

Vitamin D insufficiency and factors associated: a study with older adults people from primary health care network

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Abstract *This article aims to analyze vitamin D insufficiency and factors associated among older adults using primary health care services. This is a cross-sectional study that evaluated 533 older adults individuals (≥ 60 years old) in three cities in the state of São Paulo, Brazil. Serum level of 25-hydroxyvitamin D (25-OHD) was evaluated by chemiluminescence. The factors evaluated were sociodemographic information (sex, age group, ethnicity, education, income, marital status), health conditions (reported diseases), body composition (BMI, waist circumference), lifestyle (physical activity and smoking), and sun exposure (purpose, duration, frequency, time of exposure, exposed body parts, use of sunscreen, skin type). The prevalence of vitamin D insufficiency was 64.5%, presenting association with female participants, non-white/unreported ethnicity, low weight, high waist circumference (risk for CVD – cardiovascular disease), and physical inactivity. Negative association was observed with habitual sun exposure of hands, arms and legs, during leisure activities, daily commuting and physical activity, and between 9 am and 3 pm. The findings show the relevance of factors such as sex, ethnicity, body composition, physical activity, and sun exposure habits in the high prevalence of inadequate levels of vitamin D among older adults.*

Key words *Elder people, Vitamin D, Sun exposure, Public health.*

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Introduction

Vitamin D has a very clear role in bone mineralization; it has been highlighted for its complex activity in the body, including control of metabolic processes. Proper levels of vitamin D are suggested to help protect the body against musculoskeletal disorders, infectious diseases, cancers, autoimmune and cardiovascular diseases, diabetes mellitus, and neurocognitive dysfunctions, with low serum concentrations of vitamin D related to increased risk for development and progression of these diseases¹.

Cutaneous synthesis regulated by sunlight is the main source of vitamin D in humans, so there is a false impression that vitamin D deficiency is not a problem in sunny countries, and for this reason, serum concentrations of vitamin D in the population are not often measured. However, according to studies conducted in Brazil and other countries, hypovitaminosis D is highly prevalent, regardless of the region evaluated, with Brazilian studies showing prevalence ranging from 28.5% according to a study conducted with hypertensive older adults in João Pessoa, Paraíba, in spring and summer, to almost 96%, as reported in another study that evaluated older adults in São Paulo, SP, in winter².

A study by Cabral *et al.*³, which evaluated 234 older adults male individuals in Recife, Pernambuco, in summer, found 66.7% prevalence of vitamin D insufficiency, with mean serum concentrations of 25-hydroxyvitamin D below the recommended level (27.86 ± 13.52 ng/mL). These mean values are similar to those found in another recent study⁴ conducted with 287 older adults from a community in Palhoça, Santa Catarina (26.09 ± 9.20 ng/mL).

In addition to sun exposure, many other factors may be involved in vitamin D deficiency, such as age, skin pigmentation, obesity, multimorbidity, and use of certain medications like antihypertensive, anticonvulsant, anti-inflammatory, and sedative medications. Due to lifestyle habits, polypharmacy, multimorbidity, and reduced effectiveness of vitamin D production in the skin, the older adults are one of the most important groups at risk for vitamin D deficiency⁵.

In this context, considering that few studies have assessed representative samples of older adults in primary health care in Brazil^{6,7}, and knowing that serum vitamin D is not widely tested in the older adults population using primary health care services, factors must be identified for an early identification of vitamin D insufficiency,

enabling actions and strategies to prevent and control this condition, which has become more prevalent in Brazil and worldwide. This study aims to analyze the prevalence of vitamin D insufficiency and its association with sociodemographic variables, sun exposure habits, physical and health conditions in older adults using primary health care services.

Methods

Participants

This is a cross-sectional study, which is part of a larger study titled “Evaluation on the prevalence of micronutrient deficiency in older adults living in cities in the region of Campinas – SP,” conducted in the municipalities of Limeira, Piracicaba, and Campinas, in the state of São Paulo, Brazil.

The inclusion criteria for study participation were: aged 60 years and older, living in one of the study municipalities, and presenting proper neurological and cognitive conditions to answer the questionnaires (as assessed by the interviewer during the study presentation and invitation to participate). Exclusion criteria were: use of food supplements based on vitamins and/or minerals, participation in a home care program, and receiving chemotherapy treatment.

The study sample was estimated considering the total number of inhabitants aged 60 years and older in the municipalities of Campinas, Limeira, and Piracicaba using official data of population estimates for 2018 and considering a prevalence of 60% of older adults with deficiency of at least one of the evaluated nutrients, considering a sampling error of 10% and 95% confidence level. Then, the total sample was 600 older adults, with 250 participants from the city of Campinas, 170 from Limeira, and 180 from Piracicaba.

Data were collected from 612 participants from October 2018 to December 2019 (no data collection was performed during the winter); 17 individuals had incomplete data and 62 who reported using supplements containing vitamin D were excluded, so the final sample had 533 individuals.

The older adults volunteers were recruited through an invitation made at the basic health units (UBS) recommended by the respective Health Departments of each municipality.

This study was approved by the Ethics Committee for Research with Human Bein-

gs from Universidade Estadual de Campinas, study approval No. 2.878.652 and CAAE 95607018.8.0000.5404. All participants signed an informed consent form.

Data collection

In each selected health unit, the older adults patients were invited by the research team to participate in the study and instructed to come back on the scheduled date with at least 8-hour fasting. On the scheduled day, a blood sample was collected, anthropometric data were measured, and then an interview was conducted to answer the questionnaire. These procedures were performed by a team of undergraduate and graduate health students, previously trained by the research coordinators.

Study variables

The dependent variable of the study was vitamin D insufficiency. Serum vitamin D levels were analyzed by measuring 25-hydroxyvitamin D (25-OHD) using the chemiluminescence immunoassay technique, with the Alinity 25-OH Vitamin D reagent kit (Abbott). The tubes containing collected blood were stored in a cooler with ice, and immediately at the end of collection, they were sent to the same private laboratory hired for this study (Pasteur[®]), where the samples were processed and then analyzed (no freezing). For serum 25-hydroxyvitamin D levels, the reference values recommended by Brazilian Society of Endocrinology and Metabolism (SBEM) and Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC)⁸ were used, so values below 30 ng/ml were considered vitamin D insufficiency.

The study covariates were: sex, age group, ethnicity, monthly income, physical activity, smoking, self-reported diseases, body mass index (BMI), waist circumference, sun exposure, use of sunscreen, sun exposure time, part of the body exposed to the sun, duration of sun exposure, frequency of sun exposure, and skin types.

BMI is a person's body weight (in kilograms) divided by the square of height (in meters). Weight was measured with the individual standing on an electronic weighing scale (150 kg capacity, 50 gram accuracy). Height was measured using a professional portable stadiometer, 0.1 cm accuracy, where the individual was standing straight with heels together on the base. BMI of the participants was classified according to the cutoff

points for the older adults, as recommended by the Ministry of Health⁹, that is, up to 22 kg/m²: low weight, between 22 and 26.99 kg/m²: eutrophic, 27 kg/m² or more: overweight.

Waist circumference was measured with a non-extendable measuring tape located at the midpoint between the iliac crest and the last rib. Risk for cardiovascular disease (CVD) was considered when the male participants presented 102 centimeters or more and 88 centimeters or more for female participants¹⁰.

Regarding physical activity, individuals were considered active when they reported at least 150 minutes of moderate intensity aerobic physical activity or 75 minutes of vigorous intensity aerobic physical throughout the week¹¹.

For the assessment of smoking, individuals answered "yes" if they were currently smokers, "no" if they had never smoked, or if they were former smokers.

Skin type was assessed using the Fitzpatrick scale¹², which defines scores to skin phototypes: from 1 to 6, where type 1 is the palest skin which always burns and never tans, and type 6 is the darkest skin which never burns and always tans when exposed to the sun (the participant pointed to a printed picture matching the color of his/her skin).

Statistical analysis

For the statistical analysis of the study variables, distributions of relative frequencies, mean values and standard deviation were estimated for continuous variables, and proportions were estimated for categorical variables. Differences between groups were estimated using the Mann-Whitney test after analyzing the normality of distributions, and χ^2 test was used to analyze differences between categorical variables. Variables with p value < 0.20 in simple association were selected to calculate crude and age-adjusted odds ratios, with different models for general, physical and health characteristics and sun exposure habits, but the variables with p value < 0.05 remained in the tables. The critical level was $p < 0.05$. The analyses were performed in Stata version 14.

Results

All 533 participants included in the study had a mean age of 69.6 ± 6.7 years, with predominance of female participants. Most participants were married (60.4%), white (54.4%), had less than 8

years of education (71.8%) and income of two or more minimum wages (70.5%). When analyzing the nutritional status indicators, we found mean BMI of 28.9 ± 5.4 kg/m², with most participants classified as overweight (60.4%) and at risk for CVD according to the waist circumference assessment (58.7%). Regarding health-related behaviors, 58.7% had never smoked and 64.4% were inactive smokers (Table 1).

The mean serum concentration of 25-OHD was 28.3 ± 9.2 ng/mL, and the prevalence of insufficiency was 64.5%, higher among female participants and those who self-reported as black/brown/indigenous/yellow/unreported, categorized as “other” ethnicity (Table 1). No difference was observed in age group, marital status, income, education, those with paid work, and head of household. Table 1 shows higher prevalence of vitamin D insufficiency among individuals classified as underweight and those with a waist circumference measurement above the limits of risk for developing CVD.

Regarding health data of participants, most of them reported medical diagnosis of three or more diseases – the most prevalent disorders were: hypertension (61.9%), spine issues (50.5%), rheumatism (43.1%), diabetes (27.6%), depression (27.6%), and CVD (23.6%). No significant difference was found when associating these diseases and vitamin D insufficiency or grouping individuals by the number of pre-existing diseases.

In the evaluation of sex- and age-adjusted logistic regression of general, physical and health characteristics of participants, those of “other” ethnicity, low weight and high waist circumference were more likely to have vitamin D insufficiency (Table 2).

Table 3 shows that most participants self-reported daily sun exposure while commuting (e.g., going to the market, drugstore, etc.) without sunscreen. They also reported exposure of less than 3 times during the week, for less than 15 minutes on average, between 9 am and 3 pm, with hands, arms and face as their more frequently exposed body parts. Every participant could report more than one exposure habit, time and exposed body parts. According to the Fitzpatrick scale, the predominant skin phototype was type 4, followed by types 2 and 3.

The proportion of vitamin D sufficiency was higher among participants reporting sun exposure during leisure and physical activities ($p = 0.018$ and $p = 0.010$, respectively). A difference was also observed between participants who re-

ported exposure between 9 am and 3 pm ($p = 0.032$) and exposure of hands and arms ($p = 0.046$) (Table 3).

In the evaluation of sex- and age-adjusted logistic regression of sun exposure habits of participants, a negative association was observed between vitamin D insufficiency and individuals reporting exposure habits during leisure activities, daily commuting, practice of physical activities, exposure between 9 am and 3 pm, and hands, arms and as their more frequently exposed body parts (Table 4).

Discussion

A high prevalence of vitamin D insufficiency was reported among the older adults participants evaluated (64.5%), with sex, ethnicity, body composition and some habits of sun exposure associated with vitamin D insufficiency.

Regarding the high prevalence of vitamin D insufficiency, similar indices were found in another study conducted with the same population profile in the country^{4,13,14}, while a study performed with 81 older adults in Ribeirão Preto, São Paulo¹⁵, found a higher prevalence (76.5%) of vitamin D insufficiency, with serum 25-OHD levels of 24.63 ± 7.89 ng/mL.

Brazil has a geographic location that provides good availability of ultraviolet rays (UVB) throughout the year, which allows exposure to sunlight and cutaneous synthesis of vitamin D at proper concentrations in most seasons of the year. However, studies have reported high prevalence of vitamin D insufficiency and deficiency. This paradox can be partly explained by the concern about preventing high levels of sun exposure, regardless of the concentration of solar radiation, a precaution that leads the population to be less exposed to the sun or use physical/chemical barriers (clothes, hats, sunscreen), thus increasing the prevalence of inadequate levels of vitamin D¹⁶.

Specifically in relation to sex, the female participants had lower mean values and higher prevalence of insufficiency (adjusted OR 2.54; $p < 0.001$). A study that evaluated 359 older adults in Teresina, Piauí,⁷ found even lower mean values when analyzing serum levels (22.51 ± 8.03 ng/mL), which were also lower among female participants. Several studies¹⁷⁻¹⁹ show that women are more likely to have lower levels of 25-OHD than men, particularly due to the type of clothes and sun protection behavior of women, which makes

Table 1. General, physical and health characteristics of older adults with vitamin D sufficiency and insufficiency. Limeira, Piracicaba, and Campinas, state of São Paulo, 2018-2019.

Characteristic	Total		Vitamin D sufficiency		Vitamin D insufficiency		p-value
			(≥ 30 ng/mL)		(< 30 ng/mL)		
	n = 533		n = 189 (35.5%)		n = 344 (64.5%)		
	n	%	n	%	n	%	
Sex							0.000
Female	353	66.2	99	52.4	254	73.8	
Male	180	33.8	90	47.6	90	26.2	
Age							0.218
60-74 years	419	78.6	143	75.7	276	80.2	
75 years and older	114	21.4	46	24.3	68	19.8	
Ethnicity							0.026
White	284	53.3	113	59.8	171	49.7	
Other – black, brown, indigenous, yellow or unreported	249	46.7	76	40.2	173	50.3	
Monthly income*							0.059
< 2 minimum wages	108	20.3	29	15.3	79	23.0	
≥ 2 minimum wages	376	70.5	138	73.1	238	69.2	
unreported	49	9.2	22	11.6	27	7.8	
BMI							0.004
Low weight	44	8.3	9	4.8	35	10.2	
Eutrophy	167	31.3	74	39.1	93	27.0	
Overweight	322	60.4	106	56.1	216	62.8	
Waist circumference							0.001
With risk	313	58.7	93	49.2	220	64.0	
No risk	220	41.3	96	50.8	124	36.0	
Physical activity							0.225
Active	230	43.2	91	48.1	139	40.4	
Not active	303	56.8	98	51.8	205	59.6	
Smoking							0.749
Yes	39	7.3	14	7.4	25	7.3	
No	313	58.7	107	56.6	206	59.9	
Former smoker	181	34.0	68	36.0	113	32.8	

n: sample number; %: proportion from n; p value: Pearson's chi-square test; *minimum wage in effect at the time of the interview (2018-R\$954.00/2019-R\$998.00); BMI: body mass index.

Source: Authors.

cutaneous synthesis of vitamin D less efficient²⁰.

Regarding skin color, reduced efficiency of vitamin D synthesis in people with dark skin is well described in the literature, to the greater amount of melanin, which acts as a natural sunscreen⁵. It explains the higher prevalence of vitamin D insufficiency found among individuals reporting more pigmented skin color ethnicities.

The association between overweight and vitamin D insufficiency is also well described in the literature²¹⁻²⁷. It can be explained by biological

factors, since obesity decreases the bioavailability of vitamin D obtained by cutaneous synthesis, as vitamin D tends to be deposited on adipose tissue, changes the regulation of PTH and hepatic synthesis of 25-OHD, and involves lifestyle factors, such as lower intake of vitamin D in the diet of obese people²⁸. Although this study did not find an association with overweight according to the BMI classification, after an adjusted regression analysis, this association was observed with increased waist circumference (adjusted OR 1.57;

Table 2. Crude and adjusted odds ratios (OR) of vitamin D insufficiency in relation to sociodemographic and health characteristics of older adults. Limeira, Piracicaba, and Campinas, state of São Paulo, 2020.

Characteristic	Crude OR	p value	adjusted OR ⁺	p value
Sex				
Male	1.00		1.00	
Female	2.58	< 0.001	2.54	<0.001
Ethnicity				
White	1.00		1.00	
Other - black, brown, indigenous, yellow or unreported	1.48	0.031	1.46	0.043
Monthly income*				
< 2 minimum wages	1.00		1.00	
≥ 2 minimum wages	1.58	0.057	1.29	0.303
unreported	0.68	0.226	0.67	0.219
BMI				
Eutrophy	1.00		1.00	
Low weight	3.09	0.005	2.56	0.023
Overweigh	1.60	0.016	1.41	0.085
Waist circumference				
No risk	1.00		1.00	
With risk	1.83	0.001	1.57	0.018

OR: odds ratio; +adjustment by sex and age variables; *minimum wage in effect at the time of the interview (2018-R\$954.00/2019-R\$998.00); BMI: body mass index.

Source: Authors.

$p = 0.018$). A study conducted by Snijder *et al.*²¹ in Amsterdam with 453 older adults found an association between low levels of 25-OHD and high waist circumference. This characteristic is an important factor, as it seems to make an individual more susceptible to lower levels of 25-OHD^{29,30}.

On the other hand, we found a strong association between vitamin D insufficiency and low weight (adjusted OR 2.56; $p = 0.023$). A study conducted by Cabral³¹ that assessed older adults in Porto Alegre, Rio Grande do Sul, also found deficient levels of 25-OHD with higher prevalence among thin or malnourished older adults, suggesting vitamin D deficiency is associated with nutritional risk and malnutrition.

Several studies show higher serum levels of 25-OHD among individuals who practice physical activities^{21,28,32,33}. This characteristic is even more accentuated when this practice is performed outdoors¹⁹. In this study, we found no significant difference between groups in terms of active individuals. However, individuals reporting sun exposure during physical activity showed a lower prevalence of vitamin D insufficiency.

Among the diseases reported by participants, although the relationship between hypovita-

minosis D and various diseases such as diabetes, cardiovascular diseases, hypertension, cancer, osteoporosis, depression, among others³⁴, is well described in the literature, no association was observed between these diseases and vitamin D insufficiency. Also, no significant difference was found regarding the number of diseases reported by participants.

A correctly applied sunscreen with sun protection factor (SPF) 30 is presumed to reduce by 95% to 99% the skin ability to produce vitamin D³⁵. Despite that, we found no significant difference for vitamin D insufficiency between individuals who reported using sunscreen and those who did not have such habit. A similar result was reported by Maeda *et al.*¹⁹ for using sunscreen or not. On the other hand, in a study conducted by Cabral³¹, sunscreen was significantly associated with proper levels of 25-OHD, a fact that, according to the author, can be explained by a possible application of insufficient or uneven sunscreen.

The efficiency of sun exposure in vitamin D synthesis depends on the use of sunscreen and other factors, such as latitude, season of the year, air pollution, skin pigmentation, and age³⁶. For white adults, it is recommended to expose arms and legs to the sun (which represent 25% of the

Table 3. Sun exposure habits of individuals with vitamin D sufficiency and insufficiency. Limeira, Piracicaba, and Campinas, state of São Paulo, 2018-2019.

Variável	Total		Vitamin D sufficiency		Vitamin D insufficiency		p-value
			(≥ 30 ng/mL)		(< 30 ng/mL)		
	n = 533		n = 189 (35,5%)		n = 344 (64,5%)		
	n	%	n	%	n	%	
Sun exposure							
During leisure activities	137	25.7	60	31.7	77	22.4	0.018
During daily commuting	408	76.5	153	81.0	255	74.1	0.075
During physical activities	190	35.6	81	42.9	109	31.7	0.010
For health purposes	83	15.6	35	18.5	48	14.0	0.164
Use of sunscreen							0.207
Yes	188	35.3	60	31.7	128	37.2	
No	345	64.7	129	68.3	216	62.8	
Time of exposure							
Before 9 am	256	48.0	85	45.0	171	49.7	0.295
Between 9 am and 3 pm	274	51.4	109	57.7	165	48.0	0.032
After 3 pm	95	17.8	33	17.5	62	18.0	0.871
Exposed body parts							
Face	418	78.4	149	78.8	269	78.2	0.864
Hands and arms	474	88.9	175	92.6	299	86.9	0.046
Legs	272	51.0	107	56.6	165	48.0	0.056
Average duration							0.115
< 15 minutes	435	81.6	161	85.2	274	79.7	
≥ 15 minutes	98	18.4	28	14.8	70	20.3	
Frequency of exposure							0.718
< 3 days a week	350	65.7	126	66.7	224	65.1	
≥ 3 days a week	183	34.3	63	33.3	120	34.9	
Skin type (Fitzpatrick scale)							0.542
Type 1	70	13.1	23	12.2	47	13.7	
Type 2	142	26.6	52	27.5	90	26.2	
Type 3	107	20.1	43	22.7	64	18.6	
Type 4	144	27.0	48	25.4	96	27.9	
Type 5	39	7.3	10	5.3	29	8.4	
Type 6	20	3.8	7	3.7	13	3.8	
Not reported	11	2.1	6	3.2	5	1.4	

n: sample number; %: proportion from n; p value: Pearson's chi-square test.

Source: Authors.

body surface when wearing shorts and a short-sleeve shirt or blouse) for about 5 to 15 minutes, between 9 am and 3 pm, three times a week (depending on the latitude, season of the year, and skin pigmentation), which would be sufficient for the production of vitamin D required. However, among the older adults, cutaneous synthesis is reduced to around 25% of the capacity of a 20-year-old person³⁷.

Despite the reduced capacity of vitamin D photoproduction among older people, Brou-

wer-Brolsma *et al.*³⁶ observed significant associations between habitual sun exposure and 25-OHD in this population. Data from a study conducted by Maeda *et al.*¹⁹ suggest the amount of UVB radiation at the latitude of the city of São Paulo, SP, is sufficient for proper production of vitamin D, even for the older population, as long as they have minimal exposure. However, it should be noted that sun exposure is not enough to compensate for vitamin D insufficiency or deficiency³⁸. Data obtained in this study related to

Table 4. Crude and adjusted odds ratios (OR) of vitamin D insufficiency in relation sum exposure habits of older adults. Limeira, Piracicaba, and Campinas, state of São Paulo, 2020.

Characteristic	Crude OR	p value	Adjusted OR ⁺	p value
Sun exposure				
During leisure activities				
No	1.0		1.0	
Yes	0.62	0.018	0.63	0.030
During daily commuting				
No	1.0		1.0	
Yes	0.67	0.076	0.57	0.019
During physical activities				
No	1.0		1.0	
Yes	0.61	0.010	0.67	0.040
For health purposes				
No	1.0		1.0	
Yes	0.71	0.166	0.71	0.172
Time				
Before 9 am				
No	1.0		1.0	
Yes	1.20	0.219	1.20	0.326
Between 9 am and 3 pm				
No	1.0		1.0	
Yes	0.67	0.032	0.68	0.042
After 3 pm				
No	1.0		1.0	
Yes	1.03	0.871	1.08	0.744
Exposed body parts				
Face				
No	1.0		1.0	
Yes	0.96	0.864	0.83	0.442
Hands and arms				
No	1.0		1.0	
Yes	0.53	0.049	0.46	0.019
Legs				
No	1.0		1.0	
Yes	0.70	0.056	0.66	0.031

OR: odds ratio; +Adjustment by sex and age variables.

Source: Authors.

sun exposure agree with literature findings. Lower proportions of vitamin D insufficiency were observed among individuals who reported sun exposure during leisure activities and physical activities ($p = 0.018$ and $p = 0.010$, respectively), and between 9 am and 3 pm, the peak period of solar radiation ($p = 0.032$).

Our study limitation refers to the fact that it used the older adults participants accounts as a parameter to assess habitual sun exposure, and this information can be underestimated or over-

estimated. Also, data were not analyzed separately by season. However, data collection was not performed during the winter and, considering the incidence of UVB rays are similar during spring, summer and autumn at the latitude in question, we consider these findings to be relevant. Another study limitation is that the individuals were volunteers, and many of them came from health groups from the primary health care system in the municipalities, including outdoor physical activity groups, causing the bias of “healthy par-

participant/volunteer participant,” a fact that may have partially influenced the results. However, this is a common limitation in population studies where participants are invited in out-of-home interviews, as volunteers are usually more interested in health-related issues.

One of the strengths of our study is the assessment of a sample of older population assisted by primary health care services in the cities of Limeira, Piracicaba, and Campinas, and the investigation conducted in the same study of various factors that can be associated with vitamin D insufficiency in this vulnerable age group.

The results obtained show the relevance of the impact of factors like sex, ethnicity, body composition, physical activity, and sun exposure habits on the prevalence of inadequate levels of vitamin D among older adults, and the importance of considering these factors as vitamin D insufficiency predictors in this population, mainly as a primary care tool. The assessment of these factors is a simple, low-cost and non-invasive measure that can contribute to the early identification of vitamin D insufficiency, enabling actions and strategies to prevent and control this condition, which has become increasingly prevalent in Brazil and in the world.

Collaborations

PMD Rolizola worked on data collection, data analysis and manuscript writing. CN Freiria contributed to data collection, database preparation, data analysis and manuscript review. GM Silva acted in data collection, database preparation and manuscript review. TRP Brito and FSA Borim worked on coordinating the research and reviewing the analysis and manuscript. LP Corona contributed to the study design, analysis plan, research coordination and manuscript review. All authors read and approved the final manuscript.

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