



**ORIGINAL ARTICLE** 

# Prevalence of Dental Caries in 5-Year-Old Children in a Northeast Brazilian Capital

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Academic Editor: Burak Buldur

Received: 09 May 2022 / Review: 03 November 2022 / Accepted: 06 December 2022

**How to cite:** Saldanha KGH, Almeida JRS, Almeida MEL, Magalhães IA, Silva PGB. Prevalence of dental caries in 5-year-old children in a Northeast Brazilian capital. Pesqui Bras Odontopediatria Clín Integr. 2023; 23:e220073. https://doi.org/10.1590/pboci.2023.071

# ABSTRACT

Objective: To evaluate the prevalence of dental caries in children aged five years in a Northeast Brazilian Capital (Fortaleza, CE) and its association with sociodemographic conditions, presence of malocclusion, and gingival bleeding. Material and Methods: This is a quantitative, descriptive, observational, and crosssectional study carried out in a representative sample of 3,582 children aged five years in the city of Fortaleza-CE. Data was collected in public and private schools distributed in the city's Regional Health Coordination (CORES). Each of the six CORES worked with five field teams, participating in inter-examiner training and calibration with a final KAPPA coefficient of 0.87. Data were collected using a clinical form adapted from the SB Brasil 2010 questionnaire on sociodemographic conditions. The indices of dmft, need for treatment, malocclusion, and gingival bleeding were used for oral health conditions. The data were submitted to Pearson's Chi-square or Fisher's Exact tests, and the variables that show values of p<0.05 were submitted to a multinomial logistic regression model (forward stepwise model). Results: 57.1% of children were cariesfree, and the mean dmft-d was 1.65 (1.65±2.65). CORES I and VI were the ones that presented the most significant association with caries attacks in all primary molars. The highest percentage of caries in the 2nd upper molar (60.6%), 1st lower molar (59%), and 2nd lower molar (58.8%) were found in children with normal occlusion. There was a significant association between gingival bleeding and caries in all molars and the need for treatment. Conclusion: These results allow us to observe that the prevalence of dental caries in 5-yearold children in Fortaleza is low, although with a tendency to increase.

Keywords: Oral Health; Child; Epidemiology.





#### Introduction

In Early Childhood, caries is defined as the presence of one or more carious (cavitated or non-cavitated), lost or restored (due to caries) surfaces on any primary tooth of a child under six years. Thus as in other age groups, it is determined by biological, behavioral, and psychosocial factors related to the individual's environment [1,2]. Research has found a higher prevalence of caries in low-income groups, with the main possible causes being the influence of types of diet, worse self-care level, low demand for preventive care, worse oral hygiene conditions, and difficulty in accessing dental services, for the poorest population [3,4].

In Brazil, a decline in the prevalence curve of dental caries at age five years has been observed in recent decades. This decline can be observed in the last two epidemiological surveys carried out by SB BRASIL in 2003 and 2010, where there was a 6% increase in caries-free children. Despite this slight decrease, the oral health of preschool children in Brazil is still a concern and must be constantly monitored, and promotion and prevention actions for this age group are consistently implemented [5-8].

This reality is proven through the typical inequities of the Brazilian territory that accompany and influence the behavior of caries in early childhood. It is a fact that the North and Northeast regions of Brazil have remarkable regional and social characteristics, which often denote discrepancies when comparing oral health indices. According to a report by SB Brasil - 2010, the average values found in the dmft index (decayed teeth, indicated extraction and fillings), equivalent to the age of 5 years, stand out negatively in the Northeast, North, and Center-West regions, in detriment of the Southeast and South regions [5]. In addition, the representation of the decayed component in this group stands out in numbers. At the same time, in the South and Southeast regions, the amount of restored dental elements is significantly higher [5].

Due to the child population being part of a large portion of the people directly affected by dental caries, caries disease becomes the most common chronic disease in children. According to the 2015 Global Burden of Disease study, untreated caries in primary teeth was the tenth most prevalent health problem, affecting 7.8% of the global child population [9,10]. Thus, due to its high prevalence and the significant impacts caused on the quality of life and development, it is widely discussed and classified by several authors as a public health problem [4,11].

In Brazil, the investigation of caries in early childhood has been ongoing for some decades. However, the history of oral health in Brazil shows that the care models implemented since the 50s, such as incremental systems and models of inversion of care, and so forth, always focused on groups of children aged 7 to 14 years [12].

In the state of Ceará, specifically in the city of Fortaleza, the decline in caries is observed, according to the rest of the country. However, little is known about the prevalence of caries and other oral health problems at five years of age, as well as its distribution in the territory of this municipality, given that the capital of Ceará has high rates of social inequities in its region [5-7,13,14].

It is believed that it is essential to understand the behavior of caries in children aged five years, as well as their biological and social formation, since it is at this stage that primary care promotes the prevention of future dental caries [15]. Dental caries, characterized as being a multifactorial disease and biofilm dependent, can be influenced by gingival bleeding and/or malocclusions.

Therefore, the objective of this study is to evaluate the prevalence of caries in early childhood and its distribution within the territory of the Municipality of Fortaleza through an epidemiological survey to support the planning of local oral health policies, making them more effective and efficient, and providing greater wellbeing for this population.





## **Material and Methods**

Study Design

A cross-sectional, observational, quantitative, and descriptive study was carried out in 2019/2020 in Fortaleza.

# Sample

Following the methodology adopted in the SB Brasil 2010, in order to estimate the sample size, each of the six Regional Health Coordinations (CORES) was considered a Municipality with more than 100 thousand inhabitants in the Northeast region, considering that each CORES has over 300,000 inhabitants. For the basis of the sample size calculation, the variable dental caries attack was taken into account, which is the reference standard because it is among the most prevalent diseases of the oral cavity, measured by the dmft indexes (mean number of affected teeth per individual) to age five years. The minimum sample size for carrying out the research stipulated a standard error of 5%, a level of 95% for the confidence interval. Thus, the sample size for each CORES was the same as considered in the SB Brasil 2010 for municipalities with more than 100,000 inhab., that is, 597 children per CORES, totaling a sample of 3,582 children for the Municipality of Fortaleza.

The inclusion criteria for the exams carried out in daycare centers and schools were: Being a resident of CORES and being of the age required for this research. The exclusion criteria were: Impossibility of carrying out the exam because they used appliances with orthodontic bands or some other condition that prevented the oral exam.

#### Data Collect

The research was conducted by the oral health technical team of the Municipal Health Department in partnership with the Federal University of Ceará. Each of the six CORES worked with five field teams composed of a dental surgeon (examiner) and an oral health assistant (annotator). In order to minimize the variations between the examiners, the calibration process was carried out in 4 stages: a) Preparation of the process that consisted of organizing the entire infrastructure; b) Theoretical discussion of the variables used, codes, and examination criteria using a manual prepared by the general coordination of the research; c) Practical Discussion and finally; d) Calibration where 15 children of each age group were selected and examined by all dental surgeons. The Kappa result in the inter-examiner calibration had an average of 0.87. The clinical examination of the conditions: dental caries, gingival bleeding, and malocclusion, was performed under natural light, using a flat mouth mirror, WHO periodontal probe, in addition to gauze to dry the teeth, and the adapted clinical record from SB Brasil. The present study proposed that a maximum of 20 exams could be performed in each work shift to ensure the reliability of the data.

# Statistical Analysis

Data were tabulated in Microsoft Excel and exported to the Statistical Package for the Social Sciences (SPSS) software, version 20.0 for Windows, in which the analyzes were performed using a confidence level of 95%. The variables were categorized as follows: CORES (I, II, III, IV, V, and VI), sex (male or female); race (yellow, white, black, brown); need for treatment (yes or no), gingival alteration (absence of bleeding or presence of bleeding); occlusal alteration (normal occlusion, mild malocclusion, moderate malocclusion), gingival bleeding (no/yes). Analyzes were performed using Pearson's Chi-square or Fisher's exact tests. The dependent variable selected for the bivariate analysis was caries in deciduous molars (1st upper molar – 1st UP, 2nd upper molar – 2nd UP, 1st lower molar – 1st LM, 2nd lower molar – 2nd LM).





#### **Ethics Committee**

The Research Ethics Committee of the Federal University of Ceará approved the research through opinion nº 3,092,816. The parents or guardians of the child signed the Free and Informed Consent Form.

#### Results

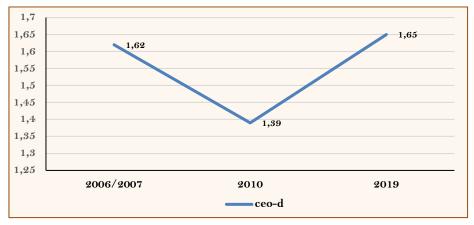
A total of 3,220 children were examined, 89.9% of the expected sample of 3,582 children. Table 1 shows the prevalence and severity of dental caries in Fortaleza, represented by the dmft index (average of decayed teeth, extraction indicated, and filled in the primary dentition). Regarding the severity of dental caries, CORES I, III, and VI exhibited mean values of dmft, respectively, of 1.9 (1.90±2.92), 1.7(1.66±2.55), and 2.0 (2.0±2.80), higher than the average dmft-d for Fortaleza, which was 1.65. The lowest values were found, respectively, in regions II - 1.4 (1.29 $\pm$ 2.49), IV - 1.3 (.1.29 $\pm$ 2.49), and V - 1.6 (1.6  $\pm$ 2.41).

Table 1. Absolute number and percentage of dmfts-d=0, dmfts-d=1 to 2, dmfts > 2, need for treatment and average dmfts at five years, according to the Regional Health Coordinations (CORES)

		Regional Health Coordination									
Variables	Fortaleza	I	II	III	IV	V	VI	p-value			
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	_			
Total	3.220	626	543	597	444	530	480				
dmfts-d (mean)	$1.65 \pm 2.65$	$1.90 \pm 2.92$	$1.4 \pm 2.61$	$1.66 \pm 2.55$	$1.29\pm2.49$	$1.6 \pm 2.41$	$2.0\pm 2.80$				
dmfts-d=0	1.840 (57.1)	339 (54.2)	346 (63.7*)	329 (55.1)	319 (71.8*)	280 (52.8)	227 (47.3)	< 0.001			
dmfts-d 1 to 2	573 (17.8)	104 (16.6)	91 (16.8)	119 (19.9*)	35 (7.9)	$123 (23.2^*)$	101 (21.0)				
dmfts-d > 2	807 (25.1)	$183 (29.2^*)$	106 (19.5)	149 (25.0)	90 (20.3)	127 (24.0)	152 (31.7*)				

<sup>\*</sup>Differences in values.

Figure 1 explains the trend of the dmft index at five years of age in the Municipality of Fortaleza between 2006 and 2019. In the epidemiological survey carried out in 2006 in the Municipality of Fortaleza, the dmft was 1.62. In the 2010 DB Brasil epidemiological survey, Fortaleza had a dmft of 1.39. In this research, the dmft found was 1.69. During the temporality, the index fell with the current growth trend.



Source: SB Fortaleza 2006/07, SB Brasil 2010, SB Fortaleza 2019.

Figure 1. The trend of the dmft index at five years, in Fortaleza, according to data from epidemiological surveys nationally based in 2010 and municipally based in 2006 and 2019.

Table 2 compares deciduous molars attacked by caries and variables related to housing CORES, sex, race, occlusion, need for treatment, and gingival bleeding. Of the 3,220 children examined, 48.7% were female and 51.3% male. As for race, 79.1% were considered brown, while 15.3% were considered white.





Regarding housing CORES, a significant difference was observed between the territories. CORES I and VI were the ones that showed the most significant association with caries attack in all deciduous molars, respectively for CORES I and VI (1st UM 23% and 21.7%, 2nd UM 21% and 21.3%, 1st LM 22.5% and 19% and 2nd LM 20.4% and 20.2%). On the other hand, CORES II and IV were the ones that showed the lowest association with deciduous molars attacked by caries (1st UM 18% and 14.9%, 2nd UM 17.6% and 14.4%, 1st LM 18.6% and 14.7%, 2nd LM 18.6% and 15.3%). CORES III and V showed variations depending on the molar attacked by caries. In CORES III, the upper 2nd molars (18.9%) and the lower 1st molars (18.9%) stand out as the least affected by caries. In CORES V, the lower molars were the most affected by caries, respectively. Regarding the sex of individuals and carious molars, there was no significant difference in the association of variables. However, regarding race, there was a significant association between mixed race and the presence of caries in maxillary first molars (83.7%) (Table 2).

Table 2. Comparative analysis between the percentage of deciduous molars with caries and variables related to housing CORES, Gender, Race, occlusion, need

for treatment, and gingival bleeding

		1	st UM caries		2	nd UM caries	S		1st LM caries	\$	2	nd LM caries	
Variables	Total	No	Yes	p-value	No	Yes	p-value&	No	Yes	p-value&	No	Yes	p-value&
	N (%)	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
CORES													
1	626 (19.4)	481 (18.6)	145 (23.0)*	< 0.001	497 (19.1)	129 (21.0)*	< 0.001	442 (18.4)	184 (22.5)*	< 0.001	450 (19.1)	176 (20.4)*	< 0.001
2	543 (16.9)	467 (18.0)*	76 (12.0)		459 (17.6)*	84 (13.7)		446 (18.6)*	97 (11.8)		438 (18.6)*	105 (12.2)	
3	597 (18.5)	478 (18.5)	119 (18.9)		493 (18.9)*	104 (16.9%)		453 (18.9)*	144 (17.6)		428 (18.1)	169 (19.6)*	
4	444 (13.8)	387 (14.9)*	57 (9.0)		376 (14.4)*	68 (11.1)		354 (14.7)*	90 (11.0)		360 (15.3)*	84 (9.8)	
5	530 (16.5)	433 (16.7)*	97 (15.4)		431 (16.5)	99 (16.1)		382 (15.9)	148 (18.1)*		377 (16.0)	153 (17.8)*	
6	480 (14.9)	343 (13.2)	137 (21.7)*		349 (13.4)	131 (21.3)*		324 (13.5)	156 (19.0)*		306 (13.0)	174 (20.2)*	
Sex													
Male	1651 (51.3)	1323 (51.1)	328 (52.0)	0.698	1316 (50.5)	335 (54.5)	0.079	1217 (50.7)	434 (53.0)	0.259	1212 (51.4)	439 (51.0)	0.836
Female	1568 (48.7)	1265 (48.9)	303 (48.0)		1288 (49.5)	280 (45.5)		1183 (49.3)	385 (47.0)		1146 (48.6)	422 (49.0)	
Race													
Mongolian	8 (0.2)	6 (0.2)	2 (0.3)	0.017	7 (0.3)	1 (0.2)	0.310	4 (0.2)	4(0.5)	0.403	4 (0.2)	4 (0.5)	0.329
White	491 (15.3)	416 (16.1)*	75 (11.9)		412 (15.8)	79 (12.9)		371 (15.5)	120 (14.7)		363 (15.4)	128 (14.9)	
Black	172 (5.3)	146 (5.6)	26 (4.1)		138 (5.3)	34(5.5)		130 (5.4)	42 (5.1)		120 (5.1)	52 (6.0)	
Brown	2545 (79.1)	2018 (78.0)	527 (83.7)*		2046 (78.6)	499 (81.4)		1894 (78.9)	651 (79.7)		1869 (79.3)	676 (78.6)	

UM: Upper Molar; LM: Lower Molar; \*Differences in values; \*Pearson's chi-square test.

Regarding occlusion, with the exception of the 1st maxillary molars, there was a difference in associations between the presence of normal occlusion and caries in deciduous molars. The highest percentage of caries in 2nd UM (60.6%), 1st LM (59%), and 2nd LM (58.8%) were found in children with normal occlusion. There is a significant difference between the absence of caries in the maxillary second molar and mild (26.8%) and moderate (19.7%) malocclusion. The same differences are repeated in mandibular first molars, where in the absence of caries, mild (27.0%) and moderate (19.6%) malocclusions are observed, and in mandibular second molars, there are relevant





differences in the absence of caries with mild malocclusion. (27.1%) and moderate (19.5%) (Table 3). Regarding the need for treatment, a significant association with the presence of caries was observed in all molars, with no significant differences in prevalence between molars, 1st UM (99.2%), 2nd UM (98.9%), 1st LM (99.1%) and 2nd LM (98.7%) (Table 3).

There was a significant association between bleeding and caries in deciduous molars. Children without gingival bleeding had the highest percentage of molars without caries, with values above 99.6% for all molars (Table 3).

Table 3. Absolute number and percentage of schoolchildren aged five years with or without caries in molars according to occlusion, need for treatment, and gingival bleeding.

		1s:	t UM caries		21	nd UM caries		18	t LM caries		2nd LM	caries	
Variables	Total	No	Yes	p-value&	No	Yes	p-value&	No	Yes	p-value&	No	Yes	p-value&
	N (%)	N (%)	N (%)		N (%)	N (%)		N (%)	N (%)		N (%)	N (%)	
Malocclusion													
Normal	1759 (54.8)	1400 (54.2)	359 (57.3)	0.313	1389 (53.5)	370 (60.6)*	0.006	1278 (53.4)	481 (59.0)*	0.018	1255 (53.4)	504 (58.8)*	0.016
Light	832 (25.9)	673 (26.1)	159 (25.4)		696 (26.8)*	136 (22.3)		645 (27.0)*	187 (22.9)		637 (27.1)*	195 (22.8)	
Moderate	617 (19.2)	508 (19.7)	109 (17.4)		512 (19.7)*	105 (17.2)		470 (19.6)*	147 (18.0)		459 (19.5)*	158 (18.4)	
Treatment Need													
No	1762 (54.7)	1757 (67.9)*	5 (0.8)	< 0.001	1755 (67.4)*	7 (1.1)	< 0.001	1755 (73.1)*	7 (0.9)	< 0.001	1751 (74.2)*	11 (1.3)	< 0.001
Yes	1458 (45.3)	832 (32.1)	626 (99.2)*		850 (32.6)	608 (98.9)*		646 (26.9)	812 (99.1)*		608 (25.8)	850 (98.7)*	
Gingival Bleeding													
No	3186 (99.3)	2570 (99.6)*	616 (97.8)	< 0.001	2585 (99.6)*	601 (97.9)	< 0.001	2386 (99.7)*	800 (97.9)	< 0.001	2340 (99.6)*	846 (98.3)	< 0.001
Yes	23 (0.7)	10 (0.4)	14 (2.2)*		11 (0.4)	13 (2.1)*		7 (0.3)	17 (2.1)*		9 (0.4)	15 (1.7)*	

UM: Upper Molar; LM: Lower Molar; \*Differences in values; \*Pearson's chi-square test.

## Discussion

The results found showed that there was no significant variation between the percentage of caries-free children in this research (57.1%) and the results of the SB Brasil 2010 epidemiological survey for the city of Fortaleza (57.3%) and the survey on the municipal base carried out in Fortaleza 2006/2007 (56.4%), at the age of five years. The minimum variation of this percentage, associated with the rise in the dmft indexes of children at this age, Fortaleza 2019 (1.65), Fortaleza in SB Brasil 2010 (1.39), Fortaleza 2006/2007 (1.62), even after more than ten years of previous research, it demonstrates factors to consider about the impact of the disease on this population and oral health actions aimed at early childhood in this municipality [5].

One of the factors to consider about early childhood caries is its significant destructive characteristic. The pain caused by tooth decay can interfere with eating behavior and cause children to grow slower, lose weight, and have more sleep disturbances. In addition, caries at this age can significantly reduce attention during activities, which affects school performance and causes learning deficits. Mainly concerning primary dentition, caries are the main reason for early loss in deciduous [4,15,16].





Another aspect to be analyzed is the distribution of caries in the population studied and its association with the place of residence. When analyzing the variables of caries in deciduous molars and CORES of residence, a significant association was observed between these two variables. Children who lived in CORES I and VI, admittedly with the worst Human Development Index (HDI; latest in Brazil was 0,755), higher rates of illiteracy and homicide in the last five years, were the ones with the highest proportion of caries in deciduous molars, while CORES II and IV, with the best HDI and lowest rates of illiteracy, had a lower percentage of caries in deciduous molars [14]. This inequality is strongly associated with this municipality's heterogeneous socio-spatial scenario, including upper-middle-class neighborhoods bordering extreme poverty neighborhoods. Despite Fortaleza accumulating stories of struggle with health and education, the municipality was considered, in 2012, the second Brazilian city with the most significant social inequality [17,18].

The National Commission on Social Determinants of Health states that a population's socioeconomic, cultural, and environmental conditions generate a stratification of individuals and population groups, thus giving them different social positions directly related to health conditions [19]. Among these inequalities marker factors, race can be mentioned. In this study, a significant association was observed between race and decayed deciduous molars, where brown-skinned children had a higher proportion of decayed molars. The same results were obtained in research conducted in Minas Gerais on caries and associated factors, where brown color is strongly associated with caries [20].

According to Osório 2021, in his text "A racial inequality in Brazil in the last three decades" even though the number of people in Brazil who consider themselves to be black and brown is increasing, as a way of combating racism and inequalities, this is not reflected in the access to health services and income distribution [21]. From 1986 to 2019, there was only a slight reduction in this inequality. Part of this reduction may have been produced by the increase in the declaration of black or brown color by relatively wealthier people, which would have displaced a portion of the inequality.

Significant associations were observed regarding the other variables, the presence of gingival bleeding, and treatment needs. Children with gingival bleeding showed a more significant association with caries in deciduous molars than those without gingival bleeding. This fact can be explained by the difficulty of children, or even parents, to clean these teeth due to pain or carelessness, increasing the retention of biofilm and gingival inflammation. The fact is that this result emphasizes the importance of parental care for children in early childhood, especially concerning oral health [22]. In the bibliographic review by Castilho et al. (2013), it was observed that, in studies related to parental perceptions of oral hygiene care and self-care, parents' behavior had a more significant specific impact on the child's oral health than their knowledge about oral hygiene caries [22].

In addition, changes in society bringing the child at a younger age as an active being in their health has changed parental relationships. In research carried out with 426 children, Nunes and Perosa [4] observed that, when taking a position on what controlled their health, primarily parents reported that it was their actions, and, when evaluating their role in the health of the child, parents believed that, in the first place, the actions of others, including the child himself, had more significant interference in the child's health/disease process. This attribution to the child in early childhood of responsibility for their health care can generate a substantial absence of parents regarding their role as caregivers.

Another aspect with a significant association with caries in deciduous molars was the need for treatment. This relationship may be associated with a probable high number of decayed molars in the population studied. According to the literature, no consensus exists regarding the tooth most frequently decayed early in children of the age group studied. The lower right second deciduous molar (85) was the tooth most affected by early loss





due to caries in studies carried out by Batista [23] and Cardoso et al. [24]. However, for Menezes and Uliana [25], the lower left first deciduous molar (74) showed a higher loss percentage. They found a higher loss frequency of the upper left second deciduous molar (65) [25]. The present study found similar results to the research carried out by Bezerra and Nogueira [26], in which the lower left second deciduous molar (75) was the tooth most affected by early loss (17.1%).

In contrast to some studies on caries related to malocclusion, where there are strong associations between these two variables, in this research, malocclusion was not strongly associated with deciduous molar caries [27].

Understanding the pattern of involvement of oral diseases in 5-year-old children in Fortaleza allowed the identification of differences in the distribution of the disease and its prevalence in territories with more vulnerable characteristics. Notably, the National Oral Health Policy (PNSB) in 2004, known as Smiling Brazil [6], recommends organizing oral health care for children aged 0 to 5 years in the system, at most from 6 months. However, it is observed that in Brazil, despite the decline in the level of dental caries in the population with the fulfillment of some goals for oral health, the age of 5 years was one of the unachieved goals evidenced by the latest national surveys, with the even in the city of Fortaleza [5-7].

Among the limitations of the present study, its transversal characteristic is highlighted, as it is not possible to establish cause-and-effect relationships between the associated factors. Among the strengths, the representative sample of this research stands out.

#### Conclusion

The prevalence of dental caries in 5-year-old children in Fortaleza is low, although with a tendency to increase. There was a significant association between decayed deciduous molars and place of residence and race. Of the children with decayed deciduous molars, the highest percentages were found in CORES VI and I. The brown color was the one that showed a significant association. There was a significant association between children with normal occlusion and caries in the deciduous second molars and between the need for treatment and gingival bleeding and decayed deciduous molars. Future studies are suggested to clarify the possible influence of socioeconomic factors associated with oral health problems in preschoolers, considering the relevant social inequalities between the Regional Health Coordinations and the health processes involved.

## **Authors' Contributions**

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KGHS	<b>D</b>	https://orcid.org/0000-0002-9449-5141	Conceptualization, Methodology, Investigation, Data Curation and Writing - Original Draft.				
JRSA	(D)	https://orcid.org/0000-0002-6736-3936	Conceptualization, Methodology, Data Curation, Writing - Original Draft and Supervision.				
MELA	(D)	https://orcid.org/0000-0001-8389-7391	Conceptualization, Investigation, Data Curation and Writing - Original Draft.				
IAM	<b>(</b>	https://orcid.org/0000-0001-8613-9326	Methodology, Software, Formal Analysis, Investigation and Writing - Review and Editing.				
PGBS	(D)	https://orcid.org/0000-0002-1513-9027	Methodology, Software, Formal Analysis, Data Curation, Writing - Review and Editing and				
Supervision.							
All authors declare that they contributed to a critical review of intellectual content and approved the final version to be published.							

# **Financial Support**

None.

### **Conflict of Interest**

The authors declare no conflicts of interest.

## **Data Availability**

The data used to support the findings of this study can be made available upon request to the corresponding author.





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