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Black stains and dental caries in Brazilian schoolchildren: a cross-sectional study

Tania Mercedes LÓPEZ MARTÍNEZ[a]
Marília Leão GOETTEMS[b]
Marina Sousa AZEVEDO[b]
Marcos Britto CORREA[b]
Flávio Fernando DEMARCO[b]
Ana Regina ROMANO[b]

[a]National Autonomos University of Nicaragua, School of Dentistry, Managua, Nicaragua.

[b]Universidade Federal de Pelotas - UFPEL, Post-Graduate Program in Dentistry, Pelotas, RS, Brazil.

Abstract: The aim of this study was to assess the prevalence of black stains (BS) and factors associated with their occurrence and to investigate the association of BS with caries experience after adjusting for other risk indicators. A school-based, cross-sectional study using multistage sampling of children aged 10–12 years from 20 private and public schools in Pelotas/Southern Brazil was conducted (n = 706). Children were evaluated for BS and dental caries (DMFT/dmft index) and socioeconomic information was collected by questionnaire. Univariate and multivariate Poisson regressions were used to assess variables associated with the prevalence of BS and with caries experience. The prevalence of BS was 5.81%. The mean DMFT/dmft was 1.54 (±1.91), with a maximum value of 10. Among the children with BS, mean dmft/DMFT was 1.22 (SD 1.68), with a maximum value of 6. After adjustments for demographics and socioeconomic variables, dental plaque and tooth brushing habits, prevalence of BS was associated with lower caries experience (RR 0.67; 95%CI 0.49–0.92). In conclusion, the presence of BS showed an association with lower dental caries experience in the evaluated population. Future studies should investigate the mechanisms behind this association.

Keywords: Epidemiology; Oral Health, Dental Caries; Cross-Sectional Studies; Child.

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Corresponding Author:
Ana Regina Romano
E-mail: romano.ana@uol.com.br

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Introduction

A marked decrease in the prevalence of dental caries has been reported worldwide[1,2], but it continues to be a major oral health problem. According to recent data, dental caries especially affects individuals in underdeveloped and developing countries, with higher prevalence in deprived populations[2].

Despite the significant improvement in oral health indicators in Brazil over the last few decades, dental caries is still a significant public health problem[3]. Recent data from a National Survey (SB 2010) demonstrated that at 12 years of age, the mean decayed, missing and filled teeth (DMFT) was 2.1 and 56% of the children presented dental caries[4]. Another important aspect in relation to the prevalence of dental caries in Brazil is the polarization of the disease, with 60% of the carious teeth found in only 20% of the 12-year-old school children[5]. The multifactorial character of caries etiology (behavioral, environmental, biological and socioeconomic) is well-established in the

literature, with the socioeconomic aspect playing a pivotal role in caries prevalence[6]. Children from low-income families[7], from parents with low schooling, with lower access to dental care and fluoride sources have an increased risk of presenting with the disease[8].

Some studies have shown that the presence of black stains (BS) could be associated with a lower prevalence of dental caries in children[9,10,11,12], possibly due to differences in microbial composition in individuals with BS[13]. A population study in a birth cohort showed that when the researchers controlled for potential confounding and mediator factors, the presence of BS could have a protective effect for the occurrence of dental caries in primary dentition (age 5)[14]. In contrast, other studies have not revealed such protective effects[15,16]. It is important to point out that these studies have not controlled for potential risk indicators that could be associated with either black stains or dental caries.

The stains are present as dots or small areas of dark coloration that can also form a line that follows the contour of the marginal gingiva. The stains may also appear in a diffuse form, covering the tooth crown. Retentive tooth areas, such as grooves, pits, and fissures can be impregnated with this type of pigmentation, being difficult to be removed in these areas[10].

There is a lack of population-based studies evaluating the prevalence of BS, but data from non-population-based investigations show a prevalence rate varying from 2.5% to 19%, depending on the region where the study was conducted, the population surveyed, or the individuals' dietary habits[9,10,17]. Until now, nostudy has investigated if BS could also have a protective effect in older children, in mixed or permanent dentition, when controlling the analysis for confounders. Therefore, the aim of this study was to investigate the prevalence of BS and factors associated with its occurrence and to assess the association of BS with caries experience, after adjusting for demographic, socioeconomic and behavioral characteristics. We tested the hypothesis that BS is associated with lower levels of dental caries after adjusting for potential confounding variables.

Methodology

Design and sampling procedures

This cross-sectional study was part of a school-based epidemiological study, which investigated different general health outcomes (obesity, physical activity) and oral health outcomes (caries, gingivitis, malocclusion, erosion, enamel defects and the prevalence of black stains), in the city of Pelotas, in 2010[18]. The study was approved by the Human Research Ethics of Federal University of Pelotas, and by the Education Department.

A two-stage sampling technique was used for subject recruitment. In the first stage, the primary units (schools) were randomly selected, considering the number of children enrolled in each school and the proportionality of public and private schools existing in the municipality. A total of 15 public and five private schools were selected. In the second stage selection in each school, three 4th to 6th grade classes were randomly selected and all children enrolled in these classes were initially eligible for the study and were invited to participate. Parents received a letter through their children explaining study aims and confidentiality and requesting parental consent for their child's participation. The study excluded children with physical and/or mental disabilities.

The survey was carried out in children aged 8–12 years old (n = 1,211), but for the potential preventive effect of black stains on dental caries in permanent dentition, children aged 10–12 were selected, because there is a higher prevalence of caries in older kids and they have a higher number of permanent teeth. The sample size required for this study, considering a prevalence of dental black stains of 6%[10], acceptable error of three percentage points, confidence level of 95%, an increase of 20% for losses and refusals and the sampling design effect estimated at 2.0, was 579 children. The minimum sample size of this study was large, because it also assessed other outcomes. The calculations were made with Epi Info 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Data collection

Data was collected using the parents' questionnaire responses, interviews, and oral clinical examinations with the children. The research team visited schools a number of times between September and November 2010 in order to ensure a sample abstention of less than 10%.

A pre-tested questionnaire was sent to the parents through the children and included socio-economics questions (maternal schooling and familial income). The interview conducted with the children contained demographic information (sex and age) and questions about oral hygiene habits.

The oral clinical examination included assessment of dental plaque, BS and dental caries in this order. After the dental plaque evaluation, teeth were cleaned with dental gauze. Examination was performed in school chairs using individual artificial lights, dental mirrors and CPI probes[19]. The criteria to assess BS presence was based on the study of Koch et al.[10], this criteria were also used in another study of *our group*[14]. *Presence of black stains on teeth was defined as firmly adherent black* dots, forming liner discoloration parallel to the gingival margin and/or covering up to one third or more of the clinical crown and the BS presence or absence was registered accordingly. At least two neighboring teeth, primary and/or permanent, should have been affected to consider the presence of black stains.

For the visible plaque index[20], the first molars, central and lateral incisors of diagonal quadrants were examined, totalizing 6 teeth evaluated. Four sites in each tooth (mesiobuccal, midbuccal, distobuccal, and lingual) were assessed. The diagonal quadrants selected for examination were randomly chosen for each selected school[20]. Dental caries in primary and permanent teeth was assessed using the dmft/DMFT index[19]. To assess dental caries experience in both dentitions, DMFT and dmft were added into one variable.

Both interviewers and examiners received prior theoretical and practical training. All the 6 examiners were Master or PhD students from Graduate Programs in Dentistry. The calibration process for dental black stains diagnosis was performed in lux. The in lux process consists of the projection of images showing different possible variations in the condition, thus testing the examiners in all examination possibilities when the condition assessed had low prevalence or large variability[14]. The process consisted of the projection of 24 images showing different clinical situations. The calibration exercise for dental caries consisted of the examination of 20 children with similar age of the sample. Kappa statistics were used to measure inter-examiner agreement, comparing with a gold-standard examiner. The mean kappa value was 0.70 (range 0.64–1.00) for dental black stains and 0.74 (range 0.62–0.79) for dental caries. Visible dental plaque index (VPI) was included in the training, but no analysis of agreement was performed due to the difficulties involved in maintaining the condition unaltered during the series of examinations.

Data analysis

Data were entered in duplicate and independently using EpiData version 3.1 [EpiData Association, Odense, Denmark] and analyzed using Stata 12.0 [Stata Corporation, College Station, TX, USA]. Descriptive analyses were initially performed.

The presence of BS was used as an outcome and as an independent variable. When BS was used as an outcome, dental caries was used as an independent variable; when dental caries was the outcome, BS presence was used as the independent variable.

First, we assessed BS as the dependent variable. To test the association of the studied variables and prevalence of black stains, Chi-square and Fisher's Exact tests were used and Multivariate Poisson regression analysis was conducted, obtaining the prevalence ratios (PR) and their 95% confidence interval (95%CI). In this analysis, dental caries was used as an independent variable and it was dichotomized as absent (dmft/DMFT = 0) or present (dmft/DMFT ≥ 1).

Next, dental caries was assessed as a dependent variable (outcome) and the mean of dmft/DMFT was used. In this analysis, BS was used as an independent variable and it was dichotomized as absent and present (at least two neighboring teeth, primary and/or permanent, affected). Data were tested for homogeneity of variance (Bartlett's test) and one-way ANOVA was used in bivariate analysis. Bonferroni comparison was used to compare the groups when

the independent variable was found to be a significant factor affecting the caries experience. Multivariate models were also performed to assess the association between BS and dental caries experience, adjusting for potential confounders (n = 706 children). Rate ratios (RR) and their 95% CI were obtained for dental caries experience.

In both analyses, the visible plaque index was used as an independent variable. For the statistical analysis, the total sites presenting dental plaque were categorized in tertiles.

Results

A total of 1,744 children were eligible for the oral health survey (aged 8–12 years), of which 418 (24%) did not present the informed consent form signed by parents. Of those children with parental consent to participate in the study, 105 (7.9%) were lost because they were absent from school during data collection. The final response rate was therefore 69.4%, yielding a total sample of 1,211 children. In the present study, 706 children aged 10–12 years were included. The prevalence of dental BS was observed in 41 (5.81%) schoolchildren (Table 1). A higher prevalence was seen in children from public schools (6.80%) and in children who reported brushing their teeth only once a day (13.56%). In the bivariate analysis, there was no significant difference among BS and dental caries (p = 0.381). In the Poisson regression analysis (Table 2), after adjustments, a protective effect was present in occurrence of pigmentation with a higher brushing frequency. Children who reported brushing their teeth twice a day had 82% of protection and children who reported three times or more a day had 62% of protection.

Regarding dental caries, the mean dmft/DMFT was 1.54 (standard deviation 1.91), with a maximum value of 10. Among the children with black stains, mean dmft/DMFT was 1.22 (SD 1.68), with a maximum value of 6. Mean dental caries experience was higher in younger children, those with mixed dentition, those with low maternal schooling and family income, and in those children from public schools. Children with higher plaque index presented higher DMFT/dmft values. The impaired perception about oral health was associated with caries experience (Table 3).

Table 3 shows the results of the association between black stains and dental caries experience. After adjustments for sex, age, family income, mothers' educational level, type of school, frequency of tooth brushing and VPI, the prevalence of black stains was associated with a lower DMFT/dmft (RR 0.67; 95%CI 0.49–0.92).

Discussion

The overall result of the present study was that children with black stains had a lower dental caries experience, when evaluating children aged 10 to 12 years after controlling for potential risk indicators. This finding corroborates the findings of a previous population-based study wherein black stains were associated with lower dental caries occurrence in primary dentition, when adjusting for social, demographic and behavioral factors[12,14]. Other non-populational studies found this association in permanent dentition[9,10]. In contrast, a few other studies failed to detect such association[11,15].

A literature review showed that in most studies, a lower prevalence of caries was present in children with black stains[17]. However, differences were not statistically significant in all of the studies, and this absence of difference can be related to the use of univariate analysis methods. In addition, most of the studies investigating this potential association had not controlled for confounding factors. In addition, considering the low prevalence of black stains in most populations, there is a need to investigate this hypothesis in large samples to confer adequate power to the study. Even though our study was a school-based survey, it can also be considered a population survey in this age, since, according to local authorities, the presence of children in the evaluated age groups in the schools is nearly 100%. Moreover, our study has a large sample size that confers enough power to the study to detect the potential association.

The reasons behind the protective effect of black stains in relation to dental caries occurrence are still unclear. It has been speculated that bacteria that cause dark pigmentation have a low pathogenicity, and these

bacteria compete with the cariogenic microbiota for the location sites, reducing the potential adhesion of these cariogenic bacteria and, as a consequence, decreasing caries development[10]. A recent study suggests that the microbial composition of the plaque might be associated with the protective effect of BS. They demonstrated an equal total number of bacterial counts in BS and non-discolored plaque samples. Therefore, significant differences in individual species of bacteria were detected with a significantly

Table 1. Association between independent variables and dental black stain in schoolchildren between 10 and 12 years old. Bivariate analysis. Pelotas, 2010 (n = 706).

Variable	Dental black stain n (%)		p-value
	No	Yes	
Sex			0.168*
Male	317 (95.48)	15 (4.52)	-
Female	348 (93.05)	26 (6.95)	-
Age			0.202*
10 years	276 (95.17)	14 (4.83)	-
11 years	242 (94.90)	13 (5.10)	-
12 years	147 (91.30)	14 (8.70)	-
Family income			0.137*
> R$ 740	272 (95.44)	13 (4.56)	-
≤ R$740	298 (92.55)	24 (7.45)	-
Mother´s education level			0.0776*
> 8 years	400 (94.12)	25 (5.88)	
≤ 8 years	247 (94.64)	14 (5.36)	-
Type of school			0.008**
Public	548 (93.20)	40 (6.80)	-
Private	117 (99.15)	1 (0.85)	-
Frequency of brushing			0.008*
Once a day	51 (86.44)	8 (13.56)	-
Twice a day	215 (96.85)	7 (3.15)	-
Three times or more a day	389 (94.19)	24 (5.81)	-
Dentition			0.264*
Mixed	385 (95.06)	20 (4.94)	-
Permanent	268 (93.06)	20 (6.94)	-
VPI (sites)			0.804*
1° tertile (0–2)	226 (93.39)	16 (6.61)	-
2° tertile (3–5)	195 (94.63)	11 (5.37)	-
3° tertile (6–20)	245 (94.59)	14 (5.41)	-
Dental Caries (DMFT/dmft)			0.381*
Absent (0)	294 (93.33)	21 (6.67)	-
Present (≥ 1)	371 (94.88)	20 (5.12)	-
Total	665 (94.19)	41 (5.81)	-

*X^2 Test; **Fisher Exact Test; 1 R$ = 0.54 US$ by data collection; Values lower than 706 are due to missing information; VPI: visible dental plaque index.

Table 2. Association of dental black stains and independent variables in schoolchildren between 10–12 years old. Poisson regression analysis. Pelotas, 2010 (n = 706).

Independent variables	PR $_{crude}$ 95%CI	p-value	PR $_{adjusted}$ 95%CI	p-value
Sex				
Male	1		1	
Female	1.54 (0.82–2.90)	0.184	1.93 (0.93–3.99)	0.077
Age				
10 years	1		1	
11 years	1.06 (0.51–2.21)	0.887	1.20 (0.54–2.65)	0.649
12 years	1.80 (0.88–3.69)	0.119	1.24 (0.54–2.84)	0.613
Family income				
> R$ 740	1		1	
≤ R$740	1.63 (0.83–3.21)	0.154	1.16 (0.56–2.41)	0.682
Mother´s education level				
> 8 years	1		1	
≤ 8 years	0.91 (0.47–1.75)	0.782	0.76 (0.37–1.58)	0.473
Type of school				
Public	1		1	
Private	0.12 (0.02–0.91)	0.040	0.17 (0.56–2.41)	0.089
Frequency of brushing				
Once a day	1		1	
Twice a day	0.23 (0.09–0.62)	0.005	0.18 (0.06–0.55)	0.003
Three times or more a day	0.43 (0.20–0.91)	0.038	0.38 (0.17–0.87)	0.021
VPI (sites)				
1° tertile (0–2)	1		1	
2° tertile (3–5)	0.81 (0.38–1.75)	0.594	0.82 (0.35–1.91)	0.645
3° tertile (6–20)	0.82 (0.40–1.68)	0.582	0.82 (0.37–1.81)	0.620

1 R$: 0.54 US$ by data collection; PR: prevalence ratio; CI: confidence interval; VPI: visible dental plaque index.

higher number of *A. naeslundii* and a significantly lower number of Lactobacillus sp. in the BS samples. The number of *S. mutans* tended to be lower in BS samples than in nondiscolored plaque samples and the numbers of *S. sobrinus* were not considerably different between the two[13].

Another hypothesis is related to a probable higher content of total calcium and inorganic phosphates in the group of children with black spots[21], which could reduce the caries occurrence by reducing the dissolution of enamel, allowing the maintenance of a more stable pH, and providing higher buffer capacity of saliva[22]. The regular consumption of iron-rich foods or iron supplements during early childhood could be reasons for favoring chromogenic microbiota development[16]. Lactoferrin, a major iron-binding protein constituent of milk and cheese, has antibacterial activity against *S. mutans*, preventing dental caries development[23]. However, a recent study has concluded that metallic ions do not seem to be the origin of BS[24].

In this sample, the prevalence of dental black stains was 5.8%. In Brazil, the prevalence figures ranged from 3.5% at 5 years[14] to 14.8%[11] in a population aged 6–12 years. Similar results were found in studies in other countries. In Italy, a prevalence of 6.0% was found among children aged 6 to 12 years[10], and in Spain, a prevalence of 7.5% was detected in children aged 4 to 11 years[25]. The only population-based study investigating black stains in primary dentition, in the

Table 3. Association between dental caries experience (DMFT/dmft) and dental black stain in schoolchildren 10–12 years. Bivariate and Poisson regression analysis. Pelotas, 2010 (n = 706).

Independents variables	Mean DMFT/dmft (sd)**	p-value	RR_{crude} 95%CI	p-value	$RR_{adjusted}$ 95%CI	p-value
Dental black stain		0.273				
Absent	1.56 (1.92)		1		1	
Present	1.22 (1.68)		0.78 (0.59–1.04)	0.092	0.67 (0.49–0.92)	0.016
Sex		0.157				
Male	1.64 (1.93)		1		1	
Female	1.44 (1.89)		0.88 (0.78–0.98)	0.030	0.95 (0.83–1.08)	0.435
Age		0.020				
10 years	1.77 (2.05)[a]		1		1	
11 years	1.34 (1.77)[b]		0.76 (0.66–0.87)	< 0.001	0.71 (0.61–0.82)	< 0.001
12 years	1.41 (1.81)[ab]		0.79 (0.68–0.93)	0.004	0.71 (0.60–0.84)	< 0.001
Family income		0.002				
> R$ 740	1.35 (1.82)		1		1	
≤ R$740	1.82 (2.02)		1.41 (1.24–1.61)	< 0.001	1.19 (1.03–1.38)	0.020
Mother´s education		< 0.001				
> 8 years	1.29 (1.84)		1		1	
≤ 8 years	1.83 (1.97)		1.35 (1.19–1.52)	< 0.001	1.10 (0.96–1.27)	0.178
Type of school		< 0.001				
Public	0.93 (1.47)		1		1	
Private	1.66 (1.96)		0.56 (0.46–0.68)	< 0.001	0.58 (0.44–0.75)	< 0.001
Frequency of brushing		0.425				
Once a day	1.85 (2.02)		1		1	
Twice a day	1.52 (1.74)		0.82 (0.66–1.02)	0.079	0.80 (0.63–1.01)	0.062
Three times or more a day	1.50 (1.98)		0.81 (0.66–0.99)	0.046	0.86 (0.68–1.07)	0.173
VPI (sites)		< 0.001				
1° tertile (0–2)	1.17 (1.71)[b]		1		1	
2° tertile (3–5)	1.56 (1.83)[ab]		1.33 (1.13–1.56)	0.001	1.26 (1.06–1.50)	0.007
3° tertile (6–20)	1.86 (2.08)[a]		1.58 (1.37–1.83)	< 0.001	1.39 (1.18–1.63)	< 0.001

*One-way ANOVA; 1 R$= 0.54 US$ by data collection; **Means followed by different letters indicate p < 0.05 (Bonferroni comparison); CI: confidence interval; VPI: visible dental plaque index; sd: standard deviation.

same city that our investigation took place, has also found a low prevalence (3.6%). This variation between studies can be attributed to differences in sample selection when the evaluation is performed at public schools[11] or rural schools[9] or as this study that evaluates public and private school population[10]. Additionally, there may be differences in data collection since information about the training and calibration of examiners is not explained in detail.

The factors associated with the occurrence of black stains were also assessed. When dental plaque was not associated with the outcome, tooth-brushing frequency was the only factor influencing in BS prevalence. Due to the design limitations, it is not possible to confirm if there is a causal relationship between a higher tooth brushing frequency and a lower prevalence of black stains, although it is possible that the presence of stains might influence oral hygiene habits. Morphological studies reported that BS are a special type of dental plaque[26]. Thus, it has also been assumed that the black stains reveal plaque at the tooth surface, resulting in different oral hygiene habits and/or demand for dental care[10].

Other possible factors have been pointed out in the literature, but none have been confirmed in this study. França-Pinto el al.[14] detected a higher frequency of black stains in children from lower socioeconomic backgrounds, and Chen et al.[12] found the association with birthplace, parents' educational level, intake frequency of soy sauce and use of nursing bottle.

The effect of dietary habits was pointed out as a possible cause of black stains, with children with traditional dietary habits (sustainable community structures, limited exchange of goods and food, and limited exposure to a Western lifestyle) being more susceptible to BS[9]. If BS is associated with biofilms composition, the fact that children with BS are exposed to lower cariogenic diet might also be connected with a lower caries occurrence, regardless of the presence of BS.

It is worth mentioning that black stains and dental caries share common co-variables such as socioeconomic and behavioral characteristics[14]. This fact can lead to a positive confusion, *i.e.* an association that was not significant in crude analysis, became statistically associated after adjustments for these co-variables, reinforcing the need to use a statistical approach with multivariable analysis to assess the possible relationship between black stains and dental caries[14].

As mentioned above, one of the strengths of this study is the representative sample, including children from private and public schools, providing different socioeconomic backgrounds. Furthermore, inter-reliability was satisfactory. The selected criteria used to detect BS was based on previous studies, allowing comparisons between the results[9,10]. In addition, this study is only the second one to perform an investigation about the relationship between dental caries and black stains, using a statistical approach that controlled for potential confounders, and it is the first one to work this in permanent/mixed dentition. Besides considering several demographic, social and behavioral factors that may have influenced the presence of BS and its caries protective effect[13], our study also included a dental plaque evaluation in the multivariable analysis. One limitation is the fact that some of the variables possibly associated both with dental caries and black stains, such as dietary habits[9] could not be assessed due to methodological issues, since the study was part of a large multidisciplinary survey. However, the inclusion of variables associated with both conditions in the model could probably reveal a stronger association between BS and dental caries. Another limitation of this study was the cross-sectional design, which impairs the establishment of causality.

Conclusions

In conclusion, in this school-based sample the prevalence of black stains was associated with lower caries experience. It is recommended that further epidemiological studies adopting an appropriate design and statistical methods assess the factors possibly involved in the phenomenon of black stains and its relationship with dental caries.

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