Prevalence of suggestive images of atheroma in panoramic radiographs: clinical profile and risk factors for cardiovascular disease

Prevalência de imagens sugestivas de ateroma em radiografias panorâmicas: perfil clínico e fatores de risco para a doença cardiovascular

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ABSTRACT
Background: In recent years, the use of panoramic radiography (PR) has been proposed as a reliable method for early diagnosis of calcified carotid artery atheroma (CCAA). The aim of this study was to determine the prevalence of images suggestive of CCAA, observed in digital PRs from users of a Brazilian military organization, and investigate the presence of cardiovascular risk factors.

Material and methods: The study analyzed a total of 2,015 digital PRs taken for dental diagnostic purposes between January and September 2019 from patients of both genders and older than 18 years. Data were analyzed regarding prevalence of CCAA and cardiovascular risk factors using the Chi-
square test at the significance level of 5%. Patients with a suggestive diagnosis of CCAA were contacted by telephone and invited to further evaluation. Results: Of 2,015 patients, 105 (6.06%; 46 women, 25 men) had CCAA detected on digital images. Of the 71 patients who agreed to further evaluation, 64.8% were women, 54.9% were 60 years or older, 38% military service, 45.1% overweight, 64.3% blood pressure alterations, 23.9% diabetics, 11.3% cardiovascular and respiratory disorders, 5.6% history of COVID-19, 12% cholesterol ≥ 240mg/dL, 26.5% triglycerides ≥ 200mg/dL and 51% glycaemia ≥ 100mg/dL. For all variables analyzed there was statistically significant difference (p<0.05), except for glycaemia (p=889). Conclusion: The presence of risk factors for stroke was meaningful in the population with CCAA, indicating the relevant role of the dentist in the early diagnosis.

Keywords: Orthopantomography, arterial hypertension, carotid atherosclerosis, stroke.

1 INTRODUCTION

Cerebrovascular accident (CVA) is the third leading cause of death in most industrialized countries (1). One-half of all strokes are believed to be the result of atherosclerotic disease at the carotid bifurcation (2) that is used to describe a group of pathologies that have in common the thickening and the loss of elasticity in the arterial walls resulting from the formation of atheroma (3). Calcified carotid artery atheroma
(CCAA) represents a maturation of atherosclerotic plaque that has been associated with an increased risk of stroke (4).

Since 1981, when CCAA s were first described in panoramic radiographs (PRs), dentistry has received increasing attention (4,5). Routine dental PR was reported to be a useful tool, low-cost, reliable, and noninvasive diagnostic medium method for identifying CCAAs (1,6).

Calcifications in the area of the carotid arteries are depicted in 3 to 15% of PR exposed in different population (7,8). Whenever CCAAs are detected on a PR, further diagnostic examination should be recommended (1). Current literature suggests that the risk of stroke should not be estimated solely on the incidental finding of CCAA on panoramic images. Other risk factors such as age, sex, history of prior stroke, obesity, diabetes mellitus, sedentary lifestyle, smoking history, total cholesterol level, rheumatoid arthritis, arterial stenosis, and hypertension should also be part of the overall risk assessment (4,6,9-13).

The majority of strokes occur without a preceding transient ischemic attack (14), therefore, early, non-invasive diagnosis can have vast clinical significance on the survival of such patient population (15). This gives dentists an additional responsibility to examine this much ignored but vital area on PR. Although calcifications may not imply significant stenosis and not all atherosclerotic lesions are calcified, it is hypothesized that presence of calcifications seen on dental radiographs may be associated with significant latent carotid disease (2).

The aim of this study was to determine the prevalence of suggestive images of CCAA, observed in digital PRs of users of the Aeronautic Dental Clinic of Recife (OARF), Pernambuco, Brazil, and to assess the presence of cardiovascular risk factors, such as age, sex, hypertension, diabetes, obesity, blood glucose, triglycerides and cholesterol.

2 MATERIAL AND METHODS
STUDY DESIGN AND PATIENT SAMPLE
The study was conducted in a Brazilian Air Force organization, OARF (Recife, Pernambuco, Brazil), and digital PRs library data from January 1, 2019 to September 30, 2019 were accessed and reviewed. The sample consisted of 2015 PRs from patients under dental treatment that uses the OARF services (military and dependents). The inclusion criteria consisted on sampling subjects, aged 18 years or older, male and
female, who presented a suggestive diagnosis of CCAA on PRs. The exclusion criteria consisted of low quality or non-evaluable radiographs and patients with specific needs. At the baseline, the reports of the 2015 radiographs were first reviewed, and 1732 attended the inclusion criteria. The objective of this first review was to select PRs with a report suggestive of CCAA and triticeous cartilage, that were found in 145. An independent, experienced examiner from the Department of Radiology, OARF, performed a second review of all the 145 PRs, with no information on medical conditions, age, or gender of the study participants. After this second appraisal, 105 patients with CCAA findings were contacted by telephone and invited to further evaluation at OARF.

STUDY GROUP

Of all 105 patients, 84 were successfully contacted and 74 agreed to participate, however 03 gave up. The study group comprised 71 patients. After consent, all the 71 patients selected for the research underwent anamnesis, as well as blood pressure (BP) measurement, to investigate risk factors related to stroke. Demographic data (sex and age) were collected in order to determine the prevalence and distribution of suggestive CCAA images. Patients were asked about the presence or absence of hypertension, diabetes, cardiovascular disorders, history of stroke, atherosclerosis, respiratory disorder, use of medication and regular physical activity. Height and weight of the participants were collected in order to obtain the body mass index (BMI). Patients who had laboratory tests performed in 2019 and 2020, triglyceride levels, total cholesterol and fasting blood glucose were evaluated. Patients were also asked about their experience of contamination by coronavirus disease (COVID-19). To measure BP, the same sphygmomanometer, digital adjustment, pulse type, Premium - LP200 was used.

Patients who had CCAA on PRs and risk factors for stroke were referred to the physician for further management.

PANORAMIC RADIOGRAPHIC EXAMINATION

Digital PRs, justified for dental treatment purposes, were performed using an Orthopantomograph OC200D – KAVO (Instrumentarium Dental, Tuusula, Finland) radiologic unit, operated at 13 mA with a peak tube potential of 66 kV. PRs were reviewed by a single senior radiologist on the same monitor 12369VM led IPS 23 " AOC, in subdued ambient light. The PRs analysis investigated radiopaque images in the
soft tissues compatible with images suggestive of CCAA. They were interpreted either as nodular or as verticolinear radiopacity below the mandibular angle, adjacent or inferior to the hyoid bone, epiglottis, and cervical vertebrae at, above, or below the intervertebral space of C3-C4, and at a 1.5–2.5-cm distance (1,2,7,10,12,16).

A complicating factor in relation to the differential diagnosis is that the age range of greatest prevalence for CCAA coincides with those of the occurrence of mineralization and/or calcifications that are reported as being responsible for erroneous diagnoses, such as ossification of the triticeous, cricoid and thyroid cartilages, sialolith, and mineralization of the stylomandibular and stylohyoid ligaments. Calcification of the triticeous cartilage is the greatest cause of erroneous diagnosis of calcified atheroma of the carotid (3). An example of a PR suitable for the assessment of the presence, or absence of a radiopaque nodular mass is presented (Fig. 1).

Figure 1: Digital panoramic radiograph with CCAA at the right side. STATISTICAL ANALYSIS

The data obtained was analyzed using Microsoft Excel and SPSS software, version 18.0 for windows, to evaluate the prevalence of CCAA in digital PRs. To assess the personal and clinical profile of the patients, frequencies percentage were calculated and the respective frequency distributions were constructed. Comparison of the percentages found in the variables categories were made using the Chi-square test for proportions comparison. All conclusions were obtained considering the significance level of 5%.
3 RESULTS

On panoramic examination of 143 PRs, 105 (6.06%) evidenced CCAA, 25 (1.44%) calcification of the triticeous cartilage, 06 (0.35%) tonsillolith and 07 (0.40%) PRs were not evaluated for obstacles with the digital file.

Results of CCAA on PR according to personal profile evaluated of all 71 patients are showed in Table 1. The factors evaluated were statistically significant, except military service.

<table>
<thead>
<tr>
<th>Factor</th>
<th>N</th>
<th>%</th>
<th>p-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>35.2</td>
<td>0.013</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>64.8</td>
<td></td>
</tr>
<tr>
<td>Age range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;49</td>
<td>13</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td>50 – 59</td>
<td>19</td>
<td>26.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;60</td>
<td>39</td>
<td>54.9</td>
<td></td>
</tr>
<tr>
<td>Min – Max</td>
<td>32.68 – 90.22</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>63.02 ± 13.51</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Military service</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27</td>
<td>38.0</td>
<td>0.044</td>
</tr>
<tr>
<td>No</td>
<td>44</td>
<td>62.0</td>
<td></td>
</tr>
</tbody>
</table>

Note: ¹p-value of the Chi-square test to compare proportions. * The number of observations differs from the sample size as 1 patient did not answer the question.

Table 2 shows the clinical evaluation distribution of the patients. The data identified that most of participants practice physical activity (n = 45, 64.3%), are overweight (n = 32, 45.1%), had BP alterations - measured (n = 45, 64.3%), with the highest frequency of stage 1 hypertensive patients (n = 26, 37.2%), followed by stage 2 hypertensive patients (n = 15, 21.4%), with a suggestion of unilateral atheroma (n = 39, 54.9% ) and did not had COVID-19 (n = 67, 94.4%). The proportion comparison test was significant for all factors evaluated (p-value <0.05), except for the laterality (p-value = 0.406), indicating that the number of patients with suggestion of unilateral atheroma is close to the number of patients with suggestion of bilateral atheroma. For the other clinical variables, the profile described is relevantly the most frequent among the evaluated patients.

Patients self-reported their comorbidities and the data identified that the significant majority of patients did not present the evaluated comorbidities (p-value <0.05), except for the BP alterations in which the majority of patients was significant
for the presence of this comorbidity (59.2%). Still, we observed that 23.9% patients had diabetes, 11.3% had cardiovascular disorder, 11.3% had respiratory disorder, 5.6% had atherosclerotic history and 1.4% had stroke history.

Our data demonstrate that the significant majority of patients use medication (78.9%, p-value <0.001). The most frequent medications are antihypertensive drugs (46.6%), hypoglycemic agents (16.5%) and hypolipemic agents (13.6%).

Table 2. Clinical evaluation distribution of participants.

<table>
<thead>
<tr>
<th>Evaluated Factor</th>
<th>N</th>
<th>%</th>
<th>p-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Practice of physical activity*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45</td>
<td>64.3</td>
<td>0.017</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>35.7</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>2</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>18</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>32</td>
<td>45.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obesity</td>
<td>19</td>
<td>26.8</td>
<td></td>
</tr>
<tr>
<td>BP alterations-measured*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45</td>
<td>64.3</td>
<td>0.017</td>
</tr>
<tr>
<td>No</td>
<td>25</td>
<td>35.7</td>
<td></td>
</tr>
<tr>
<td>BP classification*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimal</td>
<td>4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>High normal</td>
<td>14</td>
<td>20.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grade 1 hypertension</td>
<td>26</td>
<td>37.2</td>
<td></td>
</tr>
<tr>
<td>Grade 2 hypertension</td>
<td>15</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>Grade 3 hypertension</td>
<td>4</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>Laterality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>39</td>
<td>54.9</td>
<td>0.406</td>
</tr>
<tr>
<td>Bilateral</td>
<td>32</td>
<td>45.1</td>
<td></td>
</tr>
<tr>
<td>History of COVID-19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (didn’t have sequelae)</td>
<td>4</td>
<td>5.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>67</td>
<td>94.4</td>
<td></td>
</tr>
</tbody>
</table>

Note: ¹p-value of the Chi-square test to compare proportions. *The number of observations differs from the sample size as 1 patient did not answer the question.

Table 3 shows the distribution of laboratory tests classification in participants. It is verified that the statistically significant majority (p-value <0.05) of the patients had good or borderline values regarding total cholesterol (48.0% and 40.0%, respectively), had good values for triglycerides (65.3%) and prediabetes/diabetes values for fasting blood glucose (51.0%). For fasting blood glucose, the difference was not significant (p-value = 0.889).
Table 3. Classification of laboratory tests of participants.

<table>
<thead>
<tr>
<th>Evaluated Factor</th>
<th>N</th>
<th>%</th>
<th>p-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (&lt; 200 mg/dL)</td>
<td>24</td>
<td>48,0</td>
<td></td>
</tr>
<tr>
<td>Borderline (200 to 239 mg/dL)</td>
<td>20</td>
<td>40,0</td>
<td>0,005</td>
</tr>
<tr>
<td>High (≥240 mg/dL)</td>
<td>6</td>
<td>12,0</td>
<td></td>
</tr>
<tr>
<td><strong>Triglycerides</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (&lt; 150 mg/dL)</td>
<td>32</td>
<td>65,3</td>
<td></td>
</tr>
<tr>
<td>Borderline (150 to 200 mg/dL)</td>
<td>4</td>
<td>8,2</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>High (≥200 mg/dL)</td>
<td>13</td>
<td>26,5</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting blood glucose</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (70 to 99 mg/dL)</td>
<td>25</td>
<td>49,0</td>
<td></td>
</tr>
<tr>
<td>Prediabetes/diabetes (≥100 mg/dL)</td>
<td>26</td>
<td>51,0</td>
<td>0,889</td>
</tr>
</tbody>
</table>

*Note: ¹p-value of the Chi-square test to compare proportions. *The number of observations differs from the sample size as 1 patient did not answer the question.

4 DISCUSSION

In 1981 Friedlander first unveiled the presence of soft tissue calcifications in this unvisited territory of panoramic radiographs (2,5). CCAAs are closely related to cerebrovascular accidents and such calcifications can be radiographically detected while performing routine medical procedures (11). This study focused is the detection of CCAA in digital PRs, followed by the evaluation of risk factors for stroke in the OARF population. To the best of our knowledge, this is the first study designed in the OARF population to assess the prevalence of risk factors for stroke in patients with images suggestive of CCAA in digital PR.

The results of our study demonstrated a prevalence of 6.06% images suggestive of CCAA in digital PR, and they are very consistent with Gonçalves et al. (11) results, that demonstrated a prevalence of 6.90% in a study conducted in Brazilian population. In similar studies, Couto-Souza et al. (17) analyzed a group of South Brazilian population, reported a prevalence of 8.6%, and Agacayak et al. (18) detected 8.8% of atheroma in group of Turkish population. However, other studies showed a lower prevalence, such as Sisman et al. (19), that found the prevalence of 5.06% in the population of Cappadocia, Turkey; Ohba et al. (16) and Tamura et al. (20) evaluating groups of the Japanese population detected 5% and 4.13%, respectively. Bayram et al. (12) detected an even lower prevalence, just 2.10% in a group of Turkish population. It’s noteworthy that the present study evaluated patients over 18 years of age, while the studies mentioned above evaluated patients over 40 or 60 years of age, that have a higher prevalence of calcifications. It was expected that the present study would demonstrate a lower prevalence of CCAA compared to studies that assessed a population with a higher age range. These variations in prevalence may be due to sample size, patient selection, regional, dietary and lifestyle differences of the subjects (1,19). Sisman et al. (19) found...
that all patients who had a higher prevalence of CCAA compared to the normal population, were due to underlying disorders causing atherosclerosis.

The present study identified a significant difference (p = 0.013) between genders, 25 (35.2%) male and 46 female (64.8%), with the majority of female patients presenting images suggestive of CCAA, in agreement with previous studies; Santos et al. (21) (70.8% female and 29.2% male), Agacayak et al. (18) (66.7% female and 33.3% male), Ohba et al. (16) (25 female and 8 male) and Ravon et al. (22) also detected a higher prevalence in female. Tamura et al. (20) observed that the incidence rate of CCAA in females was about three times higher than the rate in males. However, Bayram et al. (12) did not find significant difference between the genders, in line with Sisman et al. (19) that observed a higher occurrence in female than in male, but the difference was not significant. The result of women having a significantly higher incidence of CCAAs suggests a relationship between CCAAs and the decline of estrogen levels in the blood that affects postmenopausal women (20).

The results of our investigation demonstrated a higher prevalence of CCAA in the age group of 60 years or more, with a mean age of 63.02±13.51. These data are in accordance with other studies that also had a higher prevalence in the elderly. Akkemik et al. (23) that found the mean age 64±8 years; Lee et al. (10) reported 72.10±7.68 years. However, Queiroz Abreu et al. (24) obtained 54.9±8.8 years and Santos et al. (21) 54±13 years.

We identified the majority of participants with a suggestion of unilateral CCAA (54.9%), nevertheless this difference was not significant (p = 0.406).

In our study, we found that most participants did not report military service (62.0%). This finding is relevant, as patients with military service are required, throughout their careers, to maintain the habit of physical activity and undergo periodic health inspection. This can interfere with the results, since this group is constantly aware of their health situation, as they are submitted to regular medical monitoring and frequent physical fitness test, every 6 months. However, we do not have this information regarding dependents. This is consistent with the “healthy warrior effect,” whereby healthier persons are more likely to be selected for military participation (25). Johnson et al. (25) provided an opportunity to investigate the long-term association of combat with subclinical atherosclerosis in a large community-based cohort with military and civilian controls. Results do not suggest that combat has a long-term detrimental effect on subclinical atherosclerosis among men.
We observed that the majority of participants practice physical activity (64.3%), although, most of them are also overweight (45.1%) or obese (26.8%). This observation is in line with other reports that found considerable obesity rates. Tamura et al. (20) reported 21.1% and Queiroz Abreu et al. (24) 42.9% of obesity in their studies.

In particular, increased BP is one of the most important elements in atheroma formation (4) and increases significantly with more severe CCAA (26). When BP was measured, most participants showed alterations (64.3%), as well as self-reported BP data (59.2%). The significant prevalence of hypertension and diabetes among patients with CCAA is in accordance with the conclusions of other studies; Queiroz Abreu et al. (24) evaluated Brazilian patients and observed 11 (52.4%) hypertensive and 7 (33.3%) diabetics. Bengtsson et al. (7) reported hypertension ranging from 30.5% to 33.5% and diabetes from 6.9% to 8.8% according to the selected population in Sweden. Agacayak et al. (18) observed hypertension in 56.4% of patients, diabetes in 10.5% and hyperlipidemia in 3.3%. Barona-Dorado et al. (6) screened 174 individuals with CCAA, using 1602 PRs, their results showed that the presence of diabetes and hypertension were, respectively, 3.18 and 2.35 times higher in the CCAA group than in the control group.

Friedlander et al. (27) demonstrated that diabetes induced/accelerated macrovascular atherosclerosis in the region of the carotid artery bifurcation. We believe that the diversity of rates of comorbidities can be due to the difference in lifestyles between countries. It is important also to consider the occurrence of white coat hypertension (WCH), when BP is high in the office, but it is normal outside it. The participants' anxiety at the time of BP measurement may influence the result.

Although the majority of patients had good values of total cholesterol and triglycerides, a considerable portion, 12% and 26.5% respectively, showed alterations, that can demonstrate a possible correlation between the diseases observed and pointing to the need for future studies. Still, in relation to fasting blood glucose, most patients had prediabetes/diabetes values (51.0%), however, it was not significant. Bayram et al. (12) evaluated 23 patients and observed 14 with high cholesterol levels (mean of 216.74 ± 24.81 mg dl⁻¹). In contrast, Ohba et al. (16) described a very little relationship between CCAAs and the patient’s general health, as there was no difference in BP, cholesterol and glucose in the groups analyzed with and without CCAA in a group of Japanese population. Ravon et al. (22) found an average cholesterol and triglyceride level of 177.1 and 141.7, respectively, indicating good values. Nevertheless, in recent study, QiaoZhen
et al. (28) found that small dense low-density lipoprotein cholesterol (sdLDL-C) was positively correlated with triglyceride. Furthermore, they explored the correlation between triglyceride and carotid atherosclerosis, and the results showed that triglyceride is correlated with stable plaques.

Our data revealed that the significant majority of patients use medication (78.9%); we found that the most frequent ones are antihypertensive (46.6%), hypoglycemic (16.5%) and hypolipemic (13.6%). Friedlander et al. (29) conducted a study with white non-hispanic men over 55 years old, and showed that 85% of the participants used antihypertensive drugs.

In a systematic review, Lim et al. (30) states that it is difficult to separate the effects of CCAAs and atherosclerosis from other risk factors due to their pathophysiological interrelationship. Still, there is heterogeneity between the studies, as there are important risk factors that were not explicitly addressed, for example, if the patients were well medicated and if they had active lifestyles.

When COVID-19 was considered, a low prevalence of patients who suffered from the disease was found. We understand that this data may be more significant in a prospective study, as the history of COVID-19 may be aggravating for these patients in the future. The inflammatory cascade induced by COVID-19 often leads to hypercoagulability and promotes the formation and progress of atherosclerosis (31). COVID-19 virus could alter the expression/activity of ACE2 (angiotensin-converting enzyme 2), consequently resulting in the disruption of renin-angiotensin system which is associated with the occurrence and progression of atherosclerosis (31).

As a limitation, COVID-19 pandemic had an impact on data collection due to difficult patient’s adherence to attend the evaluation in person. Hence, it was possible to perform only one BP measurement, which may represent a degree of bias. In addition, the current context of the pandemic reflects a state of anxiety among the population; this may also have influenced the BP data measured.

5 CONCLUSION

The detection of CCAAs in PRs performed for dental reasons can be very important for patients at risk of stroke. It is essential that the dentists are able to identify the anatomical aspects and the presence of CCAA in the PRs, as well as perform an adequate anamnesis and establish the need for additional medical evaluation. This information could contribute to the prevention of possible undesirable cardiovascular
events. It seems important to advise especially patients with hypertension and COVID-19 history, in order to receive regular medical monitoring. We believe that more extensive studies are needed in this regard.

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CONFLICTS OF INTEREST
The authors declare that they have no conflict of interest.

ETHICS
The local Research Ethics Committee of the Federal University of Pernambuco has approved the research and the study with the protocol number: 3.844.413.

SOURCE OF FUNDING
No external funding was provided in regard with this study.

AUTHORS' CONTRIBUTIONS
All authors contributed to the construction of the study, where some participated directly in the data collection, while others were responsible for coordinating the research.
REFERENCES


