one year after halting the treatment, the intervals between procedures were still longer than they were the year before.

This is the first report of use of pegylated interferon for recurrent respiratory papillomatosis in a child. Use of medication for off-label purposes is always a difficult decision. Thus, case reports on extremely severe cases such as the one described here may be of great help for colleagues caring for similar cases. Although a single case report is insufficient to determine whether the improvement can be attributed solely to adjuvant treatment and not the actual natural history of this disease, the difference in this particular case, from before to after interferon treatment, was quite striking in the eyes of the medical team that has closely followed this case for over five years.

Future studies might prove pegylated interferon to be more effective than traditional alpha-interferon, as has been demonstrated with regard to treating hepatitis C.

Table 2. Database search results regarding reports on papillomatosis treated with pegylated interferon (PEG-IFN). Search performed on January 20, 2017

Database	Search strategy	Articles found	Articles included
MEDLINE	(papillomatosis) AND (pegylated interferon) OR (peginterferon)	4	2
Cochrane Library	(papillomatosis) AND (pegylated interferon) OR (peginterferon)	0	0
	(interferon) AND (papillomatosis)	8	3
LILACS	(papillomatosis) AND (pegylated interferon) OR (peginterferon)	0	0
	(papillomatosis) AND (interferon)	13	0
Embase	(papillomatosis) AND (pegylated interferon)	2	1
	(papillomatosis) AND (peginterferon)	10	0

CONCLUSION

Pegylated interferon may be a good option for diminishing the need for surgical intervention in severe cases of RRP.

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AIM AND EDITORIAL POLICY

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São Paulo Medical Journal (formerly Revista Paulista de Medicina) was founded in 1932 and is now published bimonthly by the Associação Paulista de Medicina. It accepts articles in the fields of clinical health science (internal medicine, gynecology & obstetrics, mental health, surgery, pediatrics, epidemiology and public health). Articles will be accepted in the form of original articles, narrative reviews, case reports, short communications and letters to the editor. Papers with a commercial objective will not be accepted.

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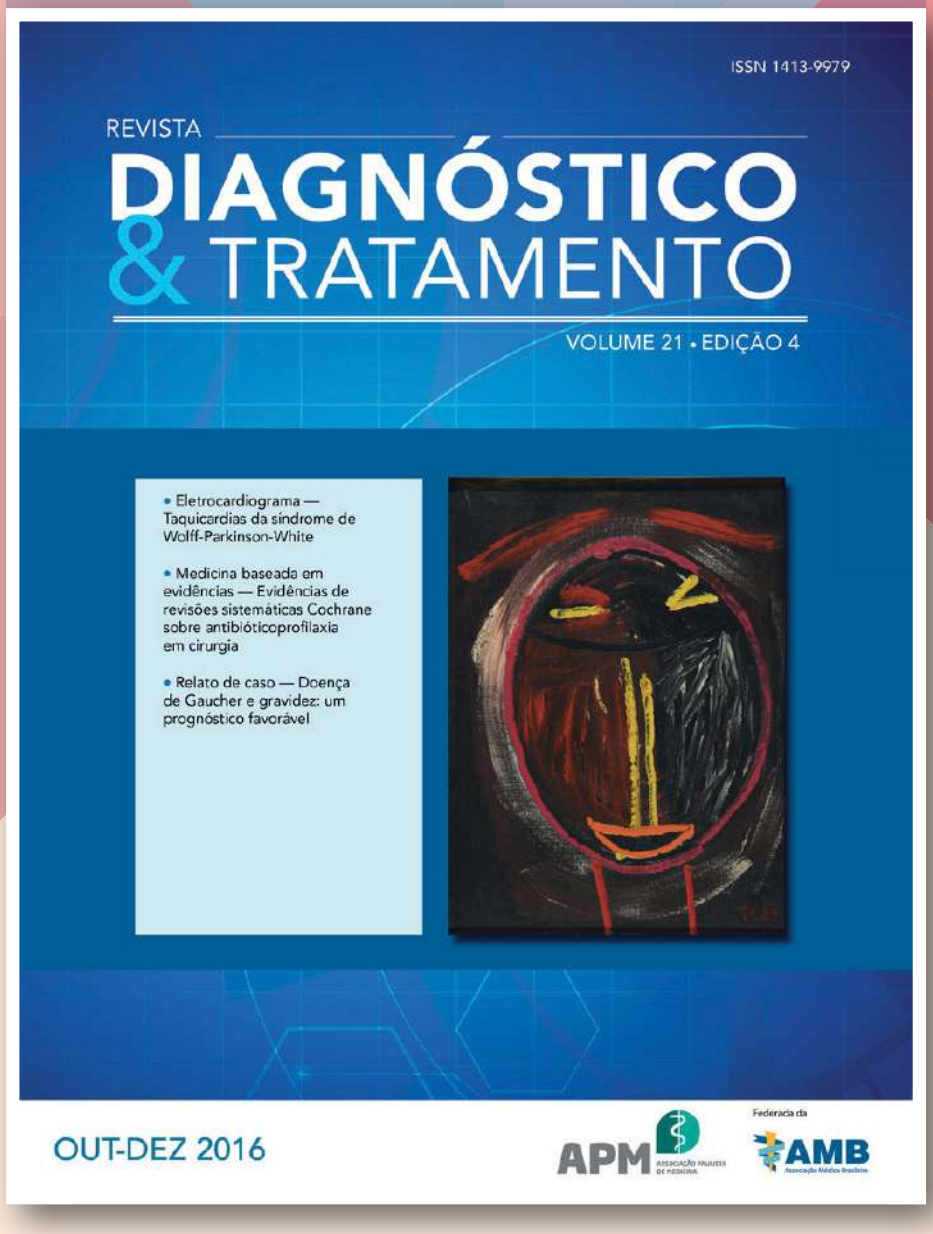

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[1]R$ 249,87 - Bradesco Saúde Efetivo III E CA Copart 6 (registro na ANS nº 480.478/18-3), da Bradesco Saúde, faixa etária até 18 anos, com coparticipação e acomodação coletiva (tabela de dezembro/2017 - SP). Planos de saúde coletivos por adesão, conforme as regras da ANS. Informações resumidas. A comercialização dos planos respeita a área de abrangência das respectivas operadoras de saúde, bem como a disponibilidade para cada entidade de classe. Os preços e as redes estão sujeitos a alterações, por parte das respectivas operadoras de saúde, respeitadas as disposições contratuais e legais (Lei nº 9.656/98). Condições contratuais disponíveis para análise. Agosto/2018.

Siga a Qualicorp: