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ORIGINAL RESEARCH

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Black stain and dental caries in primary dentition of preschool children in Qingdao, China

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Abstract

Black stain (BS) and caries are common oral issues in children worldwide. This study aimed to reveal the prevalence of BS and caries in primary dentition of children in Qingdao, China and evaluate the potential association between them. A total of 672 preschool children aged 3–5 years old from 12 kindergartens in Qingdao, northern China were enrolled in the study. The prevalences of BS and caries were counted, and their distributions across different ages and genders were analyzed. The potential protective role of BS (independent variable) on caries (dependent variable) was analyzed *via* binary logistic regression analysis. BS was observed in 103 children (15.33%), including 3 severe- (0.45%), 28 moderate- (4.17%), and 72 mild (10.71%) forms. Caries was observed in 374 children (55.65%) and it was positively associated with age. Notably, the prevalence of caries was lower in children with BS than in those without BS (42.72% *vs.* 58.00%, respectively). Children with BS were less likely to suffer from caries than those without BS. BS in mild or moderate/severe forms was associated with a low risk of caries. In summary, the prevalence of BS in primary dentition in Qingdao, China is at a relatively high level worldwide. BS is a protective factor for caries in primary dentition.

Keywords

Black stain; Dental caries; Primary dentition; Preschool children

1. Introduction

Black stain (BS) is an extrinsic discoloration characterized by a dark pigmented line on the tooth surface [1]. Generally, BS consists of ferric sulfide and is formed by the interaction between bacteria-generated hydrogen sulfide and iron in the saliva and gingival fluids [2]. Some chromogenic bacteria are involved in the initiation of BS, such as *Porphyromonas gingivalis*, *Actinomyces* and *Prevotella melaninogenic* [1, 3]. Furthermore, a high level of iron contributes to the formation of BS. Iron supplements and iron-enriched water or foods are risk factors for BS [4, 5]. Clinically, BS is common in children without gender differences, while its prevalence varies in different regions worldwide. According to uncompleted statistics, the prevalence of BS is approximately 2.4% in Greece, 3.1% in Spain, 3.5% in Brazil, 6.1% in Tunisia, 6.2% in India, 6.17% in Italy and 18.5% in Turkey [6]. The prevalence of BS in China is at a relatively high level compared with that in these countries; the prevalence of BS in primary dentition is 9.9% in Shanghai [7] and 12.1% in Wenzhou [8]. Epidemiological studies performed by our team revealed that the prevalence of BS in preschool children in Qingdao was 12.1% in 2018 [9] and 13.6% in 2019 [10]. Therefore, exploring the potential effects of BS in children is of great clinical significance in China.

Dental caries characterized by the destruction of enamel and dentin is also a prevalent health issue affecting the mouth [11]. The accumulation of lactic acid induced by an imbalance of bacteria in dental plaque biofilm contributes to tooth demineralization and is the leading cause of caries [12]. Clinically, dental caries affects people of all ages and is especially frequent in children and adolescents. The prevalence of caries is age-related among young people [13–15]. A meta-analysis in Eastern Mediterranean showed that the pooled prevalence of caries was 65% (45%–85%) for 5 year olds (primary dentition), 61% (50%–72%) for 12 year olds (permanent dentition), and 70% (64%–75%) for 15 year olds [13]. In China, two cross-sectional studies in Zhejiang (a southern region) and Liaoning (a northern region) revealed that an older age is associated with a higher risk of caries in adolescents [14, 15]. A review based on 26 articles also determined that age as a risk factor for early childhood caries (caries on primary teeth in children under 6 years old). In addition to age, extrinsic factors including poor oral hygiene habits, diets with high sugar or imbalance nutrients, and other unhealthy lifestyles are closely associated with the occurrence and progression of caries [16, 17].

Notably, a potential association exists between the prevalence of BS and caries, which is probably owing to the effects of oral microflora and saliva composition [6, 18, 19]. A meta-analysis based on research worldwide reported that the difference in caries prevalence between children with and without BS was 5.41% in Turkey (5–13-year olds), 7.63% in

Brazil (5 year olds), 12.70% in Shanghai, China (a mean of 4.55-years-old), 17.6% in Tunisia (3–5 year olds), 18.04% in Italy (6–12 year olds), and 18.50% in Germany (3–10 year olds) [6]. These results indicate the possibility that children with BS are less likely to suffer from caries. Meanwhile, a cross-sectional study in India showed that the prevalence of caries in children with BS was greater than those without BS (43.48% *vs.* 40.60%, respectively, 0–3 year olds) [20]. Since the knowledge on the effect of BS on the prevalence of caries in primary dentition is limited in northern China, this study was designed to fill this gap. Here, the prevalences of BS and caries, and the potential link between them were analyzed in preschool children. Our findings may reveal the differences in caries prevalence in primary dentition of children with and without BS in Qingdao, China.

2. Materials and methods

2.1 Study design and participant enrolment

This was a cross-sectional study on primary dentition. Preschool children aged 3–5 years old were collected from Qingdao, China between April 2022 and June 2022 using a multi-stage cluster sampling method. Specifically, two districts (Shinan and Shibei) were randomly selected from six districts in Qingdao. Subsequently, six kindergartens were randomly selected from 32 kindergartens in Shinan district, and six kindergartens were randomly selected from 48 kindergartens in Shibei district. Children in these 12 kindergartens were selected as the study participants. The inclusion criteria included: (1) children aged 3–5-years old; (2) children only with primary dentition; (3) children without systemic diseases. The exclusion criteria included: (1) children absent during the survey; (2) children with mixed dentition; (3) children who were unable to cooperate in oral health examinations. A total of 672 children were finally screened.

2.2 Sample size calculation

The prevalence of caries was used as the primary outcome. Sample size was calculated using the following formula: $N = Z^2 \times (p)(1 - p)/d^2$, where $Z = 1.96$ is the confidence level for an accuracy of 0.05, $p = 59.4\%$ is the prevalence of early childhood caries generated from an epidemiological survey in Qingdao in 2019 [10], and $d = 0.05$ is the allowable error. N was calculated as 371. Considering a 20% dropout rate, a minimum of 445 samples were required.

2.3 Dental examinations

A stomatoscope and CPI (Community Periodontal Index) probe were used to examine the primary dentition of enrolled children under natural light in a sitting position. Three examiners were qualified as dental practitioners and passed the final examination after 2 weeks of intensive training. Among them, two practitioners examined caries and BS, and the other recorded the results. Inter-examiner reliability was evaluated *via* the Cohen's kappa test, and the score was >0.85. Caries was diagnosed by the presence of carious cavity in pit-and-fissure or smooth surface, destruction of enamel, and softening of palpable bottom according to the World Health Organization (WHO) criteria. The decayed, missing and filled teeth (dmft) was calculated as the sum of decayed, missing and filled teeth. BS was diagnosed by the presence of linear discoloration formed by dark dots (diameter <0.5 mm) parallel to the gingival margin in at least two different teeth according to the criteria mentioned by Koch *et al.* [21]. It was further classified according to the criteria mentioned by Gasparetto *et al.* [22]: mild BS, presence of pigmented dots or discontinuous thin lines parallel to the gingival margin; moderate BS, presence of clear and continuous pigmented lines that do not exceed 1/2 of cervical third of the tooth surface; severe BS, presence of clear and continuous pigmented lines that exceed 1/2 of cervical third of the tooth surface. According to the WHO (World Health Organization) oral health survey method, 5% of the participants were randomly selected for duplicate examination.

2.4 Statistical analysis

Statistical analysis was performed using SPSS 23.0 (IBM, Armonk, New York, USA). Data distribution was analyzed by the Kolmogorov-Smirnov test. Categorical variables (BS, caries, age, and gender) were expressed as n (%) and statistically analyzed using the Chi-square (χ^2) test. Continuous variables (dmft index) that did not meet the normal distribution were expressed as median (Q1 and Q3), and statistically analyzed using the Kruskal-Wallis test. The protective role of BS (independent variable) on caries (dependent variable) was analyzed *via* a binary logistic regression analysis. A $p < 0.05$ represented statistically significant differences.

3. Results

3.1 Prevalence of BS in primary dentition of children aged 3–5 years old

BS was observed in 103 children (15.33%) among the enrolled children between the ages of 3 and 5, including 3 cases with severe BS (0.45%), 28 with moderate BS (4.17%), and 72 with mild BS (10.71%) (Fig. 1A). The onset of BS was not significantly associated with gender ($p = 0.071$) and age ($p = 0.188$) (Table 1).

3.2 Prevalence of caries in primary dentition of children aged 3–5-years-old

A total of 374 children with caries were observed, accounting for 55.65% of enrolled cases. The association between caries and gender was not statistically significant ($p = 0.167$). Notably, a significant association was found between caries and age ($p < 0.001$). The prevalence of caries increased with age, which was 2.98% at 3-years-old, 14.14% at 4-years-old and 38.54% at 5-years old (Table 2).

The prevalence of caries in different primary teeth presented a left-right symmetry in children between the ages of 3 and 5. The prevalence of caries on the maxilla from high to low were primary central incisors (A), primary lateral incisors (B), first

TABLE 1. Prevalence of black stain (BS) in primary dentition of children with different age and gender.

Variable	Gender		Age		
	Male	Female	3-year-old	4-year-old	5-year-old
BS					
Yes	61 (9.08%)	42 (6.25%)	11 (1.64%)	37 (5.51%)	55 (8.19%)
No	282 (41.96%)	287 (42.71%)	47 (6.99%)	164 (24.40%)	358 (53.27%)
χ^2	3.259			3.347	
p value	0.071			0.188	

Notes: The χ^2 test was used to analyze the association of BS with gender and age.

TABLE 2. Prevalence of caries in primary dentition of children with different age and gender.

Variable	Gender		Age		
	Male	Female	3-year-old	4-year-old	5-year-old
Caries					
Yes	182 (27.08%)	192 (28.57%)	20 (2.98%)	95 (14.14%)	259 (38.54%)
No	161 (23.96%)	137 (20.39%)	38 (5.65%)	106 (15.77%)	154 (22.92%)
χ^2	1.909			24.603	
p value	0.167			<0.001*	

*Notes: The χ^2 test was used to analyze the association of caries with gender and age. *Statistical significance at $p < 0.05$.*

primary molars (D), second primary molars (E), and primary canines (C). The prevalence of caries on the mandible from high to low were second primary molars (E), first primary molars (D), primary canines (C), primary central incisors (A), and primary lateral incisors (B). Maxillary primary central incisors (A) and mandibular primary molars (D and E) were more prone to suffer from caries, and the prevalences were all greater than 20% (Fig. 1B).

3.3 Association of BS with caries in primary dentition

The prevalence of caries was higher in children without BS than in those with BS (58.00% *vs.* 42.72%, respectively) (Table 3). A statistically significant relationship was found between BS and caries ($p = 0.004$). Similarly, the dmft index was slightly higher in children without BS than in those with BS ($2.86 \pm 0.15, 2\,(0, 4)$ *vs.* $2.51 \pm 0.35, 0\,(0, 5)$, respectively). However, the association between BS and the dmft index was not statistically significant ($p = 0.079$) (Table 3).

Binary logistic regression analysis was further performed to reveal whether BS is a protective factor for caries in children between the ages of 3 and 5 (Table 4). Since the age is associated with the onset of caries in children, it was adjusted during the analyses. Consequently, children with BS were less likely to develop caries compared to those without BS ($p < 0.001$, OR (Odds Ratio): 0.622, 95% CI (Confidence Interval): 0.501–0.773). The BS was classified according to different degrees and subsequently included in further analyses. Similarly, mild BS was revealed as a protective factor for caries ($p = 0.024$, OR: 0.604, 95% CI: 0.390–0.935). Additionally, moderate + severe BS was a protective factor for caries with statistical significance ($p < 0.001$, OR: 0.627, 95% CI: 0.493–0.797).

4. Discussion

BS is a frequent dental disease in children and its prevalence ranges from 2.4% to 18.5% worldwide [6]. For example, the prevalence of BS is 2.4% in children aged 3–5.5 years in Greece [23], 6.1% in children aged 3–5 years in Tunisia [24], 6.2% in children aged 0–3 years in India [20], 12.1% in children aged 3–6 years in Wenzhou, China, and 18.5% in children aged 3–5 years in Turkey [25]. BS was shown to affect 15.33% of preschool children aged 3–5 years in Qingdao, northern China. This result indicates that the prevalence of BS in primary dentition in Qingdao is at a relatively high level worldwide. Dietary differences may be the main cause for the diversification of BS prevalence in different regions [26]. Additionally, two previous studies performed by our team revealed that the prevalence of BS in preschool children in Qingdao was 12.1% in 2018 [9] and 13.6% in 2019 [10]. From these data, it can be inferred that the prevalence of BS in primary dentition presents an increasing trend in Qingdao, China.

A review based on articles from 2001 to 2014 showed that BS exhibits an equal gender distribution [27]. Consistently, this study revealed that the prevalence of BS in 3–5-year-old children was not significantly different between males and females. This finding confirms that gender cannot affect the prevalence of BS in primary dentition. Furthermore, this study found no significant differences in the prevalence of BS among different ages. Some previous studies in children under 12 years old also support this finding [7, 21]. However, evidence based on patients aged 15–25 years showed that BS is negatively associated with age [28]. Therefore, different age stages may exhibit different effects on the prevalence of BS. The prevalence of BS in primary dentition may not be affected

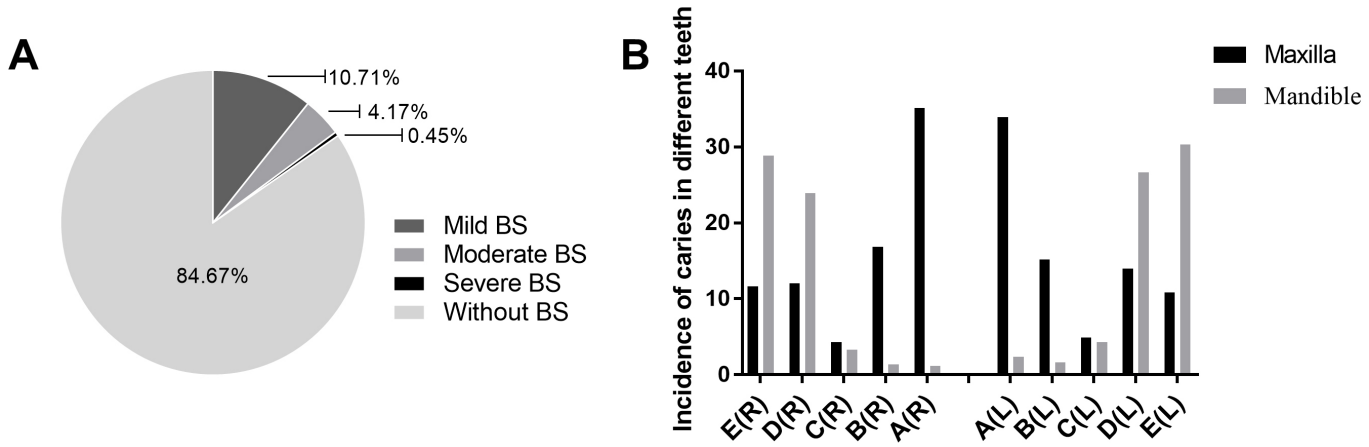

FIGURE 1. The prevalences of black stain (BS) and caries in primary dentition of children aged 3- to 5 years old. (A) The prevalences of BS in different degrees (%). (B) The prevalences of caries in different primary teeth (%). R, Right; L, Left.

TABLE 3. Association of black stain (BS) with caries in children aged 3–5 years.

BS	Total number	Caries	*p*-value	dmft index	*p*-value
Yes	103	44 (42.7%)	0.004*	2.51 ± 0.35, 0 (0, 5)	0.079
No	569	330 (58.0%)		2.86 ± 0.15, 2 (0, 4)	

*Notes: The χ^2 test was used to analyze the association of BS with caries. The Kruskal-Wallis test was used to analyze the association of BS with the dmgt index (mean ± standard error, median (Q1, Q3)). *Statistical significance at $p < 0.05$.*

TABLE 4. Binary logistic regression analysis of black stain (BS) in protecting caries in children aged 3–5 years.

Parameters	β value	SE value	Wald χ^2	*p*-value	OR (95% CI)
BS[a]	−0.475	0.111	18.413	<0.001*	0.622 (0.501–0.773)
Mild BS[a]	−0.504	0.223	5.114	0.024*	0.604 (0.390–0.935)
Moderate + severe BS[a]	−0.467	0.122	14.552	<0.001*	0.627 (0.493–0.797)

*Notes: Caries was defined as dmft ≥1. [a]adjusted for age; *statistical significance at $p < 0.05$. SE, standard error; OR, odds ratio; CI, confidence interval.*

by age.

The prevalence of caries in preschool children in Qingdao was 65.3% in 2018 and 59.4% in 2019 according to studies performed by Ji *et al.* [10] and Gao *et al.* [9]. In this study, 55.6% of preschool children in Qingdao were found to have caries in 2022. This value indicates that the prevalence of caries in primary dentition gradually decreased in Qingdao, which is contrary to the findings of BS. The possible reasons for the decrease in the prevalence of caries may include: (1) improved awareness of the parents of guardians toward protecting primary dentition; and (2) effective promotion of preventive techniques for caries, such as pit-and-fissure sealing and the use of fluoride. Subsequent analysis revealed that the prevalence of caries was not significantly different between males and females. Notably, a significant relationship was found between age and the prevalence of caries in children. This result is consistent with previous findings that the occurrence of caries is positively associated with the age [10, 29–31].

The relationship between BS and caries is a topic of interest for scholars. In this study, children with BS had a significantly lower prevalence of caries (42.7%) than those without BS (58.0%). The dmft index in children with BS was slightly lower than that in children without BS. These results indicate that BS may reduce the risk of caries, which is consistent with previous research worldwide [6]. Furthermore, binary logistic regression analysis confirmed that children with BS were less likely to develop dental caries than children without BS. Moreover, mild and moderate/severe BS were both associated with a lower risk of caries in primary dentition. These results demonstrate that BS may be a protective factor for caries. Oral microflora is involved in the underlying mechanisms of BS affecting caries since it is critical in the pathogenesis of BS and caries. For example, the predominance of *Actinomyces* and low adhesion of *Streptococcus mutans* in BS are associated with a low risk of caries [18, 19]. Furthermore, patients with BS have more calcium, sodium, copper, and inorganic phosphate in the saliva. These components may protect tooth enamel through enhancing buffering capacity and

reducing enamel solubility, thereby inhibiting the occurrence and progression of caries [6].

This study has some limitations. First, the sample size is limited, leading to a relatively small number of children suffering from moderate or severe BS. Second, the detailed effects of different degrees of BS on the initiation and progression of caries at specific tooth positions remain unclear. Third, the underlying mechanisms of BS affecting caries in primary dentition are not clearly revealed. These issues need to be addressed in future studies.

5. Conclusions

The prevalence of BS in preschool children aged 3–5 years (primary dentition) is 15.33% in Qingdao, China. Caries affected primary dentition in 55.65% of the enrolled children. Notably, the prevalence of caries was lower in children with BS than in those without BS. Thus, BS may be a protective factor for caries in primary dentition.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

CQ, RH and LM—designed the research study. CQ, RH and JY—performed the research. CQ and HH—analyzed the data. CQ and RH—drafted the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This investigation was approved by the Ethics Committee of The Affiliated Hospital of Qingdao University (QYFYWZLL25820), and informed consent was obtained from the legal guardians of enrolled children.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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