ASSESSING TESTOSTERONE LEVELS IN MEN WITH TYPE 2 DIABETES: A CROSS-SECTIONAL STUDY IN PRIMARY CARE

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Guilherme Alfonso Vieira Adami [1]
Israel Gonçalves de Carvalho Filho [2]
Caio Eduardo Alves de Oliveira Paes Leme Goulart [3]
Claudine Dullius [4]
Karen Bonete Bozza [5]
Geraldo Emílio Vicentini [6]

ABSTRACT: Objective: This study aimed to assess the prevalence of hypogonadism and its risk factors in Brazilian men with Diabetes Mellitus type 2, as well as testing the accuracy of laboratory diagnosis against The Androgen Deficiency in Aging Males (ADAM) questionnaire for the initial screening of testosterone deficiency. Methodology: A cross-sectional study was conducted with 38 type 2 diabetic men between January and May 2023 at the primary care using plasma testosterone levels for hypogonadism detection and the ADAM questionnaire comparatively. Statistical analyses were performed for normality, correlation, and association. All participants signed a free and informed consent form approved by the Ethics Committee. Results: The research identified a prevalence of hypogonadism of 47.1% among the participants. Factors such as advanced age, BMI >30.0 kg/m2, increased abdominal circumference, and high total cholesterol were associated with hypogonadism in type 2 diabetics. The ADAM questionnaire had modest accuracy (41.2%) (despite the participants' reliable response pattern (Cronbach`s alpha = 0.72). Conclusion: Hypogonadism is common in type 2 diabetics and was prevalent in almost half of the participants being associated to cardiovascular risk factors. The ADAM questionnaire had a poor performance in detecting hypogonadism in this group.

KEYWORDS: Cardiovascular Risk Factors; Hypogonadism; Testosterone; Type 2 Diabetes Mellitus.

[1] Medical Student. State University of Western Paraná – UNIOESTE – Francisco Beltrão campus.
E-mail: guilhermealfonsovieiraadami@outlook.com, ORCID: https://orcid.org/0000-0002-7478-3787
[2] Medical Doctor, MSc in Applied Health Sciences. Francisco Beltrão Health Department.
E-mail: israelc_filho@yahoo.com.br, ORCID: https://orcid.org/0000-0002-7987-3458
[3] Medical Student. State University of Western Paraná – UNIOESTE – Francisco Beltrão campus.
E-mail: caioaogoulart@hotmail.com, ORCID: https://orcid.org/0000-0002-9406-8086
[4] Nutrition Student. State University of Western Paraná – UNIOESTE – Francisco Beltrão campus.
E-mail: claudine-sk@hotmail.com, ORCID: https://orcid.org/0000-0003-4370-0341
[5] Medical Student. State University of Western Paraná – UNIOESTE – Francisco Beltrão campus.
E-mail: kareenbonete@outlook.com, ORCID: https://orcid.org/0009-0006-8252-5047
[6] Doctor of Pharmaceutical Sciences. States University of Western Paraná – UNIOESTE – Francisco Beltrão campus.
E-mail: vicentinige@gmail.com, ORCID: https://orcid.org/0000-0001-9446-0427

AVALIAÇÃO DOS NÍVEIS DE TESTOSTERONA EM HOMENS COM DIABETES TIPO 2: UM ESTUDO TRANSVERSAL NA ATENÇÃO PRIMÁRIA

RESUMO: Objetivo: Este estudo teve como objetivo avaliar a prevalência de hipogonadismo e seus fatores de risco em homens brasileiros com Diabetes Mellitus tipo 2, bem como testar a acurácia do diagnóstico laboratorial frente o questionário The Androgen Deficiency in Aging Males (ADAM) para o rastreamento inicial da deficiência de testosterona. Metodologia: Foi realizado um estudo transversal com 38 homens diabéticos tipo 2 entre janeiro e maio de 2023 na atenção primária utilizando os níveis plasmáticos de testosterona para detecção de hipogonadismo e o questionário ADAM comparativamente. Foram realizadas análises estatísticas de normalidade, correlação e associação. Todos os participantes assinaram um termo de consentimento livre e esclarecido aprovado pelo Comitê de Ética. Resultados: A pesquisa identificou uma prevalência de hipogonadismo de 47,1% entre os participantes. Fatores como idade avançada, IMC >30,0 kg/m2, circunferência abdominal aumentada e colesterol total elevado foram associados ao hipogonadismo em diabéticos tipo 2. O questionário ADAM teve acurácia modesta (41,2%) (apesar do padrão de resposta confiável dos participantes (alfa de Cronbach = 0,72). Conclusão: O hipogonadismo é comum em diabéticos tipo 2 e foi prevalente em quase metade dos participantes, estando associado a fatores de risco cardiovascular. O questionário ADAM teve um desempenho ruim na detecção de hipogonadismo neste grupo.
PALAVRAS-CHAVE: Diabetes mellitus tipo 2; Fatores de Risco Cardiovascular; Hipogonadismo; Testosterona.

EVALUACIÓN DE LOS NIVELES DE TESTOSTERONA EM HOMBRES COM DIABETES TIPO 2: UM ESTUDIO TRANSVERSAL EM ATENCIÓN PRIMARIA

RESUMEN: Objetivo: Este estudio tuvo como objetivo evaluar la prevalencia de hipogonadismo y sus factores de riesgo en hombres brasileños con diabetes mellitus tipo 2, así como probar la precisión del diagnóstico de laboratorio frente al cuestionario The Androgen Deficiency in Aging Males (ADAM) para el cribado inicial de la deficiencia de testosterona. Metodología: Se realizó un estudio transversal con 38 hombres diabéticos tipo 2 entre enero y mayo de 2023 en la atención primaria utilizando los niveles plasmáticos de testosterona para la detección de hipogonadismo y el cuestionario ADAM de forma comparativa. Se realizaron análisis estadísticos de normalidad, correlación y asociación. Todos los participantes firmaron un término de consentimiento libre e informado aprobado por el Comité de Ética. Resultados: La investigación identificó una prevalencia de hipogonadismo del 47,1% entre los participantes. Factores como edad avanzada, IMC >30,0 kg/m², circunferencia abdominal aumentada y colesterol total elevado se asociaron con el hipogonadismo en diabéticos tipo 2. El cuestionario ADAM tuvo una precisión modesta (41,2%) (a pesar del patrón de respuesta confiable de los participantes (alfa de Cronbach = 0,72). Conclusión: El hipogonadismo es común en diabéticos tipo 2 y fue prevalente en casi la mitad de los participantes, estando asociado a factores de riesgo cardiovascular. El cuestionario ADAM tuvo un desempeño pobre en la detección de hipogonadismo en este grupo.

PALABRAS CLAVE: Diabetes mellitus tipo 2; Factores de riesgo cardiovascular; Hipogonadismo; Testosterona.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) presents an escalating challenge to global public health, with an estimated average prevalence of 9.2% in Brazil and 10.5% worldwide, with projections of an increase in the coming years (Muzy *et al*., 2021; Ong *et al*., 2021). Concurrently, scientific literature establishes a correlation between the presence of T2DM and reduced testosterone levels, lower than those of the general population (Grossmann; Tang Fui, 2020). Additionally, these patients benefit from testosterone replacement therapy, which enhances the management of metabolic and sexual disorders. This includes improvements in body composition, insulin resistance, lipid and glucose profiles, among other cardiovascular risk markers, characteristic in diabetic patients with a long-standing diagnosis (Corona *et al*., 2023; Zhang *et al*., 2018).

Hypogonadism is characterized by reduced testosterone levels below normal standards, and the Androgen Deficiency in Aging Males (ADAM) questionnaire stands out as a simple, low-cost method for screening hypogonadism in the general population. It assesses a range of symptoms, including sexual alterations, mood changes, and performance decline, demonstrating 88% sensitivity and 60% specificity in its original article. However, studies attempting to replicate the original questionnaire achieved high sensitivity (ranging from 83.5 to 93.85) with moderate to low specificity (ranging from 24.3 to 77.14%), highlighting a lack of studies evaluating this screening method in diabetic men (Morley *et al*., 2000; Rabah; Arafa, 2009).

When examining the clinical features of Type 2 Diabetes Mellitus (T2DM), we find that it is generally a pathology with few symptoms, characterized by polyuria, polydipsia, polyphagia, and weight loss. However, in addition to these symptoms, increased prevalence of decreased libido, erectile dysfunction, general energy reduction, and depressive symptoms, although nonspecific, can lead to false positives in hypogonadism screening tests (Farooqi *et al*., 2022; Clark; Fox; Grandy, 2007).

Insulin resistance has been identified as a crucial link in the pathogenesis of hypogonadism in men with type 2 diabetes mellitus (T2DM). Approximately 30-40% of circulating testosterone is strongly bound to sex hormone-binding globulin (SHBG). Reduced testosterone levels are frequently observed in individuals with T2DM, obesity, and insulin resistance. Insulin plays a central role in regulating SHBG production. The

direct action of insulin on the liver inhibits SHBG synthesis, resulting in lower total testosterone concentrations. This relationship between insulin resistance and hypogonadism is robust and independent of other factors such as obesity and glycemic control (Singh *et al*., 2023; Majumdar *et al*., 2021).

In addition to its direct influence on hormone production, insulin resistance is closely associated with cardiovascular risk. Epidemiological studies demonstrate that individuals with impaired glucose tolerance and elevated insulin resistance have a significantly higher risk of adverse cardiovascular events compared to those with lower insulin resistance. Testosterone, in turn, plays a cardioprotective role. Reduced serum levels of this hormone have been associated with increased atherosclerosis and higher risk of cardiovascular morbidity and mortality. Erectile dysfunction, a common symptom of hypogonadism, emerges as an early marker of systemic atherosclerotic disease, emphasizing the intimate relationship between endocrine and cardiovascular dysfunctions (Majumdar *et al*., 2021; Ebrahimi; Christ-Crain, 2016).

In summary, insulin resistance is a multifactorial risk factor for the development of hypogonadism and cardiovascular diseases in men. Understanding this complex interaction between hormones and metabolism is essential for the development of effective therapeutic strategies for the prevention and treatment of these conditions (Ebrahimi; Christ-Crain, 2016).

In this context, the present study aimed to analyze the occurrence of hypogonadism and its related cardiovascular risk factors in Brazilian men with T2DM, as well as testing the accuracy of laboratory diagnosis against the Adam questionnaire for the initial screening of testosterone deficiency in this specific population.

2. METHODS

2.1 Study Design and ethical considerations

A descriptive cross-sectional study was conducted according to STROBE guidelines between January and May 2023. The study was approved by the Human Research Ethics Committee of the State University of Western Paraná with CAAE 61448222.2.0000.0107. All participants signed an informed consent form.

2.2 Participants

All diabetic patients eligible for the study were called for data collection being that among the 49 type 2 diabetic men each stratified in the municipal basic health unit were selected by the eligibility criteria 38 men, who agreed to participate in this study. Men diagnosed with T2DM, aged over 18, who that were accompanied in primary care in a basic health unit in Francisco Beltrão, PR, Brazil during the study period, were included. Individuals with prior genital surgeries or the use of medications affecting the synthesis or action of sex hormones were excluded.

2.3 Data collection, laboratory examinations and demographic stratification

A detailed anamnesis and general physical examination were conducted for all participants, including anthropometric, clinical and laboratory data.

Laboratory tests were conducted between 8:30 am and 10:00 am from participants while fasting at a laboratory affiliated with the municipal basic health unit including total testosterone levels (ng/dL) measured by chemiluminescence (Advia Centaur, Bayer Diagnostics), glycated hemoglobin (%), total cholesterol (mg/dL), and Vitamin D (ng/mL). This cutoff point provides a more accurate insight into the threshold in diabetic and prediabetic patients. Low testosterone levels were defined by a single total testosterone measurement below 346 ng/dL.

In the conducted study, thirty-eight male diabetic patients were included. These patients had their ages, testosterone levels, and glycated hemoglobin evaluated. However, during the assessment of BMI, abdominal circumference, total cholesterol, vitamin D level, and statin use, data from only thirty-five patients were recorded. The absence of data for three patients at this stage was attributed to a recording error of these variables. Regarding the application of the ADAM questionnaire, designed to assess symptoms of androgen deficiency, responses were obtained from 34 patients. The missing data from the remaining four patients occurred because they did not attend the second appointment necessary for applying the questionnaire. Despite attempts to reschedule and contact by phone, it was not possible to obtain responses from these patients. Patient stratification into demographic groups was based on clinical criteria and international guidelines:

Age (years): Age stratification considered the influence of age on androgenic metabolism and comorbidities associated with diabetes, varying hormonal levels and metabolic response. Body Mass Index (BMI): <30 and >30, according to WHO obesity

classification (Weir; Jan, 2023). Abdominal Circumference (cm): <102 cm and >102 cm, according to IDF guidelines (Peralta Andrade; Palacio Rojas, 2022). Vitamin D (ng/mL): <20 ng/mL and >20 ng/mL, following the Endocrine Society's Clinical Practice Guideline (Holick, *et al*., 2011). Glycated Hemoglobin (HbA1c) (%): <7% and >7%, as per ADA recommendations (American Diabetes Association, 2020). Total Cholesterol (mg/dL): <170 mg/dL and >170 mg/dL, based on AHA standards (Grundy *et al.*, 2019). Qualitative data were cross-referenced with the presence or absence of hypogonadism, defined by a single testosterone measurement below 346 ng/dL. This cutoff point was chosen based on clinical consensus and studies identifying 346 ng/dL as a threshold below which symptoms of androgen deficiency are more likely in men, aligning with international guidelines on hypogonadism (Park; Ahn; Moon, 2019).

2.4 ADAM questionnaire application and additional information collection

34 of the 37 initially selected patients responded to the ADAM questionnaire from St. Louis University to assess symptoms of low testosterone (Morley *et al*., 2000). Responses were collected in person or via telemedicine. Participants also provided additional information, including the duration of diabetes diagnosis and statin use.

2.5 Statistical analysis methods

Data were presented as means (± standard deviation) and percentages, using GraphPad Prism 8 software. The Kolmogorov-Smirnov test assessed the normality of variables. To examine the association between qualitative variables and the presence of hypogonadism, Chi-square and Fisher's tests were used. Prevalence, incidence, accuracy, and prevalence ratio were calculated for each group. The reliability of the ADAM questionnaire was assessed using Cronbach's alpha. Two-tailed $p<0.05$ were accepted as statistically significant.

3. RESULTS

The demographic and clinical characteristics of the study participants are present in Table 1. In general, the group of participants had an average age above 60 years, overweight, visceral obesity, an average diagnosis time of 10 years, glycemic control (HbA1c) imbalance, slightly elevated levels of total cholesterol, reduced levels of vitamin D and testosterone level close to the cutoff point for hypogonadism.

Table 1. Clinical Characteristics of study Participants

Variable	Mean (± Standard Deviation)	Range (minimum and maximum)
Age (years)	62.3 (±12.7)	19 - 82
Time with Diabetes Diagnosis (years)	10 (±6.2)	2 - 30
BMI (Kg/m²)	29.8 (±4.7)	19.8 – 31.1
Abdominal Circumference (cm)	105.3 (±9.5)	86 - 129
Glycated Hemoglobin (%)	7.9 (±2.0)	5.0 – 11.7
Total Cholesterol (mg/dL)	181.9 (±32.8)	114 - 252
Vitamin D (ng/mL)	25.6 (±8.4)	12.8 – 47.3
Testosterone (ng/dL)	355.1 (±92.9)	171.5 – 582.1

In the present study, results of the ADAM questionnaire (Table 2) achieved 87% sensitivity, 0% specificity, and 41.2% accuracy for screening biochemical hypogonadism. Question 7 of the ADAM questionnaire showed the highest sensitivity (87.5%), while questions 1, 4, 5, and 6 exhibited low sensitivity (ranging from 18.75% to 43.75%) and specificity (ranging from 22.2% to 61.1%). Questions 8, 2, and 7 had the lowest sensitivity rates, reaching 16.7%, 22.2%, and again 22.2%, respectively. The ADAM questionnaire demonstrated good internal consistency with a Cronbach's alpha of 0.72.

Table 2. ADAM Questionnaire Questions with percentage of responses, sensitivity and specificity values

ADAM Questionnaire Items for Hypogonadism Screening	Yes Responses n (%)	Sensitivity	Specificity
1. Do you have a decrease in libido (sexual desire)?	18 (52.9)	43.7	38.8
2. Do you have a decrease in energy?	26 (76.5)	75.0	22.2
3. Do you have a decrease in strength and/or endurance?	26 (76.5)	68.8	16.7
4. Have you lost height?	13 (38.2)	37.5	61.1
5. Have you noticed a decrease in your enjoyment of life?	11 (32.4)	25.0	61.1
6. Are you sad and/or grumpy?	11 (32.4)	18.8	55.6
7. Are your erections less strong?	28 (82.4)	87.5	22.2
8. Have you noticed a recent deterioration in your ability to play sports?	25 (73.5)	62.5	16.7
9. Do you fall asleep after dinner?	10 (29.4)	25.0	66.7
10. Has there been a recent deterioration in your work performance?	20 (58.8)	50.0	33.3

Note: Estimated Cronbach's Alpha 0.72

This suggests that the items of the questionnaire are related and measure a similar construct. However, despite this internal consistency, the questionnaire's ability to accurately identify patients with hypogonadism was limited.

Patients were stratified into demographic groups according to cutoff points for all variables and were evaluated for associations with the presence of hypogonadism. As shown in Table 3, our analysis found that a BMI below 30 kg/m², an abdominal circumference below 102 cm, and total cholesterol levels below 170 mg/dL were protective factors against hypogonadism compared to values above these cutoff points.

Table 3. Associations and prevalence ratio between hypogonadism and other variables

Participant Characteristics		Hypogonadic (n)	Non-Hypogonadic (n)	χ2 (p-value)	Prevalence Ratio
BMI (Kg/m²)	<30 >30	6 (17.14%) 12 (34.29%)	12 (34.29%) 5 (14.29%)	0.044 #	2.12
Abdominal Circumference (cm)	<102 >102	2 (5.71%) 16 (45.71%))	9 (25.71%) 8 (22.86%)	0.012 #	3.67
Vitamin D (ng/mL)	<20 >20	4 (11.43%) 14 (40%)	6 (17.14%) 11 (31.43%)	0.47 #	0.71
Glycated Hemoglobin (%)	<7 >7	10 (26.32%) 10 (26.32%)	6 (15.79%) 12 (31.58%)	0.299	0.73
Total Cholesterol (mg/dL)	<170 >170	3 (8.57%) 15 (42.86%)	9 (25.71%) 8 (22.86%)	0.035 #	2.61
Duration of Disease (years)	<10 >10	8 (23.53%) 8 (23.53%)	9 (26.47%) 9 (26.47%)	>0.99	1

Fisher's Test conducted

4. DISCUSSION

In the present study was found that 47.1% of the male diabetic patients were classified as having low testosterone levels, with an average age of 62.3 years in the studied population. This prevalence rate is higher than that found in a previous cross-sectional study, where the prevalence was 36.5% in patients with an average age of 52 years. The observed difference could be attributed to the fact that the present study included an older population and used a higher diagnostic threshold for hypogonadism, set at 346 ng/dL (Al Hayek *et al*., 2013).

It is well-established that men experience a gradual decline in total serum testosterone (TT) levels with advancing age. This phenomenon, often referred to as late-onset hypogonadism or andropause, results from a combination of both testicular and hypothalamic-pituitary factors. The decreased production of testosterone by the testes, characterized by a reduction in Leydig cell number and decreased responsiveness to luteinizing hormone (LH), significantly contributes to this decline. In parallel, a progressive increase in the serum concentration of sex hormone-binding globulin (SHBG) is observed, primarily due to increased hepatic synthesis. Although SHBG is responsible for binding testosterone, forming an inactive complex, the elevation of its levels alone does not explain the decline in serum TT. The combination of primary (testicular) and secondary (hypogonadotropic) hypogonadism results in a net reduction in serum TT. Furthermore, the increase in SHBG leads to a decrease in free testosterone fraction, which is the biologically active form of the hormone. Consequently, age-related declines in serum free testosterone are more pronounced than those observed in total testosterone. Another important aspect to consider is the altered circadian rhythm of testosterone secretion with aging. While younger individuals exhibit a pronounced morning peak in testosterone secretion, this circadian variation is attenuated in older individuals (Lisco *et al*., 2020; Theodorakis *et al*., 2024; Singh *et al*., 2023).

The coexistence of type 2 diabetes mellitus (T2DM) and aging intensifies the likelihood of observing reduced testosterone levels. This complex association can be explained, in part, by alterations in the sex hormone-binding globulin (SHBG) synthesis axis, influenced by both factors. The combination of these factors can lead to significant dysregulation of the hormonal axis, culminating in a decline in testosterone levels (Singh *et al*., 2023; Lisco *et al*., 2020).

It was observed that the ADAM questionnaire, despite its high sensitivity (87%), showed a specificity of 0%, indicating a propensity for false positives. This characteristic is consistent with previous studies in diabetic populations, where specificity varied significantly (Iranparvar; Amani; Naghizadeh, 2021; Ugwu; Ikem, 2018). The low specificity may be influenced by common factors in diabetic men, such as reduced cardiopulmonary capacity, depressive symptoms, and sexual dysfunction, contributing to the high number of false positives (Farooqi *et al*., 2022; Clark; Fox; Grandy, 2007).

Diabetes mellitus is frequently associated with depression, a comorbidity that significantly impacts patients' quality of life. Several factors may contribute to this

association. First, diabetes management requires significant lifestyle adaptations, such as adherence to restrictive diets, regular physical activity, and constant monitoring of blood glucose levels. These demands can generate stress, anxiety, and, in some cases, lead to the development of depressive episodes. Second, the metabolic dysfunction characteristic of diabetes can directly influence neurotransmission. Studies suggest that elevated levels of glutamate, an excitatory neurotransmitter, may be associated with the development of depression in diabetic patients (Abdelmageed; Mohammed Hussein, 2022).

Erectile dysfunction (ED), in turn, is another common comorbidity in men with diabetes, which can contribute to the worsening of depressive episodes. The chronic hyperglycemia characteristic of diabetes leads to the production of reactive oxygen species (ROS) and advanced glycation end products (AGEs), which damage the vascular endothelium and impair the synthesis and release of nitric oxide (NO). NO is an essential vasodilator for erection, and its deficiency contributes to erectile dysfunction. In addition, peripheral neuropathy, a common complication of diabetes, can affect the pelvic nerves, leading to retrograde ejaculation and contributing to sexual dysfunction (Andlib *et al*., 2023).

In a comprehensive cross-sectional study with 5081 participants, published in 2021, it was found that hypogonadic patients often had elevated BMI, larger abdominal circumference, and dyslipidemia (Hsu *et al*., 2021). These findings are in line with the results of the present study. It is important to note that increased total cholesterol, BMI, and abdominal circumference are established risk factors for cardiovascular diseases. Men with hypogonadism have an increased cardiovascular risk, and testosterone replacement therapy may be beneficial in reducing the risk of overall mortality without increasing cardiovascular events. Essentially, considering the existence of an already present chronic disease in the patient's life, such as Coronary Artery Disease (CAD), where low levels of endogenous testosterone can influence the progression of this disease, increasing the inflammatory response such as IL-6, TNF-alpha, and CRP. Therefore, the assessment of gonadal function in patients with type 2 diabetes assumes critical relevance, potentially leading to early diagnosis and effective treatment, improving the quality and life expectancy of patients (Fallara *et al*., 2022; Çatakoglu; Kendirci, 2017).

However, there are limitations in this study that need to be considered. The sample size may limit the ability to generalize the results. Being cross-sectional, the study does not allow for causal inferences between hypogonadism and cardiovascular risk factors.

Additionally, the diagnosis of hypogonadism was based on a single blood sample, which may have led to overdiagnosis, impacting the specificity of the ADAM questionnaire.

5. CONCLUSION

A relevant prevalence of hypogonadism was observed in the Type 2 diabetic men that was associated with others cardiovascular risk factors such as high levels of total cholesterol, weight excess and visceral obesity which further increases the risk of a fatal or non-fatal cardiac event. The ADAM questionnaire demonstrated limitations in screening for hypogonadism in diabetic men due to its low specificity in present study. Future studies could explore alternative screening tools or refine the ADAM questionnaire for diabetic men, emphasizing the importance of identifying and treating hypogonadism to improve the quality of life and minimize cardiovascular risk in these patients.

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CONTRIBUTION OF AUTHORSHIP

Guilherme Alfonso Vieira Adami: Conceptualization, Formal Analysis, Investigation, Writing – review & editing.

Israel Gonçalves de Carvalho Filho: Conceptualization, Formal Analysis, Investigation, Methodology, Project administration, Writing – review and editing.

Caio Eduardo Alves de Oliveira Paes Leme Goulart: Software, Validation, Writing – original draft.

Claudine Dullius: Data curation, Supervision, Validation, Visualization.

Karen Bonete Bozza: Research, methodology, writing -preparation of the original, writing.

Geraldo Emílio Vicentini: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft and Supervision.