Influence of different ethnicities on early childhood caries in preschool children at risk of social exclusion in Southeastern Spain

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Abstract

Background Oral diseases are a major global public health problem, with negative effects at both individual and collective levels, and there is a relationship between socioeconomic characteristics such as income, occupation and educational level, with the prevalence and severity of oral diseases.
Aim To estimate the prevalence of early childhood caries and to study a possible influence on different ethnicities in preschool children aged 3 to 5 with social exclusion risk.
Methods A cross sectional study was conducted in a sample of 288 preschool children belonging to three schools in the Southeastern Spain among other variables dmft and ICDASS index.
Results The whole sample presents a mean of 1.92 primary teeth with initial white spot enamel lesions (code 2 ICDAS), 1.54 primary teeth code 5 ICDAS and 1.44 primary teeth Code 6 ICDAS. The mean number of filled primary teeth is 0.15, restoration index of 2.1%. The dmft mean value is 3.81 (3.31-4.31 95% confidence interval). There are significant differences in dmft index between the ethnic groups (p-value=0.009). When analysing the prevalence of cavities (ICDAS index 4-6>0), there are significant differences in the prevalences (p-value<0.001) according to ethnicity, with Latin Americans having the lowest value (41.8%) and the highest value for Gypsies (88.9%). The odds of cavitated caries were 4.06 times higher in Spanish Roma than in Caucasians.
Conclusion The ethnicity of school children at risk of social exclusion is a determining factor in the increased prevalence of cavities in primary caries.

KEYWORDS Dental caries, caries prevalence, ICDAS, social exclusion.

Introduction

Oral diseases are a major global public health problem, with negative effects at both individual and collective levels, and there is a relationship between socioeconomic characteristics such as income, occupation and educational level, with the prevalence and severity of oral diseases [Paglia, 2019; Peres et al., 2019]. They affect an estimated 3.5 billion people worldwide, and are the most prevalent of the non-communicable diseases. Dental caries being the most prevalent chronic disease worldwide, with an estimated 2.3 billion people suffering from caries in their permanent dentition [Bernabe et al., 2020]. The prevalence and incidence of untreated caries vary between countries and regions within these countries, with the highest levels of caries concentrated in socially disadvantaged populations [Kassebaum et al., 2017] where the highest peaks of caries in untreated deciduous teeth are between 3-9 years of age [James et al. 2018]. Three quarters of this population live in low-income countries [Eaton et al., 2023]. Early childhood caries is defined as the presence of carious lesions on one or more surfaces of primary teeth in children aged six years or younger, and is a serious problem worldwide, currently affecting more than 600 million infants [Pitts et al., 2019]. It causes alterations in child development as important functions such as chewing, sleeping and learning are modified. Other consequences include pain, abscesses and lesions in the permanent teeth, diminishing the child's quality of life [De Grauwe et al., 2004]. Education plays an important role, as more educated people are less likely to experience poverty and social exclusion. Social exclusion is defined as "a process of loss of people integration or participation in society and in different political, economic and social spheres" [Ministerio de Derechos sociales y Agenda 2030, 2014]. The National Action Plan claims that poverty is a variable that is capable of affecting, to a lesser or greater extent, social exclusion; however, not all poor people are socially excluded and vice versa [Prada, 2020]. Population groups at risk of social exclusion have less access to services, more stress and consequently, general health is also impaired due to inadequate diet, smoking and drugs [Ministerio de Derechos sociales y Agenda 2030, 2014]. Simultaneously, oral health is compromised, as access to fluoride products, pit and fissure sealants and other preventive measures is diminished due to lack of knowledge and scarce resources [Prada, 2020]. Children at risk of social exclusion or children of immigrant origin and /or ethnic minorities are more susceptible to oral diseases, including caries, as a result of poor oral hygiene, lack of education and knowledge in families and problems at home that push oral health into the background [Mock-Muñoz De Luna et al., 2019]. The latest oral health survey in Spain [2020] has revealed a high rate of dental caries among children (5-6 years old)[Bravo Pérez et al., 2020a]. In this sense, the family environment is the basis for educating children about health and risk factors, demonstrating that there is a decrease in the caries rate in families where educational interventions have been carried out. Similarly, teachers

are of vital importance for the establishment of healthy routine practices in pupils. The prevalence of early childhood caries has been found to be increased among populations with low educational and economic status [Bravo Pérez et al., 2020a]. The Global Oral Health Action Plan (2023-2030) has recently been launched and aims to guide Member States to develop ambitious national responses to promote oral health, reduce oral diseases, other oral conditions and oral health inequalities, strengthen efforts to address oral diseases and conditions as part of the UHC, and consider the development of national and subnational targets and indicators to prioritise efforts and assess progress by 2030 and consider developing national and sub-national targets and indicators to prioritise efforts and assess progress by 2030 [Eaton et al., 2023]. The aim of the study was to determine the levels of caries in a group of schoolchildren (3-5 years old) at risk of social exclusion and the possible ethnic influence in a population of southeastern Spain.

Material and methods

Study design and population

The study has been carried out between January-May 2021 and has been approved by the Research Ethics Committee of the University of Murcia (I.D 3247/2021) and respecting the ethical principles of the Declaration of Helsinki and complying with current regulations regarding data protection. An observational, cross-sectional epidemiological study of pre-school children aged 3-5 years was conducted following the recommendations published in "The Brussels Statement on the Future Needs for Caries Epidemiology and Surveillance in Europe", for the conduct of oral health surveys [Pitts et al., 2018] and Oral Health Surveys (WHO) [World Health Organization, 2013]. The population is at risk of social exclusion and located in three early childhood education schools in a city in southeastern Spain with approximately 100,000 inhabitants (Lorca, Spain). The predominant ethnicity at the three schools were Spanish Roman, Latin American and Muslim. Oral examinations were carried out on pupils in the first, second and third year of infant school in the three schools. The examinations were carried out by two qualified dental observers in the schools themselves and were trained for data collection under the same lighting conditions and location. Cohen's Kappa index was calculated to determine the degree of inter- and intra-observer agreement. The Intra-observer Kappa was 0.91 for the first examiner and 0.93 for the second, while the Inter-observer Kappa was 0.91. Disposable mirrors sealed in individual bags of Hager Werken® number 5, WHO-type periodontal probe and gauze were used to remove possible food debris. The presence of caries has been recorded following the international system for detection and diagnosis of dental caries used is ICDAS II (International Caries Detection and Assessment System) [Gugnani et al., 2011; Pitts et al., 2013; Dikmen, 2015]. In order to compare the results obtained using the ICDAS II criteria [Gugnani et al., 2011] with studies where the index used was that of the WHO, the cut-off point has been set between level 3(d) and level 4(e) [Pitts, 2004]. Due to the difficulty of achieving optimal tooth surface dryness, the epidemiological variant of the ICDAS criteria, which approves the unification of the digits a/0 and b/1, was used [Pitts et al., 2013].

Study Size

The sample size was calculated by applying a margin of error of 10%, with a confidence level of 95% and an expected response rate of 50% of participants [Pita, 1996]. It has been considered that 10% per cohort of the Spanish child population is immigrant. Taking into account the school population of Lorca aged 3-5 years (2050 children), 10% would be included in our study.

Statistical analysis

The mean dft index and caries prevalence were estimated with a c component obtained from the E, F and G codes of the ICDAS II system. Bivariate statistics were performed for comparison of means with Student's t-test and Anova with post hoc tests (DMS) and for comparison of proportions with the chi-squared test. Multivariate statistics were performed using binary logistic regression with the Wald forward stepwise method to obtain a significant predictive model. The predictive ability of the model was measured using the ROC curve and the area under the curve. The significance level was set at a p-value <0.05.

Results

The final sample consisted of 288 pre-school children with an

Variables Sociodemographic		First Year Infants n=75	Second Year Infants n=113	Third Year Infants n=100	Total n=288
Age in years Mean (SD)		3.72 (0.28)	4.45 (0.53)	5.71 (0.33)	4,70 (0.05)
Sex n (%)	Male	n= 34 (45.3%)	n=52 (46.0%)	n=44 (44.0%)	n=130 (45.1%)
	Female	n=41 (54.7%)	n=61 (54.0%)	n=56 (56.0%)	n=158 (54.9%)
Ethnicity n (%)	Caucasian	n=3 (4.0%)	n=7 (6.2%)	n=8 (8.0%)	n=3 (6.3%)
	Muslim	n=48 (64.0%)	n=72 (63.7%)	n=60 (60.0%)	n=180 (62.5%)
	Spanish Roman	n=9 (12.0%)	n=7 (6.2%)	n=11 (11.0%)	n=27 (9.4%)
	Latin American	n=14 (18.7%)	n=24 (21.2%)	n=17 (17.0%)	n=55 (19.1%)
	Black	n=1 (1.3%)	n=3 (2.7%)	n=4 (4.0%)	n=8 (2.8%)

TABLE 1 Descriptive of the sample by grade and total (n=288) for age, sex and ethnicity.

ICDASII codes Temporary Tooth half (CI-95%)	First Year Infants n=75	Second Year Infants n=113	Third Year Infants n=100	Total N=288
ICDAS 2-C	2.13 (1.47-2.79)	2.15 (1.59-2.70)	1.50 (0.97-2.03)	1.92 (1.58-2.25)
ICDAS 3-D	1.75 (1.13-2.36)	0.55 (0.26-0.84)	1.69 (1.24-2.14)	1.26 (1.0-1.51)
ICDAS 4-E	0.41 (0.16-0.67)	0.74 (0.37-1.11)	0.83 (0.49-1.17)	0.69 (0.49-0.88)
ICDAS 5-F	1.87 (1.14-2.59)	1.50 (1.08-1.93)	1.34 (0.93-1.75)	1.54 (1.26-1.83)
ICDAS 6-G	1.45 (0.65-2.26)	0.95 (0.53-1.37)	1.98 (1.27-2.69)	1.44 (1.08-1.80)
Filled teeht	0.21 (0-0.55)	0.04 (0-0.09)	0.22 (0-0.46)	0.15 (0.0-0.27)
Total dft C-G	7.83 (6.57-9.09)	5.93 (5.03-6.83)	7.56 (6.63-8.49)	6.99 (6.41-7.57)

TABLE 2 ICDAS II caries codes, filled teeth and dft index C-G in primary dentition by year and total.

average age of 4.7 years with a range between 3 and 6 years, of whom 45.1% were male and 54.9% were female. The sample is divided by year group, with 75 children in the first year of kindergarten, 113 in the second year and 100 in the third year. The most represented ethnic group was Muslim with 180, followed by Latin American with 55 and Spanish Roman with 27. Regarding carious involvement according to ICDAS II codes (Table 2), we found that the entire sample presented an average of 1.92 primary teeth with initial lesions of white-stained enamel, corresponding to initial caries (code C), a total of 1.95 teeth with moderate caries, corresponding to code D and E. And an average of 2.98 teeth with severe caries (code F and G) were detected. The mean number of filled teeth was 0.15 deciduous teeth, which implies a restoration rate of 2.1%. The highest caries rates (7.83 teeth) have been described for children aged 3 years. Table 3 presents the dft index by grade and total according to gender and ethnicity. The dft index was calculated considering codes E, F and G as the matched component, so that the dft index was estimated at 3.81 with a 95% confidence interval between 3.31 and 4.31. No significant differences were found for gender in any grade or for the whole sample (p-value=0.493). On the other hand, there were significant differences in the dft indexE-G between ethnicities (p-value=0.009), with a statistically significant difference (p<0.005) between Latin American with a dft indexE-G of 2.23 (1.35-3.13) and Muslim with a dft indexE-G of 4.07 (3.4-3.73) and Spanish Roma with a dft indexE-G of 5.7 (3.95-7.46). On analysing the prevalence of cavitated caries (dft indexE-G >0), no significant differences were found in terms of sex (p-value=0.085), although the prevalence was higher in females with 68.9% compared to males with 59.2%. Regarding ethnicity, we found significant differences in prevalence (p-value<0.001), with Latin Americans having the lowest value (41.8%), Muslims with 67.8%, similar to Caucasians with 72.2% and the highest value corresponding to Spanish Roma with 88.9% (table 4). In a multivariate binary logistic regression analysis using the Wald forward stepwise method (omnibus test of the model p<0.001) with caries prevalence as the dependent variable and adjusted for age, Spanish Roma (p-value=0.027) and Latin American (p-value=0.001) ethnicities were found to be significant in the model. Taking Caucasian ethnicity as a reference, the Spanish Roma ethnicity showed an OR=4.06, indicating that the odds of cavities being cavitated are 4.06 times higher in Spanish Roma than in Caucasian ethnicity. In contrast, the Latin American ethnicity has an OR=0.37, indicating that the odds of cavities being cavitated are 2.7 times lower (1/0.37) in the Latin American ethnicity than in the Caucasian ethnicity (Table 5).

Discussion

The study relates the prevalence of early childhood caries in

Index cod E-G Mean (95%CI)		First Year Infants n=75	Second Year Infants n=113	Third Year Infants n=100	Total n=288
Sex	Male	3.97 (2.29-5.64)	3.27 (2.02-4.52)	4,91 (3.49-6.33)	4.01 (3.20-4.82)
	Female	3.93 (2.38-5.47)	3,20 (2.25-4.15)	3,95 (2.98-4.91)	3.65 (3.02-4.28)
	Student's t-test p-value	p=0.969	p=0.925	p=0.263	p=0.493
Ethnicity	Caucasian[1]	4.33 (2.89-5.76)	2,42 (0-6.12)	4,5 (0.98-8.01)	3.67 (1.80-5.53)
	Muslim[2]	4.19 (2.67-5.71)	3,50 (2.51-4.48)	4,65 (3.55-5.75)	4.07 (3.40-3.73)
	Spanish Roma[3]	6,22 (3.25-9.19)	7,14 (2.99-11.3)	4,36 (1.12-7.60)	5.70 (3.95-7.46)
	Latin American[4]	1.86 (0-4.01)	1.91 (0,68-3,14)	3.0 (1.21-4.79)	2.23 (1.35-3.13)
	Black[5]	Cte	Cte	5.75 (3.03-8.47)	2.88 (0.14-5.61)
	ANOVA test p-value	p=0.246	p=0.019* 1 vs 3;2 vs 3; 3 vs 4 and 3 vs 5	p=0.646	p=.,009* 2 vs 4 and 3 vs 4
Total		3.95 (2.84-5.06)	3,23 (2.47-3.99)	4.37 (3.55-5.19)	3.81 (3.31-4.31)

TABLE 3 dft indexE-G by grade and total as a function of gender and ethnicity. *Significant differences between means with p-value <0.05.

Caries prevalence (codE-G >0) % (CI-95%)		First Year Infants n=75	Second Year Infants n=113	Third Year Infants n=100	Total n=288
Sex	Male	56.3% (39.3%-71.8%	53,9% (40.5%-66.6%)	70,5% (55.8%-81.4%)	59,2% (50.6%-67.3%)
	Female	68.3% (53.0%-80.4%)	60,6% (48.1%-71.9%)	78,6% (66.2%-87.3%)	68,9% (61.4%-75.7%)
	Chi Test 2 p-value	p=0,174	p=0,465	p=0,352	p=0,085
Ethnicity	Caucasian[1]	100% (43.9%-100%)	42.9% (15.8-74.9%)	87.5% (52.9%-97.8%)	72,2% (49.1%-87.5%)
	Muslim[2]	62,5% (48.4%-74,8%)	61,1% (49,6%-71,5%)	80% (68.2%-88.2%)	67,8% (60.6%-74.2%)
	Spanish Roma[3]	100% (70,1%-100%)	100% (64.6%-100%)	72,7% (43.4%-90.3%)	88,9% (71.9%-96.2%)
	Latin American[4]	28,6% (11.7%-54.7%)	45,8% (27.9%-64,9%)	47,1% (26.2%-69.1%)	41,8% (29.75-54.9%)
	Black[5]	0% (0%-79.4%)	0% (0%-56.2%)	100% (51.0%-100%)	50% (21.5%-78.5%)
	Chi Test 2 p-value	p=0,004*	p=0,021*	p=0,042*	p<0,001*
Total		61,3% (50.0%-71.5%)	57,5% (48.3%-66,2%)	75,0% (65.7%-82.4%)	64,6% (58.9%-69.9%)

TABLE 4 Caries prevalence (dftE-G >0) by grade and total according to sex and ethnicity. *Significant differences between proportions with p-value <0.05.

Model variables	Coefficient B	p-value	Odds Ratio (CI-95%)
Age	0.305	0.035	1.35 1.02-1.80
Spanish Roma ethnicity	1.402	0.027	4,06 1.17-14.1
Latin American ethnicity	-1.004	0.001	0.37 0.20-0.68
Constant	-0.706	0.308	

TABLE 5 Logistic regression model with prevalence of cavitated caries in primary dentition (dftE-G >0) as dependent variable and significant predictor variables.

pre- schoolchildren aged 3-5 years in populations at risk of social exclusion and different ethnicities. Nevertheless, the main limitation of the study is that it is a cross-sectional survey, it does not allow a temporal relationship between exposure and outcome to be established, so these types of studies are more prone to bias. Our study population meets the characteristics of a low socio-educational level, based on the health situation analysis [Fukai et al., 2017] which indicates a population with a low cultural level, with high school dropout and absenteeism rates. It is a population dependent on social assistance and with parents with a high rate of unemployment or precarious employment. They are families with financial difficulties to meet basic needs. Few studies [Clark et al., 2019] that use ICDAS II as caries recording indices. In Spain, only the study by Almerich et al. [2020], has used the same methodology [Almerich-Torres et al. 2020]. In order to compare the results with other studies using WHO criteria, ICDAS 3 -6/C-G has been considered as the equivalent lesions [Iranzo-Cortés et al., 2013]. In addition, the indications of the Brussels Statement on the Future Needs for Caries Epidemiology and Surveillance in Europe for epidemiological studies in children have been followed, following the ICDAS diagnostic criteria [Pitts et al., 2018]. Dental caries has been considered an indicator of social exclusion [Costacurta et al., 2020]. Oral diseases such as caries and periodontal disease affect an individual's quality of life [Martins et al., 2018]. In this sense, social determinants influence people's overall health. Health and disease can be defined as the biological expression of interactions that take place at the political, economic and social levels [Costacurta et al., 2020]. The results of our study showed a dft value (caries corresponding to E-G codes) so high. No significant differences were found by sex, but there were significant differences in terms of ethnicity. Children of Roma ethnicity have a dft index (5.7) much higher than children of Latino ethnicity (2.23) or the rest of ethnicity, including Muslims. The caries prevalence (dft>0) of children of Muslim ethnicity (67.8%) is lower than those of spanish roman ethnicity (88.9%). In that sense, there are several studies carried out in Spain. One study carried out in the Western Sahara Region (Southern Algeria), found much lower caries rates in 6-7 year old children (47%) [Almerich-Silla et al., 2008] values well below those shown in our study for children of the same ethnicity [67.8%]. However a study recently published in Seville [García-Barata et al., 2023] in Roman children showed dft values of 3.3 in 5-year-old children, values slightly lower to those found in the spanish Roma group in our study (4.36). In Bilbao, another Spanish region in the north of the country, a similar study was carried out and found 70.6% of roman children with dental caries compared to 42.6% of immigrant children of similar age [Rodriguez-Alvarez et al., 2022]. The study by Cubero et al. [2019] carried out in a Spanish population of Muslim and Roman immigrants indicated caries values at 3 years of age of 46.2% compared to 61.3% in our study. However, at 5-6 years of age the values they published (77.4%) were slightly higher than those obtained in our study (75.0%). In 2006, a study was published in Spain [Paredes Gallardo et al., 2006] in an immigrant population aged 6-14 years where the caries values in the deciduous dentition were 47.23% in the immigrant population. These values indicate the incidence of childhood dental caries at discharge. However, in adolescents aged 12 and 15 years caries is decreasing considerably. It has been observed in the values of DMFT at 12 years of age,

decreasing from 4.2 since the first survey carried out in 1984, to a value below 1.0 at present [Bravo Pérez et al., 2020a], a target proposed by the Spanish Society of Epidemiology and Oral Public Health for 2015-20 [Bravo et al., 2009]. This situation is reflected by the lack of incorporation of children under 6 years of age into public oral health programs, which is where the decreases in caries rates are beginning to be observed [Cortés-Martinicorena et al., 2022]. Although immigrant populations suffer higher caries values at 12 years of age (DMFT 2.4) compared to the national population (DMFT 1) [Almerich-Silla and Montiel-Company, 2007]. The latest National Oral Survey [Bravo Pérez et al., 2020b] and Regional Survey [Almerich-Torres et al., 2020] put the prevalence of caries among Spanish schoolchildren aged 5-6 years at 28.3% and 37.4%, respectively. These values are much lower than those described by our study for a population at risk of social exclusion. In our study there were no significant differences between sex and caries prevalence, although caries values in females were higher than in males, like the results of the Spanish national survey [Bravo Pérez et al., 2020a]. The results of the logistic regression model showed that the risk of cavities in the Spanish Roma population is four times higher than in the Caucasian population, a value much higher than that described for the American Latin population, where the occurrence of cavities is 2.7 times lower than in the Caucasian population. Studies carried out in India in populations with economic low level, determined a prevalence of caries in children aged 3 to 6 years close to 80% [Jain et al. 2016]. In Egypt, 74% of children have caries in the deciduous dentition [Abbass et al., 2019]. In contrast, in Tanzania, a recently published study found a prevalence of early childhood caries of 30.2%-44.8% in school children aged 3-6 years [Ndekero et al., 2021]. Similar values (31.0%) to those detected in New Zealand with Maori ethnicity [Clark et al., 2019]. However, there are countries such as Japan where sugar consumption is very low, with caries levels in the 3-year-old population of 14.7 % [Kato et al., 2017]. This situation where developing countries have low levels of caries may explain why sugar consumption is the main cause of caries aetiology. In cultures where sugar consumption is a daily habit, caries disease is more prevalent. The rate of fillings in primary teeth has a minimum value in the schoolchildren in this study, being 0.21 in the first year, 0.22 in the second year and 0.04 in the third year. The average number of restorations is very small in relation to the large number of caries lesions present, indicating that access to a dental service is low, either due to lack of knowledge or disinterest, a possible consequence of the high rate of social exclusion of the population studied. The results are similar to the Oral Health Survey 2020, carried out by the General Council of Official Associations of Dentists and Stomatologists of Spain, which states that the greatest needs reside in children with a low socio-economic level, in which statistically significant differences can be seen with respect to children with a high level [Bravo Pérez et al., 2020b]. In Spain, the use rate of dental services by the population is low [Consejo General de Dentistas, 2023]. This is basically due to the fact that children's oral health care is not covered free of charge in Spain until the age of 6 years. Recently, new oral health services have been introduced into the Spanish Health System, so that treatment for children aged 1-5 years will be included in the portfolio of services [Ministerio de Sanidad Consejo Interterritorial del Sistema Nacional de Salud, 2022]. In families at risk of social exclusion or lower economic and educational levels, it is difficult to achieve the implementation of oral habits, such as toothbrushing and sugar reduction, thus increasing dental caries disease [Warren et al., 2016]. This can be achieved through educational programs in family and school settings [Kato et al., 2017; Ghaffari et al., 2018], targeting low-income families in particular. An increase in dental caries has been reported in Spanish schoolchildren with poor oral hygiene and cariogenic dietary intake [Obregón-Rodríguez et al., 2019]. In our study population, one of the objectives was to be introduced in the school supervised toothbrushing programs, which will ensure a minimum daily brushing, following the WHO published a document entitled "Oral Health Promotion: An essential element of a health promoting school" [Petersen, 2003] to encourage health promotion in schools and establishing healthy hygienic habits. In this sense, toothbrushing programs at school [Sekhar et al., 2014] can lead to the acquisition of skills in the child that are not performed at home specially in vulnerable target groups [Dickson-Swift et al., 2017; Natapov et al., 2019]. The 2019 Bangkok Declaration [Pitts et al., 2019], proposed a series of actions to reduce the prevalence of early childhood caries globally, focusing on the importance of raising awareness among families, caregivers, dentists, nurses and other health professionals. It proposes brushing at least twice a day with fluoride toothpaste, as well as limiting the consumption of sugars in children under two years of age and actions should be taken on healthy eating guidelines in schools [Calderón Larrañaga et al., 2019]. In Spain, there is a need for involvement of national governments, which understand the need for such interventions within national health programs, as is the case in other European countries [Damle et al., 2014].

Conclusions

The prevalence of caries in the school population aged 3-5 years at risk of social exclusion is very high, which makes it necessary to implement action programs in the family and school environment to reduce the levels of disease in vulnerable populations. The ethnicity of school children at risk of social exclusion is a determining factor in the increased prevalence of cavities in primary caries. The proportion of Spanish Roma children caries is higher than the rest of ethnicities in Spain.

Author Contributions

Conceptualisation, Y.M.B. and J.M.M.C; Data curation, J.M.M.C; Formal analysis, A.J.O.R and J.M.M.C;. Investigation, I.N.V and A.V.H.; Methodology, Y.M.B; C.S.M. and J.M.M.C.; Supervision, Y.M.V; J.M.M.C and A.J.O.R.; Validation, C.S.M. and Y.MB.; Visualisation, Y.M.B, A.V.; Writing-original draft, Y.M.B and J.M.M.C.; Writing-review & editing, A.J.O; A.V; C.S.M. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors have declared no conflicts of interest.

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